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RESEARCH ARTICLE

Exploring Nephroprotective Properties of *Wedelia chinensis: In Vitro, In Silico,* and *In Vivo* Investigations

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Abstract

The purpose of this research work is to investigate the nephroprotective efficacy of *Wedelia chinensis* leaf extracts against gentamicin-induced nephrotoxicity for *in vitro, in silico*, and *in vivo* techniques. The extracts of *Wedelia chinensis* leaf rich in flavonoids were subjected to an *in silico* method for ligands and target proteins. The results of the *in vitro* antioxidant study of extracts were tested for cytoprotective MTT assay and anti-inflammatory efficacy by protein denaturation assay using Human Embryonic Kidney cells (HEK293). The *in vivo* nephroprotective potential of the extract was evaluated with the two doses of 250mg/kg and 500mg/kg body weight in gentamicin nephrotoxicity in rats. The biochemical parameters observed for changes in the histopathology of the kidney. While comparing with other extracts of *Wedelia chinensis* Hydroalcoholic Extract (WCHAE) shows great binding affinity with bonding interactions of flavonoids and phenolics-based ligands observed with the target proteins that provided early information. The *in vitro* cell lines study revealed no cytotoxicity and better anti-inflammatory effect on HEK293 cells with cytoprotective and nephroprotective efficacy of WCHAE. The *in vivo* nephroprotective activity improved at a dose of 500mg/kg of WCHAE than *Wedelia chinensis* Ethanolic Extract (WCEE). The histopathological findings revealed the improvement in gentamicin-induced renal toxicity by the WCHAE orally treated group compared to normal and negative control groups. These results of WCHAE are more satisfactorily effective than WCEE with marked *in vitro* antioxidant, and cytoprotective effects in HEK293 cells. In *in silico* docking, it shows good interaction scores of ligands for target proteins like (kidney injury molecule) KIM-1 and Neutrophil Gelatinase-Associated Lipocalin (NAGAL) that helps to correlate nephroprotective potential benefits of antioxidants in plant extracts against gentamicin induced nephrotoxicity in rats.

Keywords: Gentamicin-induced Nephrotoxicity, HEK-293, *In Silico*, *In Vitro,* Nephroprotective Activity, *Wedelia chinensis*

1. Introduction

Globally, the disease rankings indicate that over 10% of the world's population suffers from Chronic Kidney Injury (CKI), which stands as one of the predominant non-communicable causes of death[1]. The number of patients suffering from CKI has been increasing throughout the globe and in our country[2]. In India, approximately 38% of deaths increase in proportion due to kidney failure[3]. Nephrotoxicity and kidney disorders are affected due to many risk factors like obesity diabetes mellitus, hypertension, and renal failure due to drug therapies[4]. The common drawback associated with the renal system is the toxicity of drugs

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and chemicals, which may arise due to exposure to several medications and environmental substances that result in either temporary or permanent renal failure[5]. Gentamicin, a potent aminoglycoside antibiotic, is extensively utilized in the treatment of severe and critical infections caused by gram-negative bacteria. However, its usage is restricted due to its significant nephrotoxic side effects[6]. The prevalence of gentamicin-used nephrotoxicity is approximately 13–30% and incidences are increasing every year. The actual mechanism of Gentamicin-Induced Nephrotoxicity (GIN) is not understood well, and the mechanism behind the development of nephrotoxicity by the formation of Reactive Oxygen Species (ROS), which develops toxicity to the renal system[5]. In addition to ROS, Reactive Nitrogen Species (RNS), Hydroxyl Radicals (HOR), and Superoxide Anions (SOA) factors for the appearance of nephrotoxicity[7].

Furthermore, gentamicin diminishes the concentration of reduced glutathione and suppresses the functionality of antioxidant enzymes such as superoxide dismutase, catalase, and glutathione peroxidase may contribute to renal toxicity and failure[8]. This is accompanied by a gradual rise in the level of creatinine, urea, blood urea nitrogen, and uric acid levels with a rise in proteinuria and impairment in electrolyte balance[8,9].

Acute kidney toxicity confines a range of kidney-related conditions characterized by a sudden decline in renal function and GFR[10]. Acute Kidney Injury (AKI) can be attributed to various factors, with microbial sepsis, blood volume depletion, lower renal blood supply, and the administration of nephrotoxic medications being among the most common causative factors[11,12].

The occurrence and frequency of drug-induced kidney injury have risen over the past two decades due to changes in healthcare practices (frequent use of possible nephrotoxic drugs like NSAIDs, and antimicrobial agents recently. Pre-existing comorbidities and parameters directly related to the illness are among the many causes of the increased risk of long-term consequences and early death following AKI[13]. Apoptosis, necrosis, autophagic cell death, tubular injury, reduced GFR, and endothelial and vascular injury may be important components in the development of AKI along with the factors indicated above[14].

1.1 Pathogenesis of Aminoglycosides Induced Nephrotoxicity (PAIN)

Aminoglycoside (AG)-Induced Nephrotoxicity (AIN) is a direct toxic effect on the proximal tubule cells that results in Acute Tubular Necrosis (ATN) and is accompanied by glucose, protein, enzyme, potassium, calcium, and magnesium losses. (Figure 1)[15]. Gentamicin is not metabolized by the liver and most of the drug gets excreted in an unchanged form and eliminated by the kidneys[16], but when it is clinically significant, a small but harmful amount accumulates in proximal renal tubular cells and induces apoptosis[17].

Renal toxicity leads to the formation of free radicals, which inflict damage on the tubular and glomerular basement membranes, resulting in cellular injury and necrosis via multiple intricate pathways. These pathways encompass membrane lipid peroxidation, protein carbonylation, and DNA degradation[18]. Furthermore, gentamicin reduces the efficacy and protective role of antioxidant enzymes in the renal system and kidney tissues[19-21]. Reactive Species (RS) are free radicals that

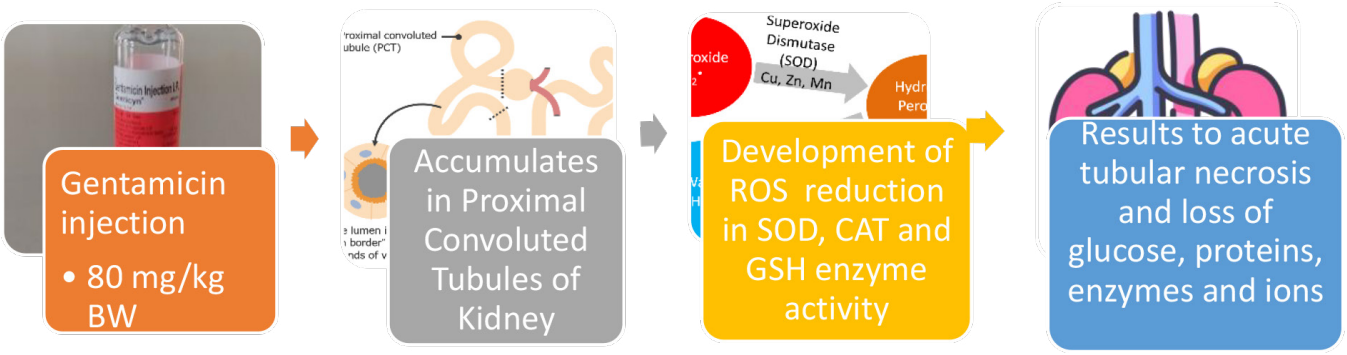

Figure 1. Pathogenesis of Gentamicin-Induced Nephrotoxicity (PGIN).

