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Original Article

Ethanolic Leaf Extract of *Centella asiatica* L. Possesses Nephroprotection in an Animal Model of Nephrotoxicity

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ABSTRACT

Background: Nephrotoxicity is a significant adverse effect of gentamicin, which is thought to be related to reactive oxygen species in the kidney. The purpose of this study was to look at how gentamicin-induced nephrotoxicity in rats was affected by a leaf extract from *Centella asiatica* L. **Materials and Methods:** Adult Wistar rats were divided into following groups: normal saline, gentamicin group injected with gentamicin sulfate (100 mg/kg, i.p) for 8 days, *Centella asiatica* L. groups administered with ethanolic leaf extract (200 mg and 400 mg/kg, p.o) daily for 8 days concurrently with gentamicin. Superoxide dismutase and catalase activity, urea, blood urea nitrogen, urea, serum, and urine creatinine were measured. **Results:** The administration of gentamicin resulted in nephrotoxicity as evidenced by significant elevations in blood urea nitrogen, serum creatinine, and urea as well as urine creatinine and urea. Interestingly, the ethanolic leaf extract of *Centella asiatica* decreased these indicators, minimizing the degree of gentamicin-induced kidney impairment. In rats administered with gentamicin, histopathological analysis revealed epithelial loss along with severe granular degeneration, and *Centella asiatica* leaf extract restored morphological changes in the kidney. **Conclusion:** This study has shown that gentamicin-induced kidney damage can be prevented by using a leaf extract from the *Centella asiatica* plant.

Keywords: Nephroprotective activity, *Centella asiatica*, Gentamicin, Nephrotoxicity.

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INTRODUCTION

Acute renal injury is a serious condition brought about by exposure to chemicals from drugs, the workplace, or the environment.[1] Gentamicin is clinically used to treat gram negative bacterial infection and has good bactericidal effect with low cost. Gentamicin induced renal damage accounts for 10-15% of total cases worldwide.[2] Even though the precise mechanism by which gentamicin produces nephrotoxicity is unknown, earlier studies showed increased reactive oxygen species and lipid peroxidation, nephron necrosis and possible nephrotoxicity.[3]

Herbal plants are enriched with bioflavonoids and possess powerful antioxidant activity. *Centella asiatica* Linn. (Family: Apiaceae; locally known as *Brahmi, Gotu kola*) is widely found throughout the Asia including India, especially in wetlands.[4] *Brahmi* is used extensively in indigenous system of medicine like Ayurveda, Siddha, Unani and Naturopathy. Traditionally, leaves are used to treat asthma, ulcers, skin conditions, memory impairment, leprosy, depression, wounds. *Centella asiatica* is utilised as an anti-bacterial, anti-depressant, anti-emetic, anti-neoplastic, antioxidant, anti-thrombotic, anxiolytic, gastroprotective, and immunological modulator.[5,6] Previous studies indicate the antioxidant actions of *Centella asiatica* in different animal models. Aqueous *Brahmi* extract significantly reduced the levels of MDA and increased glutathione and catalase in rats subjected to STZ-induced oxidative stress. The anti-oxidant enzymes Superoxide Dismutase (SOD), catalase, and Glutathione Peroxidase (GSHPx) were enhanced in lymphoma-bearing mice by a methanolic extract of *Centella asiatica*.[7-9]

Previous studies have found that *Centella asiatica* has a number of antioxidant substances that have shown to protect various organs. However, there are no data on how well it works in preventing nephrotoxicity brought on by gentamicin. In light of this, the goal of the current investigation was to evaluate the nephroprotective effects of *Centella asiatica* ethanolic leaf extract using a rat model of gentamicin-induced kidney injury.

MATERIALS AND METHODS

Plant Material

Centella asiatica plant was procured from local market, Bengaluru district in the state of Karnataka, India. Plant material was cleaned

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and washed thoroughly with water. Plant was authenticated by Dr. Vijay from Vriksha Vijnan Private. Ltd., Bengaluru.

