

Ameliorative efficacy of quercetin against cisplatin-induced mitochondrial dysfunction: Study on isolated rat liver mitochondria

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Abstract. The present study aimed to investigate the hepatoprotective effects of the bioflavonoid quercetin (QR) on cisplatin (CP)-induced mitochondrial oxidative stress in the livers of rats, to elucidate the role of mitochondria in CP-induced hepatotoxicity, and its underlying mechanism. Isolated liver mitochondria were incubated with 100 μ g/ml CP and/or 50 μ M QR *in vitro*. CP treatment triggered a significant increase in membrane lipid peroxidation (LPO) levels, protein carbonyl (PC) contents, and a decrease in reduced glutathione (GSH) and non-protein thiol (NP-SH) levels. In addition, CP caused a marked decline in the activities of enzymatic antioxidants and mitochondrial complexes (I, II, III and V) in liver mitochondria. QR pre-treatment significantly modulated the activities of enzymatic antioxidants and mitochondrial complex enzymes. Furthermore, QR reversed the alterations in LPO and PC levels, and GSH and NP-SH contents in liver mitochondria. The results of the present study suggested that QR supplementation may suppress CP-induced mitochondrial toxicity during chemotherapy, and provides a potential prophylactic and defensive candidate for anticancer agent-induced oxidative stress.

Introduction

Chemotherapy is an important cancer treatment and numerous anti-cancer agents have been developed. However, the adverse effects and systemic toxicity induced by these agents limits their application (1,2). Cisplatin (cisdiamminedichloroplatinum II; CP) is a platinum-containing anticancer therapeutic

that is used for the treatment of various human carcinomas, including head, oral, lung and neck cancer, metastatic tumors of testis and ovaries, progressed bladder cancer and other solid tumors. The exact mechanism underlying CP-induced toxicity remains to be fully elucidated. Previous studies have demonstrated that the anticancer behavior of CP originates from its capacity to attach to the N-7 position of purine bases of cellular DNA causing mono-adducts formation, which are converted into inter- and intra-strand cross links with a reaction at the secondary reactive site of drug together with the second nucleobase (3-5). Mitochondria have been revealed to be the cellular power plants (6,7). A direct association between mitochondrial dysfunction and the toxicity of chemotherapeutic agents has been demonstrated (8), and mitochondria are now considered anticancer drug targets. Previous *in vitro* studies have revealed that CP-treated rat hepatic cells undergo alterations to mitochondrial structure and function (9,10). These alterations may be crucial in strengthening various aspects of CP hepatotoxicity. Natural antioxidants have been investigated as potential nutraceuticals to minimize the adverse effects and increase the efficacy of chemotherapeutic agents (11). Quercetin (3,3',4',5,7-pentahydroxyflavone; QR) is a large class of polyphenolic compounds ubiquitously present in plants and food sources. It is primarily present in vegetables, fruits, red wine, tea and other aromatic plants (12). QR has been investigated as a therapeutic agent to ameliorate various toxicities, including nephrotoxicity (13), cardiotoxicity (14), neurotoxicity (15) and hepatotoxicity (16).

In addition, QR has been reported to contribute to various pharmacological and biological activities, including antimicrobial (17), antioxidant (18), anti-inflammatory (19) and anticancer activities (20). It has been demonstrated to inhibit oxidative stress-induced mitochondrial damage (21). The present study aimed to investigate the effects of QR on CP-induced mitochondrial dysfunction using an *in vitro* model.

Materials and methods

Chemicals. 4-amino-3-hydroxy-1-naphtalenesulfonic acid (ANSA), bovine serum albumin (BSA), butylated hydroxy toluene (BHT), 1-chloro-2, 4 dinitrobenzene (CDNB), 2,6, dichlorophenol indophenols (DCIP), 2,4-dinitrophenyl

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hydrazine (DNPH), 5,5'-dithiobis (2-nitrobenzoic acid) (DTNB), ethylenediaminetetraacetic acid (EDTA), ethylene glycol-O, -O'-bis, (2-Aminoethyl) tetraacetic acid epinephrine, reduced glutathione (GSH), hydrogen peroxide (H_2O_2), nicotinamide adenine dinucleotide reduced (NADH), nicotinamide adenine dinucleotide phosphate reduced tetra sodium salt (NADPH), o-phosphoric acid (OPA), thiobarbituric acid (TBA) and trichloro acetic acid (TCA) were purchased from Sigma-Aldrich; Merck Millipore (Darmstadt, Germany). CP and QR were obtained from Dr Reddy's Laboratories, Ltd. (Hyderabad, India) and HiMedia Laboratories Pvt. Ltd. (Mumbai, India), respectively. General chemicals were purchased from Sigma-Aldrich; Merck Millipore, Sisco Research Laboratories Pvt. Ltd. (Mumbai, India) and Merck Ltd. India (Mumbai, India).