are composed of various types of activated oxygen or nitrogen. Free Radicals Originate from Oxygen (ROS) and Nitrogen (RNS)[22] leading to changes in a cellular membrane or intracellular molecules with a relationship imbalance between RS and the antioxidant defence system depicted in Figure 2[23]. RS is constantly produced during normal physiological processes, although it is possibly removed by the antioxidant defense system[24]. The treatments for drug-induced nephrotoxicity are dialysis, renal transplantation, and other costlier treatments are available.

In this research work, we evaluated the unexplored antioxidant potential benefits of phytoconstituents in *Wedelia chinensis* hydroalcoholic leaf extracts for nephroprotective activity. The presence of sufficient levels of flavonoids and phenolic substances in leaf extracts, that could be useful for the diminution of nephrotoxicity in the rats, served as the consideration for the selection of this plant. This study highlighted the *in vitro* antioxidant, *in vitro* cell line assays, possible molecular docking studies, and histopathological findings for the nephroprotective activity of hydroalcoholic leaf extracts of *W. chinensis* in experimental animals.

2. Materials and Methods

2.1 Plant Materials

The *Wedelia chinensis* (aerial parts) leaves were collected in January 2021 from the Western Ghats of the Konkan region, Maharashtra, India. The plants were identified and authenticated with reference number 23-K/425/2021 by Professor Dr. Anil Dethe, HOD, at the Department of Botany, SPK College of Science Sawantwadi Dist. Sindhudurg, Maharashtra India.

2.2 Preparation of Extracts[25]

The leaves were first cleaned and then shade-dried at room temperature and ground separately in coarse powder. A 500gm of each ground plant material was subjected to continuous extraction by the Soxhlet apparatus. The solvents petroleum ether, chloroform, and ethanol are used with increasing polarity in the ratio of 1:4 (drug:solvent). The hydroalcoholic solvent in the ratio of 70:30 water and ethanol was used for extraction shown in Figure 2[26,27].

The filtrate of extract was filtered out by using Whatman filter paper and then the filtrate was dried using a rotary evaporator at 40°C. The dried filtrate extracts were then stored in a refrigerator for subsequent experimentation[28].

2.3 Drugs and Chemicals

A gentamicin sulfate injection of Abbott Pharmaceutical Pvt. Ltd. was purchased and procured from the market. The standard drugs and chemicals like quercetin, rutin, DPPH, and Griess reagent were purchased from Sisco Research Laboratories Pvt. Ltd., India. The remaining other chemicals like ascorbic acid, gallic acid, and all other chemicals were purchased from Research Lab Pvt. Ltd., Mumbai. The chemicals and drugs were used in the experimental procedures of analytical grades. The cell lines used in *in vitro* experimentation i.e. Human Embryonic Kidney cell-293 were procured from the NCCS Pune, Maharashtra, India.

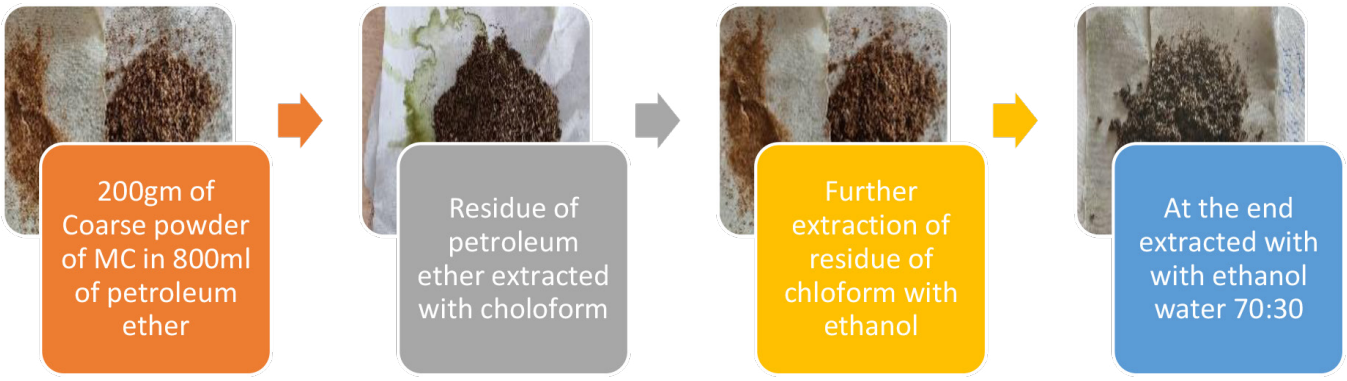

Figure 2. Schematic illustration of collection of extractive residues.

2.4 Phytochemical Screening of Plant Extracts[29,30]

Plant extracts were subjected to a first phytochemical analysis to detect the presence of various compounds such as proteins, carbohydrates, tannins, flavonoids, phenols, and alkaloids. Out of all these 4 extracts we found flavonoid and phenolic content was more in hydroalcoholic extract than ethanol>chloroform>petroleum ether. The hydroethanolic extract of *Wedelia chinensis* was subject to an *in vitro* antioxidant study.

2.5 Analysis of Extracts

2.5.1 Quantification of Total Phenolic Content (TPC)

The extract of *Wedelia chinensis* was analyzed phenolic content using the Folin-Ciocalteu reagent calculated as Gallic acid equivalent (GAE) in mg/g of the extract). The absorbance values of samples were measured at 765 nm and test samples were compared with a calibration curve using standard gallic acid to calculate the phenolic content in the extract. The gallic acid equivalents in mg/100gm of sample(mg GAE / 100 g sample) were used to determine the results[31].

2.5.2 Quantification of Total Flavonoid Content (TFC)

The Total Flavonoid Content (TFC) of the *Wedelia chinensis* extract was determined using a standard method. The absorbance was taken at 420 nm against a blank solution and different samples of standard drugs and extracts. TFC was quantified in terms of grams of quercetin equivalents (QE/mg) of dry extracts using a calibration curve prepared with quercetin[32].

