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Impact of Catechin and Quercetin against Cisplatin-Induced *in vitro* Cytotoxicity and Altered Antioxidant Status in Renal Epithelial Cells

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Abstract

Strategies to minimise the nephrotoxicity of platinated antineoplastic drugs without affecting their antitumour efficacy are strongly recommended to improve the therapeutic profile of these drugs. The natural flavonoids have been reported to afford nephroprotection without affecting the cisplatin antitumour effect. The purpose of the present study was to assess the protective mechanisms of flavonoids (catechin and quercetin) on cisplatin (cDDP) induced cytotoxicity in the NRK-52E cell line. MTT, Resazurin, and Trypan blue assays were performed to determine cytotoxicity and correlate with the alterations in the levels of antioxidants to assess the role of oxidative stress in the mechanism of cisplatin-induced toxicity. Different doses of catechin and quercetin increased the dose-dependent proliferation of the cell line. Exposure to cDDP (200 µM) resulted in significantly ($p<0.05$) reduced cell viability and disturbances in antioxidant homeostasis. Dose-dependent significant changes were seen in antioxidant enzymes and indicators of lipid and protein peroxidation (MDA and AOPP), indicating oxidative insult. However, our results showed that catechin and quercetin could effectively protect renal cells from cDDP-induced oxidative stress. Both phytoingredients offered protection against cDDP toxicity in renal epithelium, in all likelihood due to their richness in ROS quenching potential. The underlying antioxidant and anti-apoptotic properties in both compounds contributed to the normalisation of cell survivability in renal epithelium exposed against cDDP-induced oxidant stress. These results suggest that catechin and quercetin may shield cDDP-induced nephrotoxicity in patients undergoing anticancer chemotherapy.

Major Findings: Exposure of cDDP (200 µM) significantly reduced cell viability may be due to reduced cellular antioxidant status. Quercetin and catechin produces dose dependent proliferation of NRK 53E cell line.

Keywords: Catechin, Cisplatin, Cytotoxicity, NRK-52E Cell Line, Quercetin, Resazurin

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1. Introduction

Cisplatin (cis-Diamminedichloroplatinum (II)) (cDDP) is an inorganic platinum complex that is frequently employed as a cytostatic drug for the treatment of various solid malignant tumors[1]. cDDP is eliminated via glomerular filtration and tubular secretions primarily through organic Cation Transporter Proteins-2 (OCT2) and Copper Transporter-1 (CTR1). Higher accumulation (five times greater than blood) and metabolism of cDDP in renal proximal tubular epithelial cells lead to acute renal injury in exposed animals[2,3]. The straight section of the proximal tubules is the primary renal target of cDDP, leading to cell death either by necrosis or apoptosis of renal cells[4]. The cDDP induced nephrotoxicity impacts several biochemical pathways alongside nuclear as well as cytoplasmic macromolecules including lipids, proteins and nucleic acids which is mediated by generation of reactive oxygen/nitrogen species (RONS) in response to interaction of intracellular cDDP and its metabolites with organellar constituents, cytoskeleton, intracellular transport systems, severely impairing cell function and viability[5-7]. Even though DNA damage is believed to be the primary cause of cDDP-induced adverse effects, research involving enucleated cells suggested that nucleus-independent signaling could impact calcium homeostasis and protein folding in the endoplasmic reticulum[8].

Catechin (CTC) and Quercetin (QRT) are among the most well-known naturally occurring flavonoids found in fruits, vegetables, tea, and some conventional herbal remedial formulations [9,10]. They exhibit robust bioactivity and possess several formidable health benefits. These flavonoids protect against cellular damage from oxidative insults by directly scavenging free radicals and indirectly by enhancing the cellular defense system *via* stimulating Nrf2 signalling. They have been shown to mitigate oxidative insults in renal tissues and prevent acute kidney damage caused by drugs by blocking the Protein Kinase R-like ER Kinase (PERK) signaling pathway [11,12]. Studies have also shown that quercetin-3-O-glucuronide, a metabolite of QRT, partially prevented the effect of cDDP in NRK-52E cells[13] whereas catechin reduced both apoptosis and ferroptosis, in gentamicin-induced nephrotoxicity by knocking down Nrf2 signaling[14]. The rodent origin renal tubular epithelial cell line (NRK-52E) was effectively used as a model for the chemical-induced renal toxicity and its prevention [1,15]. Therefore, the present study aimed to compare the antioxidant abilities of QRT and CTC mechanisms in imparting protection against cDDP-induced cytotoxicity and the role of the antioxidant system in countering cytotoxicity using the NRK-52E cell line of rat origin.

