Ind. J. Pharm. Edu. Res., 2025; 59(3s):s1-s8.
https://www.ijper.org

Original Article

Mitigating Cisplatin-Induced Nephrotoxicity with Morin: A Flavonoid's Role in Nrf2/HO-1 Pathway Activation in HEK 293 Cells

Jianliang Qu[1], Wei Song[2,*], Abdullah A. Alarfaj[3], Abdurahman Hajinur Hirad[3]

[1]Department of Nephrology, Affiliated Hospital of Hanzhong Vocational and Technical College, Hanzhong, CHINA.
[2]College of Medicine, Shandong Medical College, Shandong, CHINA.
[3]Department of Botany and Microbiology, College of Science, King Saud University, Riyadh, SAUDI ARABIA.

ABSTRACT

Objectives: Cisplatin is a widely used chemotherapeutic agent used in various cancers, but its clinical use is limited due to its dose-dependent toxicity, especially nephrotoxicity. Flavonoids are a class of natural compounds that often show kidney-protecting function. The current study aimed to investigate the probable mechanism of morin, a flavonoid in cisplatin-induced nephrotoxicity and the involvement of Nrf2 pathways in managing the stress induced in HEK 293 cells. **Materials and Methods:** Cytotoxicity and nephroprotection of morin in cisplatin-treated HEK 293 cells were evaluated by the Alamar Blue assay. DCFDA analysis evaluated the morin efficacy in suppressing ROS generated with cisplatin treatment. Molecular docking tools were used to predict the affinity of morin for the Nrf2 binding site on the Keap1 protein. Later, real-time PCR was used to estimate gene expression levels related to oxidative pathways, especially Nrf2 and HMOX1 gene expression. **Results:** The results showed that Morin has minimum or no cytotoxicity in the HEK 293 cells up to 100 µg/mL, establishing its safety profile for further analysis. There was significant nephroprotection by morin, even at lower doses, with reduced ROS levels and protection from cellular damage. Molecular docking studies revealed a strong affinity of morin for the Nrf2 binding site on the Keap1 protein, with a docking score of -7.7 kcal/mol. This indicates its potential to disrupt Keap1-Nrf2 interaction. Subsequent real-time PCR analysis confirmed upregulation of Nrf2 and HMOX1 gene expression, suggesting activation of antioxidant pathways.**Conclusion:** These findings highlight morin as a promising candidate for mitigating cisplatin-induced nephrotoxicity via the Nrf2/HMMOX-1 axis, warranting further preclinical and clinical investigations.

Keywords: Antioxidant, Cisplatin, HEK 293 cells, Morin, Nephrotoxicity, Nrf2 pathway.

Correspondence:
Dr. Wei Song
College of Medicine, Shandong Medical College, Shandong-250000, CHINA.
Email: songwei0862697@Outlook.com

Received: x-x-x;
Revised: x-x-x;
Accepted: x-x-x.

INTRODUCTION

Nephrotoxicity is defined as any injury to kidneys caused by drug administration leading to acute kidney injury or chronic kidney disease, tubulopathies, and glomerulopathies. It is a common characteristic associated with chemotherapy treatment. Among the various chemotherapeutic agents, cisplatin, a platinum-based agent, is most implicated in nephrotoxicity.[1] Despite its efficacy in treating multiple types of cancers, such as pancreatic cancer, lung cancer, bladder cancer, cervical cancer, etc., the therapeutic efficacy of cisplatin is often limited due to the nephrotoxicity it causes.[2] It is observed that just about 20-30% of patients receiving cisplatin therapy develop acute renal injury, which affects the quality of life of the patient.[3] The first sign of toxicity after cisplatin administration is visible after 10 days in clinical practice, manifested as a reduction in glomerular filtration rate, elevated serum creatinine, and hyperkalemia. The mechanism by which it exerts the side effects is studied well. Cisplatin-induced nephrotoxicity usually happens due to proximal tubular damage, oxidative stress, and inflammatory responses.[4] Since the toxicity of cisplatin still exists and it is an effective drug of choice, there needs to be more studies to invent molecules to protect against the toxicity exerted by these drugs.

Natural products have consistently gained attention due to their potential as a starting point for drug discovery and as an excellent choice to mitigate the harmful effects of existing drugs. Flavonoids, a class of secondary metabolites with variable phenolic structures, have been discovered as a prominent group of natural products.[5] They are available in many fruits, leaves, bark, roots, stems, and other different parts of the plants. Apart from their medicinal value, they are also used as cosmetics and nutraceuticals.[6] They

DOI: ["DOI"].11.2.1

are identified for their antioxidant, anti-inflammatory, and cytoprotective effects, which makes them an ideal candidate for mitigating drug-induced system toxicities. Flavonoids are famous for their anti-cholinesterase activity, anti-inflammatory activity, xanthine oxidase modulators, countering antibiotic resistance, etc.[7,8] Morin, 3,5,7,2′,4′-pentahydroxyflavone, is a flavanol isolated from different plants, mostly available in plants with the Moraceae family.[9] Morin exhibits a variety of pharmacological activities, including anti-inflammatory, antioxidant, anticancer, and protective effects against kidney injury, among others. Earlier studies have shown the capacity of morin to reduce cisplatin-induced nephrotoxicity.[10] However, the precise mechanism involving the gene that regulates it remains incompletely understood.

An antioxidant is a common mechanism by which the plant product protects against the toxicities in the biological system. The reason behind this is the fact that most of the toxicities are produced due to the excess production of free radicals, which can be effectively prevented by using antioxidant compounds.[11] In normal physiological conditions, there will be a well-balanced redox state, which means equilibrium between pro-oxidants and antioxidants. However, if there is an increase in redox status, the excessive production of free radicals induces injury to the target organ.[12,13] It is well established that free radicals are key factors in the generation of nephrotoxicity. The excessive generation of free radicals leads to mitochondrial dysfunction and an energy crisis that forms nephrotoxicity.[14] A major mechanism responsible for the cellular defence against the excessive production of free radicals is the activation of Nrf2, an antioxidant response, by the induction of various transcriptional factors in the nucleolus, which includes Heme Oxygenase-1 (HMOX1).[15] Morin has been shown to induce the effect by Keap1/Nrf2 pathways.[16] There is no clear evidence regarding the role of the Nrf2/HMOX-1 stress response mechanism in protecting against cisplatin-induced nephrotoxicity.[10] Hence, this study aims to evaluate the effect of Morin in inducing the nephroprotective effect and the probability of involvement of the Nrf2/HO-1 mechanism.

