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MULTI-MODAL ASSESSMENT OF NEPHROPROTECTIVE PROPERTIES OF MICHELIA CHAMPACA: IN VITRO AND IN VIVO INVESTIGATIONS

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Abstract

This research aims to investigate the in silico, in vitro, and in vivo nephroprotective activity of *Michelia champaca* leaves extracts in gentamicin induced nephrotoxicity rats. The flavonoid and phenols-rich fractions of *Michelia champaca* leaves extract were subjected to *in silico* method with 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 at a lower dose of 250 mg/kg and a higher dose of 500 mg/kg body weight in gentamicin nephrotoxicity in rats with histopathological investigations. The *Michelia champaca* hydroalcoholic extract (MCHAE) shows remarkable 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 MCHAE. The *in vivo* nephroprotective activity improved at a dose of 500mg/kg of MCHAE than *Michelia champaca* ethanolic extract (MCEE). Histopathological investigations revealed the improvement in gentamicin-induced renal toxicity by

the MCHAE orally treated group compared to other groups. These results of MCHAE are more effective than MCEE and have a marked *in vitro* antioxidant, and cytoprotective effect in HEK293 cell lines, with good interaction scores of ligands in molecular docking studies with nephroprotective potential benefits in gentamicin-induced nephrotoxicity in rats.

Keywords: Nephroprotective activity, *Michelia champaca, In silico,* HEK-293 cells, gentamicin-induced nephrotoxicity.

1. Introduction

In the world, the global ranking of diseases shows that more than 10% of the population worldwide is affected by extremely common one of the leading noncommunicable causes of death chronic kidney disease (CKD).[1] The number of patients suffering from CKD has been increasing throughout the globe.[2] In India, approximately 38% of deaths increase in proportion due to kidney failure.[3] Nephrotoxicity & kidney disorders are affected due to many risk factors like obesity and diabetes mellitus, and renal failure due to drug therapies.[4] One of the more common problems associated with the renal system is renal toxicity, which may arise due to exposure to several medications and/or environmental substances and may result in either temporary or permanent renal failure.[5] Gentamicin is one of the most effective aminoglycoside antibiotics used widely in severe and detrimental infections caused by gram-negative bacteria. The use of gentamicin is limited as it has severe nephrotoxic adverse effects.[6] The prevalence of gentamicin-used nephrotoxicity is approximately 13–30%. The actual mechanism of gentamicin-induced nephrotoxicity (GIN) is not understood well, and the mechanism behind the development of nephrotoxicity by stimulating the generation of reactive oxygen species (ROS), which leads to toxicity to the renal system.[5] In addition to ROS, reactive nitrogen species (RNS), hydroxyl radicals (HOR), and superoxide anions (SOA) for the appearance of nephrotoxicity.[7]

In addition to this gentamicin reduces the level of glutathione (GSH) and the activity of antioxidant enzymes including superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPO).[8] GIN decreases the level of antioxidant enzymes including reduced glutathione (GSH), superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPO) resulting in renal failure, with a slower increase in creatinine (CR), blood urea nitrogen (BUN), urea and uric acid level along with proteinuria and electrolytes impairment.[8,9]

Acute Kidney Injury (AKI) is a condition that includes a variety of kidney-related disorders. A rapid reduction in kidney function is known as AKI.[10] There are several causative factors responsible for AKI, but the most often ones include microbial sepsis, blood volume loss, instability of blood flow toward the renal system, and the use of nephrotoxic medications.[11,12]

The incidence and prevalence of drug-induced kidney injury (DIKI) have increased in the last 20 years due to changes in healthcare practices (frequent use of possible nephrotoxic drugs, contrast media, and increase in high-risk interventions) and expansion of medical awareness recently. There are multiple reasons for the high risk of long-term complications and premature mortality after AKI, including pre-existing comorbidities and factors directly associated with the condition.[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]

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.[15] Gentamicin is not metabolized by the liver and most of the drug gets excreted in unchanged form and eliminated by the kidneys,[16] but when it is clinically significant, a small but harmful amount builds up in the lysosomes of the proximal renal tubular cells and induces apoptosis.[17]

In renal toxicity, free radicals damage in the tubular and glomerular basement membranes, causes cellular injury and necrosis through several complicating pathways, including the

peroxidation of membrane lipids, protein carbonylation, and deterioration of DNA.[18] In addition to this, gentamicin minimizes the effectiveness of antioxidant enzymes present in the kidney such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX), and glutathione (GSH).[19,20,21] Reactive species (RS) are free radicals that 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.[23,24] RS is constantly produced during normal physiological processes, although it is possibly removed by the antioxidant defence system.[25,26] The treatments for drug-induced nephrotoxicity are dialysis, renal transplantation, and other costlier treatments available.

