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Original article

Network pharmacology and *in vivo* validation of *Manilkara hexandra* bark bioactives against cisplatin-induced nephrotoxicity and cardiac dysfunction in Wistar rats

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Abstract

Objective: The current study aimed to evaluate the protective role of *Manilkara hexandra* methanolic bark extract (MHBE) against cisplatin-induced renal injuries and its effects on the cardiovascular system in Wistar rats using *in-silico* and *in-vivo* approaches.

Materials and methods: *In-silico* studies, including network pharmacology and molecular docking, identified key MHBE phytochemicals and their target proteins. Nephrotoxicity was induced with a single intraperitoneal dose of cisplatin (5 mg/kg), followed by oral MHBE treatment at low (100 mg/kg) and high (200 mg/kg) doses for 21 days. Renal and cardiac biochemical markers were measured weekly, alongside urine analysis and ECG monitoring. Histopathological examinations of kidney and heart tissues were conducted.

Results: In-silico findings revealed that bioactives such as taxifolin, catechin, and quercetin effectively targeted poly (ADP-ribose) polymerase 1 (PARP1), tumor necrosis factor ligand superfamily member 11 (TNFSF11), and interleukin-6 (IL-6), providing protection against cisplatin-induced damage. Cisplatin administration elevated serum creatinine, blood urea nitrogen (BUN), uric acid, lactate dehydrogenase (LDH), and creatine kinase-myocardial band (CK-MB), increased urinary sodium and potassium, and reduced serum total protein and albumin. Electrocardiogram (ECG) showed prolonged PR, QT, and QRS intervals with bradycardia in cisplatin-treated rats. MHBE treatment significantly attenuated these biochemical and electrophysiological alterations. Histopathological observations confirmed MHBE ability to restore renal and cardiac architecture.

Conclusion: The findings of in silico and in vivo studies demonstrate that *Manilkara hexandra* methanolic bark extract mitigates cisplatin-induced renal and cardiac toxicities.

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Introduction

The kidney, which receives approximately 25% of the cardiac output, serves as a vital excretory organ that eliminates xenobiotics and metabolic waste products through urine, while also regulating water, electrolyte, and acid–base homeostasis, processes that are fundamental to the maintenance of blood pressure (Sands and Verlander 2018). Acute nephropathy, chronic kidney disease, and end-stage renal disease (ESRD) are all primarily caused by medication-induced nephrotoxicity. Estimates suggest that drug-induced renal failure contributes to about 25% of all cases of acute renal failure (Wu and Huang 2018). It is not easy to diagnose drug-induced nephrotoxicity at the early stage because, in the initial stage, there will be slight renal damage and subtle irregularities in urinary sediment. The functional integrity of the kidney can be severely compromised by exposure to nephrotoxic agents, particularly chemotherapeutic drugs such as cisplatin. A platinum-based anticancer medication called cisplatin (cis-diamminedichloroplatinum (II)) has been approved for use in the treatment of several cancers since the 1970s, however, its adverse reactions like nephrotoxicity and significant negative impact on heart function with an increase in myocardial enzymes and inflammatory markers restrict the use of this medication (De Jongh et al. 2003; Al-Majed et al. 2006; Yousef et al. 2009). Anorexia, polyuria, decreased clearance of waste products, hypomagnesemia, hypokalemia, hyponatremia and alterations in the heart rate are some manifestations of cisplatin-induced nephrotoxicity (CIN). The mechanism of renal cell damage has been the subject of intense investigation for some time. Recent research suggests that some of this damage may be explained by oxidative stress, apoptosis, and inflammation.

Manilkara hexandra Roxb. Dubard is native to South Asian nations and a member of the Sapotaceae family. It has traditionally been employed as an anti-helminthic and to treat diarrhea, fever, and stomach illnesses. *Manilkara hexandra* methanolic bark extract has been shown in earlier studies to have good free radical scavenging activity (Annamalai and Prince 2021). The primary objective of the present investigation was to comprehensively evaluate the protective potential of *M. hexandra* bark extract (MHBE) against cisplatin-induced nephrotoxicity, with particular emphasis on its modulatory effects on renal function, histopathological alterations, and associated biochemical markers. Furthermore, this study also aimed to delineate the extract influence on CIN - mediated cardiovascular disturbances in Wistar rats, thereby providing integrated insights into its therapeutic efficacy and mechanistic basis.

Materials and Methods

Reagents and chemicals

Cisplatin (CELON Labs), ketamine (NEON Laboratories), xylazine (Indian Immunological Ltd.), formalin (NICE chemicals Pvt. Ltd), standard creatinine (SRL Pvt. Ltd.), 0.04 M picric acid, 0.75 N sodium hydroxide, sodium chloride, potassium chloride and reagents kits (Erba) for estimation of serum creatinine, blood urea nitrogen (BUN), uric acid, albumin, total protein, CK-MB, and Lactate dehydrogenase (LDH).

In-silico studies

Mining of active compounds

Active phytochemicals present in bark of *M. hexandra* were retrieved using online databases like IMPPAT (https://cb.imsc.res.in/imppat/home), Dr. Duke's Phytochemical and Ethnobotanical Databases (https://phytochem.nal.usda.gov/) and reviewing previously published GCMS, HPTLC, and LCMS studies using Google Scholar (https://scholar.google.com/) and PubMed

(https://pubmed.ncbi.nlm.nih.gov/) servers (Alamri and Tahir ul Qamar 2023).

Drug likeness property of phytochemicals

Molecular weight, molecular formula and canonical SMILES were collected from PubChem chemistry database. The number of Hydrogen bond donors (HBD), hydrogen bond acceptors (HAD), Blood-brain barrier (BBB) score and Drug likeness model score (DLS) of phytochemicals were predicted using Molsoft server (https://molsoft.com/mprop/). SwissADME (http://www.swissadme.ch/) was used to identify the compounds passing the Lipinski Rule of 5. Compounds with DLS>0.18 and satisfied Lipinski Rule of 5 were considered candidates for further studies (Dwivedi et al. 2021; Mir et al. 2023).

Target mining

Potential targets of active phytochemicals from MHBE were obtained using the Swiss Target Prediction Database (http://swisstargetprediction.ch/) and STITCH Database (http://stitch.embl.de/)

Screening of CIN targets

The OMIM database (https://www.omim.org/) and the Gene Cards database (https://www.genecards.org/) were employed for identifying the genes that trigger CIN. Terms like “Cisplatin-induced nephrotoxicity”, “Cisplatin-induced kidney damage”, and “Cisplatin-induced kidney injury” were used to obtain disease-related targets (Jia et al. 2021).

