

Protective effects of N-acetylcysteine against cadmium-induced damage in cultured rat normal liver cells

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Abstract. In this study, the protective effects of N-acetylcysteine (NAC), a precursor of reduced glutathione, were studied by measuring the viability, the levels of antioxidant enzymes, and by analyzing the cell cycle in cadmium (Cd)-treated rat liver cells. The cells were treated with 150 μ M CdCl₂ alone or co-treated with 150 μ M CdCl₂ and 5 mM NAC (2 h pre-, simultaneous or 2 h post-treatment) for 24 h. The viability of the cells treated with 150 μ M CdCl₂ alone decreased to 40.1%, while that of the cells co-treated with 5 mM NAC (pre-, simultaneous and post-treatment) significantly increased to 83.7, 86.2 and 83.7%, respectively in comparison to the control cells (100%). The catalase enzyme level decreased to undetectable level in the cells treated with CdCl₂ alone, while it significantly increased in the co-treated cells (pre-, simultaneous and post-treatment) to 40.1, 34.3 and 13.2%, respectively. In the cells treated with CdCl₂ alone, the glutathione peroxidase enzyme level decreased to 78.3%, while it increased in the co-treated cells (pre-, simultaneous, and post-treatment) to 84.5, 83.3 and 87.9%, respectively. The glutathione reductase enzyme level decreased to 56.1% in the cells treated with cadmium alone, but significantly increased in the cells co treated with NAC (pre-, simultaneous and post-treatment) to 79.5, 78.5 and 78.2%, respectively. Cd caused cell cycle arrest at the S and G2/M phases. The co-treatment with NAC inhibited cell cycle arrest by shifting the cells to the G1 phase. These results clearly show the protective effects of NAC against Cd-induced damage in rat liver cells.

Introduction

Cadmium (Cd) is one of the environmental, industrial and hazardous toxicants and it is classified as a type I carcinogen (1,2). It is a highly reactive metal affecting many human

organs depending on the dose, route and duration of exposure (3,4). Furthermore, Cd is implicated in the development of cancer in various organs, such as the lung, liver and kidney (5,6). Human exposure occurs mainly through inhalation (cigarette smoke, occupation), oral (food and water) and dermal absorption (occupational exposure) (7). Cd is widely used in smelting, zinc refining, electroplating, galvanizing, nickel-Cd battery production and welding.

Cd induces oxidative stress by generating free radicals and thereby increasing lipid peroxidation and altering the antioxidant status in the liver cells (8,9). Although the general level of Cd exposure is normally quite low, due to its long biological half-life of >20 years, it accumulates inside the body parts (10,11). Cd is considered to be a multi-target toxicant and it mainly accumulates in the liver and kidney after absorption (12). Many substances that act as chelators and/or antioxidants have been used to prevent and/or treat Cd-induced cytotoxicity in animal models or cell lines. Substances, such as selenium (13), diallyl tetrasulfide (14) and picroliv (15), have been experimentally proven to provide protection against Cd toxicity.

N-acetylcysteine (NAC), a small molecule containing a thiol group (16) and a precursor of reduced glutathione (GSH) (17), has also been clinically used for >30 years as a mucolytic agent with a broad application for the treatment of HIV, cancer, heart disease (18,19) and the management of acetaminophen poisoning due to its hepatoprotective activity (20). Furthermore, it also has clinical usefulness in the treatment of acute heavy metal poisoning (21) both as a protective agent of the liver and kidney due to its antioxidative properties and as a chelating agent in the elimination of metals due to its thiol group. Previously, we studied the effect of Cd on the antioxidative enzyme status in the cytoplasm and mitochondria of the normal rat liver cell line, CRL-1439 (9).

The primary objective of this study was to investigate the protective effects of NAC on the cytotoxicity, antioxidative enzyme status, and cell cycle phases in the Cd-induced cytotoxic rat liver CRL-1439 cell line.