2. Materials and Methods

2.1 Cell Line Culture

Renal epithelial cells (NRK-52E, rat origin) were procured from the National Centre for Cell Science (NCCS), Pune, Maharashtra, India. The NRK-52E cells were visualized (Nikon, ECLIPSE-TS 100, Japan) and cultured using DMEM media containing fetal bovine serum and penicillin–streptomycin solution and were kept at 37°C with 5% CO_2 in the carbon dioxide incubator (HERA cell 150, Thermo Fisher Scientific, USA).

2.2 Chemicals Used

The various chemicals used during the experiments like Dulbecco's modified Eagle's medium (DMEM) (CAT No. D5671), Dimethyl Sulfoxide (DMSO) (CAT No. D2650), fetal bovine serum (CAT No. F7524) Dulbecco's Phosphate Buffered Saline (DPBS) (CAT No. D5537), trypan blue (CAT No. T8154) penicillin–streptomycin (CAT No. P4333), trypsin–EDTA (TCL006), and L-glutamine (CAT No. TCL012), were obtained Sigma-Aldrich, USA whereas 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide (MTT) (CAT No. MB 186-1G) was procured from Hi media, India. Chemicals viz. cisplatin (CAT No. PHR 1624), quercetin (CAT No. S1BB8166V), catechin (CAT No. C1251) were purchased from Sigma-Aldrich Pvt. Ltd., USA.

2.3 Cytotoxicity Testing

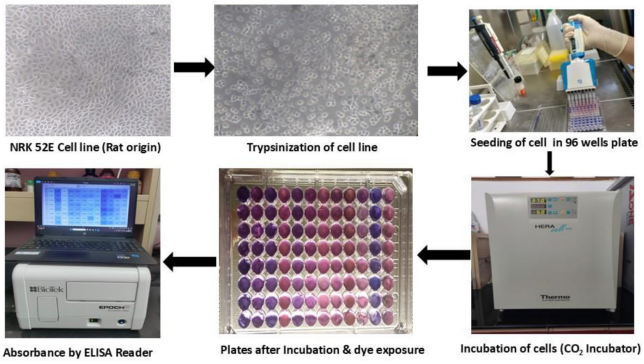
The MTT, Resazurin and Trypan blue assays were used in the present study to measure the cell viability in the cell culture laboratory of the division[16,17]. For MTT and Resazurin assay, cells were cultured in 96-well plates with 5000-8,000 cells/well and were exposed to cDDP alone and in combination with QRT and CTC for 24 h. For the selection of cytotoxic dose of cDDP, different concentrations from 50 µM to 800 µM were selected for exposure. 75-85% cell death was observed at 200 µM and therefore, 200 µM of cDDP was used for further study[18]. Additionally, the effect of different concentrations (50, 25, 12.5, 6.25 and 3.125 µg/ml) of QRT and CTC was used alone and also or in combination with cisplatin (200 µM) to study their effect on the NRK-52E cell line.

2.4 MTT Assay

MTT assay was used to measure cell viability[19,20]. Cultured cells were seeded in 96-well plate and incubated for 24 h. Following the incubation period, the cells were treated with cDDP and QRT alone and in combination, and likewise, in a separate plate, cDDP and CTC were used alone and also in combination with each other. After discarding the medium, 20 µl MTT (5 mg/ml) was added per well, and cells were incubated with MTT dye for 4 h. After the incubation period, the cell media was replaced with 100 µl DMSO to dissolve the formazan crystals. The absorbance was recorded at 570 nm by an ELISA microplate reader ($EPOCH_2$, BioTek, USA). Results were calculated as the mean percentage of cell proliferation.

2.5 Resazurin Assay

Resazurin assay was used to detect the cytotoxicity induced by cDDP in NRK-52E cells[21,22]. The 96-well plates were plated with 100 µl of cells and test substances as described above. The plate was incubated for a period of 24 h, and after that, 20 µl resazurin solution was added to each well and kept in an incubator for 4 to 6 h at 37°C. The fluorescence was recorded using a 560 nm excitation/590 nm emission filter set. Results were analysed similar to those for the MTT assay. In brief, the flow chart of the procedure is displayed as follows:



2.6 Trypan Blue Assay

For the trypan blue assay, cells were exposed to cDDP in 6-well plates and then were treated with QRT in different concentrations. The same protocol was followed for CTC treatment also. After exposure of 24 h, the cells were trypsinised and the aliquot was collected in a micro centrifugation tube, which was then stained with trypan blue dye and cells were counted using Neubauer's chamber. The counting of dead and live cells was done manually with the assistance of a camera attached to a microscope (MLX with OLYMPUS PEN Lite, Japan). The percentage cell viability was calculated using the standard formula = (total no. of viable cells per ml/total no of cells per ml) × 100.