Identification of overlapping genes

Using Venny 2.1.0, the intersection between targets related to CIN and those associated with active phytoconstituents was identified. The intersection targets were then considered potential candidates for CIN treatment (Szklarczyk et al. 2023).

Protein-protein interaction analysis

Potential targets were subsequently evaluated for Protein-Protein Interaction (PPI) through the utilization of the STRING database (https://string-db.org/) while restricting the species to "*Homo-sapiens"* and *“Rattus norvegicus”*

Gene set enrichment analysis

Utilizing the STRING database (https://string-db.org/), a compilation was made of the target protein(s) responsible for the pathogenesis of CIN that pharmaceutical agents modulate. The data about the enriched pathways was assembled using the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway (https://www.genome.jp/kegg/). A network involving compounds, targets, and pathways was established and examined by utilizing Cytoscape 3.6.1 (https://cytoscape.org/).

Molecular docking

3D structures of all targets were obtained from the PubChem database, while protein X-ray crystallographic structures came from the RCSB PDB database. Missing loops and termini in PDB structures were modelled utilizing Swiss-Model (https://swissmodel.expasy.org/). Using AutoDock, the protein was prepared by removing heteroatoms, adding polar hydrogens, and applying Kollman charges for structural enhancement. The optimized structure was then saved in PDBQT format. A docking grid box was generated, and the results were analyzed to identify the best binding poses and calculate the binding affinity. The protein-ligand complex with the highest binding affinity was visualized using Discovery Studio Visualizer 2021 (Meng et al. 2011).

In-vivo studies

Plant material

The fresh bark of *M. hexandra* was collected from Kanpur, Uttar Pradesh, India. It was authenticated by H.S. Kotambari Science Institute, Hubballi, Karnataka, India (Ref. No.: SKAHSKScl/

Authentication/ 23-24). The bark was washed thoroughly with water, crushed into small pieces and shade-dried for 36 days. Dried bark was pulverized into coarse powder.

Extraction

The Soxhlet extraction method was employed to isolate bioactive compounds from dried *M. hexandra* bark powder using 75% methanol as the solvent (Abd El-Mordy et al. 2020; Garabadu et al. 2021). The obtained yield was found to be 10.21%.

Phytochemical screening was performed to detect the presence of secondary metabolites like alkaloids, flavonoids, tannins, saponins, polyphenols, carbohydrates and proteins (Garg et al. 2019) (Abubakar and Haque 2020).

Carbohydrates were identified using the Molisch test, where the addition of alcoholic α-naphthol followed by 0.2 ml of concentrated sulphuric acid along the tube wall produced a violet ring.

Alkaloids were detected by observing a brown precipitate after adding Wagner's reagent (potassium bismuth iodide) to the acidified extract.

Flavonoids were confirmed by the appearance of a crimson red color upon treatment with magnesium turnings and concentrated hydrochloric acid.

The presence of saponins was indicated by a stable froth formed after vigorously shaking 1 ml of the aqueous extract for 15 minutes.

Tannins were detected by the formation of a white precipitate upon the addition of 10% lead acetate.

Finally, proteins and free amino acids were confirmed using Millon's test, where heating the extract with an equal volume of Millon's reagent yielded a white precipitate that subsequently turned red.

Animals

Male Albino Wistar rats of 250-280 g were procured from KLE College of Pharmacy, Hubballi, Karnataka. Animals were housed in four groups in polypropylene cages maintained at room temperature (25±2°C) under 12 hours light and 12 hours dark cycles with free access to a standard pellet diet and water *ad libitum* (Arung et al. 2011). The IAEC committee of KLEU's College of Pharmacy, Hubballi reviewed and approved the animal experimentation protocol. (Reference No. Mph/NC0222003/KLECoPH/23)

Experimental model

A total of 24 rats were divided into four groups, six animals in each group. Group I (Normal Control) received a standard diet and water *ad libitum* throughout the study. Nephrotoxicity was induced in Group II, III and IV rats by administering a single i.p. injection of 5 mg/kg of cisplatin (Iqbal et al. 2021), on the first day of the study. Group II (cisplatin group) was considered intoxicated group and remained untreated, whereas Group III and Group IV were treated with 100 and 200 mg/kg of MHBE, respectively, administered daily via oral gavage for 21 days.

The food intake, water intake and Body weight (BW) were measured daily for 21 days. On the 7th, 14th, and 21st days of the experiment, the rats were anesthetized with a ketamine–xylazine combination (70 and 8 mg/kg, respectively). Blood samples were then collected via the retro-orbital route and centrifuged at 5000 rpm for 10 min to separate the serum which was subsequently used for biomarker analysis. Renal biomarkers were assessed weekly, while cardiac biomarkers, including Lactate Dehydrogenase(LDH) and Creatine Kinase-Myocardial Band(CK-MB), were evaluated on the last day of the study. At the end of every week, rats were kept in the metabolic cage for 24-hr urine collection to estimate the urine output. Subsequently, urine samples were subjected to centrifugation at 2000 rpm for 10 min to get a clear supernatant for the estimation of urine content, including sodium and potassium levels, whereas urine creatinine levels and creatinine clearance were estimated on the last day of the study.

Electrocardiogram (ECG) analysis was also done at the end of the study, i.e. on the 21st day.

Estimation of serum biomarkers

The amounts of serum creatinine, Blood Urea Nitrogen (BUN), uric acid, total protein, albumin, LDH, and CK-MB were estimated using commercially available Erba kits. The estimate of these biomarkers was done using the Erba Mannheim Chem-7. Each of the reagents used throughout this investigation was of analytical grade.

Assessment of urinary creatinine, electrolytes, and creatinine clearance

Urine samples from experimental rats were collected and appropriately diluted following standardized protocols reported in earlier studies (Soltani Hekmat et al. 2021). Quantitative estimation of urinary creatinine was performed using a colorimetric method (ELICO Model CL 223 colorimeter), while sodium and potassium levels were determined by flame photometry (Systronics Model 126). The creatinine clearance was subsequently calculated in accordance with established procedures described in previous literature (Wang et al. 2019; Iqbal et al. 2022; Dugbartey et al. 2025).

Electrocardiogram analysis

ECG recordings were obtained using the lead II method employing a BIOPAC MP36 system manufactured by Inc Santa Barbara, USA. Signals were examined in terms of PR interval, QT interval, QRS complex and Heart rate (Koti et al. 2009; Ahmed et al. 2021).

Histopathological study

The heart and kidney were isolated, sectioned into small fragments, and fixed in 10% formalin for two days. Subsequently, tissue pieces were treated with different concentrations of ethanol, i.e. 50%, 70%, 99% and at last with absolute ethanol. Subsequently, tissues were treated with acetone and xylene and dipped in the melted paraffin wax for 2 hr. After completion of tissue processing, embedding was performed by using L-blocks. A microtome (Leica) was then used to slice the blocks into sections with a thickness of 3 micrometers, followed by staining with hematoxylin and eosin and examination under a microscope for any histopathological changes. A semi-quantitative scoring system was employed to assess the severity of histopathological alterations, wherein (+) represented mild changes, (++) indicated moderate changes, and (+++) denoted severe changes.