MATERIALS AND METHODS

Chemicals and materials

Morin, with >98% purity, was procured from Sigma Aldrich, USA. All other chemicals were purchased from commercial suppliers. Cell culture media DMEM, fetal bovine serum, DMSO, and antibiotics were purchased from Themo Fisher Scientific USA.

Cell culture and maintenance

HEK 293 (human embryonic kidney) cells were purchased from ATCC. They were cultured in T25 flasks at 37°C in a humidified 5% CO_2 incubator (NBS Eppendorf, Germany) using the DMEM media supplemented with FBS and 1% antibiotics. Upon completion of the formation of the monolayer, cells were used for further experiments.

Cytotoxicity analysis

Since the study aimed to find the nephroprotective effect of the compounds, we conducted cytotoxicity to find out the sub cytotoxic concentration of morin. Hence, the confluent cells were trypsinized and plated into 96-well plates (5×10^3 cells/well) and allowed to grow for 24 hr. Then, the plates were added with morin at different concentrations, decreasing the order of concentration starting at 100 to 6.5 µg/mL. The plates were kept in the incubator for 24 hr, and then the plates were observed under an inverted microscope to capture images and further analysis. Cell cytotoxicity-associated morphology changes such as cell shrinking, blebbing, detachment and vacuole formation in the cytoplasm were carefully monitored. Each well was added with Alamar reagent to the treated and control wells.[17] The plates were then kept again in the CO_2 incubator for 4 hr. Then, the fluorescence generated was measured with a fluorimeter at 530-560 nm excitation and emission at 590 nm. The values were measured arbitrarily. The percentage of cell death was calculated using the following formula:

$$\% \text{ of viability} = \frac{\text{Mean Intensity of Samples}}{\text{Mean Intensity of the control}} \times 100$$

Nephroprotective assay

The earlier cytotoxic studies proved no cytotoxicity to the selected cell line until 100 µg/mL. Hence, the nephroprotective studies were carried out till the max dose of 12.5 µg/mL. Briefly, the confluent cells were trypsinized and plated into 96-well plates (5×10^3 cells/well) and allowed to grow for 24 hr. After attaining sufficient growth, cisplatin (25 µM) was added to induce toxicity and incubated for 1 hr. After incubation, the freshly prepared morin was added at concentrations of 12.5, 6.25, 3.1, 1.5, and 0.75 µg/mL. The plates were kept in the CO_2 incubator for 24 hr, and then the plates were observed under a microscope, and images were captured to analyze any changes in cell morphology. Each well was added with Alamar reagent to the treated and control wells. The plates were then kept again in the CO_2 incubator for 4 hr. Then, a fluorimeter was used to quantify the fluorescence generated at 530-560 nm excitation and emission at 590 nm. The percentage of cell death was calculated using the following formula discussed above.

ROS analysis

Confluent cells were transferred to the T25 flask at a concentration of 1 x 10^6 cells/flask. The flasks were kept for 24 hr in an incubator to adhere the cells to the flasks. Then the flasks were treated with cisplatin (25 µM) and incubated for 1 hr to induce the cell toxicity, and then 12.5 µg/mL of morin was added to the test flask while keeping the untreated control and cisplatin-treated positive control. After 24 hr, the flasks were washed with PBS

2 times and 50 µL of DCFDA was added and incubated for 30 min in the dark.[18] After incubation, the excess dye was removed by PBS washing, and the cells were captured using a fluorescent microscope. Simultaneously, the excitation was quantified at 470 nm, and the emission was 635 nm.

Molecular docking

Missing hydrogen atoms and restrained electrostatic potential charges[19] were added to Keap1 protein (PDBID: 7K2L)[20] using the leap module of AmberTools2023[21] software using amberff99SB[22] force field. Keap1 monomer (chain A of crystal structure) was selected for the docking studies. Morin was optimized using 6-31G* basis set using Gaussian 03 software.[23] The restrained electrostatic potential on atoms were generated using antechamber module of AmberTools.[21] The restrained electrostatic charges on each atom were preserved during docking using AutoDock Vina.[24] The grid box (box dimensions 40, 40, 24 on x, y, and z directions and with a grid centre 23.73, 29.48, and -25.18 on x, y and z directions was generated on the active site of the protein containing the amino acid residues TYR-9, ARG-55, ASN-57, ARG-89, ARG-90, ARG-158, SER-183, GLY-184, TYR-200, GLN-205, ALA-231, TYR-247, PHE-252, SER-277 and GLY-2782. The grid spacing used for docking was 1.0.

Real-Time PCR

HEK 293 cell line was cultured as per the standard procedure mentioned earlier. The cultured cells were placed in a T25 flask for overnight incubation, and the next day, the cells were pretreated with Ciplstain (25 µM) and incubated for 1 hr to induce the cell toxicity, then 12.5 µg/mL of Morin was added to the test flask while keeping the untreated control and cisplatin-treated positive control. After 24 hr, the cells were removed from the flask and proceeded with RNA isolation. The RNA isolation has been proceeded by us using RNA isolation reagent produced from (Invitrogen-product code 10296010). Briefly, 1 mL of TRIzol reagent was added to the flask and incubated for 5 min. Then, the cell contents were transferred to a fresh, sterile Eppendorf tube, and 200 µL of chloroform was added at room temperature, and the contents were centrifuged at 14000 rpm at 40°C for 15 min. The aqueous layer was collected from the centrifuge content into a fresh tube and added with 500 µL of 100% isopropanol. This mixture was again incubated at room temperature and then centrifuged at 14000 rpm at 40°C for 15 min. The supernatant was discarded, and the pellets were collected and washed with 200 µL of ethyl alcohol. This was again centrifuged at 14000 rpm at 40°C for 15 min in a refrigerated centrifuge. The RNA pellets were dried and suspended in TE buffer for the cDNA synthesis.