Animals. Male Wistar rats ($n=24$; weight, 180-250 g) were acquired from the Central Animal House of Jamia Hamdard (New Delhi, India). The rats were acclimatized for a week prior to the initiation of the experiments. Animals were housed at a temperature of $22\pm 2^\circ\text{C}$ and a relative humidity of $65\pm 10\%$ under a 12-h light/dark cycle, and had *ad libitum* access to standard rodent food and deionized water. Experiments were performed according to the standard guidelines of Institutional Animal Ethics Committee of Jamia Hamdard (New Delhi, India). The study was approved by the Institutional Animal Ethics Committee of Jamia Hamdard.

Mitochondrial preparation. Mitochondria were isolated by differential centrifugation, as previously described (22). Briefly, liver from anaesthetized (Nembutal, 150 mg/kg, *i.p.*, Sigma-Aldrich) adult rats were excised and homogenized using a mechanical Potter Elvehjem homogenizer in an ice-cold isolation buffer containing 0.25 M sucrose, 1 mM EDTA adjusted with Tris to pH 7.4, and centrifuged at $800 \times g$ for 5 min at 4°C . The supernatant was centrifuged at $5,100 \times g$ for 4 min at 4°C . Subsequently, the obtained pellet was resuspended in a 0.25 M sucrose solution adjusted with Tris to pH 7.4, and centrifuged at $12,300 \times g$ for 2 min at 4°C . Finally, the pellet was resuspended in a 0.25 M sucrose solution adjusted with Tris to pH 7.4, centrifuged at $12,300 \times g$ for 10 min (4°C) and resuspended in a buffer containing 0.25 M sucrose, 0.5 mM EDTA adjusted with Tris to pH 7.4. The protein concentration of the stock mitochondrial stock preparation was 4.5 mg/ml, as determined by Waseem and Parvez (22).

Experimental design (pre-incubation). For *in vitro* investigations of CP-induced hepatic mitotoxicity and its modulation by QR, mitochondrial samples were analyzed as following: Group I (untreated control), group II (QR), group III (CP) and group IV (CP with QR pre-treatment). In group IV, liver mitochondria were pre-treated with $50 \mu\text{M}$ QR at 37°C for 1 h prior to exposure to $100 \mu\text{g/ml}$ CP for 1 h (22). The concentration of QR was selected according to previous *in vitro* studies on hepatic and non-hepatic cells (17,21). The schedule was designed so that the end point of all groups occurred concurrently.

Evaluation of mitochondrial lipid peroxidation (LPO). LPO was quantified using the protocol described by Waseem and

Parvez (22). The reaction mixture consisted of 0.01 M BHT, 6.7 mg/ml TBA, 1% chilled OPA and 250 μl mitochondrial preparation. The rate of LPO was determined as nmoles thiobarbituric acid reactive substances formed/h/g of tissue using a molar extinction coefficient of $1.56 \times 10^5 \text{ M}^{-1}\text{cm}^{-1}$.

Estimation of mitochondrial protein oxidation (PC). PC content was assessed using the protocol described by Waseem and Parvez (22). Mitochondria (2 mg/ml) were mixed with 0.01 M DNPH in 2 M HCl for 1 h at room temperature and precipitated with 60 mg/ml TCA. The protein pellet was washed two or three times with a solution of ethanol/ethyl acetate (1:1 ratio, v/v). Proteins were subsequently solubilized in 6 M guanidine hydrochloride and 50% formic acid, and centrifuged at $10,000 \times g$ for 5 min at 4°C . The carbonyl level was quantified spectrophotometrically at a wavelength of 360 nm. Results were expressed as nmoles DNPH incorporated/mg protein using a molar extinction coefficient of $21,000 \text{ M}^{-1}\text{cm}^{-1}$.