Materials and methods

Chemicals. F12K medium, penicillin-streptomycin anti-biotic solution (100X), fetal bovine serum (FBS), trypsin-EDTA solution (1X), phosphate buffered saline (PBS), CdCl₂,

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glutaraldehyde, crystal violet, 5'5-dithiobis-(2-nitrobenzoic acid) (DTNB), NADPH, GSH reductase (GR), oxidized GSH, sodium azide, Tris-HCl, propidium iodide, and RNase were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). Potassium phosphate, EDTA, D-glucose, ethanol, SDS, sodium chloride and sodium citrate were purchased from Thomas Scientific Co. (Swedesboro, NJ, USA).

Maintenance of cell line. The rat normal liver CRL-1439 epithelial cell line was purchased from the American Type Culture Collection (ATCC, Rockville, MD, USA) and cultured as per the supplied guidelines. The cells were maintained in F12K medium containing 100 units of penicillin/ml, 100 μ g of streptomycin/ml, 2 mM L-glutamine, and 10% fetal bovine serum in T-75 cm² flasks at 37°C in a 5% CO₂ incubator.

Treatment of cells with Cd. The rat liver cells were plated at a density of 10x10⁴ cells per well in polystyrene, flat-bottomed 24-well microtiter plates (Corning Costar, Rochester, NY) in F12K medium containing 10% FBS and were allowed to stabilize overnight in a CO₂ incubator at 37°C. Following this, the cells were treated with 0, 50 and 150 μ M CdCl₂ alone or co-treated with 5 mM NAC (2 h pre-, simultaneous, or 2 h post-treatment) in a final volume of 1 ml per well in triplicate wells for each treatment for 24 h at 37°C in a 5% CO₂ incubator. In the simultaneous treatment, CdCl₂ and NAC were added to the cells simultaneously and in the post treatments, the cells were first treated with CdCl₂ before NAC. All the experiments were repeated at least twice. The untreated cells served as the control (0 μ M CdCl₂).

Evaluation of cell viability. At the end of the incubation period, cell viability was evaluated by the dye uptake assay according to Badisa *et al.* (22). Glutaraldehyde (400 μ l of 0.25%) was added to each well and incubated for 30 min at room temperature to fix the cells. The glutaraldehyde (0.07% final concentration in the well) in the crystal violet dye staining assay procedure fixed the viable cells after the treatment with the compound. The plates were rinsed with water to wash off the dead cells and dried under airflow inside the laminar hood for 5-10 min. Crystal violet (400 μ l of 0.1%) was added to each well, incubated for 15 min, washed and dried. To solubilize the dye, 1 ml of 0.05 M sodium phosphate solution (monobasic) in 50% ethyl alcohol was added to each well and the plates were read at 540 nm in a plate reader (Bio-Tek EL800 Plate Reader).

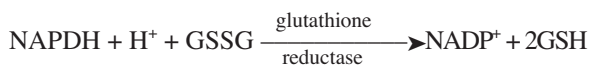
Preparation of enzyme extracts. Crude enzyme extracts were prepared as per the method of Ikediobi *et al.* (8). Approximately 3.9x10⁶ cells per T-75 flask were plated and stabilized overnight. Following this, the cells were treated with 0, 50 and 150 μ M CdCl₂ alone or co-treated with 150 μ M CdCl₂ and 5 mM NAC (2 h pre-, simultaneous, or 2 h post-treatment) in a final volume of 10 ml per flask in triplicate for 24 h at 37°C in a 5% CO₂ incubator. At the end of incubation, the cells were trypsinized and pelleted by centrifugation at 2,500 rpm for 5 min. The cell pellets were suspended in 1 ml of 50 mM PBS, pH 7.0 and homogenized with Polytron homogenizer in a glass vial on ice for 1 min at intervals of 15 sec. The homo-

genates were transferred to Eppendorff tubes and centrifuged at 3,000 rpm for 10 min at 4°C to remove the lysed cell membrane debris. The supernatants were transferred to new tubes, stored at 4°C and used for enzyme assay experiments.

Catalase enzyme assay. Catalase activity was assayed according to the method described by Aebi (23). The assay volume (450 μ l) contained 50 μ l of cell extract and 250 μ l of 50 mM PBS pH 7.0. The reaction at 37°C was started by the addition of 150 μ l of 30 mM H₂O₂. The decrease in absorbance at 240 nm was monitored for 1 min in a Beckman DU 7500 spectrophotometer. The enzyme activity level was calculated using the extinction coefficient of 0.00394 L mmol⁻¹ mm⁻¹ and the unit of enzyme activity was expressed as mmoles H₂O₂ decomposed per min.