Prior to the cDNA synthesis, the purity and integrity of the RNA that had been obtained were analyzed. Template cDNA was prepared using a cDNA kit (G BIOSCIENCES, MO, USA). Briefly, 2 µL of RNA template (0.5 µg of total RNA), 5 µL of RT easy mix, and 0.5 µL of oligo dT were added in an RNAse-free PCR tube. Sterile distilled water was used to make up the volume of the reaction mixture to 10 µL, and the mixture was mixed gently up and down without the formation of bubbles. The thermal cycler (Eppendorf Master Cycler) was performed to synthesize the cDNA at a cycling condition of cDNA synthesis at 42°C for 20 min and one cycle. Inactivation was performed at 850°C for 5 min in one cycle.

The expression levels of genes representing the HMOX and Nrf2 in control and treatments were evaluated using real-time qRT-PCR using SYBR Green Master Mix (G BIOSCIENCES, MO, USA) and LightCycler 96 (Roche). All reactions were performed in triplicates, and the data were analyzed as per the ΔΔCt method. The PCR reaction was carried out as follows: an initial activation step for 2 min at 95°C; 3-step cycling of denaturation for 10 sec at 950°C, annealing for 1 min at 560°C, and extension for 1 min/kb at 72°C, all for 40 cycles. The end of PCR cycling was kept at 40°C for an indefinite time period. The primers used for the experiments are HMOX (sense primer: 5′-CTCAAACCTCCAAAAGCC-3′; antisense primer: 5′-TCAAAAACCACCCCAACCC-3′), Nrf2 (sense primer: 5′-CACATCCAGTCAGAAACCAGTGG-3′; antisense primer: 5′-GGAATGTCTGCGCCAAAAGCTG-3′), and GAPDH (sense primer: 5′-ACTCAGAAGACTGTGGATGG-3′; antisense primer: 5′-GTCATCATACTTGGCAGGTT-3′). The finished PCR product was also run in an electrophoresis tank, and the gel was visualized.

Statistical analysis

All the experiments were performed in triplicate. The statistical analysis was run using GraphPad Prism software, version 6.0. Significance was measured using one-way ANOVA and Duncan's multiple-range test. The statistical significance was set at $^{*}p < 0.1$ compared to the control group.

RESULTS

Morin exerts nephrotoxicity protection without causing cytotoxicity

The study's first aim was to rule out any chances of cellular toxicity from morin to the selected cell lines. Hence, we have conducted cytotoxicity studies. As shown in Figure 1, there is no sign of toxicity to the cells, even at higher concentrations. There was still 75% of cells live at 100 µg/mL. This showed that the compound morin is safer to study nephroprotective less than this dose concentrations. The viable cells were quantified and presented in the Figure 1G. All experiments were carried out in triplicates. Results were presented as Mean±SE. $^{***}p<0.001$ compared to the control group, $^{*}p<0.1$ compared to control group.

The viable cells were quantified and presented in the Figure 2. All experiments were carried out in triplicates. Results were presented as Mean±SE. $^{***}p<0.001$ compared to the control group, $^{*}p<0.1$ compared to control group

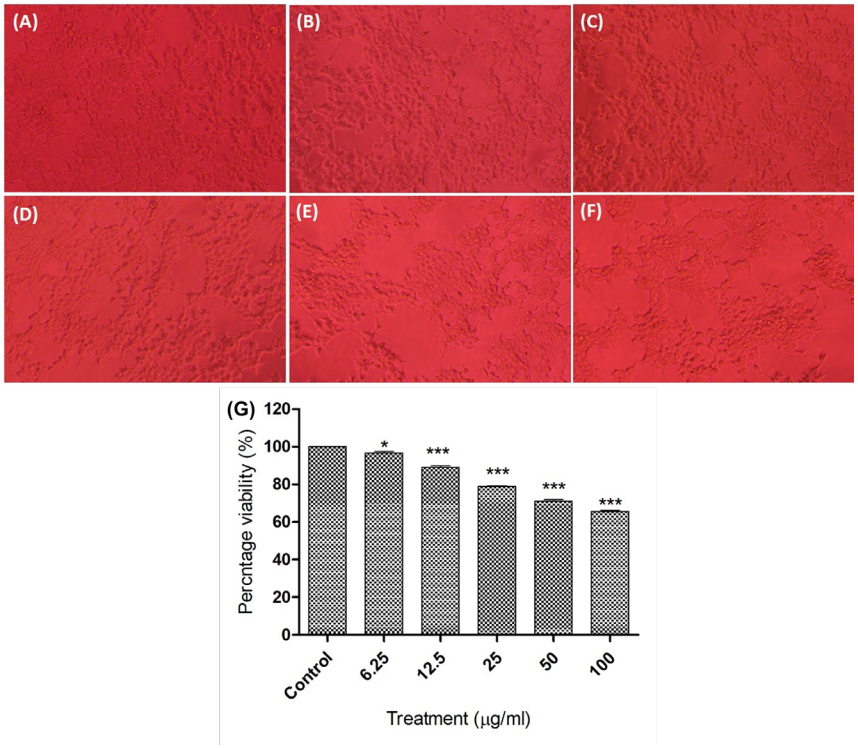

Figure 1: HEK 293 cells were treated with Morin, and the cytotoxicity was tested using Alamar blue staining. (A) Control group, (B-F) morin treatment 6.25, 12.5, 25, 50 and 100 µg/mL respectively.

Morin-induced antioxidant effect via protecting the ROS generation

In order to evaluate if Morin can protect the cell from toxicity induced by ROS generation, a DCFDA assay was performed. As shown in Figure 3, cisplatin has significantly induced the excess production of ROS, which was effectively and significantly protected by the morin. The results 3D showed that 12.5 µg/mL Morin has significantly protected the oxidative damage at $^{*}p<0.05$.