Determination of mitochondrial GSH. The GSH level was assessed according to the procedure of Tabassum *et al.* (23). Mitochondria were primarily precipitated with 40 mg/ml sulphosalicylic acid and were maintained at 4°C for 1 h, followed by centrifugation at $1,500 \times g$ for 15 min at 4°C . The reaction mixture (total volume, 3 ml) consisted of 100 mM sodium phosphate buffer (pH 7.4), 0.01 M DTNB and 400 μl mitochondrial stock preparation. The absorbance of the reacted product was measured at a wavelength of 412 nm on a dual-beam spectrophotometer. The GSH content was expressed as μmoles GSH/g tissue.

Estimation of mitochondrial non-protein-bound thiols (NP-SH). NP-SH levels were measured according to the protocol of Waseem and Parvez (22), with certain minor modifications. Mitochondria (1-2 mg/ml) were precipitated with 400 mg/ml TCA and subsequently centrifuged at $3000 \times g$ for 15 min at 4°C . Following this, 400 mM Tris buffer (pH 8.9) and 10 mM DTNB were added to the supernatant. Absorbance was measured at a wavelength of 412 nm, and results were expressed as μmoles NP-SH/g tissue using a molar extinction coefficient of $13,100 \text{ M}^{-1}\text{cm}^{-1}$.

Activity of mitochondrial glutathione S-transferase (GST). The method of Waseem and Parvez (22) was used to evaluate GST activity, with certain minor modifications. The reaction mixture consisted of 100 mM sodium phosphate buffer (pH 7.4), 10 mM GSH, 10 mM CDNB and 100 μl mitochondrial suspension. Absorbance was measured at a wavelength of 340 nm at 30 sec intervals for 3 min, and results were expressed as μmoles CDNB conjugate formed/min/mg protein using a molar extinction coefficient of $9.6 \times 10^3 \text{ M}^{-1}\text{cm}^{-1}$.

Kinetics of mitochondrial glutathione peroxidase (GPx). The method of Waseem and Parvez (22) was used to evaluate GPx activity, with certain minor modifications. The reaction mixture consisted of 100 mM sodium phosphate buffer, 1 mM EDTA, 1 mM sodium azide, 10 mM GSH, 2 mM NADPH, 10 μl H_2O_2 (10.32 M) and 4-5 mg/ml mitochondrial suspension, in a final volume of 2 ml. NADPH oxidation was measured

kinetically at a wavelength of 340 nm at 30 sec intervals for 3 min. The enzyme activity was calculated as nmoles NADPH oxidized/min/mg protein, using a molar extinction coefficient of $6.22 \times 10^3 \text{ M}^{-1}\text{cm}^{-1}$.

Activity of mitochondrial manganese-superoxide dismutase (Mn-SOD). Mn-SOD activity was assessed according to the procedure of Waseem and Parvez (23). Mitochondria (180 μl stock preparation) were treated with 0.05 M glycine buffer (pH 10.4) and 20 mg/ml epinephrine. The enzymatic activity was measured kinetically at a wavelength of 480 nm at 30 sec intervals for 3 min. The activity was expressed as nmoles epinephrine protected from oxidation/min/mg protein using a molar extinction coefficient of $4,020 \text{ M}^{-1}\text{cm}^{-1}$.

Activity of complex I (NADH-dehydrogenase). The procedure of King and Howard (24) was used to measure NADH-dehydrogenase activity, with certain minor modifications. The reaction mixture consisted of 600 μM DCIP, 2 mM glycyl glycine buffer, 600 μM NADH and 100 μl mitochondrial stock preparation. The absorbance was measured at a wavelength of 600 nm. The enzyme activity was expressed as nmoles NADH oxidized/min/mg protein using a molar extinction coefficient of $21,000 \text{ M}^{-1}\text{cm}^{-1}$.

Activity of complex II (succinate dehydrogenase). Succinate dehydrogenase activity was assessed according to the protocol of Waseem and Parvez (22). The reaction mixture consisted of 100 mM phosphate buffer (pH 7.4), 10 mg/ml BSA, 0.0015 M potassium ferricyanide, 15 mM sodium succinate and 100 μl mitochondrial stock preparation. The absorbance was measured for 3 min at a wavelength of 420 nm. The enzyme activity was expressed as nmoles succinate produced/min/mg protein using a molar extinction coefficient of $1,000 \text{ M}^{-1}\text{cm}^{-1}$.