Statistical analysis

One-way ANOVA followed by Tukey's Multiple Comparison Test were the statistical methods used to analyze the experimental data. The findings are presented as Mean ± SEM, with $p<0.05$ designated as the significance level. 8.0.1 version of GraphPad Prism® software was used to conduct the analysis. GraphPad p value range $p<0.05$(*), $p<0.01$(**), $p<0.001$(***) and $p<0.0001$(****) were utilized.

Results

In-silico findings

Prediction of MHBE effect against CIN by an *in-silico* approach

A total of 93 phytochemicals were identified in the bark of MHBE. (Lawrence and Prakash 2019; Abd El-Mordy et al. 2020; Annamalai et al. 2021; Chaudhary et al. 2023). Among 93 compounds, 13 compounds met the criteria i.e. DLS>0.18 and out of 13 compounds, only 4 compounds passed the Lipinski rule of five. We acquired 120 targets associated with 4 active phytochemicals and identified 4163 pertinent gene targets associated with CIN. The Venn diagram illustrated the presence of 71 overlapping targets between MHBE and nephrotoxicity induced by Cisplatin (shown in Figure_1 (A)). Protein-protein interaction studies also revealed that the key pathways and proteins involved in

cisplatin-induced nephrotoxicity remained consistent in both *Homo sapiens* and *Rattus norvegicus*. The network analysis showed that quercetin had the highest edge count and targeted most of the genes responsible for CIN, while taxifolin and catechin targeted Interleukin (IL)-6 and TNFSF11 which are responsible for causing inflammation and apoptosis (shown in Figure_1(B)). Quercetin was docked with PARP1, AKT1 and PIK3R1; taxifolin was docked with TNFSF11 and catechin was docked with IL-6. Compound –protein binding energies. quercetin was found to have the highest binding energy of -8.9 kcal/mol with the target gene PARP 1. Figure 2(A), 2(B) and 2(C) show the docking results.

In-vivo findings

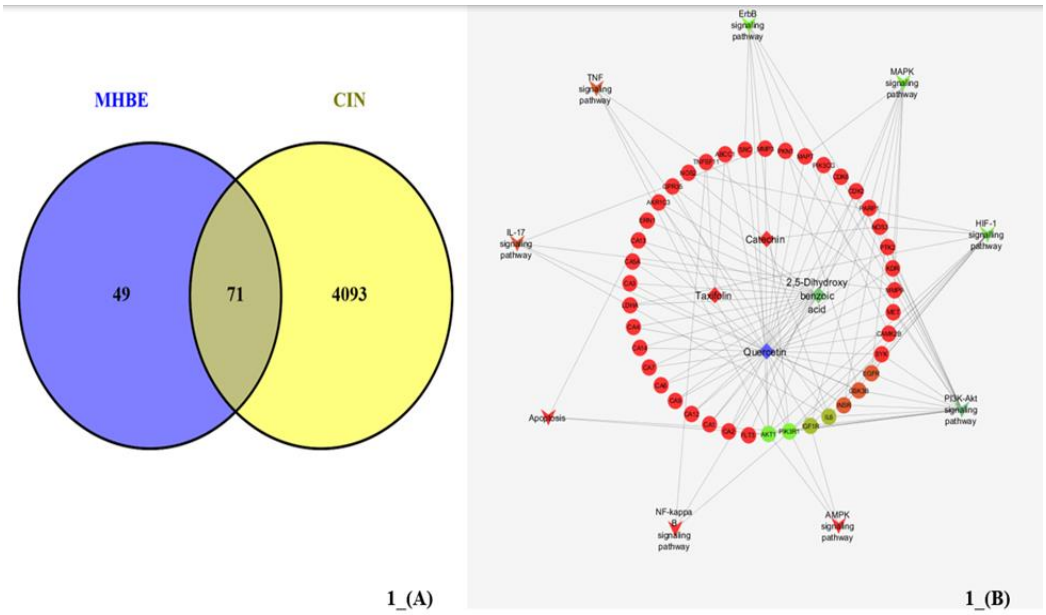

Figure 1 (A). Illustration of overlapping targets between MHBE and cisplatin-induced nephrotoxicity. (B). The compound-target-protein network of MHBE against cisplatin-induced nephrotoxicity.

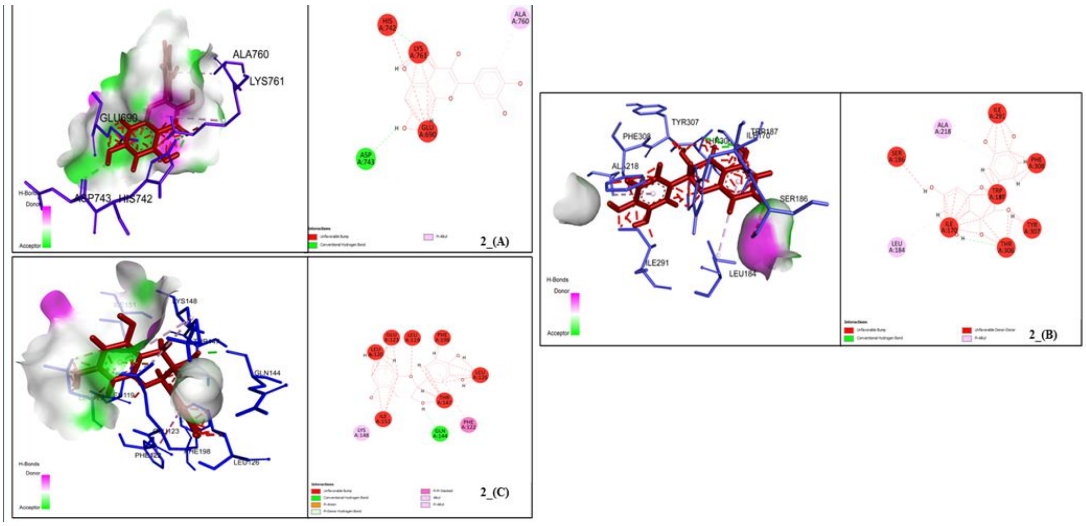

Figure 2 (A), 2(B) and 2(C): 3D and 2D illustration of the interaction of Quercetin, Taxifolin and Catechin with PARP1, TNFSF11 and IL-6, respectively.