GSH peroxidase (GPx) enzyme assay. The GPx activity level was assayed as described by Yang *et al.* (24). The reaction mixture (500 μ l) contained 3.2 mM GSH, 0.32 mM NADPH, 1 unit GR, 1 mM sodium azide and 0.82 mM EDTA in 0.16 M Tris-HCl, pH 7.0. The sodium azide was added to the reaction mixture to inhibit endogenous catalase activity level. The reaction mixture was incubated with 50 μ l of sample at 37°C for 5 min and the reaction was started by the addition of H₂O₂ at a final concentration of 100 μ M. The rate of NADPH consumption was monitored at 340 nm for 3 min. One unit of GPx activity level was defined as the amount of enzyme required to consume 1 μ mol of NADPH/min in the coupled assay.

GR enzyme assay. GR activity was assayed as described by Smith *et al.* (25). This assay is based on the following reactions:



We measured the change in absorbance at 412 nm due to the formation of TNB. The assay volume (2 ml) contained 1 ml of 0.2 M potassium phosphate with 1 mM EDTA buffer pH 7.5, 500 μ l of 3 mM DTNB in 0.01 M PBS pH 7.0, 250 μ l water, 100 μ l of 2 mM NADPH in water, 50 μ l cell extract, as well as 100 μ l of 20 mM oxidized GSH. The increase in absorbance at 412 nm was monitored for 3 min in a Beckman DU 7500 spectrophotometer at 24°C. The enzyme activity level was calculated using the extinction coefficient of TNB ($E_{412}=13.6$ L mmol⁻¹ cm⁻¹). One unit of enzyme activity was defined as the amount of enzyme that catalyzed the oxidation of 1 μ mole NADPH per min.

Cell cycle analysis by flow cytometer. The protective effect of NAC on Cd-induced cell cycle arrest in the rat normal liver cell line, CRL-1439, was studied by using the FACScalibur flow cytometer (BD Biosciences, San Jose, CA) as described by Badisa *et al.* (26). Cells at a density of 1.3x10⁶ cells per T-25 flask were plated overnight. The following day, the cells were treated with 0, 25, 50 and 75 μ M CdCl₂ alone or co-treated with 5 mM NAC simultaneously in triplicate flasks for 24 h in a 5% CO₂ incubator at 37°C. At the end of incubation, the cells were trypsinized and centrifuged at

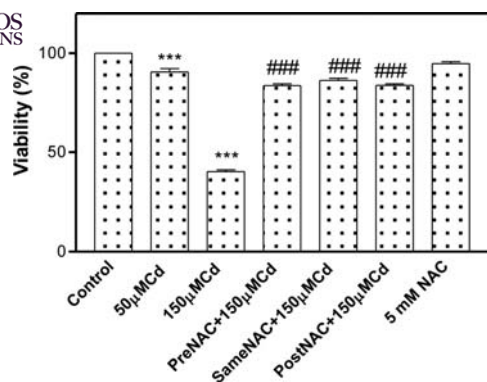


Figure 1. Effect of NAC on Cd-induced cell toxicity in rat normal liver cells. All values are means \pm SD (n=6). Statistical (Dunnett's multiple comparison tests) differences between the control (***p<0.001) and the 150 μ M Cd alone-treated cells (###p<0.001), are marked.

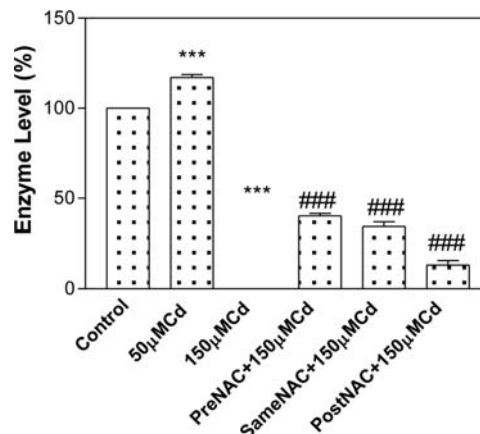


Figure 2. Effect of NAC on catalase enzyme in Cd-induced cytotoxic rat normal liver cells. All values are means \pm SD (n=3). Statistical (Dunnett's multiple comparison tests) differences between the control (***p<0.001) and the 150 μ M Cd alone-treated cells (###p<0.001), are marked.