Morin induced nephroprotective effect through modulating Nrf2/HMOX-1 mechanism

Many studies have shown that Nrf2/HMOX-1 pathways are the most important endogenous mechanism of antioxidant protection. In the event of oxidative stress, Nrf2 will be activated and translocated from the cytoplasm to the nucleus, and there, it will initiate the translational upregulation of HMOX-1. Hence, we evaluated the involvement with docking studies at the beginning. Docking is a powerful tool that accelerates drug discovery. As per the results, the complex of Keap1 with the Nrf2 domain shows that the key amino acids involved in the binding process are TYR-9, ASN-57, ARG-89, ARG-90, ARG-158, SER-183, GLY-184, TYR-200, GLN-205, ALA-231, TYR-247, PHE-252, SER277 and GLY-278[2]. So, for docking, grid was generated on this site to know the affinity of Morin towards the Nrf2 binding sites of Keap1 protein. The docking score of Morin in the active site of Keap1 is -7.7 kcal/mol which shows good affinity of Morin towards the Nrf2 binding site. The best docked structure is shown in Figure 4. In Figure 4A, the Nrf2 domain is also aligned to understand whether Morin binds to the binding site with similar orientation as that of the Nrf2 domain. Morin binds to the active site with a favourable binding affinity and also binds to active site with similar orientation as that of Nrf2 domain of Keap1 protein. Figure 4B shows the key amino acid residues which interact with Morin. This includes TYR-9, SER-13, SER-277, ARG-55, ASN-57, ASN-89, ARG-90, TYR-200, GLN-205, ALA-231 and TYR-247. The amino acids TYR-9, ARG-55, ASN-89, ARG-90. GLN-205, ALA-231 and TYR-247 are shared by both Morin and the domain of Nrf2. Figure 4C shows the ligand interaction with the surrounding amino acids of the enzyme. Here, SER-277, ARG-55 and SER-230 form hydrogen bond interactions with the hydroxyl groups of Morin. Also, TYR-247 forms pi-pi stacking interaction with the aromatic ring of Morin. These observations suggest that Morin could be potential lead molecule as Keap1 inhibitor. Gene expression research was carried out following the positive results we got from docking studies (Figure 5). In this study, the expression of Nrf2 and HMOX has been evaluated using real-time RT-PCR. The results showed a significant elevation in the mRNA expression of both Nrf2 (1.99 folds) and HMOX (1.85 folds). At the same time, a negative regulation was observed in the cisplatin-alone group. This suggests that the nephroprotective effect of Morin has been largely activated through Nrf2/HMOX-1 pathways.

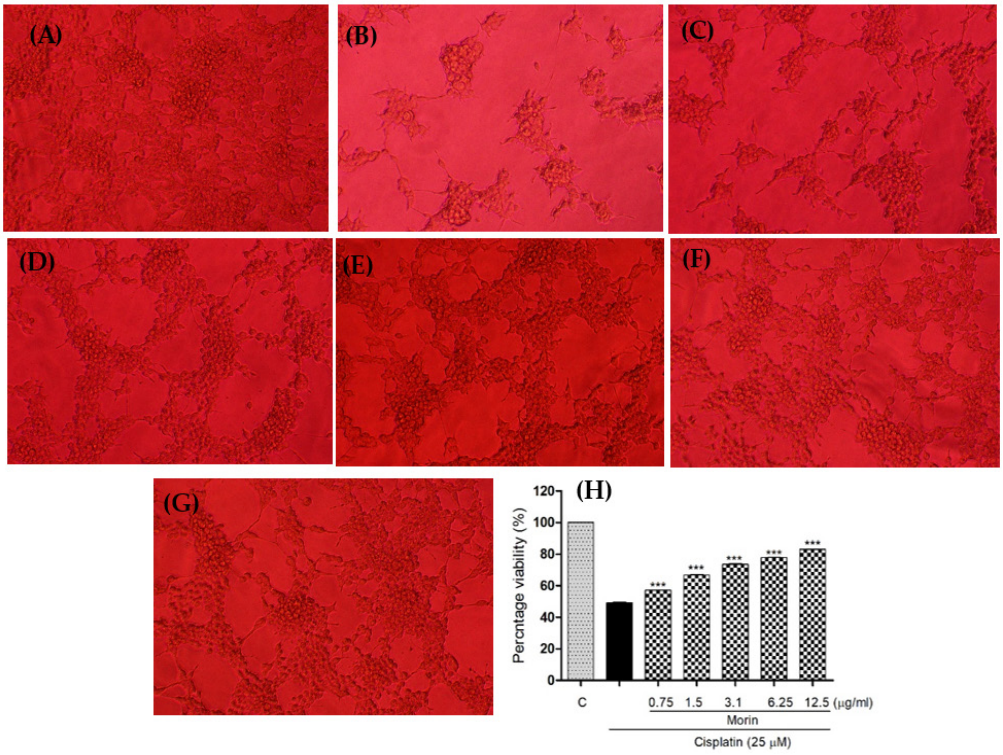

Figure 2: HEK 293 cells were treated with cisplatin and morin, and the cytotoxicity was tested using Alamar blue staining. (A) Untreated control group, (B) cisplatin-treated cells, (C-F) Morin treatment 0.75, 1.5, 3.1 6.25 and 12.5 µg/mL, respectively.