2.7 Antioxidant Biomarkers in NRK-52E cells

Cells were treated in 6-well plates as above**.** Cell lysate of NRK-52E cells was prepared using a sonicator probe (2 min at 20KHz). The lysate was cleared by centrifugation at 12000 rpm for 5 min. The supernatant was used for the determination of cellular antioxidant biomarkers and enzymatic activities in cell lysates. The substrate phenyl acetate was used to measure the activity of AE (U/ml), where one unit equals µmol of phenol produced per min[23]. Using established standard procedures, reduced Glutathione (GSH), Total Thiols (TTH), and Total Antioxidant Status (TAS) were assessed[24-26]. The levels of Catalase (CAT) and glutathione Peroxidase (GPx) were measured by documented methods[27,28]. The procedures described elsewhere were used to assess the activity of Superoxide Dismutase (SOD) and glutathione reductase (GR), respectively[29,30]. Similarly, the levels of malondialdehyde (MDA) and Advanced Oxidation Protein Product (AOPP) were assessed using accepted techniques[31,32]. The percentage of cell viability was calculated using the formula: Percentage of cell viability = (The absorbance of the sample/control) × 100. The half maximal inhibitory concentration (IC_{50}) was determined from the absorbance versus concentration curve. The IC_{50} value was calculated, and the experiments were repeated three times.

2.8 Statistical Analysis

The values of antioxidant biomarkers were presented as mean ± standard error. The data was analyzed by one-way Analysis of Variance (ANOVA), followed by Tukey's post hoc test was used to statistically analyze the data. Statistical differences were considered significant for $p<0.05$ (IBM, statistics SPSS 21.0).

3. Results

3.1 Effect on Per Cent Amelioration

A dose-dependent ameliorative effect of QRT against cDDP-induced toxicity was noticed. Similarly, ameliorative

effects of CTC against cDDP-induced toxicity were also observed at different dose levels (Figure 1). The trypan blue assay revealed an increase in per cent dead cells and a decrease in cell viability on exposure to cDDP in a dose-dependent manner, while cDDP treatment along with both CTC and QRT reduced cDDP-induced renal cell death in a dose-dependent manner (Figure 2).

3.2 Effect on NRK-52E Cells Proliferation

The potential effect of CTC at different doses and cDDP toxicity on NRK-52E cells was evaluated after an exposure period of 24 h. At the end of the exposure period, proliferation % was calculated using MTT, Resazurin and trypan blue assay. The media control group was used as a baseline for growth (100%); accordingly, the percentage was calculated and further statistically compared between different doses. The cDDP alone exposure caused a significant decrease ($p<0.05$) in per cent proliferation when compared to the control group. Catechin alone-treated groups showed a dose-dependent increase in per cent proliferation of NRK-52E cells when compared to the control group, except the CTC-3.125µg dose. When CTC was given in the presence of cDDP exposed groups, all the doses showed significantly higher ($p<0.05$) per cent proliferation in combination groups when compared with the cDDP alone treated group.

The potential effect of QRT at different doses and cDDP alone and in combination on the NRK-52E cell line was evaluated after the completion of the exposure period of 24 h. At the end of the exposure period, per cent proliferation was calculated using MTT and resazurin assay. Media control cells were used as a baseline for growth