2,500 rpm for 10 min at room temperature. Each pellet was re-suspended in 100 μ l PBS and formed singlet cells by passing 3 times through a 25 G needle. The cells were fixed in pre-cooled 95% ethanol (5 ml were added in a drop-wise manner to each tube while vortexing) and incubated at 4°C for at least 24 h. The cells were then harvested and re-suspended in ethanol (100 μ l of 95%). The cell suspensions were transferred into BD Falcon tubes and were shielded from light. Staining solution (1 ml) containing final concentrations of 1.25 mg/ml ribonuclease A, 1 mg/ml D-glucose and 50 μ g/ml propidium iodide was added to each tube in the dark. The tubes were incubated at room temperature for 1 h in the dark with occasional stirring. The distribution of cells in each phase was analyzed within 2 h with the FACScalibur flow cytometer. In each sample, a total of 10,000 events from the gated subpopulation were analyzed separately. CellQuest software was used for the acquisition and analysis of the data, and the percentage of cells in each phase was determined with ModFit 3.0 software (Verity Software House, Topsham, ME).

Statistical analysis. The viability and enzyme assay results were presented as the means \pm SD (n=3). The data from all the treated cells were presented in a percentage value in comparison to the untreated control cells (100%). The data were analyzed for significance by one-way ANOVA, and then compared by Dunnett's multiple comparison tests, using GraphPad Prism software, version 3.00 (San Diego, CA). The test values, p<0.05 and p<0.01, were considered significant and highly significant, respectively in comparison to the respective untreated control.

Results

Protective effect of NAC on Cd-induced cytotoxicity. The toxic effects of Cd at different concentrations on various cell cultures have been evaluated previously (9,21). However, little is known about the protective effect(s) of NAC on Cd-induced hepatotoxicity in normal rat cells. Therefore, we studied the protective effects of 5 mM NAC on CdCl₂-induced toxicity after 24 h. It was observed that the cell

viability of the Cd-treated cells was dose-dependent. The cell viability in the 50 and 150 μ M CdCl₂-treated cells decreased to 90.5 \pm 3.05 and 40.1 \pm 1.6%, respectively (p<0.001, Fig. 1) in comparison to the control (100%). In order to observe the protective role of NAC, we selected 150 μ M CdCl₂ in our subsequent experiments. It was observed that in the cells co-treated with 150 μ M CdCl₂ and 5 mM NAC (2 h pre-, simultaneous, or 2 h post-treatment), the viability increased significantly (p<0.001) to 83.7 \pm 1.5, 86.2 \pm 1.9 and 83.7 \pm 1.5%, respectively in comparison to the cells treated with 150 μ M CdCl₂ alone. In the cells treated with 5 mM NAC alone, the cell viability decreased to 94.8 \pm 1.7%, which does not show much toxic effect on the cells in comparison to control (100%). These results clearly demonstrate the protective effect of NAC on Cd-induced cell death.

Protective effect of NAC on catalase enzyme. Fig. 2 shows the protective effect of 5 mM NAC on the levels of catalase enzyme in the Cd-treated cells after 24 h. In the 50 μ M CdCl₂-treated cells, the catalase enzyme level increased to 117 \pm 3% (p<0.001) in comparison to the control cells. However, in the 150 μ M CdCl₂-treated cells, the catalase enzyme level decreased drastically to an undetectable level (p<0.001). In cells co-treated with 150 μ M CdCl₂ and 5 mM NAC (2 h pre-, simultaneous, or 2 h post-treatment), there was a significant increase in the level of catalase enzyme to 40.1 \pm 2.4, 34.3 \pm 4.6 and 13.2 \pm 4.3%, respectively (p<0.001) in comparison to the 150 μ M CdCl₂-treated cells (0%). Within the co-treated cells, the 2 h post-treated cells had a significantly (p<0.001) decreased catalase enzyme level compared to the other 2 groups (simultaneous and 2 h pre-treatment). These results clearly demonstrate the protective effect of NAC and treatment with NAC at various time-points on the level of catalase enzyme in Cd-induced toxicity in normal liver cells.