2.6 *In Vitro* Antioxidant Study

2.6.1 DPPH Antioxidant Assessment[33]

The *in vitro* antioxidant activity of *Wedelia chinensis* Hydroalcoholic Extract (WCHAE) and the standard drug is used as ascorbic acid assessed using the DPPH antioxidant assay method[34]. The antioxidant capacity of WCHAE to directly interact with DPPH radicals was evaluated using a decolourization test conducted with a UV-visible spectrophotometer at 517 nm. The standard ascorbic acid served as a positive control in similar test conditions. The percentage of DPPH radical scavenging activity of the extract was calculated[35].

2.6.2 Reducing Power Assessment[36]

The determination reducing antioxidant assay of *Wedelia chinensis* extracts was calculated and compared with ascorbic acid following the procedure outlined by Oyaizu. The absorbance of the solution was taken at 700 nm using a spectrophotometer and reducing power antioxidant activity was demonstrated by the reaction mixture's[37].

2.6.3 Hydroxyl Radical Scavenging Activity[38]

The hydroxyl radical antioxidant activity of the extract was assessed using the 2-deoxyribose assay as described by Halliwell and Gutteridge. The absorbance of the supernatant was measured at 532 nm using a UV spectrophotometer[39,40].

2.6.4 Nitric Oxide Radical Scavenging Activity[41]

The Garratt *et al*., reported procedure was followed while conducting this antioxidant scavenging assay[41]. The Griess reagent was used for nitric oxide radical scavenging antioxidant assay and the absorbance was measured at 546 nm. Ascorbic acid was used as the standard drug to compare with a test sample of extract. The percentage of inhibition was subsequently measured and calculated[42].

2.7 *In Vitro* Cell Line Study[13,43]

2.7.1 Maintenance of (Human Embryonic Kidney) HEK Cells

The HEK cell lines were purchased and procured from NCCS Pune, Maharashtra, India. The data sheet with sixteen Short Tandem Repeat (STR) loci proved to be a 100% match with the ATCC STR profile. After procuring the cell lines, maintenance and subculturing of the cells were done by preparing 100 of complete media comprising DMEM 89ml (Himedia Ref: AL250A) Foetal Bovine Serum (FBS) 10 ml (Himedia RM 10432, LOT 573421 and antibiotics 1ml (Himedia A002, LOT 5392281). The cells were developed and cultivated in 96 well plates maintained in a 5% CO_2 incubator and observed under an inverted light microscope. All the procedure of the cell culture was performed in the class-II cabinet by considering all the

aseptic conditions. On cells reaching 85% confluency, trypsinization was performed using trypsin (TCL007, LOT 536691), and subculturing was done as per the standard protocol. The cell line study was carried out in collaboration with the Cell Culture Lab, Dr. Prabhakar Kore BSRC, Belagavi, Karnataka.

2.7.2 Viability and Cell Cytotoxicity Tests

2.7.2.1 Microculture Tetrazolium (MTT) Assay[44]

The cell cytotoxicity of WCHAE on HEK-293 cell lines was evaluated. The 96 well plates marking was done by considering negative control without adding compound and test compound. The serial dilutions of test compound extracts in 1% DMSO in triplicates of various concentrations to avoid any bias. A trypan blue assay was performed and the live viable cells number was counted and calculated. The compounds of serial dilutions were added to wells and kept for 24 hours for incubation. Subsequently, 20 µL of tetrazolium (MTT) dye mixed with media was introduced and kept for 24 hours for further incubation. Later, the supernatant was carefully removed without disturbing the formazan crystals. Following this, to dissolve the crystals 100 µL of Dimethyl Sulfoxide (DMSO) was added up, and the absorbance was taken at 570 nm using a UV spectrophotometer. The proliferative index was calculated by dividing the test's Optical Density (OD) by the OD of the control, then multiplying by 100.

2.7.2.2 Protein Denaturation Assay[45]

Test tubes were prepared corresponding to the number of concentrations used in the MTT assay. Each set included a negative control and a positive control using diclofenac. To each tube, 2.8 ml of sterile buffer solution (Phosphate buffer) with a pH of 6.3-6.4 was added. Subsequently, 0.2 ml (200 µL) of egg albumin was added to all tubes. For the negative control, 2 ml of PBS was added, while for the positive control, 2 ml of Diclofenac was added. After that, each test tube was incubated for 15 minutes at 37°C in a water bath, and then for an additional 5 minutes at 70°C. After cooling, the absorbance was measured at 660 nm using a spectrophotometer to calculate the percentage inhibition of protein denaturation.

2.8 Molecular Docking[46-48]

In silico interaction of ligands and target molecules, i.e., docking study was carried out using the Auto Dock Pyrex software to determine the likely interaction of certain expected flavonoids from extracts with the specific standard markers for proximal tubules transmembrane protein, i.e., Kidney Injury Molecule-1(KIM-1)[49]. In the docking study, we tried to predict binding affinity and potential intermolecular non-covalent bonding which provide early and predictive information on the binding mechanisms of ligand molecules in and around receptor pockets. Discovery Studio was used as a visualization tool for viewing and analyzing protein and modelling data. The 3D structures of apigenin, rutin, and quercetin were retrieved in SDF format, and the ligands were sourced from the public chemical information database. The Protein Data Bank provided the targeted protein molecules' structures for the *in silico* docking study.

2.9 Toxicity Study

2.9.1 Acute Oral Toxicity (LD_{50}) Determination[50]

The acute oral toxicity study was conducted following the guidelines given by the Organization for Economic Cooperation and Development (OECD) and the Committee for Control and Supervision of Experiments on Animals (CCSEA). The protocol JKKMMRAFCP/IAEC/2019/002 was sanctioned and approved by the Institutional Animal Ethical Committee (IAEC) with registration number 1158/PO/Re/S/07/CPCSEA of JKKMMRF Annai JKK Sampooorani Ammal College of Pharmacy, Ethirmedu, Komaraplayam Namakkal District, Tamil Nadu, India[51].

2.10 Study Design and Grouping of Animals[52,53]

For the *in vivo* nephroprotective assessment of plant extract, Wistar strain albino rats were used. In the study, both male and female rats weighing 175-225g body weight were used. The animals were kept under careful attention, with a temperature of 25°C, relative humidity in between the range of 30% to 70%, and a 12-hour light-dark cycle. Throughout the animal experimentation, ad libitum of food and water were available to the animals. All animals were given a time of 15 days to familiarize and adjust the environment

of the laboratory before starting experimentation in rats. A total of five experimental groups, each with six rats, were designed and grouped for animal study. The institutional animal ethics committee's recommendations were closely followed to uphold ethical standards.