Activity of complex III (mitochondrial dehydrogenase, MTT). The MTT reduction rate was used to measure the mitochondrial respiratory complex activity according to the method of Kamboj *et al* (25), with certain minor modifications. Briefly, 100 μg mitochondrial preparation was suspended in 1.5 ml eppendorf tubes with ice cold buffer C, and incubated at 37°C in the presence of 20 μl of MTT (0.1 mg/ml). After 30 min incubation period, tubes were centrifuged at $1,000 \times g$ for 10 min, and the blue formazan crystals were solubilised in 1 ml DMSO. The absorbance was measured at 595 nm. The results were expressed as nmoles formazan formed/min/mg protein using a molar extinction coefficient of $51,000 \text{ M}^{-1}\text{cm}^{-1}$.

Activity of complex V (total ATPase). Total ATPase activity was quantified by measuring the hydrolysis rate of ATP to ADP and inorganic phosphate (Pi), according to the protocol of Waseem and Parvez (22), with certain minor modifications. Mitochondria (0.2 mg stock preparation) were incubated in ATPase buffer (50 mM Tris and 5 mM MgCl_2 , pH 7.5) at 37°C with 5 mM ATP for 10 min. The reaction was terminated via the addition of 100 mg/ml TCA. The suspension was subsequently centrifuged at $3,000 \times g$ for 20 min at 4°C , and the supernatants were mixed with 0.5 ml distilled water. The reaction measuring Pi production was initiated by adding a mixture containing 720 mM sodium bisulfite, 41.6 mM sodium

sulfite and 10 mM ANSA. The enzyme activity was measured at 660 nm and expressed as μg Pi liberated/min/mg protein.

Protein content determination. The protein content of mitochondria was assessed according to the protocol described by Lowry *et al* (26). BSA (1 mg/ml) served as the standard.

Statistical analysis. Results are expressed as the mean \pm standard error. Data were analyzed using one-way analysis of variance followed by Tukey's post hoc test. $P < 0.05$ was considered to indicate a statistically significant difference. Statistical analyses were performed using GraphPad Prism software version 5 (GraphPad Software, Inc., La Jolla, CA, USA).

Results

QR inhibits the CP-induced increase in LPO and PC levels. CP treatment (group III) significantly increased LPO (Fig. 1A) and PC (Fig. 1B) levels ($P < 0.001$, respectively) compared with the control group (group I). QR pre-treatment (group IV) significantly decreased LPO and PC levels ($P < 0.001$) compared with CP treatment alone (group III). QR treatment alone (group II) significantly decreased LPO levels compared with the control group ($P < 0.01$); however, it had no significant effect on PC levels.

QR attenuates the CP-induced decrease in GSH and NP-SH levels. CP treatment significantly decreased GSH (Fig. 2A) and NP-SH (Fig. 2B) levels in liver mitochondria compared with the control group ($P < 0.001$). QR pre-treatment significantly attenuated these effects ($P < 0.001$). QR alone significantly increased GSH levels compared with the control group ($P < 0.001$); however, it had no significant effect on NP-SH content.

QR protects against the CP-induced decrease in glutathione metabolizing enzyme levels. CP treatment significantly decreased GST (Fig. 3A) and GPx (Fig. 3B) activities compared with the control group ($P < 0.01$ and $P < 0.001$, respectively). QR pre-treatment significantly attenuated these effects. QR alone had no significant effects on GST or GPx activities.

QR modulates the CP-induced decrease in Mn-SOD activity. CP treatment significantly decreased Mn-SOD activity (Fig. 4) in liver mitochondria compared with the control group ($P < 0.01$). QR pre-treatment significantly abrogated this effect ($P < 0.01$). QR alone had no significant effect of Mn-SOD.

QR ameliorates the CP-induced decrease in complex I and II enzyme activities. CP treatment significantly reduced NADH dehydrogenase (Fig. 5A) and succinate dehydrogenase (Fig. 5B) activities compared with the control group ($P < 0.01$ and $P < 0.001$, respectively). QR pre-treatment significantly attenuated these effects ($P < 0.001$). QR alone significantly increased succinate dehydrogenase activity compared with the control group ($P < 0.01$); however, it had no significant effect on NADH dehydrogenase activity.