Body weight, food intake, water intake and urine output

Cisplatin treatment resulted in a significant decline in body weight and food intake, accompanied by polydipsia and polyuria compared to the normal control group. Administration of MHBE mitigated these alterations, as illustrated in Figures 3(A)–3(D).

Serum biomarkers

Administration of cisplatin significantly elevated serum creatinine and BUN levels in the cisplatin control group with $p< 0.0001$ and $p<0.0001$, respectively, when compared to the normal control group. However, treatment with 100 and 200 mg/kg of MHBE significantly reduced the serum creatinine with $p<0.0001$ and $p<0.0001$, respectively, along with BUN with $p<0.01$ and $p<0.0001$. Substantial drops in the serum albumin and total protein concentration were reported in the cisplatin control group ($p<0.01$ and $p<0.01$), respectively) when compared to normal control. Treatment with 100 mg/kg of MHBE showed no significant changes in serum albumin and total protein levels when compared to the cisplatin control group. However, treatment with 200 mg/kg exerted a little significant rise in serum albumin and total protein levels ($p<0.05$ and $p<0.05$), respectively) when compared to the cisplatin control group. The level of uric acid was reported to be significantly elevated in the Cisplatin control group ($p<0.01$) when compared to the normal group. However, treatment with 100mg/kg and 200mg/kg of MHBE substantially drops the uric acid with $p<0.001$ and $p<0.001$, respectively, when compared to the Cisplatin control group. LDH and CK-MB levels were reported to be elevated in the Cisplatin control group ($p<0.0001$ and $p<0.0001$, respectively) when compared to the normal group, which was mitigated by treatment with 100 and 200 mg/kg of MHBE. Figures 4(A), 4(B), 4(C), 4(D), 4(E), 4(F) and 4(G) show the effect of MHBE on serum creatinine, BUN, Uric acid, albumin, total protein, LDH and CK-MB levels, respectively, of different treatment groups. All biomarkers that were evaluated on a weekly basis have their corresponding values presented.

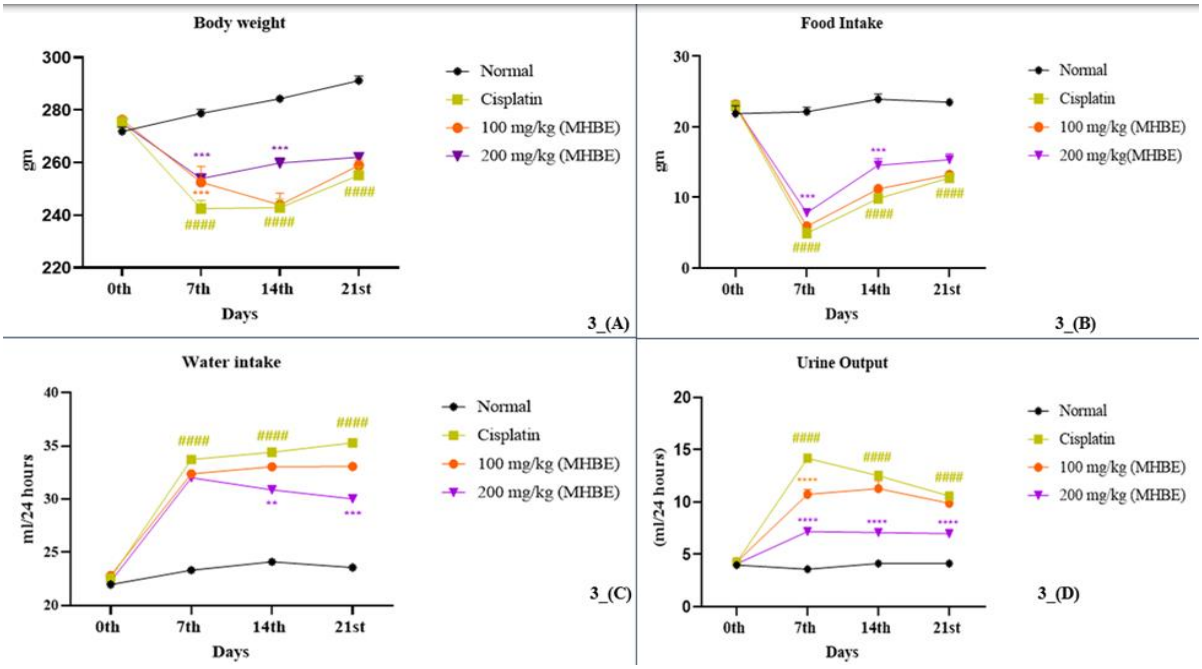

Figure 3. Illustration of the effect of MHBE on cisplatin-induced alterations in body weight, food intake, water intake and urine output. All values are expressed as mean ± SEM; n=6 in each group, ####$p<0.0001$ when compared to normal control group, ****$p<0.0001$, ***$p<0.001$, and **$p<0.01$ when compared to Cisplatin control group.