2.10.1 Experimental Setup

The random selection of 30 Wistar strain rats, both male and female, were selected and used in the study protocol. For the study, a total of five Groups I to V each containing six animals were chosen. The gentamicin injection was used in the negative control Group II by intraperitoneal route and a normal saline solution was given to other groups. Gentamicin was given consecutively from day 8 to day 14, for a total of seven days in normal control. In Group III, the standard drug quercetin was administered, while Groups IV-V received low and high doses of phenolic/flavonoid-rich WCHAE extracts from day 1 to day 14. Subsequently, gentamicin was administered from day 8 to day 14. On day 15, 24 hours after the last dose of extracts, the animals were euthanized under ether anaesthesia for histopathological and biochemical investigations[54,55].

Group I received 5ml/kg body weight of distilled water orally for 14 days and intraperitoneally (i.p.) from day 8 to day 14. Group II received 5ml/kg body weight of distilled water orally for 14 days and gentamicin 80mg/kg body weight i.p. from day 8 to day 14. Group III was administered quercetin 50mg/kg orally and gentamicin 80mg/kg body weight i.p. from day 8 to day 14. Group IV received WCHAE 250mg/kg orally for 14 days and gentamicin 80mg/kg body weight i.p. from day 8 to day 14. Group V received WCHAE 500mg/kg orally for 14 days and gentamicin 80mg/kg body weight i.p. from day 8 to day 14.

2.10.2 The Preparation of Tissue Homogenate

The clear supernatant generated from centrifuging kidney homogenate at 5,000 rpm for 10 minutes at 5°C using a buffer solution with a pH of 7.4 was used to measure the levels of superoxide dismutase, catalase activity, glutathione peroxidase, reduced glutathione, and lipid peroxidation.

2.11 *In vivo* Antioxidant Assays

2.11.1 Super Oxide Dismutase (SOD)

The superoxide dismutase antioxidant activity in kidney tissue homogenate was assessed following a modified procedure outlined by Kakkar *et al.* The change in optical density per minute was measured at 480 nm using a double-beam UV-VIS spectrophotometer. SOD activity was expressed as unit/min/mg of protein[56].

2.11.2 Reduced Glutathione (GSH)

The reduced glutathione antioxidant assay was measured and calculated by using Ellman's method[57,58]. The absorbance at 412 nm was measured. A standard curve was constructed using glutathione reduction (1.0 mg/mL), and the glutathione content for the sample was determined by interpolation. The glutathione content was expressed as g/mg of protein[59].

2.11.3 Catalase (CAT)

The catalase activity was measured using the colorimetric technique described by Sinha. The green colour produced by the sample after the reaction was calculated and measured at 570 nm using a UV spectrophotometer. Catalase activity was expressed in mol of hydrogen peroxide absorbed per minute per milligram of protein[60].

2.12 Histopathological Examination[61]

Kidney tissue sections from each group of rats were isolated and preserved in 10% formalin before being processed in paraffin wax. Thin sections were obtained for histopathological examination and stained with hematoxylin followed by eosin for enhanced visualization. Images were captured using a light microscope. A minimum of two sections were examined from each kidney sample, and analyses were conducted to identify any morphological alterations associated with gentamicin-induced nephrotoxicity. Histological changes in glomeruli, proximal tubules, and interstitial components were observed under the binocular light microscope.

2.13 Statistical Analysis

The statistical data is revealed as mean values with standard error of the mean (±SEM). Statistical analysis was performed using InStat 10.0.2 software. The

one-way Analysis of Variance (ANOVA) followed by the Dunnett test for multiple group comparison. The normal group was compared with Group II and the rest of the group compared with Group II. Statistical significance value was calculated and considered at a p-value of ANOVA is < 0.05.

3. Results

3.1 *In vitro* Antioxidant Study

The hydroalcoholic extract of *W. chinensis* showed good phenolic and flavonoid content compared to that of other extracts. The TFC of WCHAE and MCEE was 15mg/ ml and 13 mg/ml respectively. The TPC was obtained higher for WCHAE than other extracts shown in Figures 3 and 4. The *in vitro* antioxidant assay was performed for WCHAE and observed that the plant extract was highly effective in the prevention of DPPH, reducing power, hydroxy radical, and nitric oxide radicals with scavenging properties compared with standard ascorbic acid shown in Figures 5-8.

3.2 Molecular Docking Study

The molecular docking analysis was performed for the interaction of known flavonoids and phenolic compounds present in the plant extract. The better interaction in 2D and 3D with KIM-1, NGAL, and serine kinase was observed for flavonoid and phenols in the plant extract with quercetin, rutin apigenin, gallic acid, and ferulic acid that was considered for the invitro cell line study and *in vivo* study. The results are shown in Figures 9 and 10.

3.3 *In Vitro* Cell Lines Study

3.3.1 MTT Assay

The percentage cell viability of WCHAE extract at various dilutions, namely 12.5, 25, 50, 100, and 200µg/ mL, was recorded as 89.7%, 91.4%, 93.1%, 96.9%, and

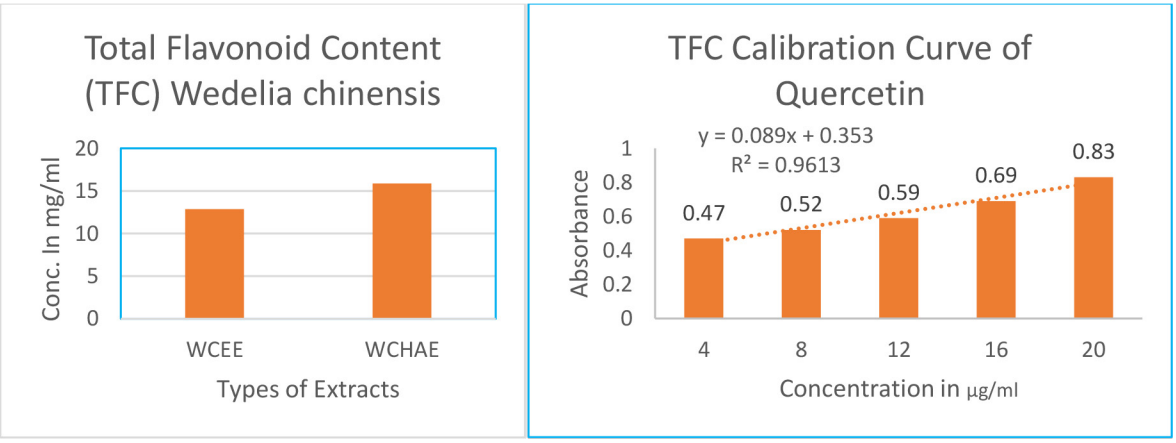

Figure 3. Measurement of TFC of WCHAE.