QR modulates the CP-induced inhibition of complex III and V enzyme activities. CP treatment significantly decreased MTT

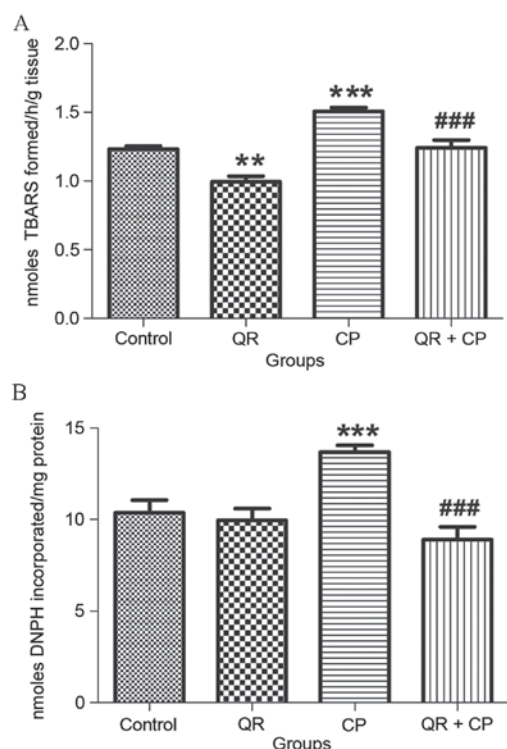


Figure 1. Effect of 100 µg/ml CP and 50 µM QR on LPO and PC levels in rat liver mitochondria. QR inhibited the CP-induced increase in (A) LPO and (B) PC levels. Results are expressed as nmols TBARS formed/h/g protein and nmols DNPH incorporated/mg protein. Data are expressed as the mean \pm standard error (n=6). **P<0.01 and ***P<0.001 vs. control; ###P<0.001 vs. CP. CP, cisplatin; QR, quercetin; LPO, lipid peroxidation; PC, protein oxidation; TBARS, thiobarbituric acid reactive substances; DNPH, 2,4-dinitrophenyl hydrazine.

ability (Fig. 6A) and total ATPase activity (Fig. 6B) compared with the control group (P<0.01, respectively). QR pre-treatment significantly abrogated these effects (P<0.01 and P<0.001, respectively). QR alone significantly increased MTT ability compared with the control group (P<0.05); however, it had no significant effect on total ATPase activity.

Discussion

CP is a platinum-based heavy metal that is a chemotherapeutic agent used for the treatment of various cancers (22). However, the application of CP is limited as a result of the hepatotoxicity that has been demonstrated to develop following treatment in various animal models. Our previous study (27) was the first to observe that CP significantly elevated LPO levels in isolated rat liver mitochondria. LPO has been suggested as a primary underlying mechanism by which free radicals damage cells. The significant increase in LPO levels may be due to its poor antioxidant defenses or the non-stimulation of antioxidant enzymes, due to oxidative stress. In addition, increased LPO levels or a reduction in antioxidants have been associated with complex IV activity decrease, which may ultimately result in mitochondria-dependent apoptosis (28). In the present study, QR pre-treatment was observed to significantly restore LPO levels and alter antioxidant status. QR decreased the oxidative stress marker LPO via reactive oxygen species (ROS) scavenging (29). In