Protective effect of NAC on GPx enzyme. The protective effect of 5 mM NAC on the level of GPx enzyme in the Cd-treated cells after 24 h is shown in Fig. 3. In both the 50 μ M

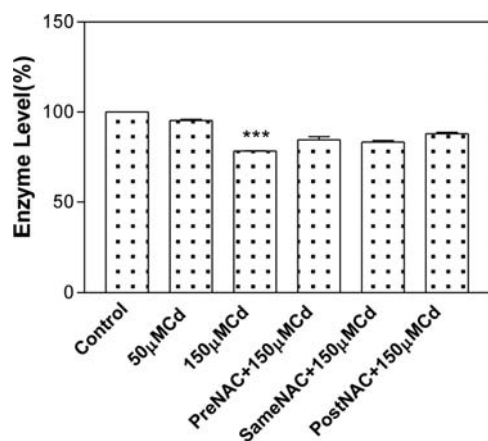


Figure 3. Effect of NAC on GPx enzyme in Cd-induced cytotoxic rat normal liver cells. All values are means \pm SD (n=6). Statistical (Dunnett's multiple comparison tests) differences compared to the control (***p<0.001), are marked.

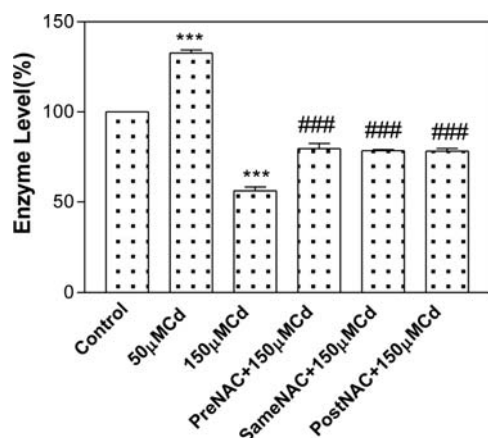


Figure 4. Effect of NAC on GR enzyme in Cd-induced cytotoxic rat normal liver cells. All values are means \pm SD (n=3). Statistical (Dunnett's multiple comparison tests) differences between the control (***p<0.001) and the 150 μ M Cd alone-treated cells (###p<0.001), are marked.

and 150 μ M CdCl₂-treated cells, the GPx enzyme level decreased to $95.4 \pm 0.9\%$ and $78.3 \pm 0.26\%$, respectively (p<0.001) in comparison to the control cells (100%). However, in the cells co-treated with 150 μ M CdCl₂ and 5 mM NAC (2 h pre-, simultaneous, or 2 h post-treatment), the level of GPx enzyme insignificantly increased (p>0.05) to 84.5 ± 3.1 , 83.3 ± 1.6 , and $87.9 \pm 1.35\%$, respectively. The treatment with NAC at various time-points had no effect on the GPx enzyme. These results clearly demonstrate that NAC does not have a significant effect on the level of GPx enzyme in order to protect Cd-induced cytotoxicity in liver cells.

Protective effect of NAC on GR enzyme. Fig. 4 shows the protective effect of 5 mM NAC on the level of GR enzyme in the Cd-treated cells after 24 h. In the 50 μ M CdCl₂-treated cells, the GR enzyme level significantly increased (p<0.001) to $132.7 \pm 2.99\%$ in comparison to the control cells (100%). However, in the 150 μ M CdCl₂-treated cells, the GR enzyme level decreased to $56.1 \pm 3.8\%$ (p<0.001). In the cells treated with

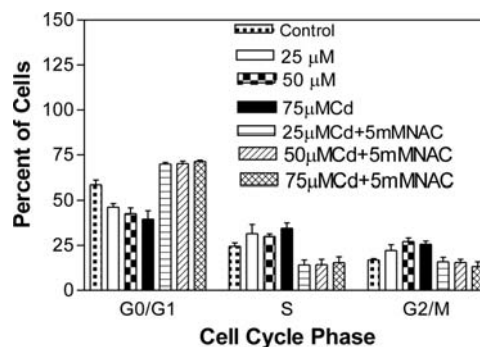


Figure 5. Effect of NAC and Cd on cell cycle phase in Cd-induced cytotoxic rat normal liver cells. All values are means \pm SD (n=6). Statistical (Dunnett's multiple comparison tests) differences between the control (*p<0.001) and the respective Cd alone-treated cells (*p<0.001), are marked.