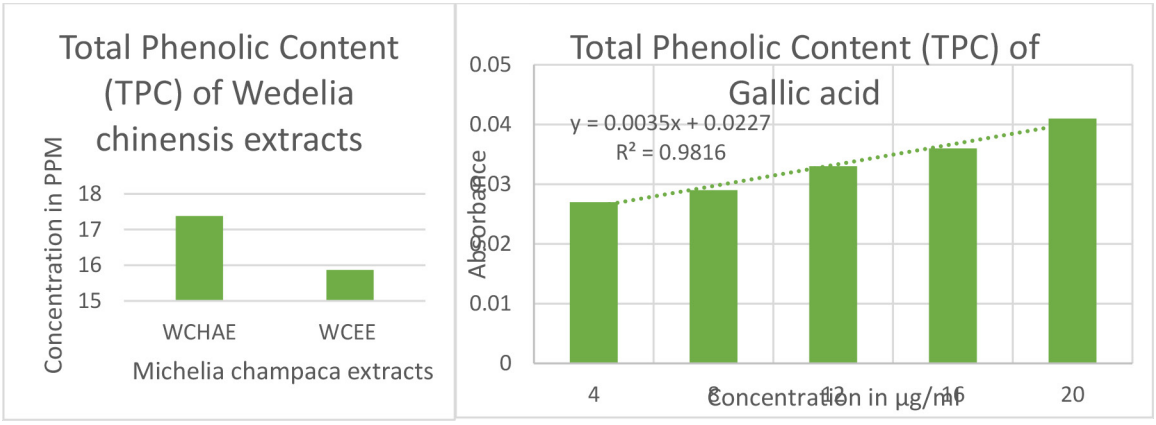

Figure 4. Measurement of the TPC in hydroalcoholic extracts of *W. chinensis.*

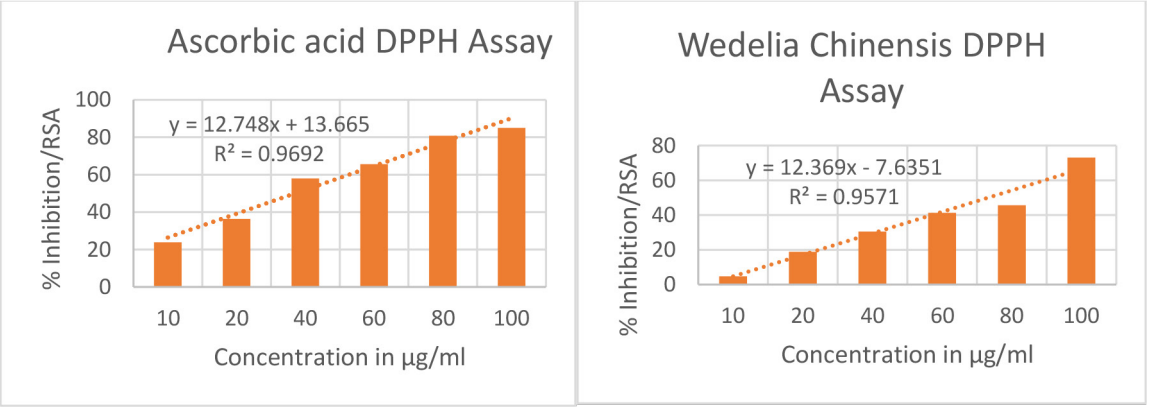

Figure 5. DPPH antioxidant assay of ascorbic acid and WCHAE.

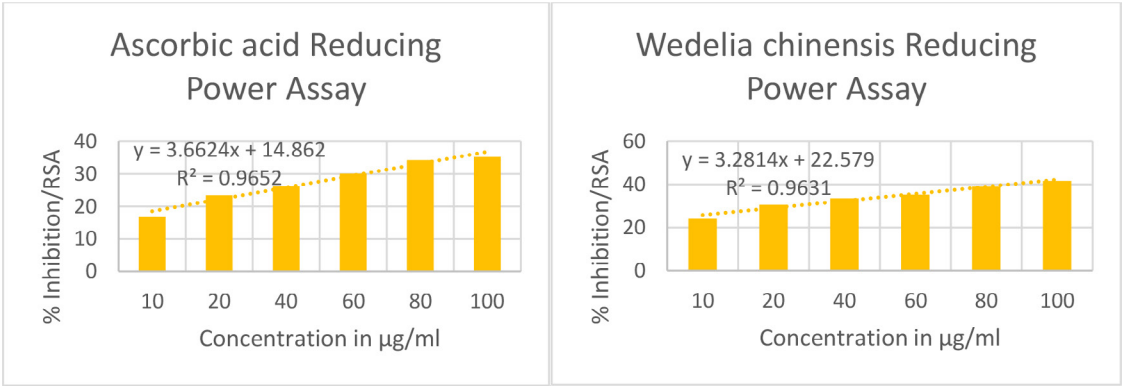

Figure 6. Reducing power antioxidant assay of ascorbic acid and WCHAE.

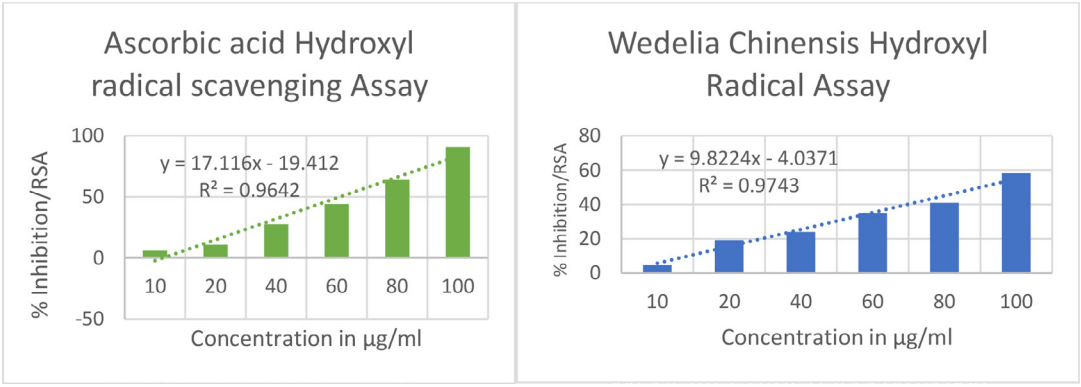

Figure 7. Hydroxyl Radical scavenging assay of ascorbic acid and WCHAE.

87.6%, respectively. For WCEE, the corresponding percentages were 87.8%, 81.4%, 91.9%, 97.4%, and 90.3%. Notably, a significant percentage of cell viability was observed for WCHAE (91.5%) and WCEE (97.4%) at 100µg/ml concentration compared with the control and standard quercetin and rutin preparations. The mean optical density of various test compounds, as well as WCHAE and WCEE extracts, along with the percentage cell viability depicted in Tables 1 and 2.