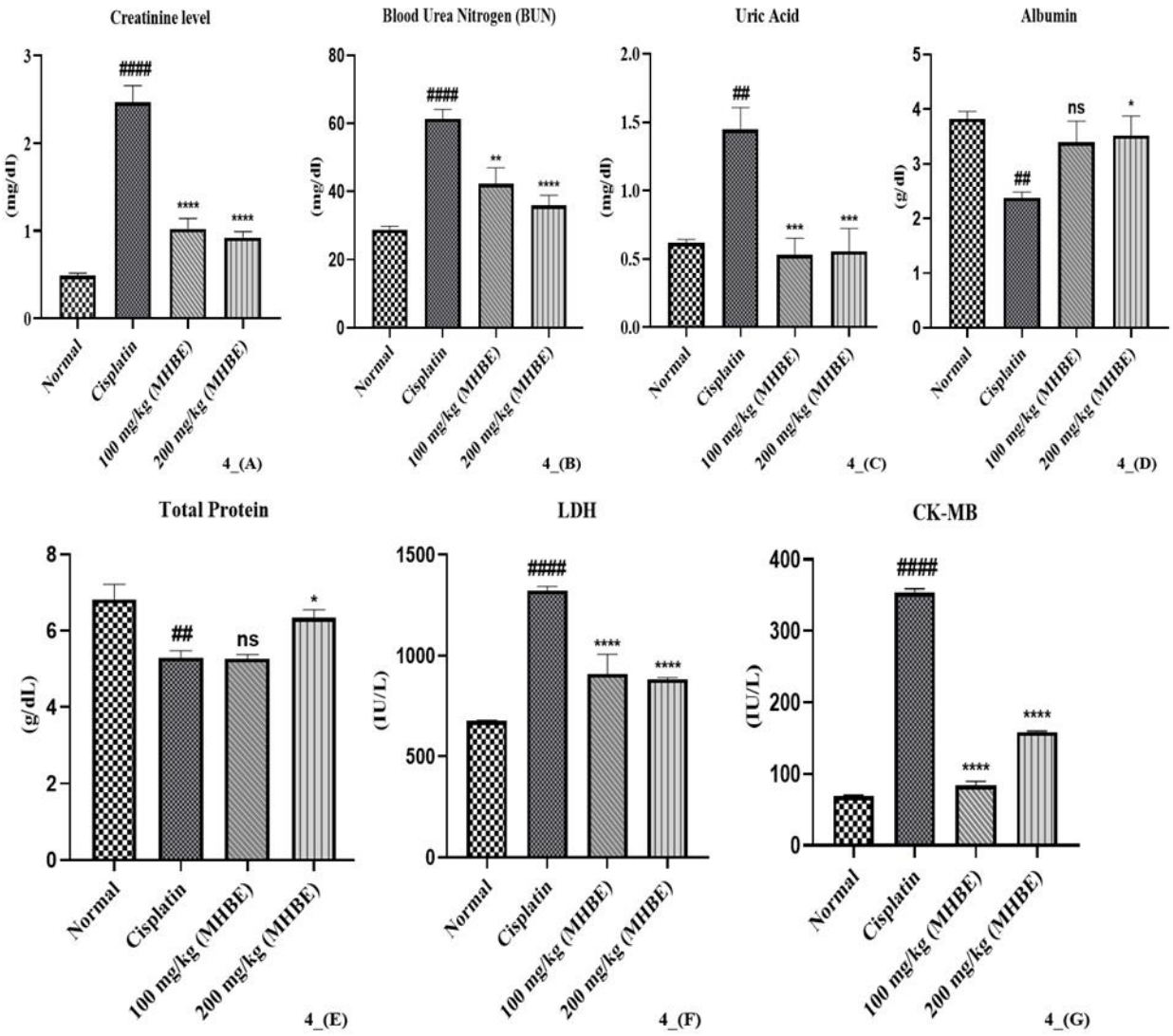

Figure 4. Effect of MHBE on cisplatin-induced alterations in renal and cardiac biomarkers. All values are expressed as mean ± SEM; n=6 in each group, ####p<0.0001, and ##p<0.01, when compared to normal control group, ****p<0.0001, ***p<0.001, **p<0.01 and *p<0.05 when compared to Cisplatin control group.

Urine parameters

Cisplatin was found to exert a significant drop in the urine creatinine concentration of the Cisplatin control group (p<0.0001) compared to the normal group. However, treatment with 100 and 200mg/kg of MHBE notably elevated the creatinine levels in urine (p<0.0001 and p<0.0001), respectively) as shown in Figure 5 (A). Creatinine clearance was also found to be decreased in Cisplatin control group (p<0.0001) as compared to normal control group; however, treatment with 100 and 200mg/kg of MHBE elevated the creatinine clearance with p<0.0001 and p<0.001, respectively as compared to Cisplatin control group, depicted in Figure 5 (B). Urine sodium and potassium levels were significantly raised in the Cisplatin control group (p<0.0001and p<0.0001, respectively) as compared to normal control group. However, treatment with 100 and 200mg/kg of MHBE resulted in the mitigation of overexcretion of sodium and potassium through urine, as depicted in Figure 5 (C) and 5 (D).

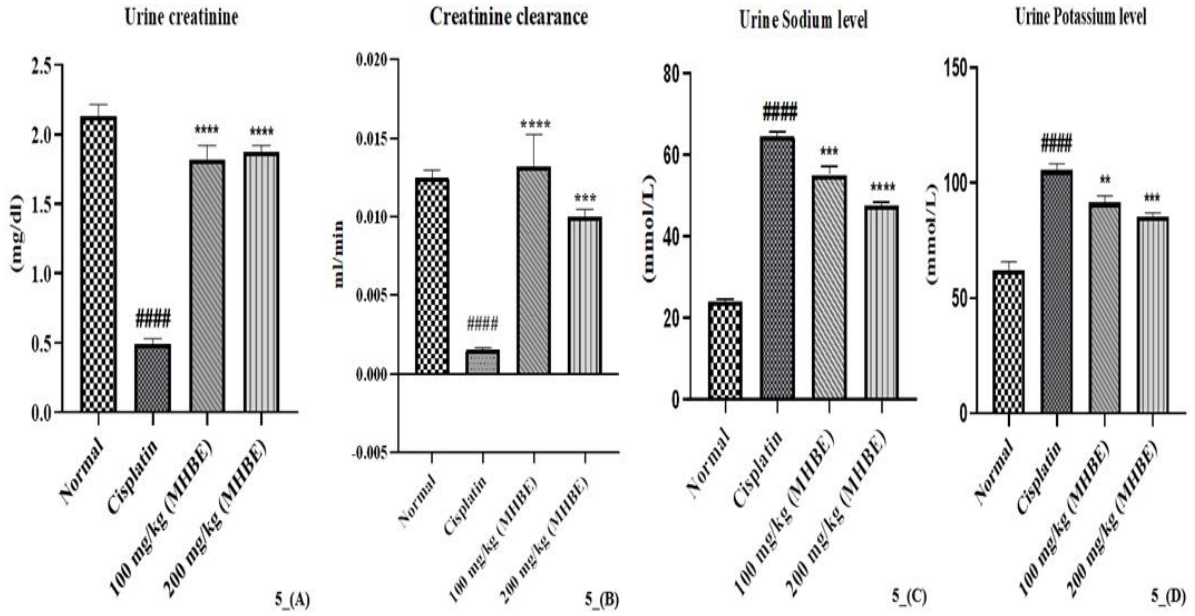

Figure 5. Effect of MHBE on cisplatin-induced changes in urinary creatinine, creatinine clearance sodium, and potassium levels. All values are expressed as mean ± SEM; n=6 in each group, ####p<0.0001, when compared to normal control group, ****p<0.0001, ***p<0.001, and **p<0.01 when compared to Cisplatin control group.

Electrocardiogram analysis

Administration of cisplatin caused a notable rise in the intervals of QT and PR of the Cisplatin control group with p<0.0001 and p<0.0001, respectively, when compared to the normal group. When compared to the Cisplatin control group, treatment with 100 mg/kg of MHBE resulted in a substantial decrease in the QT interval (p<0.0001), although the PR interval showed no significant difference. Treatment with 200 mg/kg of MHBE showed a substantial reduction in QT and PR interval with respectively p<0.0001 and p<0.01 when compared with the Cisplatin control group. QRS (amplitude) of the Cisplatin control group was found to be slightly elevated, p<0.0001, when compared with the normal group. However, treatment with 200 mg/kg of MHBE showed a significant decrease in the amplitude with p<0.0001. Still, treatment with 100 mg/kg of MHBE showed no significant difference in QRS (amplitude) compared to the Cisplatin control group. Heart rate was found to be lower in the Cisplatin control group (p<0.0001) when compared with the normal group. However, treatment with 100 and 200 mg/kg of MHBE showed restoration of the heart rate with p< 0.001 and p<0.0001, respectively when compared to the Cisplatin control group. Figures 6(A), 6(B), 6(C) and 6(D) show the ECG of the Normal group, Cisplatin-control group, low-dose treatment group and high-dose treatment group, respectively. Figures 7(A), 7(B), 7(C) and 7(D) show the effect of MHBE on PR interval, QRS complex, QT interval and heart rate, respectively, of different treatment groups.