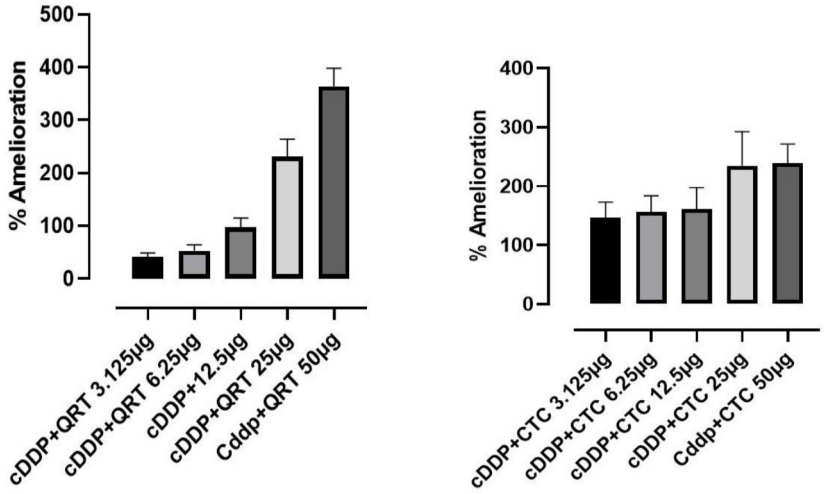


Figure 1. Per cent amelioration of NRK-52E cell line on exposure to different concentrations of QRT and CTC alone and along with cDDP.

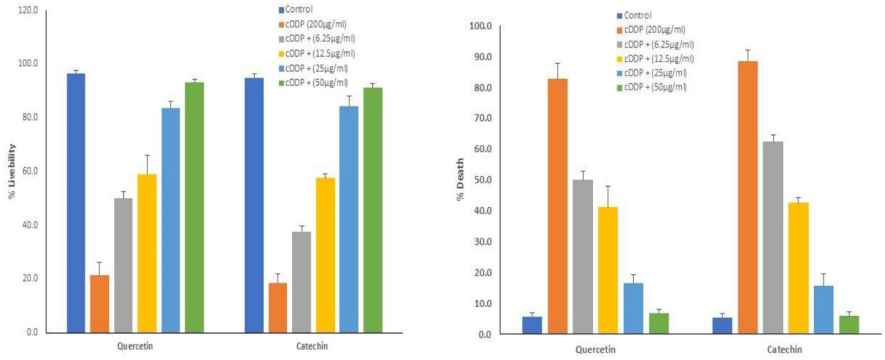


Figure 2. Per cent viability (% viability) and per cent cell death (% death) of NRK-52E cell line on exposure to different concentrations of QRT and CTC along with cDDP, on the basis of trypan blue analysis.

(100%); accordingly, the percentage was calculated, and a comparison was made. Statistical comparisons were made between different groups. The cDDP alone treatment caused a significant decrease ($p<0.05$) in proliferation rate as compared to the control group. The QRT alone-treated cells showed a dose-dependent increase ($p<0.05$) in the proliferation per cent of the NRK-52E cell line when compared to the control. When QRT was given in the presence of cDDP, all the doses showed a significantly higher ($p<0.05$) proliferation rate in combination groups when compared with cDDP alone-treated cells (Figure 3).

3.3 Antioxidant Status of NRK-52E Cell Line

Total antioxidant status of cDDP-treated cells significantly ($p<0.05$) increased as compared to the corresponding levels in the control group. These levels were effectively brought down only by 50µg/ml CTC, whereas all other doses were ineffective. In contrast, all the doses of QRT significantly reduced the cDDP-induced increase in TAS values. Similarly, the levels of GSH were reduced but non significantly after cDDP exposure as compared to control. All the doses of QRT and all doses of CTC except the 6.25 µg/ml dose of either treatment significantly ($p<0.05$) increased the GSH levels as compared to the respective levels in the control (Figure 4).

3.4 The Cellular Activities of Antioxidant Enzymes

The activities of GST, GR, GPx, SOD, CAT and AE were determined in control and different treatment groups and alterations in their activities are presented in Figure 4. As compared to the control, cDDP raised GST and GPx levels significantly. All the doses of CTC, as well as those of QRT except the treatment with 6.25 µg/ml of any of them, were able to significantly reduce GST and GPx activities as compared to the corresponding levels of cDDP only application. cDDP significantly increased GR and AE activity in comparison to the control. On the contrary, CTC only when used at 50 µg/ml, but QRT at 50, 25 and 12 µg/ml significantly reduced GR levels. All doses of CTC except that of 6.25 µg/ml significantly reduced AE activity; however, QRT at any dose level could not bring about any significant change in the altered AE activity. cDDP significantly enhanced CAT and SOD activities when compared to the control. Catechin, as well as QRT at 50 and 25 µg/ml, significantly increased CAT levels. At 12.5 µg/ml, both CTC as well as QRT reduced the CAT levels, but the difference was non-significant. Likewise, CTC as well as quercetin only at 50 µg/ml significantly reduce SOD activities. At 25 µg/ml, there was a non-significant reduction with both the agents but at lower doses there was no reduction in SOD levels.