3.3.2 Protein Denaturation Assay

Tables 1 and 2 demonstrate that none of the test compounds exhibited cytotoxic effects on HEK293 cell lines. Additionally, no activity was observed regarding the percentage of protein denaturation for

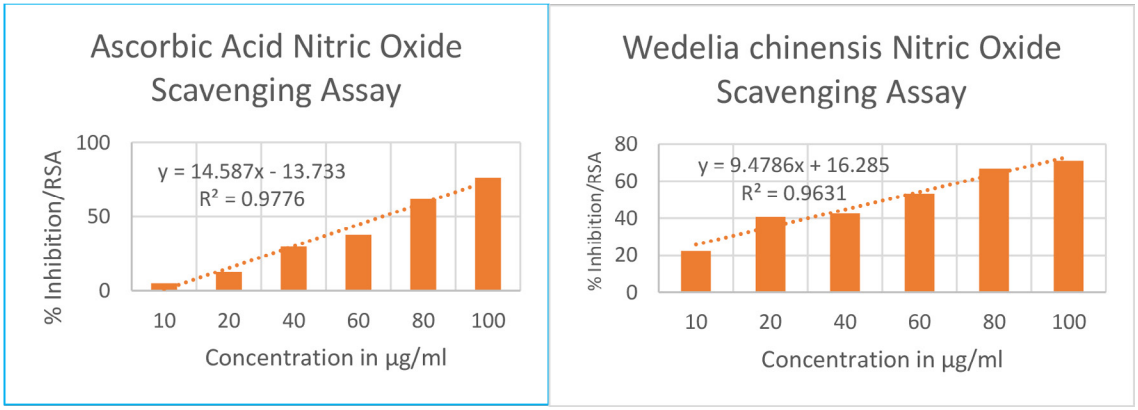

Figure 8. Nitric oxide antioxidant assay of ascorbic acid and WCHAE.

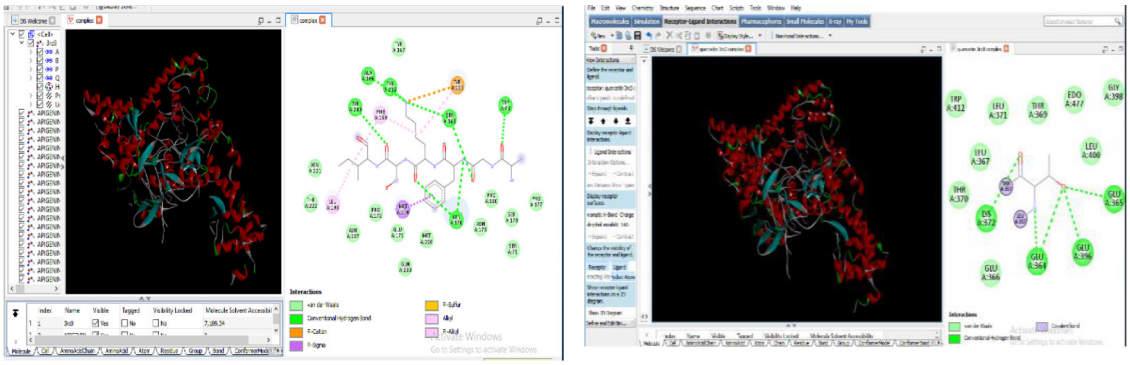

Figure 9. 3D and 2D interactions of apigenin and quercetin with KIM-1 molecule.

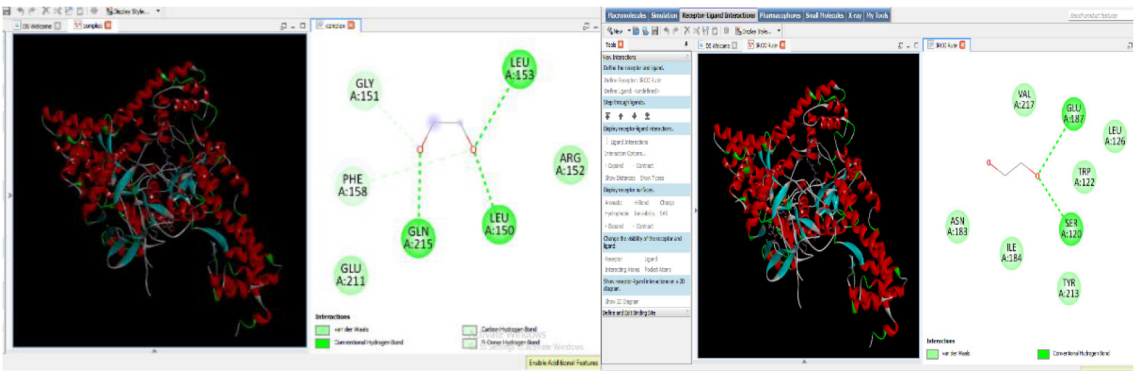

Figure 10. 3D and 2D interactions of rutin for KIM-1 and NGAL molecules.

all concentrations of the test compound WCHAE. In contrast, the positive control diclofenac displayed 30% inhibition of protein denaturation. Therefore, WCHAE demonstrated anti-inflammatory properties, suggesting its potential utility in mitigating gentamicin-induced nephrotoxic inflammation. Tables 3 and 4 present the data on mean optical density and Percentage Cell Inhibition (PCI) of the protein denaturation assay respectively.

3.4 Renal Biochemical Examination

3.4.1 The Results of Wedelia chinensis Hydroalcoholic Extract (WCHAE) on Biochemical Parameters

The animal administered with gentamicin-induced nephrotoxicity Group II decreased biochemical parameters level of urinary as well as serum total protein and albumin levels in rats compared to the normal control Group I. However, the levels improved

Table 1. MTT assay: Mean Optical Density (MOD) of *W. chinensis* extract

Test concentration µg/ml	Mean Optical Density (MOD)				
	Rutin	Quercetin	Gentamicin	WCEE	WCHAE
200	0.421	0.449	0.528	0.528	0.544
100	0.505	0.511	0.583	0.551	0.586
50	0.593	0.488	0.561	0.516	0.553
25	0.528	0.513	0.55	0.531	0.49
12.5	0.565	0.563	0.54	0.501	0.529
Mean	0.5224	0.5048	0.5524	0.5254	0.5404
SD	0.026393	0.0166	0.008403	0.007422	0.01403

Table 2. MTT assay: Percentage Cell viability (PCV) of *W. chinensis* extract

Test concentration µg/ml	% Cell viability (PCV)				
	Rutin	Quercetin	Gentamicin	WCEE	WCHAE
200	69.9	74.5	87.6	87.6	90.3
100	83.9	84.8	96.9	91.5	97.4
50	98.6	81.1	93.1	85.7	91.9
25	87.8	85.2	91.4	88.3	81.4
12.5	93.9	93.5	89.7	83.2	87.8

Table 3. Protein denaturation assay: Mean Optical Density (MOD)

Test concentration µg/ml	Mean Optical Density (MOD)				
	Rutin	Quercetin	Gentamicin	WCEE	WCHAE
200	0.1596	0.188	0.139	0.13	0.181
100	0.166	0.174	0.121	0.342	0.305
50	0.165	0.282	0.127	0.201	0.234
25	0.216	0.312	0.147	0.178	0.336
12.5	0.176	0.255	0.144	0.261	0.168
Mean	0.17652	0.2422	0.1356	0.2224	0.2448
SD	0.00914	0.02384	0.004469	0.032718	0.029683

significantly in the standard quercetin Group III and the animal group of WCHAE with a dose of 500mg/kg, while the improvement observed in Group IV with a dose of 250mg/kg was insignificant. The results on biochemical parameters are given in Tables 5 and 6 with statistical graphical images in Figure 1S (Supplementary file).