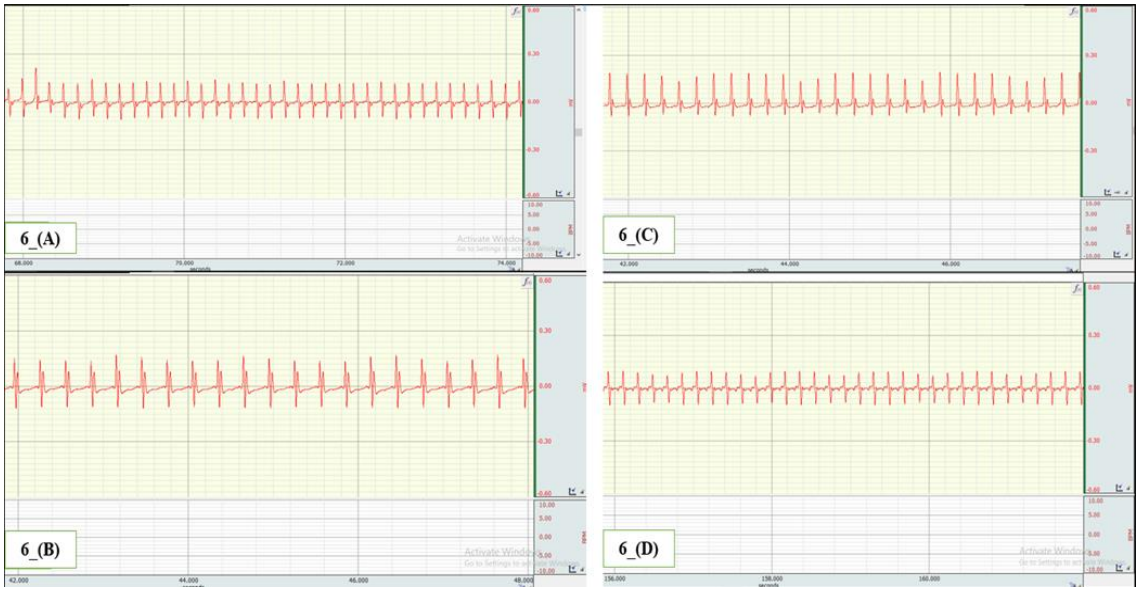

Figure 6. Illustration of ECG of rats of the Normal group (6 (A)), Cisplatin-control group (6 (B)), low-dose treatment group (6 (C)) and high-dose treatment group (6 (D)).

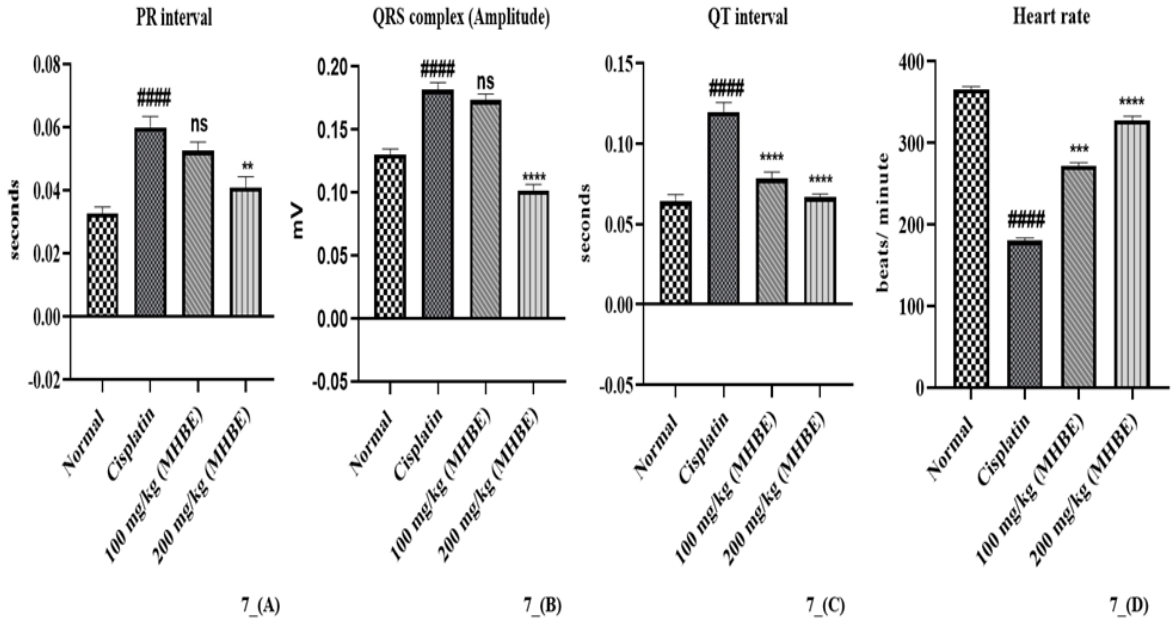

Figure 7. Effect of MHBE on cisplatin-induced alterations in PR interval, QT interval, QRS complex and heart rate. All values are expressed as mean ± SEM; n=6 in each group, ####p<0.0001 when compared to normal control group, ****p<0.0001, ***p<0.001, and **p<0.01 when compared to Cisplatin control group.

Histopathological outcomes

The normal rat's kidney and heart did not exhibit any pathological abnormalities. However, significant tubular congestion, mild tubular desquamation, tubular degeneration, Glomerular tuft atrophy, crystal deposits in the kidney and moderate congestion in the heart were all observed in the Cisplatin control group. Treatment with MHBE extract mitigated these pathological changes and exhibited a clear protective effect, as shown in Figures 8 and 9.