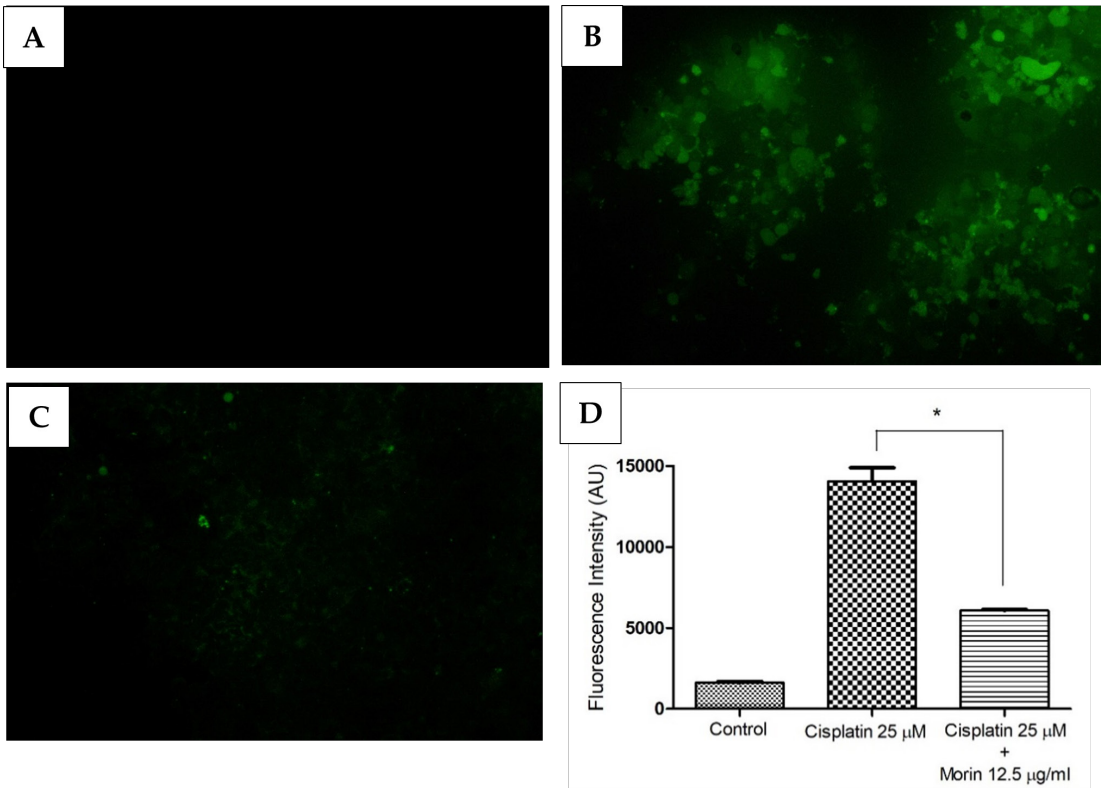

Figure 3: Determination of ROS generation by DCFDA staining. (A) Control, (B) Cisplatin 25 µM alone, (C) cisplatin 25 µM and morin 12.5 µg/mL and (D) quantitative analysis of data. All experiments were done in triplicates and results were represented as Mean±SE. One-way ANOVA was performed to analyse data. *p<0.05 compared to the control group.

DISCUSSION

In the current study, we have attempted to analyse the effectiveness of morin to protect against the nephrotoxicity induced by the chemotherapeutic drug cisplatin in human kidney cells *in vitro*. Cisplatin is a metal coordination compound, an effective and well-used drug in chemotherapy, either alone or in combination with other drugs. Cisplatin works in cancer by forming DNA adducts to distort DNA by acting on both intrastrand and interstrand crosslinks and subsequently inducing apoptosis in cancer cells.[25] But the dose-related toxicity, especially nephrotoxicity, limits its use. There have been many attempts made in the past to incorporate natural products in preclinical studies to overcome these issues. But there need to be many more good attempts to find better choices. Even though many studies have been conducted in order to establish the mechanism behind cisplatin-induced nephrotoxicity, the major findings in this area clearly indicate that there is a decrease in antioxidant defence systems due to ROS generation and stress, which includes the role of enzymatic and non-enzymatic molecules and reduced GSH.[26] Hence, finding an antioxidant to protect against the toxicity, especially from natural sources, would be the ideal choice.

Morin is a polyphenolic compound that belongs to the flavanol class of flavonoids. This compound has been found in many edible plants, such as figs, chestnuts, jackfruit, coffee, apples, etc.[27] This compound has a shorter half-life and delay in absorption while administered orally. Since it is a medically active compound, it has been shown to induce anti-inflammatory, anticancer, and gastroprotective effects.[28] Therefore, it is proven to be a potential resource to mitigate multiple disease conditions. In addition, Morin's safety features are in a highly acceptable range, according to earlier studies.[29] In the current research, we were first interested in knowing whether the compound induces any kind of cellular toxicity in the chosen cell line. It is because we have considered the fact that some compounds, while exerting beneficial effects in the cellular system, also cause potential traits to some extent, which is not an acceptable model. In our work, we have shown that up to 100 µg/mL doses of morin have excellent safety potential. Hence, all other studies, including the nephrotoxic studies we have carried out, have used much lower doses than that, which has improved the significance of this compound to this study plan.

The nephroprotective role was initially accessed by cytotoxicity screening, in which we used cisplatin initiation and additional morin application. As we can see from the results, cisplatin directly induces cellular toxicity. Morin protects the induced toxicity from 7.5 to 12.5 µg/mL. It is to be noted that even lower doses of morin could protect the cell from injury, which shows the significance of using morin at lower doses, probably in clinical trials later. This could be due to the general protective functions of flavonoids in general.[30] Earlier studies done on many similar compounds from flavonoids showed very good activity in nephroprotection. For instance, apigenin, flavone glycosidase, catechin, quercetin, etc., are some of those examples.[31,32] All these compounds have been exhibited to have either an antioxidant effect, an anti-apoptotic effect, or an anti-inflammatory effect in order to protect the cells. Compared to other phytochemicals, flavonoids have a higher potential in exerting these actions, maybe due to their ability to modify cellular enzymes along with antioxidant activity.

ROS has always been an interesting area of research, as it plays a bimodal role in cellular homeostasis maintenance. The favourable nature of ROS belongs to their action on redox potential and in cell signaling, while it has much more adverse events resulting from excess production of free radicals or inadequate functioning of endogenous antioxidants.[33] ROS plays a significant role in nephrotoxicity. Either by environmental pollutants or by cisplatin-kind chemotherapeutic agents, ROS levels are increased, leading to increased lipid peroxidation, DNA damage, protein modification, and depletion of ATP, etc. Since ROS has the ability to trigger apoptosis, there are many drugs designed to kill cancer drugs through ROS.[34] In the case of cisplatin, the administered