3.5 Cellular Damage Indicators

To determine the intensity of cellular macromolecular damage, *viz.,* membrane lipids and proteins, the cellular levels of malondialdehyde (end product of lipid peroxidation) and AOPP were determined and presented in Figure 5. The MDA and AOPP levels saw a significant increase upon cDDP administration in comparison to the control levels. Catechin and QRT significantly reduced MDA levels when employed at 50 µg/ml and 25 µg/ml. Quercetin at 50, 25 and 12.5 µg/ml significantly reduced AOPP, whereas catechin only at 50 µg/ml showed similar effectiveness.

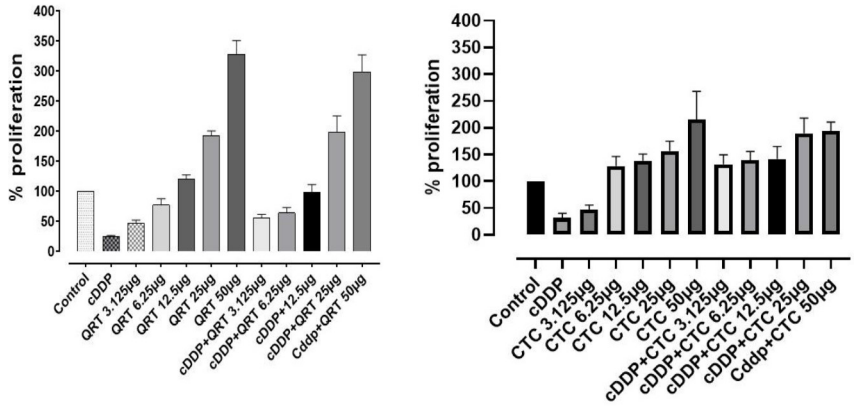


Figure 3. Per cent proliferation of NRK-52E cells on exposure to different concentrations of QRT and CTC alone and along with cDDP.