In this research work, we evaluated the unexplored antioxidant potential benefits of phytoconstituents in *Michelia champaca* hydroalcoholic leaves extracts for the nephroprotective activity. The basis for the selection of this plant is the presence of plentiful flavonoids and phenolic compounds rich in leaf extract that could be helpful in the prevention of gentamicin-induced nephrotoxicity (GIN) in rats. This study highlighted the vitro antioxidant, *in vitro* cell line assays, possible molecular docking studies, and histopathological findings for the nephroprotective activity of hydroalcoholic leaves extracts of *Michelia champaca* in rats.

Material and Methods

Collection of Plant materials

The leaves of *Michelia champaca* (aerial parts) 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.

Preparation of Extract:[27]

The plant materials were cleaned and shade-dried at room temperature and ground separately in coarse powder. A 500 gm 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 ethanol & distilled water was used for extraction.[28,29] These extracts were filtered by Whatman filter paper and filtrate was evaporated to dryness by rotary evaporation at 40^0C and stored in the refrigerator for further experimentation.[30]

Phytochemical Investigation of Plant Extracts [31,32]

All four extracts of the plant were tested for preliminary phytochemical investigation for the presence of alkaloids, tannins, flavonoids, phenols, carbohydrates proteins, etc. 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 *Michelia champaca* was subject to an *in vitro* antioxidant study.

Quantitative screening of *Michelia champaca*

Total Phenolic Content (TPC)

Estimation of total polyphenol contents in *Michelia champaca* extracts was performed by the Singleton and Rossi method using the Folin Ciocalteu method (Gallic acid equivalent (GAE) in mg/g of the extract). The absorbance was measured a λmax nm. A calibration curve was prepared using standard gallic acid and used to calculate the total phenolic content of the extract and the results were expressed as gallic acid equivalents (mg GAE / 100 g sample).[33,34]

Total Flavonoid Content (TFC)

An established technique was used to determine the total flavonoid content (TFC) of *Michelia champca* extract. The absorbance was measured at 510 nm after 30 minutes in comparison to the

blank solution. The flavonoid compound's estimate was done in three separate steps. Using the standard quercetin and results are expressed in equivalents (mg GAE / 100 g sample).

In vitro antioxidant assay

DPPH Antioxidant Assessment: [35]

An evaluation of the *in vitro* antioxidant activity of *Michelia champaca* hydroalcoholic extract (MCHAE) and standard ascorbic acid by the DPPH radical scavenging assay was performed according to the literature with standard procedure.[36,37] The antioxidant (AH) ability of MCHAE to directly react with DPPH radicals was determined by a decolorization test, which was performed using a UV-visible spectrophotometer (EQUIPTRONICS Mumbai, India). Trolox equivalents (mol TE/g) were used to express the amount of DPPH radical scavenging activity. Under the same test conditions, ascorbic acid was used as a positive control.

Reducing Power Assessment:[38]

The reducing power of extracts and positive controls were determined according to the method described by Oyaizu.[39] The absorbance was measured at 700 nm after the top surface of the solution was mixed with distilled water and 0.1% FeCl3 at a ratio of 1:1:2. The reaction mixture's enhanced absorbance was a sign of its improved reducing power.[40]

Hydroxyl Radical Scavenging activity:[41]

The scavenging activity of the extract on hydroxyl radical was determined according to the 2-deoxyribose assay reported by Halliwell and Gutteridge. A hydrogen peroxide, EDTA, and Fe3+-ascorbate system produced a hydroxyl radical. The supernatant's absorbance was calculated at 532 nm.[42,43]

Nitric Oxide Radical Scavenging Activity: [44,45]

The Garratt et al.-described procedure was followed while conducting this antioxidant scavenging assay.[44] The Griess-Ilosvay reagent was modified with the use of naphthyl ethylenediamine dihydrochloride (0.1 w/v) rather than 1-naphthyl amine (5%). The absorbance was determined at 546 nm. The standard was used as ascorbic acid and the percentage of inhibition was calculated.[46]

In vitro Cell Line Study [47,13]

Maintenance of (Human Embryonic Kidney) HEK cells:

Cell lines were procured from NCCS Pune, Maharashtra. The data sheet with sixteen short tandem repeat (STR) loci proved to be 100% matching with ATCC STR profile. After procuring the cell lines, maintenance & subculturing of the cells 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.

Cell viability and cell Cytotoxicity Tests.

Microculture Tetrazolium (MTT) Assay:[48,49]

Cell cytotoxicity analysis of MCHAE & *Michelia champaca* ethanolic extract (MCEE) against human embryonic kidney 293 (HEK 293) cell lines was determined. A trypan blue assay was performed and the number of viable cells was counted and calculated. The compounds of serial dilutions were added to wells and incubated for 24 hours. Later 20µL of tetrazolium (MTT) dye with media was added and incubated for 24 hrs. The supernatant was discarded without disturbing formazan crystals, 100µL of DMSO was added to dissolve the crystals and the reading was taken at 570 nm using a spectrophotometer. The proliferative index was calculated by dividing the OD of the test by the OD of the control multiplied by 100.