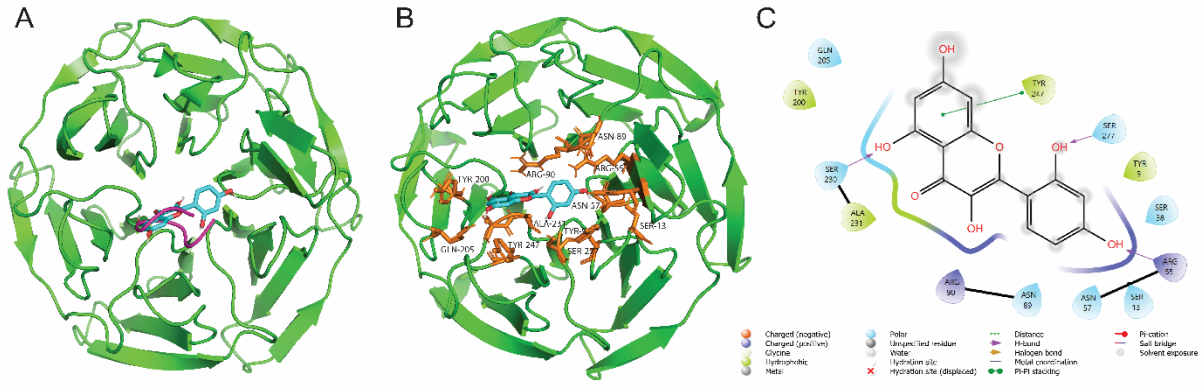

Figure 4: A) Docked complex of Keap1 with Morin in the Nrf2 binding site. Keap1 is shown as green cartoon representation and purple colour represents the Nrf2 domain. Morin is represented by the sticks with cyan colour. The Nrf2 domain is also shown to visualize that Morin also binds to the Nrf2 binding site of Keap1. B) The complex of Keap1 with Morin showing the key amino acids responsible for binding. The orange-coloured sticks represent the amino acids within 4.0 Å from Morin. C) Keap1-Morin interactions depicting various kinds of non-covalent interactions between them.

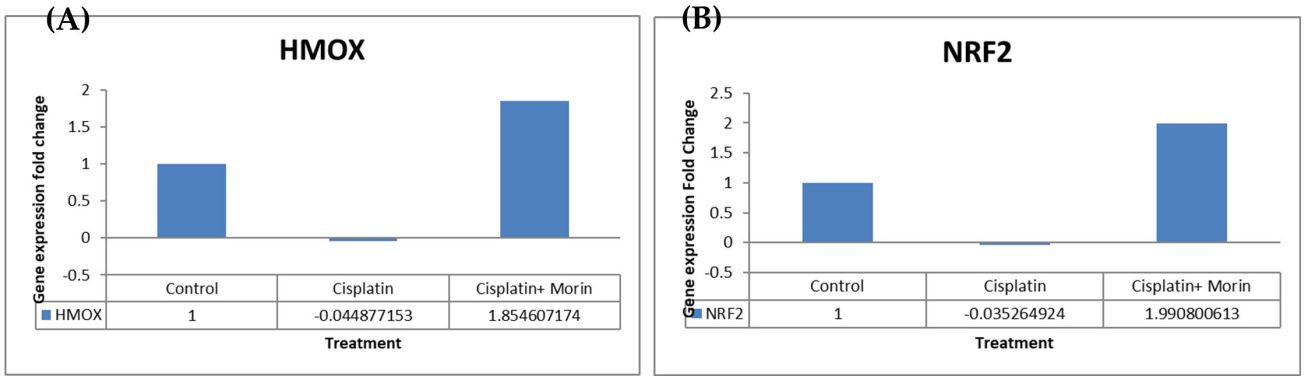

Figure 5: Graphical representation depicting the expression pattern of (A) HMOX and (B) NRF2.

cisplatin enters the renal epithelial cells either through OCT2 or Crt1 transporters, damages the DNA, and produces ROS. Morin is a well-known natural product that induces protection against mitochondrial ROS and direct cellular ROS generation.[35] Our results show that cisplatin significantly reduced the ROS produced by cisplatin through the DCFDA assay. The results are in agreement with previous studies in which morin has displayed ROS scavenging activity in many cell-based assays.

Nrf2 pathways are one of the major pathways related to oxidative stress and redox potential. Under normal physiological conditions, Keap1 holds Nrf2 in the cytoplasm and promotes ubiquitination. However, in pathological conditions, Nrf2 gets detached from the bond and translocates to the nucleolus, where it accelerates the transcriptional gene activation for the antioxidant action.[36] This pathway is a potential target for cytoprotective agents because stress is the main functional agent for toxicity generation. Novel phytochemicals that can act as antagonists in the binding site, where Nrf2 binds with Keap1, could be the fundamental behind the theory. We have observed that in the docking studies, Morin has a good affinity towards the Nrf2 binding site, with a docking score of -7.7 kcal/mol. Later in the mRNA gene expression studies, support for this finding was seen, where we have seen increased expression of Nrf2 and their downstream target HMOX, which is an enzyme that protects the cells from oxidative stress.[37] Studies have earlier shown that when cells are exposed to stress-inducing cisplatin-like drugs, Nrf2/HMOX-1 helps alleviate damage. Hence, we can clearly suggest that cisplatin-induced nephrotoxicity-based oxidative stress damage can be significantly prevented by using morin.

CONCLUSION

To conclude, our results suggest that morin is a potential compound that can be the best candidate to develop for clinical trials to use together with cisplatin chemotherapy. Our study made the mechanism of Morin in nephroprotection, which was through protecting the cells from harmful free radical stresses. The study also suggests that there is a clear evasion that the anti-oxidation of morin is through Nrf2/Hmox pathway regulation. We have shown this interaction through molecular docking and mRNA studies. Further animal-based and clinical-based studies are thus strongly warranted with a special focus on more anti oxidative gene expressions.

ACKNOWLEDGEMENT

The authors Jianliang Qu and Wei Song would like to thank the Medical Research General Project Supported by Xi 'an Science and Technology (2024JH-YLYB-0445). The authors extend their appreciation to the Researchers Supporting Project number (RSPD-2025R677), King Saud University, Riyadh, Saudi Arabia for financial support.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

FUNDING SOURCES

The authors Jianliang Qu and Wei Song would like to thank the Medical Research General Project Supported by Xi 'an Science and Technology (2024JH-YLYB-0445). The authors extend their appreciation to the Researchers Supporting Project number (RSPD-2025R677), King Saud University, Riyadh, Saudi Arabia for financial support.

ETHICAL STATEMENT

This research in not associated with any ethical concerns.