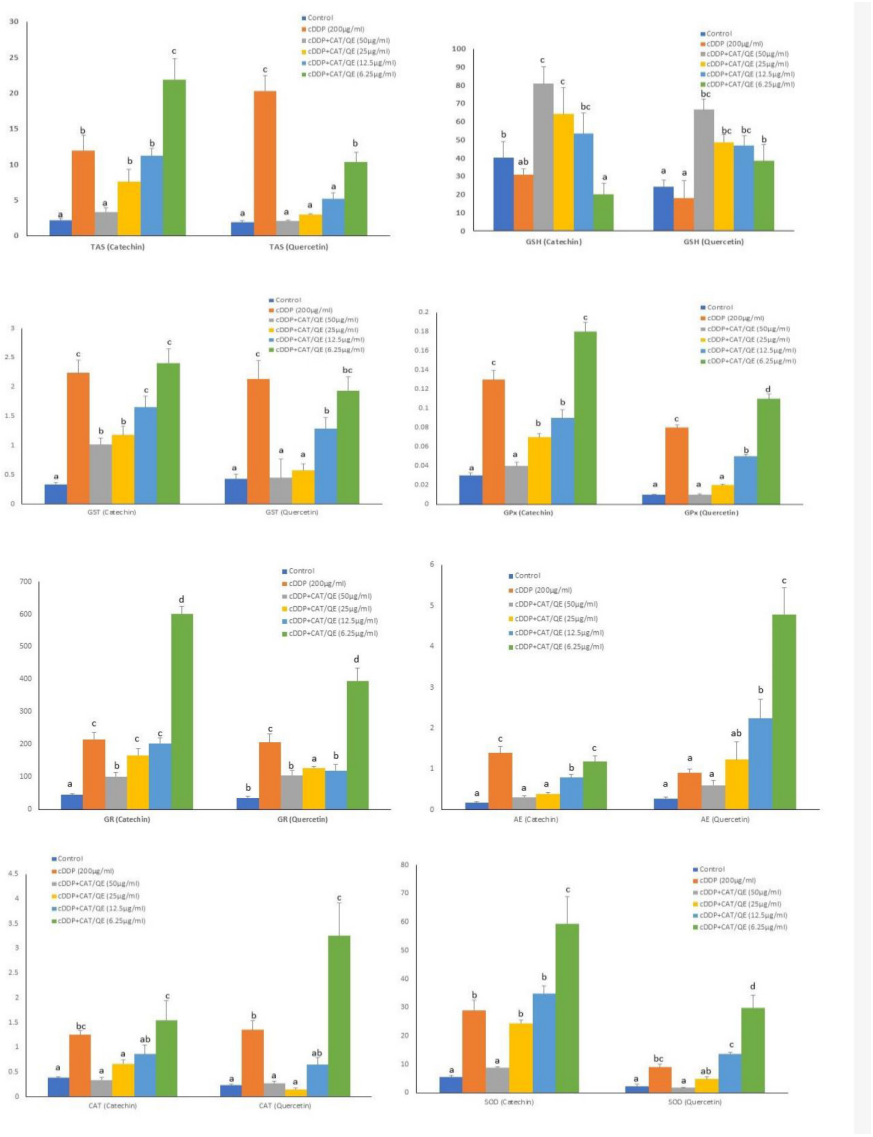


Figure 4. Effects of different doses of CTC and QRT exposure on total antioxidant status (TAS); reduced glutathione (GSH); glutathione peroxidase (GPx); glutathione-s-transferase (GST); glutathione reductase (GR); arylesterase (AE); superoxide dismutase (SOD) and catalase (CAT) activities in control and cDDP exposed NRK-52E cell line.

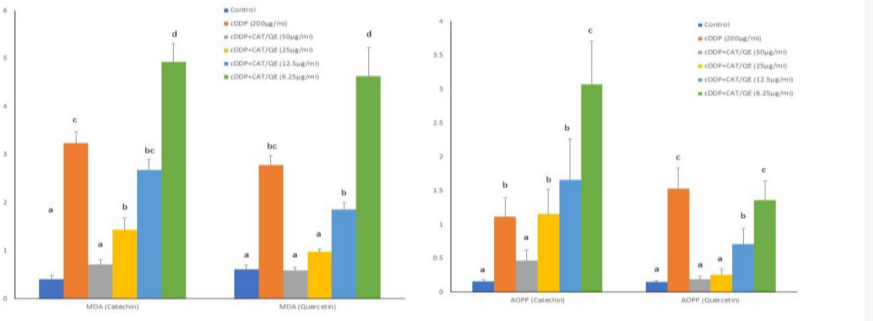


Figure 5. Effects of catechin and quercetin exposure on levels of malondialdehyde (MDA) and Advanced Oxidation Protein Products (AOPP) in renal tubular cell line (NRK-52E) treated with cisplatin.

4. Discussion

Kidneys are highly susceptible to extraneous toxicants, including chemotherapeutic drugs, because they handle high concentrations of drugs and their metabolites present in urinary filtrate. The cDDP, despite possessing an excellent anti-tumorigenic potential against a number of tumors, has limited clinical usage because it also causes acute renal damage simultaneously.

Various epidemiological and experimental studies have convincingly shown that supplementation of natural polyphenolic compounds confers renoprotection, which can significantly cut down oxidative damage fomented by exposure to a large variety of chemicals[33-35]. The beneficial effects of flavonoids such as CTC and QRT abundantly present in fruits, vegetables, and teas include anti-cancer, anti-oxidative, anti-inflammatory, anti-apoptotic, and anti-proliferative properties[35,36]. These natural compounds effectually counter harmful active byproducts of drug metabolites and induce enhanced expression and activities of detoxification enzymes, and hence nullify oxidative stress and suppress inflammatory pathways, which in turn make these flavanols attractive modalities to be employed in neutralising renal dysfunction incurred in response to nephrotoxic drugs. The present study analysed the impact of polyphenolic compounds to counter renal tubular damage in NRK-52E cells exposed to cDDP. In the present study, varying concentrations of polyphenolic compounds alone and in combination with cDDP were used to compare their impact on cell viability, proliferation, and antioxidant levels against cDDP. The cDDP induced a significant reduction in cell viability, and cells proliferated at a rate less than half that of the control. Earlier studies have also reported that cDDP causes deficient cell proliferation, for instance, cDDP reduced the viability of human embryonic tubular epithelial cells (AD293) in a time-dependent manner[37]. The CTC and QRT, when used at higher doses, reversed the cDDP-induced reduction in cell proliferation. One of the phytoingredients, thrombomodulin, a natural anticoagulant factor also known for its clinical anti-inflammatory properties, attenuated ROS levels, thereby reducing oxidative stress-induced apoptotic cell death and endoplasmic reticulum stress in renal proximal tubular epithelial cells after cDDP treatment, as well as in kidneys of mice[38]. In another study, AD293 cells preconditioned with hyperoxigenic conditions had a significant reduction in apoptosis due to lowering of caspase 3 levels and normalization of Bax/BCl2 ratio as compared to the corresponding conditions of no hyperoxia before cDDP toxicity and it was inferred that activation of endogenous antioxidant mechanisms which could quash ROS could be the key in conferring protection against cDDP toxicity in renal epithelium[37]. Similarly, cDDP reduced viability and induced apoptosis in glomerular cells (SV40 MES-13), NRK-52E cells and renal fibroblasts (NRK-49F); however, sodium acetate ameliorated cDDP-induced reduced cell survivability[39].