Protein Denaturation Assay: [50,51]

The test tubes were taken according to the number of concentrations similar to the MTT assay. One negative control and one diclofenac which was a positive control. 2.8 ml of sterile Phosphate Buffer Solution (PBS) having pH 6.3-6.4 was added to all tubes. Further, 0.2ml i.e., 200 µL of egg albumin was added to all respective tubes for negative control 2ml of PBS was added, and for positive control group 2ml of Diclofenac was added. All the test tubes were incubated in a water bath at 37^0C for 15 mins and further for 5 mins at 70^0C. After cooling the OD was taken at 660nm by using a spectrophotometer. The % inhibition of protein denaturation was calculated.

Toxicity Study[52,53]

The acute oral toxicity study was carried out as per the guidelines 423 set by the Organization for Economic Cooperation and Development (OECD) and the Committee for Control and Supervision of Experiments on Animals (CCSEA). The protocol was sanctioned and approved by the Institutional Animal Ethical Committee (IAEC) with registration number 1158/PO/Re/ S/07/ CPCSEA of JKKMMRF Sampooorani Ammal College of Pharmacy, Ethirmedu, Komaraplayam Namakkal District, Tamil Nadu India.[54]

Animal Study

Experimental Setup[55,56]

A random selection of 30 Wistar strain rats of either sex was used in the study protocol. A total of 05 groups labelling with, I-V with 06 animals in each group were divided for the study. The gentamicin injection used in the negative control group was given intraperitoneally and normal saline solution was given orally. Gentamicin was given consecutively from day 7 to day 14, for a total of seven days in normal control. In group III standard drug quercetin[57] and the remaining groups IV-V were administered low and high doses of phenolic/flavonoid-rich MCHAE extracts from day 1 to day 14, followed by gentamicin from day 8 to day 14. At the end of the study on day 14 after 24 hrs. of the last dose of extracts, animals were sacrificed under ether anesthesia.[58]

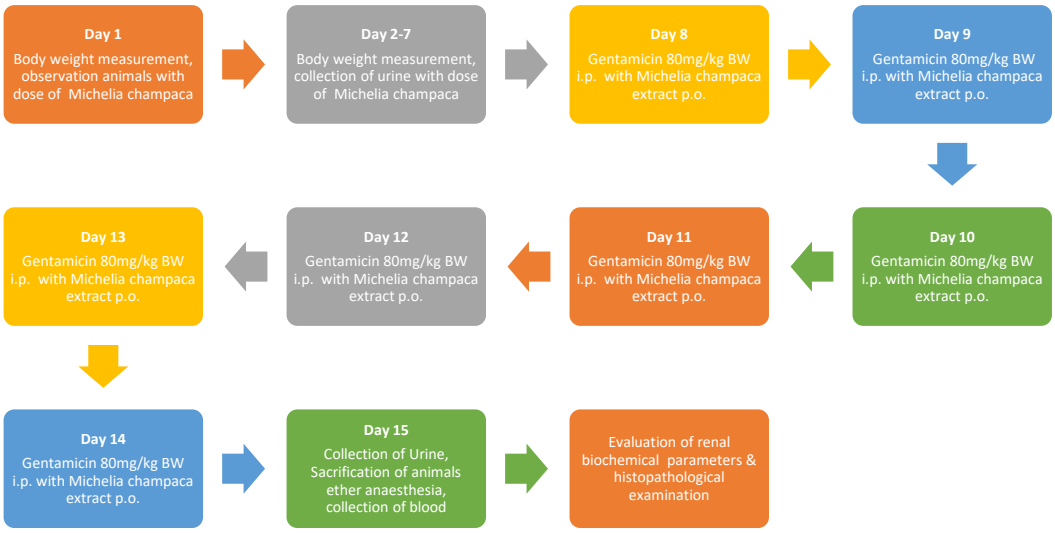


Figure 1. Schematic presentation of animal experimentation protocol.

Histopathological Examination

The histopathological investigation and stained with haematoxylin followed by eosin for clear visualization and images were captured with a light microscope. A kidney sections and samples were analysed to find out the changes in the morphological features associated with gentamicin-induced nephrotoxicity. The histological changes of glomeruli, proximal tubules, and interstitial components were observed in the light microscope.[59-60]

Statistical Analysis

The data are presented as the mean± SEM. The InStat 10.0.2 software was used for the statistical analysis. One-way Analysis of Variance (ANOVA) and Dunnett test were applied. The difference was considered significant if the p-values < 0.05.

Results

***In-vitro* Antioxidant Study:**

The hydroalcoholic extract of *Michelia champaca* showed good phenolic and flavonoid content compared to that of other extracts. The TFC of MCHAE and MCEE was 15mg/ ml and 13 mg/ml respectively. The TPC was obtained higher for MCHAE than other extracts. The *in vitro* antioxidant assay was performed for MCHAE 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 to standard ascorbic acid results are shown below in Figure 2.