ABBREVIATIONS

cDNA: Complementary DNA; **DCFDA:** 2'-7'-Dichlorodihydrofluorescein diacetate; **DMEM:** Dulbecco's Modified Eagle Medium; **FBS:** Fetal Bovine Serum; **HEK:** Human embryonic kidney; **HMOX:** Heme oxygenase; **Keap1:** Kelch-like ECH-associated protein 1; **Nrf2:** Nuclear factor erythroid 2-related factor 2; **PBS:** Phosphate Buffered Saline; **PCR:** Polymerase chain reaction; **Rpm:** Rotation per minute; **TE:** Tris EDTA buffer.

SUMMARY

Cisplatin-induced nephrotoxicity remains a major struggle in chemotherapy. It happens majorly due to free radical-mediated cell damage. This manuscript investigated the protective effect of morin, a flavonoid, in combating the side effects exerted by cisplatin using HEK 293 cells as an *in vitro* model. The study established morin's safety at sub-cytotoxic concentrations and highlighted its efficacy in mitigating oxidative damage. The protective mechanism was established through the Nrf2/HMOX-1 antioxidant pathway, supported by molecular docking and real-time PCR. These findings not only advance our understanding of morin's nephroprotective potential but also highlight the therapeutic possibilities of natural products in addressing drug-induced toxicities.