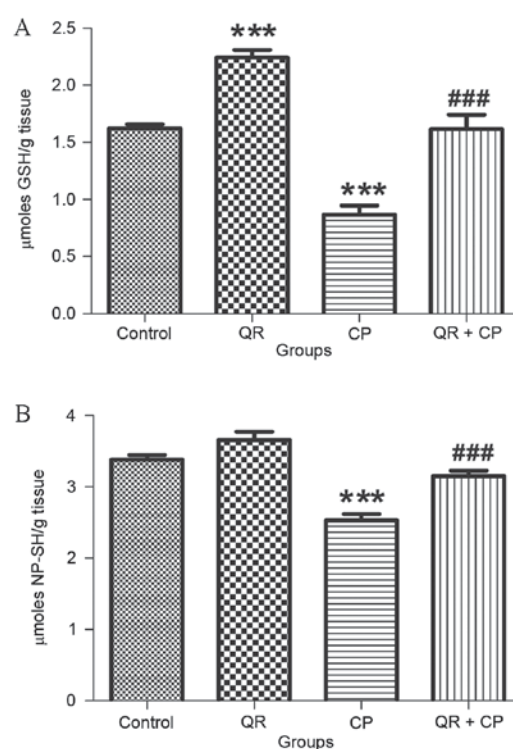


Figure 2. Effect of 100 µg/ml CP and 50 µM QR on GSH and NP-SH contents in rat liver mitochondria. QR attenuated the CP-induced decrease in (A) GSH and (B) NP-SH levels. Results are expressed as µmoles GSH/g tissue and µmoles NP-SH/g tissue. Data are expressed as the mean \pm standard error (n=6). ***P<0.001 vs. control; ###P<0.001 vs. CP. CP, cisplatin; QR, quercetin; GSH, reduced glutathione; NP-SH, non-protein-bound thiols.

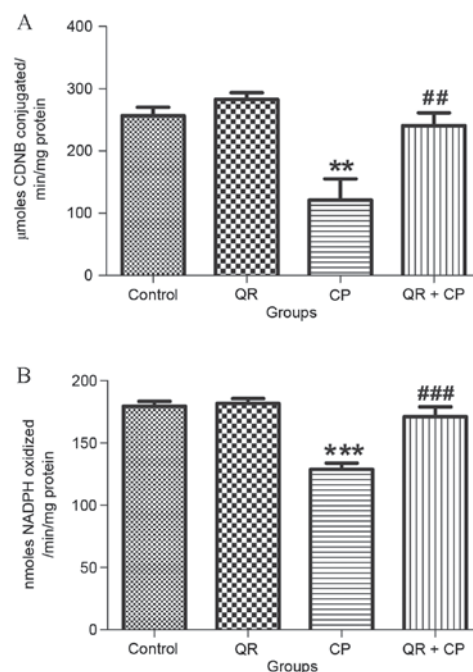


Figure 3. Effect of 100 µg/ml CP and 50 µM QR on GST and GPx activities in rat liver mitochondria. QR protected against the CP-induced decrease in (A) GST and (B) GPx activities. Results are expressed as µmoles CDNB conjugated/min/mg protein and nmols NADPH oxidized/min/mg protein. Data are expressed as the mean \pm standard error (n=6). **P<0.01 and ***P<0.001 vs. control; ##P<0.01 and ###P<0.001 vs. CP. CP, cisplatin; QR, quercetin; GST, glutathione S-transferase; GPx, glutathione peroxidase; CDNB, 1-chloro-2, 4 dinitrobenzene; NADPH, nicotinamide adenine dinucleotide phosphate reduced tetra sodium salt.

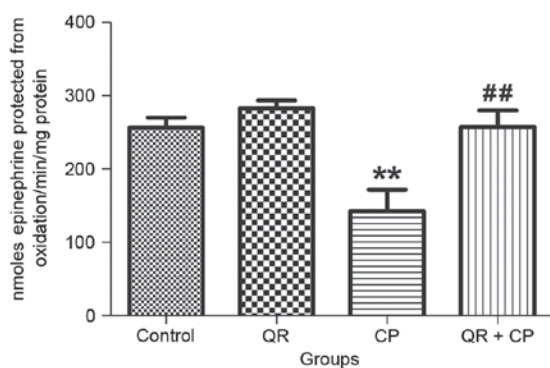


Figure 4. Effect of 100 $\mu\text{g/ml}$ CP and 50 μM QR on Mn-SOD activity in rat liver mitochondria. QR modulated the CP-induced decrease in Mn-SOD activity. Results are expressed as nmol epinephrine protected from oxidation/min/mg protein. Data are expressed as the mean \pm standard error ($n=6$). ** $P<0.01$ vs. control; ## $P<0.01$ vs. CP. CP, cisplatin; QR, quercetin; Mn-SOD, manganese-superoxide dismutase.