((Abs control - Abs test) / Abs control) X 10 = percentage inhibition (%)

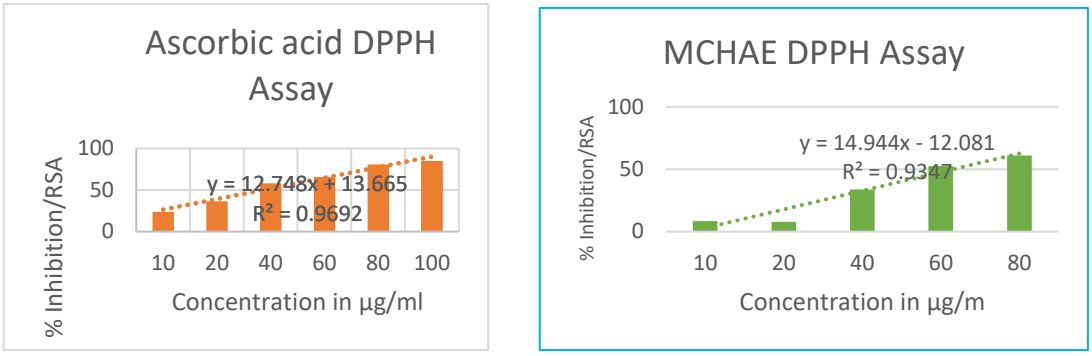


Fig. No. 2(A) Ascorbic acid and MCHAE DPPH Assay

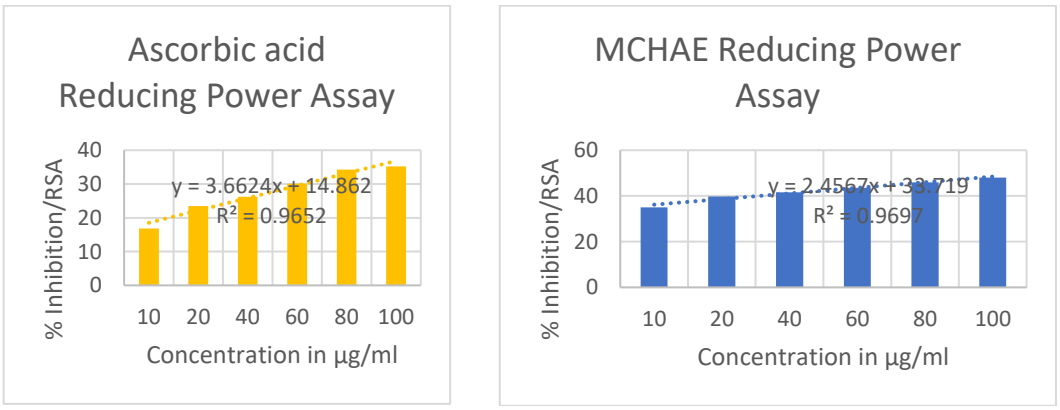


Fig. No. 2 (B) Ascorbic acid and MCHAE Reducing Power Assay

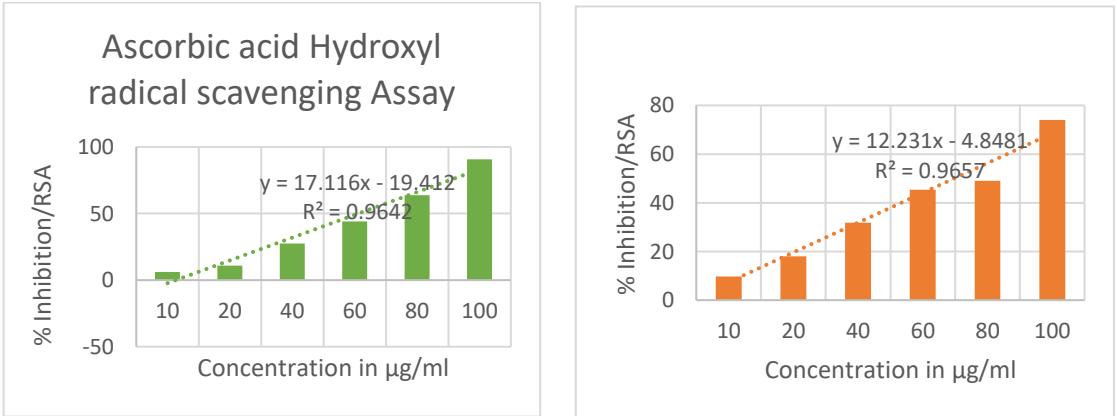


Fig. No. 2(C) Ascorbic acid and MCHAE Hydroxyl Radical Scavenging Assay

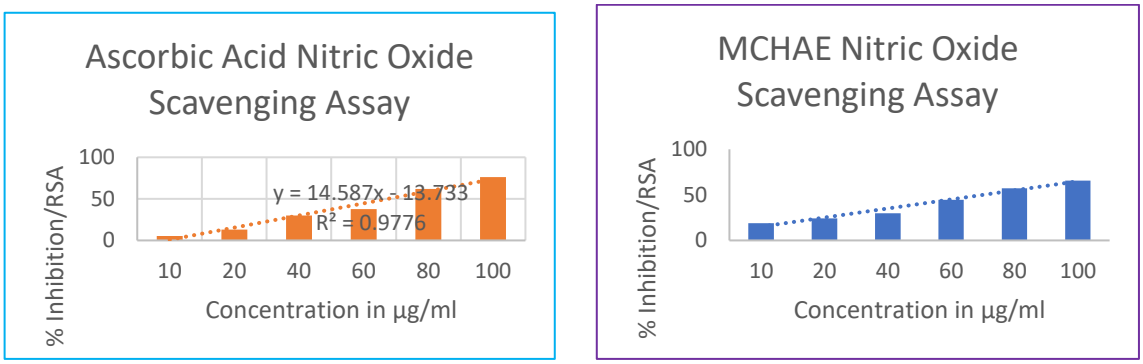


Fig. No. 2 (D) Ascorbic acid and MCHAE Nitric Oxide Assay

In vitro cell lines study

MTT Assay

The percentage cell viability of MCHAE extract at different dilutions at 12.5, 25, 50, 100, and 200 µg/mL showed 89.7%, 91.4%, 93.1%, 96.9%, 87.6%, while MCEE 87.8%, 81.4%, 91.9%, 97.4%, 90.3% cell viability respectively. A significant percentage of cell viability of MCHAE 91.5% and MCEE 97.4% at 100 µg/ml concentration was observed, compared to control and standard quercetin & rutin. The mean optical density of different test compounds and MCHAE and MCEE extract and percentage cell viability are shown in Table No. 1.

Table 1: MTT Assay: Mean Optical Density (MOD)

Test concentration µg/ml	Mean Optical Density (MOD)				
	Rutin	Quercetin	Gentamicin	MCHAE	MCEE
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
Percentage Cell viability (PCV)					
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

Protein Denaturation Assay

All the test compounds had no cytotoxic effect on HEK293 cell lines shown in table 1. There was no extractive activity of the percentage of protein denaturation was found with all the concentrations of test compound MCHAE. The positive control of diclofenac showed 30% inhibition of protein denaturation. Thus, the test compound MCHAE was found to have anti-inflammatory properties and can be helpful in the prevention of gentamicin nephrotoxic inflammation. The data of mean optical density and percentage cell inhibition (PCI) of protein denaturation assay are shown in Tables 2.

Table 2: Protein denaturation assay: Mean Optical Density (MOD)

Test concentration µg/ml	**Mean Optical Density (MOD)**				
	Rutin	Quercetin	Gentamicin	MCHAE	MCEE
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