150 μ M CdCl₂ and 5 mM NAC (2 h pre-, simultaneous, or 2 h post-treatment), the enzyme levels increased to 79.5 ± 4.94 , 78.5 ± 1.04 and $78.2 \pm 2.42\%$, respectively (p<0.001). The decrease in the level of GR enzyme seen in the cells treated with Cd alone was dose-dependent, and treatment with NAC at various time-points did not have an effect on the GR levels. These results clearly demonstrate the protective effect of NAC on the level of GR enzyme in Cd-induced cytotoxic liver cells.

Protective effect of NAC on cell cycle arrest. Rat normal liver cells (1.3×10^6) were treated with 0, 25, 50 and 75 μ M CdCl₂ alone or co-treated with 5 mM NAC (simultaneous treatment) for 24 h and subjected to flow cytometer analysis. Fig. 5 shows the flow analysis data of the cells (10,000 events). The decrease in cell population in the G0/G1 phase and the increase in the S and G2 phases in the cells treated with CdCl₂ alone, were dose-dependent. As the Cd concentration increased, the percentage of cells in G1 decreased in the cells treated with CdCl₂ in comparison to the untreated control group. However, the co-treatment with NAC increased the cell population in G1 at all 3 CdCl₂ concentrations. The cells treated with 25 and 75 μ M CdCl₂ alone were arrested mainly at the S phase while those treated with 50 μ M CdCl₂ alone were arrested at both the G2 and S phases. The population of cells co-treated with NAC was neither arrested in the S nor G2 phase. It was observed that NAC inhibited cell cycle arrest at all Cd concentrations by shifting to the G1 phase from the G2 and S phases.

Discussion

Previously, we studied the effect of Cd on the levels of anti-oxidative enzymes in the mitochondria and cytoplasm of CRL-1439 rat normal liver cells (9). NAC is a well-known antioxidant which increases the intracellular GSH levels in erythrocytes, liver and lung cells (18). In the present study, the protective effects of NAC against Cd-induced cell damage were evaluated in normal rat liver cells. The cells were either treated with CdCl₂ alone or co-treated with NAC (2 h pre-, simultaneous, or 2 h post-treatment) in order to study the protective effect and treatment with NAC at various time-



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against Cd-induced cytotoxicity. Cytotoxicity was assayed by a simple and reproducible crystal violet dye staining assay (22). In our previous study (27), we showed that the IC_{50} for Cd chloride in CRL-1439 rat liver cells was 125 μ M. In this study, we used 150 μ M $CdCl_2$ for better visualization and pronounced effect of NAC on Cd-induced toxicity. The treatment of cells with 50 and 150 μ M $CdCl_2$ alone resulted in 9.5 ± 3.05 and $59.9 \pm 1.56\%$ cell death, respectively (Fig. 1) in comparison to the untreated control cells (100%). These results are consistent with our previous observations. The co-treatment with NAC (2 h pre-, simultaneous, or 2 h post-treatment) showed almost equal protection against Cd-induced cytotoxicity (83.7 ± 1.53 , 86.2 ± 1.9 and $83.7 \pm 1.54\%$, Fig. 1). The protective effect of NAC could be as a result of NAC acting as a chelator due to its disulfide bonds as well as acting as an antioxidant by quenching the free radicals. NAC can also form mixed- and single-ligand complexes with Cd (28). As NAC is a well-known antioxidant, it protects the cells from Cd toxicity by quenching the free radicals or by increasing the antioxidant enzyme levels.