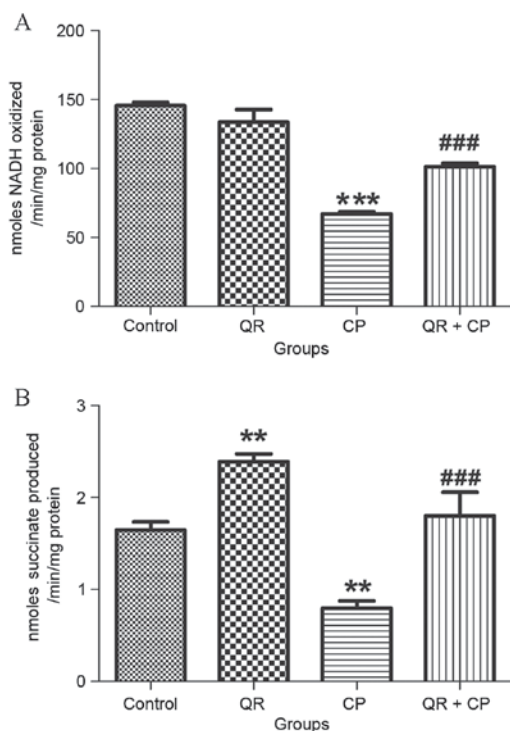


Figure 5. Effect of 100 $\mu\text{g/ml}$ CP and 50 μM QR on NADH-dehydrogenase and succinate dehydrogenase activities in rat liver mitochondria. QR attenuated the CP-induced decrease in (A) NADH-dehydrogenase and (B) succinate dehydrogenase activities. Results are expressed as nmol NADH oxidized/min/mg protein and nmol succinate oxidized/min/mg protein. Data are expressed as the mean \pm standard error ($n=6$). ** $P<0.01$ and *** $P<0.001$ vs. control; ### $P<0.001$ vs. CP. CP, cisplatin; QR, quercetin; NADH, nicotinamide adenine dinucleotide reduced.

the present study, QR prevented the CP-induced increase in LPO levels, which may sustain cellular integrity and defense against damage due to free radicals.

PC is an extensively used biomarker and the predominant indicator of protein carbonyl accumulation and protein oxidation. The results of the present study support this, as PC content increased in isolated liver mitochondria on exposure to CP. The biomarker of oxidative protein damage is protein

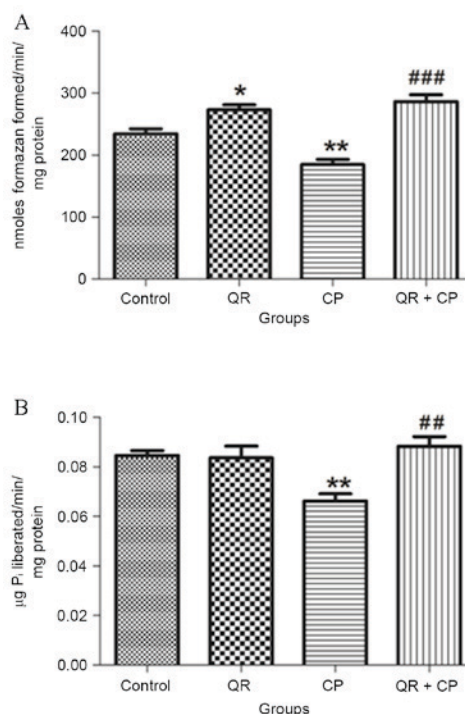


Figure 6. Effect of 100 $\mu\text{g/ml}$ CP and protective effect of 50 μM QR on MTT level and total ATPase activity in rat liver mitochondria. QR abrogated the CP-induced decrease in (A) MTT ability and (B) total ATPase activity. Results are expressed as nmol formazan formed/min/mg protein and μg Pi liberated/min/mg protein. Data are expressed as the mean \pm standard error ($n=6$). * $P<0.05$ and ** $P<0.01$ vs. control; ## $P<0.01$ and ### $P<0.001$ vs. CP. CP, cisplatin; QR, quercetin; Pi, inorganic phosphate.

carbonylation, as a result of xenobiotically-induced oxidative stress. QR pre-treatment significantly restored PC contents in isolated liver mitochondria. The modulatory role of natural compounds on protein carbonylation has been demonstrated by previous studies (30).