Protein denaturation assay: Percentage Cell Inhibition (PCI)					
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

Renal Biochemical Parameters Examination

Effect of *Michelia champaca* Hydroalcoholic Extract (MCHAE) on renal biochemical parameters

The group of gentamicin-induced nephrotoxicity significantly ($P < 0.001$) increased the levels of urinary and serum total protein and albumin levels compared to that of the normal control group. The level in the standard quercetin group and higher doses of MCHAE (500 mg/kg) group improved the level significantly compared to the lower dose of 250 mg/kg was insignificant shown in Table 3.

Table 3: Effect of MCHAE on urinary and serum total protein and albumin levels and

Urine parameters (Unit)	**Group I Normal control**	**Group II GM 80 mg/kg**	**Group III GM+ QRTN 50 mg/kg**	**Group IV GM+MCHAE 250 mg/kg**	**Group V GM+MCHAE 500 mg/kg**
Total protein (g/dL)	3.49±0.085****	4.47±0.082****	3.40±0.01****	4.26±0.07^{ns}	4.04±0.03***
Albumin (g/dL)	0.58±0.018****	0.75±0.013****	0.57±0.016****	0.71±0.019^{ns}	064±0.013**
Effect of MCHAE on serum protein and albumin in gentamicin-induced nephrotoxicity in rats					
Serum parameters (Unit)	**Group I Normal control**	**Group II GM 80 mg/kg**	**Group III GM+ QRTN 50 mg/kg**	**Group IV GM+MCHAE 250 mg/kg**	**Group V GM+MCHAE 500 mg/kg**
Total protein (g/dL)	7.19±006***	7.15±003	7.19±005***	7.17±005^{ns}	7.18±005**
Albumin (g/dL)	4.36±0.003****	4.33±0.003	4.37±0.003****	4.34±0.003^{ns}	4.36±0.002***

Effect of the *Michelia champaca* hydroalcoholic extract (MCHAE) on the renal biochemical parameters (A) Total Protein in urine, (B) Urine albumin. Data are presented as the mean ± SEM, n = 6. One-way analysis of variance (ANOVA) and the Dunnett test were used to evaluate the significant differences among all the groups. A statistically significant difference (*** GM Vs MCHAE) was found in all data 500 mg/kg MCHAE and 500 mg/kg dose except at 250 mg/kg dose of MCHAE, were nonsignificant. ****P < 0.001, highly significant; ***P < 0.01, more significant; **P < 0.01, satisfactory significant; ns, nonsignificant; NC: negative control (Gentamicin administered group); and STD: Standard quercetin group.