Interestingly, in our investigation, CTC and QRT at lower doses by themselves also negatively impacted cell proliferation. However, QRT at 12.5 µg/ml and above and CTC at 6.25 µg/ml and above caused greater cell proliferation rates. All doses of QRT, when given with cDDP, inhibited cDDP-induced toxicity and cells so treated showed higher rates of proliferation as compared to cDDP-only-treated cells in a dose-dependent manner. In a nutshell, QRT counterbalanced cDDP induced reduced proliferation in a dose-dependent manner. The CTC alone, as well as when co-treated with cDDP, had a similar effect, except that at 6.25 µg/ml, it alone was not toxic to cells, in contrast to the effects of the similar dose of QRT. Also, even the lowest dose of CTC completely offset cDDP-induced reduction in cell proliferation, whereas QRT at a similar dose could not fully ameliorate cDDP-induced reduced cell proliferation. At higher doses, *i.e.*, 25 µg/ml and 50 µg/ml, the cell proliferation rates induced by polyphenols were significantly higher than that of the control, but proliferation induced by CTC was significantly less as compared to that of QRT at these doses.

It could also be inferred from our results that lower doses of CTC displayed more effective amelioration potential as compared to the similar doses of QRT, whereas the maximum dose of QRT showed a higher amelioration rate than that of the corresponding dose of CTC. Previous studies showed that sodium acetate reduced cell cDDP-induced cellular senescence and apoptosis by blocking cDDP-induced oxidative stress[39]. Similarly, in the present study, while the majority of cells treated with cDDP were dead, the treatment with CTC as well as QRT significantly reduced cell death in a dose-dependent manner. Following cDDP treatment, cell viability was only one-fifth of that of the control, whereas both agents significantly enhanced cell viability in a dose-dependent manner. However, QRT, when given at 6.25 µg/ml, showed significantly higher per cent viability as compared to catechin treatment. Suman

and coworkers, using human renal proximal tubular cells, provided evidence of the involvement of extracellular signal-regulated protein kinases 1/2 (ERK) pathway in cDDP-induced nephrotoxicity. During the initial stages of toxicity, the ERK pathway protected against cell death; however, it enabled apoptosis during the later stage. This pathway also promoted the initiation of mitophagy and autophagy in later stages of cDDP toxicity. They also propounded that ERK inhibitors like mirdametinib could be promising drugs to mitigate AKI induced in the aftermath of cDDP chemotherapy[40].

The cDDP exposure caused a significant reduction in GSH but a significant enhancement in the activities of GR, GPx and GST in test cells. The activities of CAT, SOD, AE and TAS were also significantly raised after cDDP treatment as compared to sham treated wells. The cDDP suppressed genes governing metabolic pathways such as glycolysis, tricarboxylic acid and enhanced levels of p53 alongside mitochondrial ROS, which collapsed energy production, leading to cell death in proximal tubular epithelial cells. However, ROS quenching and p53 inhibition resurrected the involved pathways and ameliorated cDDP toxicity; therefore, ROS was the foremost player in cDDP-induced toxic effects[41]. The QRT and CTC displayed significant ameliorative potential against cDDP-induced toxicity in renal epithelial cells. Significant increases in MDA, AOPP were caused by the treatment of cells with cDDP alone. Complete resurrection of MDA was recorded when cells subjected to cDDP were co-treated with QRT and CTC at 50 µg/ml. Honokiol, a polyphenolic compound derived from the extract of *Magnolia officinalis,* was used to counteract cDDP-induced injury in renal epithelial cells[42]. Previous research employing mouse renal epithelial cells has suggested that epicatechin may stall cDDP-induced nephropathy by preventing damage to mitochondria and by reducing ERK activity, resurrection of manganese containing SOD and alleviation of oxidative stress in renal epithelium[43]. Honokoi reduced oxidative stress, promoted strengthening of cellular antioxidant machinery as well as that of epithelial cytoskeleton by favouring actin and tubulin polymerization, besides the stabilization of localization of occludin as well as E-Cadherin, which preserved cellular polarity and prevented cDDP-induced disruptions in epithelial cell morphology and induced damage to the epithelium. It was shown that anticancer agent and copper chelator, ammonium tetra-thiomolybdate (TM), could protect against cDDP-induced damage in HK-2 cells by blocking ubiquitination of Nrf2 and activation of downstream antioxidant pathways, but the mitigation of oxidative stress upon TM treatment was compromised when the Nrf2 gene was knocked down. Moreover, TM also activated Nrf2-responsive genes in the liver and kidney[44].