3.4.2 Results of Wedelia chinensis Hydroalcoholic Extract (WCHAE) on Biochemical Parameters

In Animal Group II, which was administered with gentamicin, the serum creatinine level, urea level, and uric acid level were elevated, indicating signs of acute renal toxicity in rats. However, compared to that of elevated creatinine levels, urea and uric acid levels in Group II received gentamicin drug, and normal Group I, the WCHAE with a dose of 250mg/kg body weight administered in Group IV observed insignificant changes. Alternatively, with a higher dose of plant extract at 500mg/kg body weight, efficacy was observed compared to that with standard quercetin at 50mg/kg and showed marked improvement in the above parameters. Some exception is seen in blood urea levels, where the effects of both the lower and higher doses of WCHAE were more effective, significantly

Table 4. Protein denaturation assay: Percentage Cell Inhibition (PCI)

Test concentration µg/ml	% Cell Inhibition (PCI)				
	Rutin	Quercetin	Gentamicin	WCEE	WCHAE
200	18.55	12.79	22.53	24.38	14.31
100	17.29	15.707	26.24	-17.82	-10.46
50	17.36	-5.82	25.05	10.322	3.64
25	7.35	-11.72	21.09	14.84	16.56
12.5	15.17	-0.52	21.6	-1.72	16.9

Table 5. The results of WCHAE on total protein and albumin levels in the urine of experimental rats

Urine parameters (Unit)	Group I Normal	Group II Gentamicin 80mg/kg	Group III Gentamicin + Quercetin 50mg/kg	Group IV Gentamicin+WCHAE 250mg/kg	Group V Gentamicin+WCHAE 500mg/kg
Total protein (g/dL)	$3.49\pm0.0^{85**}**$	$4.47\pm0.0^{82**}**$	$3.40\pm0.^{01**}**$	$4.26\pm0.^{07}$ns	$4.05\pm0.^{03*}**$
Albumin (g/dL)	$0.58\pm0.01^{8***}*$	$0.75\pm0.01^{3***}*$	$0.57\pm0.01^{6***}*$	0.74 ± 0.00^{9n}s	$0.6\pm0.01^{8*}*$

Table 6. Results of WCHAE on total protein and albumin level in serum of experimental rats

Serum parameters (Unit)	Group I Normal	Group II Gentamicin 80mg/kg	Group III Gentamicin + Quercetin 50mg/kg	Group IV Gentamicin +WCHAE 250mg/kg	Group V Gentamicin +WCHAE 500mg/kg
Total protein (g/dL)	$7.19\pm006^{***}$	7.15±003	$7.19\pm005^{***}$	7.17 ± 00^{ns}	$7.18\pm005^{**}$
Albumin (g/dL)	$4.36\pm0.003^{****}$	4.33±0.003	$4.37\pm0.003^{****}$	4.34 ± 0.003^{ns}	$4.35\pm0.003^{***}$

Table 7. Results of WCHAE on serum creatinine urea and uric acid levels in Gentamicin induced nephrotoxicity in rats

Serum biochemical parameters (Unit)	Group I Normal	Group II Gentamicin 80mg/kg	Group III Gentamicin +Quercetin 50mg/kg	Group IV Gentamicin +WCHAE 250mg/kg	Group V Gentamicin +WCHAE 500mg/kg
Creatinine (mg/dL)	$1.06\pm0.018^{****}$	1.76±0.0358	$1.12\pm 0.005^{****}$	1.71 ± 0.022^{ns}	$1.64\pm 0.021^{**}$
Blood Urea (mg/dL)	$30.5\pm 0.026^{****}$	38.91 ± 0.096	$34.88\pm 0.164^{****}$	$37.99\pm 0.201^{**}$	$37.73\pm 0.219^{***}$
Uric acid (mg/dL)	$2.46\pm 0.109^{****}$	3.59 ± 0.094	$2.076\pm 0.020^{****}$	3.51 ± 0.122^{ns}	$3.32\pm 0.064^{***}$

reducing the elevated biochemical parameters results are detailed in Table 7, and graphs are shown in the supplementary file Figure 2S.

3.4.3 The Results of Wedelia chinensis Hydroalcoholic Extract (WCHAE) on Antioxidant Enzymes

The Group II animals are administered with gentamicin and develop acute toxicity in renal tubules in addition to acute tubular damage of the first-line defensive antioxidant system of kidney tubules and tissues. The levels of antioxidant enzymes like superoxide dismutase, glutathione peroxidase, and catalase were observed significantly ($P < 0.005$) reduced in the gentamicin-induced toxicity Group II. Groups IV and V administered with WCHAE (250mg/kg and 500mg/kg) improved satisfactorily with gradual improvement in the levels of antioxidant enzyme stress parameters

Table 8. Effect of WCHAE on oxidative stress parameters SOD, GSH, and CAT in rats

Serum biochemical parameters (Unit)	Group I Normal	Group II Gentamicin 80mg/kg	Group III Gentamicin + Quercetin 50mg/kg	Group IV Gentamicin +WCHAE 250mg/kg	Group V Gentamicin +WCHAE 500mg/kg
SOD U/min/mg of protein	8.52 ± 0.175****	3.23± 0.0377	7.94± 0.075****	4.24± 0.061**	7.19± 0.029****
GSH µmoleGSH/mg protein	1.33± 0.147**	0.891± 0.027	1.36± 0.052**	1.22± 0.078^{ns}	1.38± 0.057**
CAT µmolesofH2O2consumed/ min/mg protein	2.095± 0.081***	1.43± 0.168	2.17± 0.071****	1.81± 0.280^{ns}	2.01± 0.054**

with an increased dose of plant extract, the results and Figures 3S and 4S (Supplementary file) *in-vivo* antioxidant enzyme levels are shown in Table 8.

3.5 Histopathological Findings

The histopathological examination of the normal group I is compared with the gentamicin Group II and other groups of WCHAE are shown in Figure 5S (Supplementary file). According to biochemical and antioxidant analysis, the kidney tissue of rats in the normal group did not exhibit any outward symptoms of renal toxicity or degeneration. However, the rats treated with gentamicin alone showed extensive renal tubule damage, degeneration, tubular necrosis, and toxicity (Figure 5 SB) (Supplementary file). The tubular toxicity pattern in the rats treated with 250mg/kg is optimum WCHAE and satisfactory in terms of prevention of necrosis and degranulation (Figure 5 SD) (Supplementary file), but renal tubule morphology has not improved as compared to the normal group. The group-V of rats administered with a dose of 500mg/kg WCHAE, observed minor necrosis, cellular damage, and renal toxicity compared to the gentamicin-treated and normal control groups.