In the 50 μ M $CdCl_2$ -treated cells, the catalase enzyme significantly ($p < 0.001$) increased in comparison to the control cells and was responsible for higher viability. The catalase enzyme level decreased drastically to an undetectable level in the cells treated with 150 μ M $CdCl_2$ alone. However, the level of catalase increased in the cells co-treated with NAC (2 h pre-treatment, $40.1 \pm 2.4\%$; simultaneous treatment, $34.3 \pm 4.64\%$). This result is consistent with an earlier report where it was shown that NAC inhibited Cd-induced apoptosis by the up-regulation of catalase in HepG2 cells (29). In the cells co-treated with 150 μ M $CdCl_2$ and 5 mM NAC (2 h post-treatment), the catalase enzyme level significantly decreased ($p < 0.001$) compared to the other 2 groups (simultaneous and 2 h pre-treatment). The decrease in enzyme level could be due to the toxic effect of Cd which was added prior to NAC. However, cell or organ specificity played a role in the effect of NAC on catalase level. In another study, Cd intake did not change catalase enzyme levels in rat kidney cells (21). Furthermore, it was also observed that neither co- nor post-treatment with NAC and Cd changed the catalase levels in the above cell line (21). From the above studies, it is clear that the effect of NAC on the level of catalase enzyme shows specificity to certain types of tissue.

In the cells treated with 50 μ M $CdCl_2$ alone for 24 h, the Gpx enzyme was insignificantly reduced to $95.4 \pm 0.9\%$ in comparison to the control cells (100%). The GPx enzyme level in this study decreased to $78.3 \pm 0.26\%$ in the cells treated with 150 μ M $CdCl_2$ alone for 24 h compared to the untreated control cells. There was an increase in the GPx level in the cells co-treated with NAC and 150 μ M $CdCl_2$ compared to the cells treated with 150 μ M $CdCl_2$ alone. However, this was not significant. This increase in the GPx level was supported by an earlier report where an increase in the GSH-Px activity was observed in rat kidney tissue co-treated with NAC and Cd (21). It is clear that NAC also had a tissue-specific effect on the GPx enzyme as was shown in the kidney cells (21) as well as in this study, as we observed no effect on the liver cells. Further studies are required in

order to confirm the tissue-specific effect of NAC on the catalase and GPx enzymes.

In the 50 μ M $CdCl_2$ -treated cells, the GR enzyme increased in comparison to the control cells and was responsible for higher viability. When the cells were treated with 150 μ M $CdCl_2$ alone for 24 h, the GR activity level drastically decreased to $56.1 \pm 3.8\%$ ($p < 0.001$) in comparison to the untreated control cells (100%). In the cells treated with 150 μ M $CdCl_2$ and 5 mM NAC (simultaneously, 2 h pre-, and 2 h post-treatment), the enzyme levels increased to 79.5 ± 4.94 , 78.5 ± 1.04 and $78.2 \pm 2.42\%$, respectively ($p < 0.001$). It has also been reported that NAC increases the intracellular GSH levels (30). Several reports have also indicated that GSH can form a complex with Cd and that it decreases the uptake of Cd into the cells and prevents toxicity of this metal (31,32).

Interesting results were observed when the effect of Cd on the cell cycle arrest at different concentrations was studied in the presence or absence of NAC in rat normal liver cells. Each $CdCl_2$ concentration induced a certain type of cell phase arrest. The 25 and 75 μ M $CdCl_2$ -treated cells were arrested mainly at the S phase (Fig. 5). The 50 μ M $CdCl_2$ -treated cells were arrested both at the G2 and S phases (Fig. 5). It has also been reported that Cd caused cell arrest at G1 in kidney distal epithelial cells (33). Therefore, it is evident that the Cd effect on the cell cycle depends on the tissue type. It was also reported that a low concentration of Cd (0.9-1.5 μ M) treatment for 3 months in human lung embryo fibroblast cells caused cell cycle arrest at the S phase (34). Hence, the cell cycle arrest depends on the type of cells, Cd concentration and duration of the treatment. In our study, the protective effect of NAC was observed with the removal of cell arrest at the G2 and S phases and with the increase in cell population at the G1 phase (Fig. 5). Consistent with these observations, it was also previously reported that NAC inhibited signal-regulated kinase activation by shifting to the G0/G1 from the G2/M phase in murine macrophages (35).

In conclusion, NAC shows a protective effect on viability, antioxidative enzymes, particularly on catalase and GR, as well as on cell cycle arrest in Cd-induced cytotoxic rat liver CRL-1439 cells.

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