REFERENCES

1. Tang C, Livingston MJ, Safirstein R, Dong ZJ. Cisplatin nephrotoxicity: new insights and therapeutic implications. Nat Rev Nephrol. 2023;19(1):53-72. doi: 10.1038/s41581-022-00631-7, PMID 36229672.
2. Tsvetkova D, Ivanova SJ. Application of approved cisplatin derivatives in combination therapy against different cancer diseases. Molecules. 2022;27(8):2466. doi: 10.3390/molecules27082466, PMID 35458666.
3. Alqahtani MM. Cisplatin-induced renal failure measured by glomerular filtration rate (GFR) with 99mTc-DTPA scans in cancer patients: A systematic review and meta-analysis. Diagnostics (Basel). 2024;14(22):2468. doi: 10.3390/diagnostics14222468, PMID 39594134.
4. Aladaileh SH, Al-Swailmi FK, Abukhalil MH, Ahmeda AF, Mahmoud AM. Punicalagin prevents cisplatin-induced nephrotoxicity by attenuating oxidative stress, inflammatory response, and apoptosis in rats. Life Sci. 2021;286:120071. doi: 10.1016/j.lfs.2021.120071, PMID 34688692.
5. Shen N, Wang T, Gan Q, Liu S, Wang L, Jin B. Plant flavonoids: classification, distribution, biosynthesis, and antioxidant activity. Food Chem. 2022;383:132531. doi: 10.1016/j.foodchem.2022.132531, PMID 35413752.
6. Čižmárová B, Hubková B, Tomečková V, Birková AJ. Flavonoids as promising natural compounds in the prevention and treatment of selected skin diseases. Int J Mol Sci. 2023;24(7):6324. doi: 10.3390/ijms24076324, PMID 37047297.
7. Roy A, Khan A, Ahmad I, Alghamdi S, Rajab BS, Babalghith AO, *et al.* Flavonoids a bioactive compound from medicinal plants and its therapeutic applications. BioMed Res Int. 2022;2022(1):5445291. doi: 10.1155/2022/5445291, PMID 35707379.
8. Dias MC, Pinto DC, Silva AM. Plant flavonoids: chemical characteristics and biological activity. Molecules. 2021;26(17):5377. doi: 10.3390/molecules26175377, PMID 34500810.
9. Goyal A, Dubey N, Agrawal A, Verma A. Sharma RJPR-MCM. Therapeutic potential of morin: special focus on cognitive dysfunction, diabetes mellitus and cancer. Mod Chin Med. 2023;9:100318.
10. Ijaz MU, Shahzadi S, Zahra Z, Samad A, Ehsan N, Afsar T, et al. Ameliorative effects of morin on cisplatin-induced toxicity in renal mitochondria isolated from rats. J King Saud Univ Sci. 2021;33(8):101655. doi: 10.1016/j.jksus.2021.101655.
11. Chaudhary P, Janmeda P, Docea AO, Yeskaliyeva B, Abdull Razis AF, Modu B, et al. Oxidative stress, free radicals and antioxidants: potential crosstalk in the pathophysiology of human diseases. Front Chem. 2023;11:1158198. doi: 10.3389/fchem.2023.1158198, PMID 37234200.
12. Martemucci G, Costagliola C, Mariano M, D'andrea L, Napolitano P, D'Alessandro AG. Free radical properties, source and targets, antioxidant consumption and health. Oxygen. 2022;2(2):48-78. doi: 10.3390/oxygen2020006.
13. Unsal V, Cicek M, Sabancilar İ. Toxicity of carbon tetrachloride, free radicals and role of antioxidants. Rev Environ Health. 2021;36(2):279-95. doi: 10.1515/reveh-2020-0048, PMID 32970608.
14. Zhou J, Nie Rc, Yin Yx, Cai Xx, Xie D. Cai M-yJDm. Protective effect of natural antioxidants on reducing cisplatin-induced nephrotoxicity. Dis Markers. 2022;2022(1):1612348.
15. Jin W, Botchway BO, Liu XJ, Repair N. Curcumin can activate the Nrf2/HO-1 signaling pathway and scavenge free radicals in spinal cord injury treatment. Neurorehab Neural Repair. 2021;35(7):576-84. doi: 10.1177/15459683211011232, PMID 33980059.
16. Hsieh CY, Jayakumar T, Lin KC, Yen TL, Hsia CW, Huang WC, *et al.* Morin hydrate suppresses lipoteichoic acid-induced oxidative stress-mediated inflammatory events in macrophages via augmenting Nrf2/HO-1 and antioxidant defense molecules. Eur J Inflamm. 2023;21:1721727X231199414. doi: 10.1177/1721727X231199414.
17. Longhin EM, El Yamani N, Rundén-Pran E, Dusinska M. The alamar blue assay in the context of safety testing of nanomaterials. Front Toxicol. 2022;4:981701. doi: 10.3389/ftox.2022.981701, PMID 36245792.
18. Ng NS, Ooi LJ. A simple microplate assay for reactive oxygen species generation and rapid cellular protein normalization. Bio Protoc. 2021;11(1):e3877. doi: 10.21769/BioProtoc.3877, PMID 33732765.
19. Bayly CI, Cieplak P, Cornell W, Kollman PA. A well-behaved electrostatic potential based method using charge restraints for deriving atomic charges: the RESP model. J Phys Chem. 1993;97(40):10269-80. doi: 10.1021/j100142a004.
20. Ortet PC, Muellers SN, Viarengo-Baker LA, Streu K, Szymczyna BR, Beeler AB, *et al.* Recapitulating the binding affinity of Nrf2 for KEAP1 in a cyclic heptapeptide, guided by NMR, X-ray crystallography, and machine learning. J Am Chem Soc. 2021;143(10):3779-93. doi: 10.1021/jacs.0c09799, PMID 33683866.
21. Case DA, Aktulga HM, Belfon K, Cerutti DS, Cisneros GA, Cruzeiro VW, *et al.* AmberTools. J Chem Inf Model. 2023;63(20):6183-91. doi: 10.1021/acs.jcim.3c01153, PMID 37805934.
22. Hornak V, Abel R, Okur A, Strockbine B, Roitberg A, Simmerling C. Comparison of multiple Amber force fields and development of improved protein backbone parameters. Proteins. 2006;65(3):712-25. doi: 10.1002/prot.21123, PMID 16981200.
23. Gaussian 09 citation. Gaussian.com. Available from: https://gaussian.com/g09citation/.
24. Eberhardt J, Santos-Martins D, Tillack AF, Forli S, AutoDock V. AutoDock Vina 1.2.0: New docking methods, expanded force field, and python bindings. J Chem Inf Model. 2021;61(8):3891-8. doi: 10.1021/acs.jcim.1c00203, PMID 34278794.
25. Jamieson ER, Lippard SJ. Structure, recognition, and processing of cisplatin– DNA adducts. Chem Rev. 1999;99(9):2467-98. doi: 10.1021/cr980421n, PMID 11749487.
26. Choi YM, Kim HK, Shim W, Anwar MA, Kwon JW, Kwon HK, *et al.* Mechanism of cisplatin-induced cytotoxicity is correlated to impaired metabolism due to mitochondrial ROS generation. PLOS One. 2015;10(8):e0135083. doi: 10.1371/journal.pone.0135083, PMID 26247588.
27. Rajput SA, Wang XQ, Yan HC. Morin hydrate: A comprehensive review on novel natural dietary bioactive compound with versatile biological and pharmacological potential. Biomed Pharmacother. 2021;138:111511. doi: 10.1016/j.biopha.2021.111511, PMID 33744757.
28. Arab HH, Saad MA, El-Sahar AE, Al-Shorbagy MY. Mechanistic perspective of morin protection against ketoprofen-induced gastric mucosal injury: targeting HMGB1/RAGE/NF-κB, DJ-1/Nrf2/HO-1 and PI3K/mTOR pathways. Arch Biochem Biophys. 2020;693:108552. doi: 10.1016/j.abb.2020.108552, PMID 32860758.
29. Kuzu M, Yıldırım S, Kandemir FM, Küçükler S, Çağlayan C, Türk E, *et al.* Protective effect of morin on doxorubicin-induced hepatorenal toxicity in rats. Chem Biol Interact. 2019;308:89-100. doi: 10.1016/j.cbi.2019.05.017, PMID 31100273.
30. Zeng X, Xi Y, Jiang W. Protective roles of flavonoids and flavonoid-rich plant extracts against urolithiasis: a review. Crit Rev Food Sci Nutr. 2019;59(13):2125-35. doi: 10.1080/10408398.2018.1439880, PMID 29432040.
31. Hassan SM, Khalaf MM, Sadek SA, Abo-Youssef AMPb. Protective effects of apigenin and myricetin against cisplatin-induced nephrotoxicity in mice. Pharm Biol. 2017;55(1):766-74. doi: 10.1080/13880209.2016.1275704, PMID 28064632.
32. Kratochwil C, Giesel FL, Leotta K, Eder M, Hoppe-Tich T, Youssoufian H, *et al.* PMPA for nephroprotection in PSMA-targeted radionuclide therapy of prostate cancer. J Nucl Med. 2015;56(2):293-8. doi: 10.2967/jnumed.114.147181, PMID 25613537.
33. Kim H, Kim IY, Lee SY, Jeong D. Bimodal actions of reactive oxygen species in the differentiation and bone-resorbing functions of osteoclasts. FEBS Lett. 2006;580(24):5661-5. doi: 10.1016/j.febslet.2006.09.015, PMID 16996506.
34. Ezhilarasan D, Apoorva VS, Vardhan A. NJJoOP, medicine. Syzygium cumini extract induced reactive oxygen species-mediated apoptosis in human oral squamous carcinoma cells. J Oral Pathol Med. 2019;48(2):115-21.
35. Herrera-Pérez Z, Gretz N, Dweep HJ. A comprehensive review on the genetic regulation of cisplatin-induced nephrotoxicity. Curr Genomics. 2016;17(3):279-93. doi: 10.2174/1389202917666160202220555, PMID 27252593.
36. Li M, Huang W, Jie F, Wang M, Zhong Y, Chen Q, *et al.* Discovery of Keap1– Nrf2 small–molecule inhibitors from phytochemicals based on molecular docking. Food Chem Toxicol. 2019;133:110758. doi: 10.1016/j.fct.2019.110758, PMID 31412289.
37. Wu W, Tang J, Bao W, Feng Q, Zheng J, Hong M, *et al.* Thiols-rich peptide from water buffalo horn keratin alleviates oxidative stress and inflammation through co-regulating Nrf2/Hmox-1 and NF-κB signaling pathway. Free Radic Biol Med. 2024;223:131-43. doi: 10.1016/j.freeradbiomed.2024.07.023, PMID 39084576.

Cite this article: Qu J, Song W, Alarfaj AA, Hirad AH. Mitigating Cisplatin-Induced Nephrotoxicity with Morin: A Flavonoid's Role in Nrf2/HO-1 Pathway Activation in HEK 293 Cells. Indian J of Pharmaceutical Education and Research. 2025;59(3s):s1-s10.