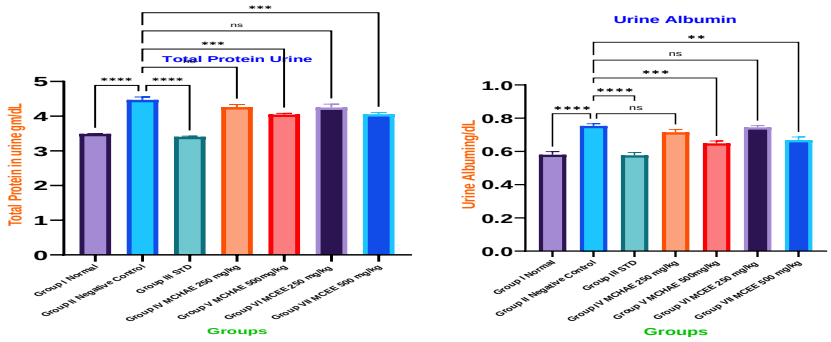


Figure 3: Effect of MCHAE and MCEE on Total Protein (A) and albumin (B) in urine

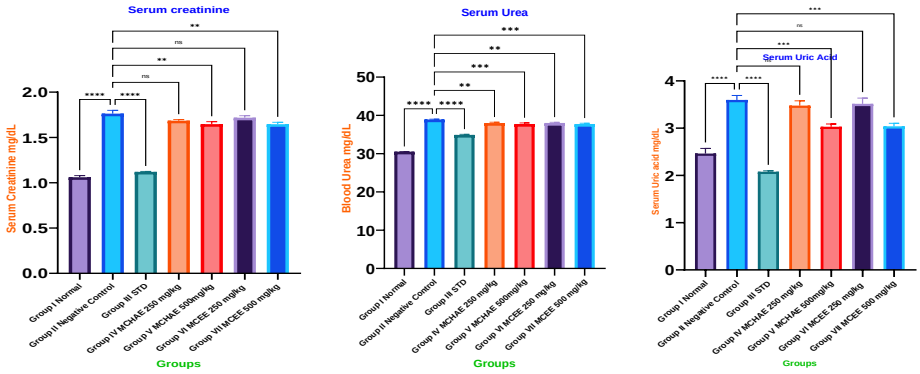
Effect of *Michelia champaca* hydroalcoholic Extract (MCHAE) on biochemical parameters

The levels of serum creatinine, urea, and uric acid in the negative control group were elevated with signs of acute renal toxicity. Compared to these elevated creatinine and uric acid in the negative control group the MCHAE at a lower dose of 250mg/kg body weight extracts was insignificant. However, a dose-dependent effect of standard quercetin 50mg/kg and higher doses of extracts 500 mg/kg significantly ($P < 0.001$) improved all of these parameters. The only exception is blood urea level, where the effects of both the lower and higher doses are better with significantly reduced elevated biochemical parameters and results are shown in Table 4.

Table 4: Effect of MCHAE on serum creatinine urea and uric acid levels in Gentamicin induced nephrotoxicity in rats

Serum parameters (Unit)	Group I Normal control	Group II GM 80 mg/kg	Group III GM+ QRTN 50 mg/kg	Group IV GM+MCHAE 250 mg/kg	Group V GM+MCHAE 500 mg/kg
Creatinine (mg/dL)	1.06± 0.018****	1.76± 0.0358	1.12± 0.005****	1.68± 0.012^{ns}	1.64± 0.027**
Blood Urea (mg/dL)	30.5± 0.026****	38.91± 0.096	34.88± 0.164****	37.99± 0.220**	37.74± 0.282***
Uric acid (mg/dL)	2.46± 0.109****	3.59± 0.094	2.076± 0.020****	3.47± 0.102133^{ns}	3.02± 0.059***

Effect of the *Michelia champaca* hydroalcoholic extract (MCHAE) on serum creatinine, urea, and uric acid. Data are presented as the mean ± SEM, n = 6. One-way analysis of variance (ANOVA) and the Dunnett test were used to evaluate the significant differences among all the groups. A statistically significant difference (*** GM Vs MCHAE) was found at a dose of 500 mg/kg of MCHAE of serum creatinine, blood urea, and uric acid except at a dose of 250 mg/kg dose of MCHAE, was nonsignificant. ****P < 0.001, highly significant; ***P < 0.01, more significant; **P < 0.01, satisfactory significant; ns, nonsignificant; NC: negative control (Gentamicin administered group); and STD: Standard quercetin group.



A B C

Figure 4: Effect of MCHAE on A: Serum Creatinine B: Urea and C: Uric acid.

Effect of the Michelia champaca hydroalcoholic extract (MCHAE) on the renal biochemical parameters (A) Serum Creatinine in urine, (B) Urea (C) Uric acid. Multiple correlation data are presented as the mean ± SEM, n = 6. One-way analysis of variance (ANOVA) and the Dunnett test were used to evaluate the significant differences among all the groups. A statistically significant difference (*** GM Vs MCHAE) was found in all data 500 mg/kg MCHAE sets except serum urea at 250 mg/kg dose of MCHAE, which were nonsignificant. ****P < 0.001, highly significant; ***P < 0.01, more significant; **P < 0.01, satisfactory significant; ns, nonsignificant; NC: negative control (Gentamicin administered group); and STD: Standard quercetin group.