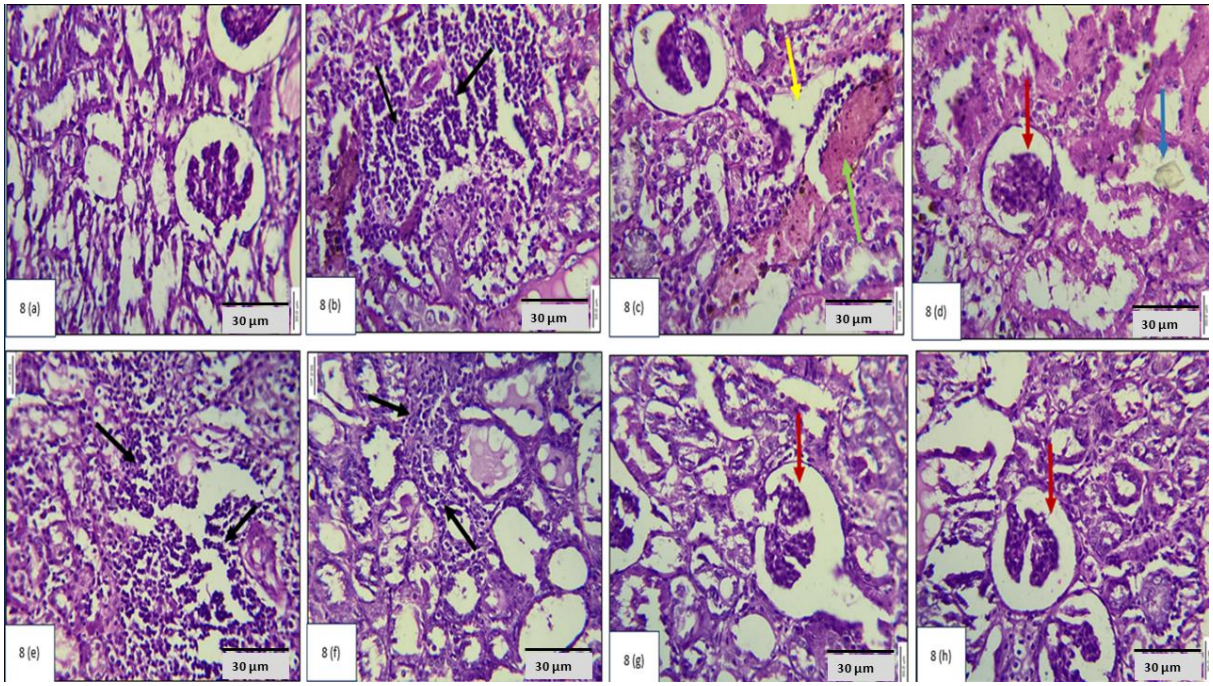

Figure 8. Histopathological findings of stained kidney sections of rats of different treatment groups at 40x magnification: (a) Normal control, (b, c, and d) Cisplatin control, (e and g) 100 mg/kg MHBE, and (f and h) 200 mg/kg MHBE: black arrow indicates tubular congestion, yellow arrow indicates tubular degeneration, green arrow represents tubular desquamation, red arrow marks glomerular tuft atrophy and blue arrow indicates crystal deposition.

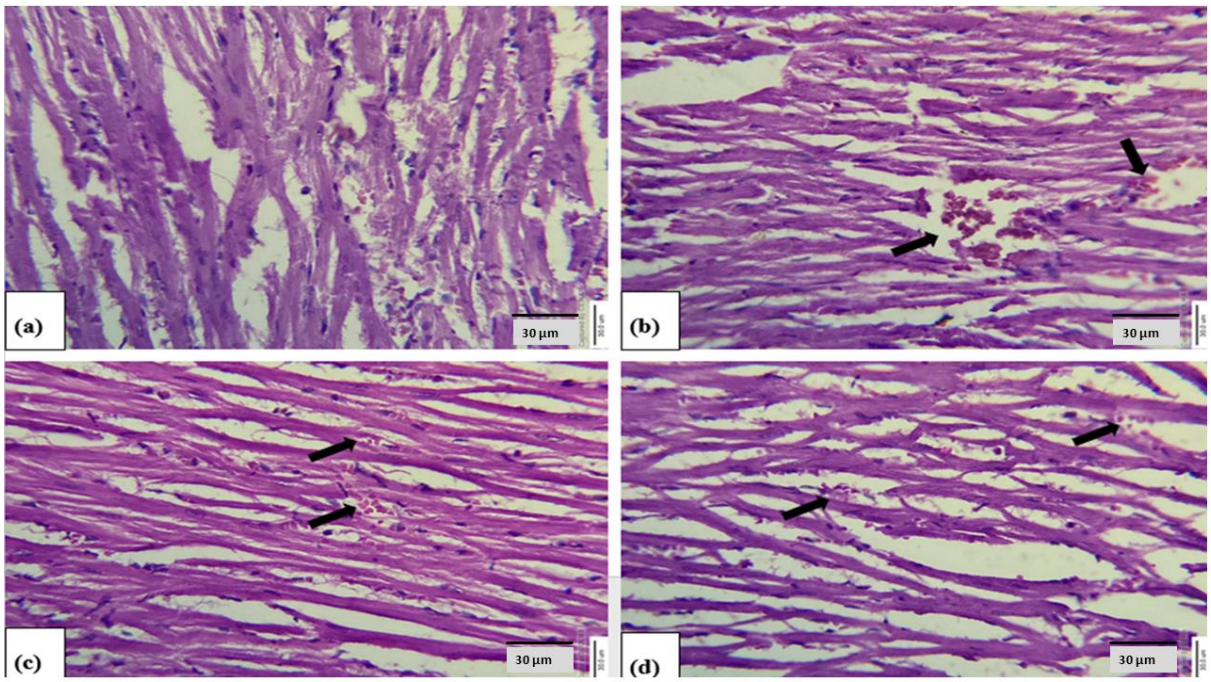

Figure 9. Histopathological findings of stained heart sections of rats of different treatment groups at 40x magnification: (a) Normal control, (b) Cisplatin control (c) 100 mg/kg MHBE and (d) 200 mg/kg MHBE: black arrow indicates congestion.

Discussion

Uncontrollably growing or proliferating cells are the defining characteristics of cancer, a complex illness. Platinum-coordinating chemicals are the drugs that are commonly used in the chemotherapy of solid malignancies. While cisplatin is a well-known and effective anti-cancer

therapy, adverse drug reactions (ADRs) such as nephrotoxicity, arrhythmia, and hyperuricemia limit the use of this medication. The current study aims to evaluate the effect of MHBE against CIN and its effect on the cardiovascular system using *in-silico* and *in-vivo* approaches.

We built a network involving the interactions of the phytoconstituents, their targets, and potential pathways. The findings showed that flavonoids like quercetin, taxifolin and catechin are possible phytoconstituents that could interact with several protein molecules involved in the molecular mechanism of CIN. KEGG pathway analysis indicated the role of MHBE in the management of CIN by modulating major nine pathways i.e., TNF-α, ErbB, HIF-1, P13K-Akt, AMPK, NF-kB, IL-17 signaling pathway and Apoptosis (Volarevic et al. 2019; Cheng et al. 2019; Li et al. 2021; Ali et al. 2021; Chen et al. 2022).