Although both phytoingredients used in the current research ameliorated oxidative stress, there was also variation in their potential. At 50 µg/ml, QRT completely restored all antioxidant enzyme levels (TAS, GSH, GPx, GR, AE, CAT and SOD) to the basal levels but CTC at the same dose show partial resurrections of GR and GST while complete normalization of the rest. At 25 µg/ml, QRT significantly restored all except AE but CTC failed in restoring TAS, GR and SOD levels at this dose. At 12.5 µg/ml, CTC only improved GSH, GPX, AE and CAT whereas QRT improved all except AE and SOD. At 6.25 µg/ml, CTC failed to stop cDDP-induced antioxidant enzymatic alterations, while at this dose, QRT still improved cDDP-induced lowering of GSH levels.

Even though significant reductions in MDA were orchestrated by 50 µg/ml CTC but complete normalization of MDA was noted at the same dose of QRT. Lower doses of both were ineffective in reducing lipid oxidation. Significant reduction of AOPP occurred only upon treatment with the highest dose of CTC (50 µg/ml). On the contrary, only the lowest dose (6.25 µg/ml) of QRT was ineffective, but the rest were effective in significantly lowering cDDP-induced peroxidation. Wang and coworkers demonstrated that NAcetylcysteine (NAC) administration protected against hepatocyte damage induced by cDDP treatment of human derived HepG2 cells[45]. The NAC prevented cDDP-inflicted oxidative stress-mediated apoptosis of HepG2 cells by significantly reducing levels of MDA, minimising DNA damage and restoring GSH levels. HEK293 cells exposed to cDDP witnessed a down regulation of PI3K/Akt and mTOR pathway, however, simultaneous maltol treatment increased AMPK (AMP-activated protein kinase) expression, a sensor of energy kinetics of cells, decreased Bax and caspase 3 expression, and reduced the activity of p53 in these cells and enhanced the cell viability[46]. These *in vitro* findings in the above study corroborated the parallel *in vivo* data wherein maltol pre-treatment in mice caused repression of inflammatory mediators NF-κB, IL-1β, reduced BUN, CRE and oxidative stress parameters *viz*. lipid peroxidation. Inherent antioxidant and anti-apoptotic effects were beneficial in reducing renal tubular oxidative damage induced by toxic

metabolites by promoting mitochondrial health in renal tubular cells facing cDDP toxicity[47]. By boosting mitochondrial resilience, CTC contributed to the overall cell survival[43]. Catechin inhibits pro-inflammatory pathways via suppressing NF-κB (nuclear factor kappa-light-chain-enhancer of activated B cells), which reduces the production of inflammatory cytokines, *e.g.*, TNF-α, IL-6 and prevents renal injury, which makes CTC a promising candidate for lessening cDDP-induced nephrotoxicity[48]. In the same way, QRT promoted cytoprotection of renal epithelium subjected to cDDP toxicity by unleashing its antiapoptotic, antioxidant, and anti-inflammatory potential[49]. In a nutshell, both QRT and CTC demonstrated tremendous anti-radical activity against cDDP toxicity in rat renal epithelium.

5. Conclusions

In the present study, an assessment of the nephroprotection abilities of QRT and CTC was carried out. Exposure of rat renal tubular epithelial cells to cDDP resulted in significantly reduced cell viability and disturbances in antioxidant homeostasis. The latter was manifested as significant alterations in values of physiological indicators of oxidative stress like GSH, SOD, *etc*. Additionally, significant enhancement was recorded in lipid and protein oxidation. However, our results showed that CTC and QRT can effectively protect renal cells from cDDP-induced oxidative stress and cell damage. Both phytoingredients offered protection against cDDP toxicity in renal epithelial cell line, all likelihood due to their richness in ROS quenching potential. The underlying antioxidant and anti-apoptotic properties in both compounds contributed to the normalization of cell survivability in renal epithelium exposed to cDDP-induced oxidant stress. These results suggest that CTC and QRT may shield cDDP-induced nephrotoxicity in patients undergoing anticancer chemotherapy.

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