Histopathological findings:

In the histopathological examination of the normal group compared with the gentamicin alone group, two chosen doses of gentamicin and MCHAE are shown in figure 5 (A-D). According to biochemical and antioxidant analyses, the kidney tissue of rats in the normal group did not exhibit any outward symptoms of renal toxicity or degeneration (figure 3 and 4). The rats treated with gentamicin alone showed extensive renal tubule damage, degeneration, and tubular necrosis (figure 5B). The tubular toxicity pattern in the rats treated with 250 mg/kg optimum MCHAE was satisfactory in terms of necrosis and degranulation (figure 5D), but renal tubule shape did not improve as much. The rats that received 500 mg/kg MCHAE, however, showed (figure 5D), very

minor necrosis, cellular damage, and renal toxicity compared to the gentamicin-treated and normal control groups.[61,62]

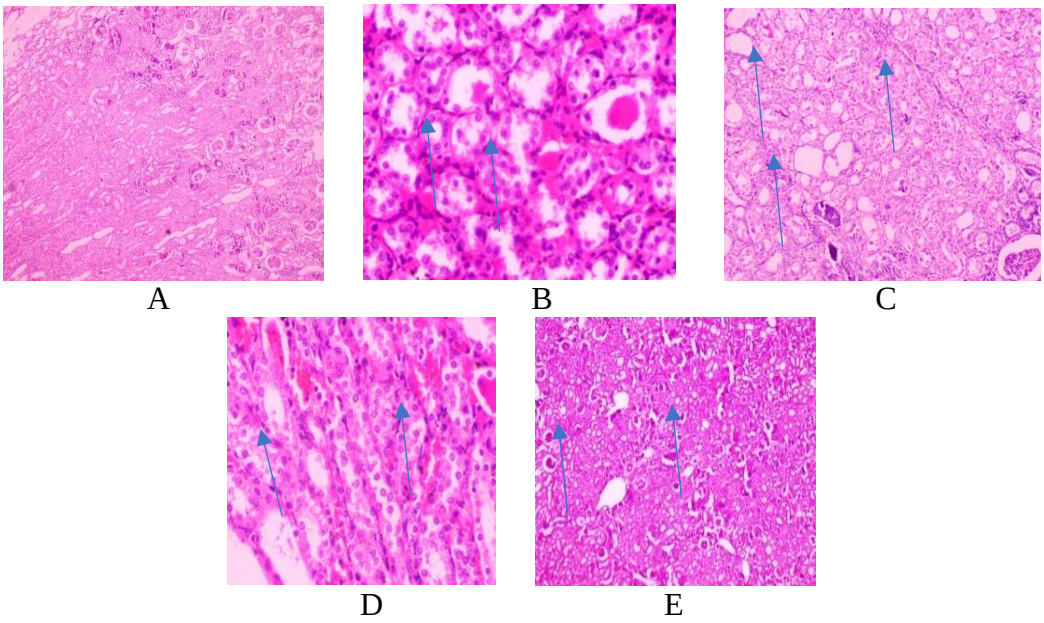


Figure 5: Histopathological findings of effects of MCHAE extract on gentamicin-induced nephrotoxicity in rats. A: Normal group-I: No visible sign of acute tubular toxicity; B: Group-II Negative Control: Clear sign of tubular toxicity by accumulated gentamicin; C: Group-III STD QRTN: Comparatively lesser toxicity in renal tubules; D: Low Dose MCHAE: Toxicity reasonable toxicity by gentamicin; E High Dose MCHAE: Indicating the comparatively less cellular toxicity.

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.[63] The antioxidant property of phenols and flavonoids is mainly due to their possible redox activity. The total phenolic content (TPC) and total flavonoid content (TFC) were quantified in MCHAE and ascorbic acid as standard.[64] In addition to quantification of TFC and TPC *in vitro* antioxidant assay was performed to evaluate the antioxidant potential of MCHAE extract. The DPPH scavenging activity of MCHAE[65] was found to be significant as compared to ascorbic acid, with notable IC50 values. Similarly, Reducing power assay, hydroxyl radical scavenging assay and nitric oxide scavenging assay were performed, and significantly effective.[66] Therefore, a ROS scavenger with antioxidant molecules of MCHAE may have the capacity to partially reduce or eliminate the nephrotoxic effects induced by gentamicin. As already mentioned, this antioxidant potential might be due to the presence of various phytoconstituents, especially flavonoids and phenolic compounds.