PARP1 and IL-6 play a crucial role in the pathogenesis of CIN. Kim et al. found that animals lacking PARP1 experience less kidney damage, tubular necrosis, and oxidative stress. IL-6 levels were found to be significantly increased in CIN. Previous studies indicated that nicotinamide and quercetin can reduce CIN by inhibiting the PARP1 protein (Kim et al. 2012; Engen et al. 2015; Wu et al. 2021). Cheng et al. found that catechin can downregulate IL-6 and other pro-inflammatory cytokines like IL-1α and IL-1β (Cheng et al. 2019). *In silico* analyses suggested that quercetin, catechin, and taxifolin may interact with critical genes involved in the molecular pathways contributing to the pathogenesis of CIN. These bioactive compounds exhibited potential binding affinity towards key regulatory genes, which play a pivotal role in renal disease progression. Furthermore, *in vivo* studies validated the nephroprotective potential of MHBE against CIN, which may be attributed to the therapeutic properties of the mentioned bioactive constituents.

Cisplatin is known to cause anorexia and gastrointestinal issues, potentially involving serotonin and oxytocin. Blocking the 5-HT2 and OXTR-A receptors may alleviate cisplatin-induced anorexia. (Arase et al. 2018) The current study showed that cisplatin led to anorexia, weight loss, and polydipsia in all groups, however, these effects were significantly mitigated in rats treated with a high dose of MHBE (200 mg/kg).

Reduced blood flow and Glomerular Filtration Rate (GFR) limit the excretion of creatinine, BUN, and uric acid. This study found that the diseased group had substantially higher blood serum levels of creatinine, uric acid, BUN, and LDH, while albumin and total protein were found to be lower in the serum, however, treatment with MHBE attenuated these effects. Additionally, urinary creatinine levels dropped sharply in the Cisplatin control group, but treatment with both doses of MHBE increased the excretion of creatinine through urine.

Wong et al. and Kishore et al. found that cisplatin-induced polyuria may be due to the downregulation of aquaporin channels AQP1, AQP2, and AQP3. (Wong et al. 1993; Kishore et al. 2000) Downregulation of these water channels results in reduced water reabsorption, causing increased urine output and wasting of electrolytes. Rutin, a flavonoid that is also present in MHBE bark extract, has been shown to significantly upregulate AQP1 levels in rats (Caglayan et al. 2019). In the current study, it is evident that the administration of MHBE mitigated the cisplatin-induced polyuria.

CIN is also associated with electrolyte imbalance. In our study, sodium and potassium levels in the urine were found to be elevated in the diseased group, however, treatment with MHBE significantly decreased the levels of electrolytes in the urine. Wang et al. found that electrolyte imbalance is responsible for causing changes in normal ECG patterns. Hypokalemia is associated with prolongation of PR and QT and decreased

heart rate (arrhythmia), whereas chemotherapeutic agents have been reported to induce hypertrophic cardiomyopathy (ventricular hypertrophy) characterized by increased QRS amplitude (Wang et al. 2020; Abbasi et al. 2024). Kozlucaa et al. proposed that quercetin and catechin have been found to provide a protective effect against this arrhythmia by shortening the QT interval and increasing heart rate (Kozlucaa et al. 1996). These two phytochemicals in the MHBE may help to treat the prolonged QT interval and reduced heart rate caused by cisplatin. Consequently, in our study, it was evident that treatment with both low and high doses of the MHBE significantly shortened the QT interval and increased heart rate.

Histopathological studies showed no abnormalities in the kidneys or hearts of normal rats. In contrast, the Cisplatin control group exhibited significant tubular congestion, mild tubular desquamation, tubular degeneration, glomerular tuft atrophy, crystal deposits in the kidney, and moderate heart congestion. Treatment with MHBE mitigated these pathological changes, demonstrating an apparent protective effect.

Taxifolin, catechin and quercetin, bioactives of MHBE, interacted with selected protein targets viz, PARP 1, TNFSF 11 and IL-6 were shown to be nephroprotective as per the gene set enrichment analysis and docking assessment. *In vivo* study showed that the administration of 100 and 200 mg/kg of MHBE attenuated the effects of cisplatin on kidney as well as cardiac biomarkers, urine content, and electrocardiogram. The MHBE was found to ameliorate CIN. Near-future bioactives from *M. hexandra* bark could be isolated and further studied pre-clinically to establish the molecular mechanism to support the *in-silico* studies.

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Conflicts of interest

The authors had no competing interests.

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Ethical Considerations

All animal care and experimental protocols were conducted in strict compliance with the guidelines established by the Committee for Control and Supervision of Experiments on Animals (CCSEA). The experimental study was reviewed and approved by the Institutional Animal Ethics Committee (IAEC) of KLE College of Pharmacy, Hubballi, Karnataka, India (Approval No. Mph/NC0222003/KLECoPH/23).

Code of Ethics

This original manuscript is neither previously published nor under concurrent review. The laboratory is registered with the Committee for the Control and Supervision of Experiments on Animals (CCSEA), Government of India (Registration No. 126/PO/ReBi/S/99/CPCSEA). All animal experiments were conducted in strict accordance with CCSEA guidelines and relevant national regulations.

Authors' Contributions

Manas Mishra: Methodology, Investigation, data curation and writing original draft. Laxmi Pattanashetti: Investigation, formal analysis and supervision. Santosh Patil: Writing and Reviewing manuscript draft. Girish A. Hampannavar: Methodology, Formal analysis. Sinchana MA: Investigation and methodology.Goutami G Kulkarni and Khushi J Soni: Resources, Conceptualization.

Abbreviations

MHBE: *Manilkara hexandra* bark extract
ESRD: End-stage renal disease\
CIN: Cisplatin-induced Nephrotoxicity
BUN: Blood Urea Nitrogen
CK-MB: Creatine Kinase- Myocardial Band
LDH: Lactate dehydrogenase
DLS: Drug likeness score
OMIM: Online Mendelian Inheritance in Man
IMPPAT: Indian Medicinal Plants, Phytochemistry And Therapeutics
RCSB PDB: Research Collaboratory for Structural Bioinformatics Protein Data Bank
PPI: Protein-Protein Interaction
KEGG: Kyoto Encyclopedia of Genes and Genomes
PDBQT: Protein Data Bank, Partial Charge (Q), & Atom Type (T)
i.p: Intra-peritoneal
ECG: Electrocardiogram
PARP 1: Poly [ADP-ribose] polymerase 1
TNFSF 11: TNF Superfamily Member 11
IL-6 Interleukin-

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