4. Discussion

The herbal pants have a diverse group of flavonoids and phenolic contents like ferulic acid, caffeic acid, gallic acid, quercetin, rutin, kaempferol, apigenin have antioxidant potential in the prevention of nephrotoxicity. The antioxidant properties of phenols and flavonoids are mainly due to their possible redox activity. The total phenol and flavonoid content were measured and calculated for WCHAE using ascorbic acid and quercetin as standard[62]. The *in vitro* antioxidant assay was performed to find out the antioxidant potential of the WCHAE extract. The WCHAE exhibited significant DPPH scavenging activity[63] compared to ascorbic acid, demonstrating notable IC50 values and % inhibition. Similarly, the reducing power assay, hydroxyl radical scavenging assay, and nitric oxide scavenging assay also showed significant effectiveness. Hence, the presence of antioxidant molecules in WCHAE may have the capacity to partially mitigate or eliminate the nephrotoxic effects induced by gentamicin. The presence of several phytoconstituents in the plant extracts, particularly flavonoids and phenolic compounds are most likely responsible for its antioxidant capacity[64].

The nephroprotective efficacy of WCHAE was further validated using HEK-293 cell lines in the MTT assay and protein denaturation assay, based on the data of the *in vitro* antioxidant activity of plant extracts. The results indicated that the extracts of WCHAE did not exhibit any cytotoxic properties, suggesting their potential safety and effectiveness for nephroprotection[65]. In addition to this expected and desired flavonoids in the *Wedelia chinensis* extract were performed in the molecular docking studies[66] as ligands with target proteins from the kidney such as kidney injury molecule (KIM-1)[67] and Neutrophil gelatinase-associated lipocalin (NAGAL)[68]. The *in silico* results indicated that the secondary phytoconstituents like quercetin, rutin, and gallic acid, have better binding affinities with target molecules of kidney like NGAL and KIM-1. We expect the *in-vivo* nephroprotective potential of WCHAE extracts in animal models based on the outcomes of cell lines and molecular docking

studies. This study proposes that gentamicin-induced nephrotoxicity can be recognized by elevated kidney weight, elevated serum creatinine, urea, and uric acid, as well as decreased levels of superoxide dismutase, catalase, and reduced glutathione in the kidney tissues of experimental animals. In the experimental animals gentamicin-induced nephrotoxicity[69], can be minimized gradually by providing WCHAE and improvement of renal function. Renal cell damage caused by inflammation is most likely the reason for an increase in kidney weight blood flow to the inflamed area. That enables inflammatory cells like neutrophils, monocytes, and fluids, to leak out from the vascular compartment into the renal tissue, a layer of tissue, that likely increases kidney weight[70]. In the course of WCHAE treatment, rats' kidney weight was substantially decreased by the higher dose. The process by which aminoglycoside antibiotics, such as gentamicin, cause acute renal tubular toxicity is mediated by the production of inflammatory mediators and reactive oxygen species[71]. Thus, it is proposed that the use of secondary metabolites including flavonoids and phenolic compounds, in herbal supplements could significantly reduce kidney toxicity through their antioxidant characteristics[72].

The nephroprotective potential of *Wedelia chinensis* was examined in an acute kidney toxicity rat model. In the group of animals administered with gentamicin, levels of renal biochemical parameters such as urea, uric acid, and creatinine in both plasma and urine were elevated[73]. The 80mg/kg dose of gentamicin significantly reduced the level of stress antioxidant enzymes[74], as discussed in the results. The selected doses of *Wedelia chinensis* extract compared with the gentamicin group II effectively reduced the elevated levels of biochemical parameters such as creatinine level, serum urea, and uric acid level. Furthermore, the levels of renal biochemical parameters associated with oxidative stress, particularly superoxide dismutase, glutathione peroxidase, and catalase enzymes were assessed. The *Wedelia chinensis* leaf extracts significantly enhanced the activity of antioxidant enzymes in renal tissues showcasing significant *in vitro* antioxidant benefits, except for glutathione and catalase enzymes at the lower dose of 250mg/kg[75]. These findings could be associated with the antioxidants present in WCHAE and suggest that reactive oxygen species play a significant role in gentamicin-induced nephrotoxicity. The renal toxicity can be minimized by preventing oxidation with flavonoids and phenolic phytoconstituents of WCHAE leaf extract.

The histopathological findings reveal acute tubular necrosis and that assures the nephrotoxicity[76]. In this study, an assessment of renal sections in the gentamicin-treated group revealed more pronounced renal tubular damage and necrosis compared to the control group. Additionally, swelling was observed in the glomerular region[77]. Likewise, in this investigation, we found structural impairments in the kidneys of rats treated with gentamicin as minimized to those treated with WCHAE extract in the histopathology. The animals treated with the hydroalcoholic extract of WCHAE with doses of 250 and 500mg/kg reveal mild glomerular and tubular epithelial alterations, indicating a repairment and gradual development of renal physiology. The gentamicin administered to Group II rats after 7 days of treatment exhibits obvious damage with blue-coloured patches, which are signs of drug-induced toxicity. The hydroalcoholic extract of *Wedelia chinensis* at a lower dose of 250mg/kg indicates some improvement in tubular necrosis and degeneration, although slight leukocyte infiltrations in the intratubular area were noted. Rats treated with a dose of 500mg/kg body weight of WCHAE, exhibited a significant improvement in cellular morphology and a decrease in tubular damage as compared to rats with a dose of 250mg/kg body weight. The WCHAE extract doses provided mild to moderate protection in the renal tubules and epithelium, which could help minimize nephrotoxicity.

5. Conclusion

Previous studies on *Wedelia chinensis* leaf extract have suggested the presence of flavonoids and phenols, but none have explored its nephroprotective efficacy through *in vitro, in silico*, and *in-vivo* studies. This study's findings conclude that the hydroalcoholic extract of *Wedelia chinensis* (WCHAE) exhibits significant cytoprotective and anti-inflammatory properties on HEK293 cell lines, potentially reducing gentamicin-induced nephrotoxicity in rat models. Molecular docking studies have demonstrated good binding interactions with target kidney proteins.

These protective effects of WCHAE are attributed to its secondary metabolites, including flavonoids and phenolic compounds in the plant extracts. Consequently, the hydroalcoholic extract of *Wedelia chinensis* presents a promising good observation for further molecular-level research following the isolation and purification of various known and unknown phytoconstituents.

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