Considering *in vitro* antioxidant activity observations, for confirmation of the nephroprotective efficacy of MCHAE, were tested for MTT assay and protein denaturation assay using HEK293 cell lines. The results showed that no cytotoxic property was observed in the extracts of MCHAE and could be safely effective for nephroprotective efficacy.[67]

Based on traditional medicine uses of *Michelia champaca*[65] this investigation indicated that gentamicin-induced nephrotoxicity can be assessed from the increased kidney weight, high serum levels of creatinine, urea, and uric acid, and decreased levels of superoxide dismutase, catalase, and reduced glutathione in the tissues of the kidneys of the experimental animals (Wistar strain rats). In the rats' gentamicin-induced nephrotoxicity,[68] can be minimized by providing MCHAE. In the previous studies observed that gentamicin-treated experimental animals have considerably greater kidney weight, our research findings also showed that gentamicin-treated experimental animals have considerably greater kidney weight. Renal cell damage caused by inflammation is most likely the

reason for an increase in kidney weight after gentamicin injection. Blood flow to the area increases when an organ or tissue is inflamed, and there is a brief stasis of blood in the area. That enables inflammatory cells like neutrophils, monocytes, and fluids, to leak out from the vascular compartment into the interstitial, a layer of tissue, that likely increases kidney weight.[69] The course of treatment rats' kidney weight was substantially decreased by the higher dosages of MCHAE. Their anti-inflammatory properties can be used to clarify the reduction in kidney weight after MCHAE during treatment.

The mechanism behind the development of acute renal tubular toxicity by aminoglycoside antibiotics like gentamicin is the production of ROS and inflammatory mediators.[70,71] Thus, it is postulated that the use of antioxidants secondary metabolites like flavonoids and phenolic compounds-rich herbal supplements can be effective in minimization of renal toxicity by antioxidant potentials.[72]

The *in vivo* activity of *Michelia champaca* was investigated to find out the nephroprotective potential in an acute kidney toxicity rat model. The renal biochemical parameters levels of urea, uric acid, and creatinine, in plasma as well as urine, are increased in the gentamicin-administered group of animals.[73] The 80 mg/kg dose of gentamicin significantly reduced the level of antioxidant enzymes,[74] as discussed in the results. The selected doses (250 and 500 mg/kg) of *Michelia champaca* extract are investigated and compared with the gentamicin group. The *Michelia champaca* leaf extract decreased the elevated levels of serum biochemical parameters like serum creatinine, serum urea, and uric acid. The levels of the biochemical renal parameters of oxidative stress, mainly SOD, GSH, and CAT, were analysed. Several compounds with antioxidant activity, flavonoids, and phenolic compounds have been successfully used to prevent or minimize gentamicin-induced nephrotoxicity. The *Michelia champaca* leaves extracts significantly improved the activity of antioxidant enzymes like SOD, GSH, and CAT with significant invitro antioxidant benefits with the exception of GSH and CAT at a lower dose of 250mg/kg.[75,76] These results may be obtained due to the availability of the antioxidant potential of MCHAE. This shows that the role of ROS in gentamicin-induced nephrotoxicity can enable the renal toxicity that prevents oxidation with adequate flavonoids and phenolic compound-rich phytoconstituents in MCHAE leaf extract.

The histopathological studies reveal acute tubular necrosis and that assures the nephrotoxicity.[77] In the previous findings for evaluation of renal sections in the gentamicin-treated group, compared to that of the control group observed more renal tubular damages and necrosis in renal tubules as well as swelling in the glomerular region.[78] Similarly, in histopathological findings in the present study, we observed damages in the structures of the kidneys of gentamicin-administered rats concerning MCHAE extract-administered group. Glomerular and tubular epithelial changes were mild in the groups treated with the hydroalcoholic extract of MCHAE (250 and 500 mg/kg), and remodelling of normal histopathology was seen. During seven days of gentamicin injection exhibit clear damages and bluish spots that indicated accumulated drug toxicity. The hydroalcoholic extract at a lower dose of 250 mg/kg with little improvement in tubular necrosis and tubular degeneration observed in histopathological investigation. However, slight leukocyte infiltrations in the intratubular area were present. The case of rats treated with hydroalcoholic extract of *Michelia champaca* at a dose of 500 mg/kg revealed a notable reduction of tubular damage and improvement of cellular morphology than the lower dose of MCHAE 250 mg/kg body weight. The MCHAE extract doses provide moderate to good protection closer to normal physiology of epithelial and tubular renal physiology.

Conclusion:

The earlier finding in *Michelia champaca* leaves extract revealed the availability of an ample quantity of flavonoids and phenols but none of the investigators performed the *in vitro*, *in silico*, and *in vivo* study of *Michelia* for nephroprotective efficacy. This study concludes that the hydroalcoholic extract of *Michelia champaca* (MCHAE) significantly has cytoprotective benefits

and good anti-inflammatory properties on HEK293 cell lines that can improve and minimize the gentamicin-induced nephrotoxicity in rat models, which might provide the rationale for antioxidant and nephroprotective efficacy. The protective benefits MCHAE revealed due to the presence of secondary metabolites like flavonoids and phenolic compounds, as mentioned in the prior section. Thus, the hydroalcoholic extract of *Michelia champaca* opens a new research area for carrying out further molecular-level research after the isolation and purification of various known and unknown secondary phytoconstituents.

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