GSH is the primary antioxidant molecule and detoxifies various types of endogenous and exogenous toxicants, including CP, via GSH adducts formation (22). Additionally, the redox cycle of GSH, consisting of GSH, GPx and glutathione reductase, is crucial in scavenging the ROS generated by CP, to protect cells from potential toxicity and carcinogenesis (31). In the present study, CP significantly reduced the levels of GSH in liver mitochondria. QR treatment increased mitochondrial GSH levels and pre-treatment with QR inhibited the CP-induced reduction in GSH levels. These results suggested that cells protected by QR pre-treatment are less susceptible to CP-induced mitochondrial oxidative stress.

In the present study, NP-SH levels were significantly decreased in isolated liver mitochondria by CP treatment, which is in accordance with the GSH results, as GSH levels in liver tissue comprise >90% of the NP-SH pool. It is recognized that the total cellular thiol pool is integral in homeostasis and is additionally important in oxidative physiology. Treatment with QR increased the mitochondrial NP-SH levels (32) and QR pre-treatment significantly attenuated the CP-induced reduction in NP-SH levels, suggesting that cells protected by QR pre-treatment are less susceptible to CP-induced mitochondrial oxidative stress.

GSTs are a group of enzymes that conjugate GSH to structurally diverse electrophilic compounds. GST catalyzes the conjugation of GSH via a sulfhydryl group to electrophilic centers on a wide variety of substrates. GST is responsible for scavenging organic peroxides, and endogenous and exogenous electrophiles. Diverse forms of GST have been revealed to bind CP *in vivo* and *in vitro* (33). In the present study, it was observed that CP significantly decreased GST activity in isolated liver mitochondria. Reduced mitochondrial GST activity may be associated with an elevation in ROS generation following tissue injury (22). In the present study, QR pre-treatment restored the activity of GST. This may provide protection from oxidative stress due to excess O₂ and H₂O₂. Mitochondrial oxidative damage occurs as a results of the respiratory chain; complex I and III are the foremost sources of superoxide anion (O₂⁻) (22). Energy or ATP production by oxidative phosphorylation takes place in mitochondria and is catalyzed by membrane-bound protein complexes, namely NADH-dehydrogenase (complex I), succinate dehydrogenase (complex II), cytochrome c oxidoreductase (complex III) and total ATPases. Succinate dehydrogenase contributes only by transferring electrons to the electron transport chain (ETC), whereas NADH-dehydrogenase is associated with proton translocation and electron transfer. The defect in any of the enzyme complexes responsible for oxidative metabolism may lead to mitochondrial cytopathy (22) and to the opening of the mitochondrial permeability transition pore that allows the membrane potential to dissipate resulting in uncoupling of oxidative phosphorylation and therefore impaired cellular ATP production. Previous studies have demonstrated that mitochondrial dysfunctions are involved in mitochondrial toxicity induced by platinum-based chemotherapeutic agents, including CP (2,22). The results of the present study suggested that CP inactivated mitochondrial complex enzymes, as indicated by a reduction in NADH-dehydrogenase and succinate dehydrogenase activities, MTT ability and ATPase activity. QR pre-treatment significantly protected these complex enzymes. QR has been revealed to modulate mitochondrial dysfunction in rodents and its property as an antioxidant is hypothesized to be responsible for its protective effects in mitochondria (5,9).

The alterations in the activities of mitochondrial complex enzymes may be involved in hepatotoxicity. This may be as a result of free radicals, as well as a reduction in mitochondrial transcription and translation. Furthermore, it has been revealed that mitochondrial activity interference is associated with effects on complex enzymes, particularly complexes I and III of the ETC, which result in increased mitochondrial electron leakage. QR pre-treatment restored the activity of complex enzymes in liver mitochondria. This may be due to the increase in the scavenging and inactivation of H₂O₂ and hydroxyl radicals caused by QR (34). Previous studies have demonstrated that QR protects against mitochondrial oxidative stress and actively increases biologically active mitochondria in cells, following treatment with the concentration used in the present study (5,9).

In conclusion, the results of the present study suggested that CP exerts hepatotoxic effects through the induction of oxidative stress, indicated by the alterations in the concentrations of mitochondrial complex enzymes and non-enzymatic antioxidants. The protective effect of QR was associated with

its antioxidant potential, as it potentially acts as a free radical scavenger, LPO inhibitor and GSH activator. Further studies, particularly molecular experiments, are required to investigate the mitigatory effect of QR on mitochondria-mediated anti-cancer drug toxicities.

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