Preparation of Extract

The ethanolic leaf extract of *Centella asiatica* was prepared by using Soxhlet apparatus.[10,11] The *Centella asiatica* fresh leaf was dried in the shade, and the dried leaf was ground into a coarse powder using a mixer. 220 g of coarse powder were utilised for the extraction using the Soxhlet equipment, which was then subjected to continuous hot percolation using 95% v/v of ethanol while keeping the temperature at 50-55°C. The collected extract was evaporated using a rotary evaporator, and the yield of the crude extract was 56 g.

Phytochemical Investigation of Extracts

The ethanolic leaf extract of *Centella asiatica* underwent a preliminary phytochemical examination.[12]

Experimental Animals

Wistar rats weighing 150-200g were purchased from Adita Biosys Private. Ltd., in Tumkur, Karnataka (Reg. No. 237/99/CPCSEA). Rats were kept in plastic cages under standard conditions (room temperature of 27±4°C, humidity of 45-55%, and a cycle of 12 hr of light and 12 hr of darkness), with free access to food and water. Animals were acclimatised to laboratory conditions prior to the experimental protocol. All experiments were conducted in accordance with the CPCSEA recommendations. The Institutional Animal Ethical Committee approval (IAEC/NCP/90/2015) was obtained from Nargund College of Pharmacy in Bengaluru, Karnataka.

Acute Oral Toxicity Study

In order to investigate the acute oral toxicity of *Centella asiatica* ethanolic leaf extract, Wistar rats (both male and female) were utilised. Doses ranging from 100 to 2000 mg/kg body weight were used, and the LD_{50} was calculated.[13] The leaf extract showed no mortality at a dose of 2000 mg/kg body weight. Therefore, two doses of 200 and 400 mg/kg, p.o., were chosen for further experiments.

Gentamicin-Induced Nephrotoxicity

Wistar rats (Male and female; 150-200 g) were used for the study. They were randomly divided into following groups. Control: Each rat received 2 mL of distilled water for 8 days. Gentamicin: Each rat injected gentamicin sulphate (100 mg/kg; i.p. for 8 days). Gentamicin standard drug, Gentamicin+*Centella asiatica*-200 and Gentamicin+*Centella asiatica*-400: animals received cystone 5 mL/kg; p.o.; *Centella asiatica* ethanolic leaf extract 200 and 400 mg/kg p.o.; once daily for 8 days respectively along with gentamicin. *Centella asiatica*-400: control rats received *Centella asiatica* ethanolic leaf extract 400 mg/kg p.o.; once daily for 8 days.

Renal Function Tests

Following the treatment schedule, the animals were kept separately in the metabolic cages for 24 hr in order to collect urine. Urine samples have been collected, rapidly evaluated for volume and pH, centrifuged for 10 min at 1500 g to remove debris, and then preserved at -4°C for analysis. Urine samples were acidified to pH 2 using 5M HCl.[2] Urine samples were analysed for various biochemical parameters.

Blood Sample Collection and Serum Separation

After collecting the urine, animals from all 6 groups were anaesthetized with halothane. Blood was drawn from the retro orbital plexus of each rat, collected in centrifuge tubes, and allowed to coagulate for 30 min at 37°C. The blood was centrifuged in a micro centrifuge for 10 min at 2500 rpm. The produced serum samples were kept at -20°C and utilised for biochemical estimation.

Body Weight Measurement

Animals' body weights were recorded both before and after the experiments.

Urine and Serum Analysis

Estimation of numerous biochemical parameters of urine (urea, creatinine) and serum (urea, creatinine, Blood Urea Nitrogen (BUN)) was done using a semi-auto analyser (Robonik India Pvt. Ltd.[2]

Estimation of Endogenous Antioxidant Levels in Kidney

An overdose of anaesthetic was used to sacrifice the animals, and the kidneys were then removed from the surrounding connective tissues and fat before being instantly weighed. Extracted tissue was homogenised in cooled phosphate buffer (pH 7.0). The homogenate was centrifuged at 5000 rpm for 20 min at 4°C. The resulting supernatant was used to measure the activity of antioxidant enzymes including Superoxide Dismutase (SOD) and Catalase (CAT) using a colorimetric method.[14,15]

Estimation of Superoxide Dismutase (SOD) Activity

1 mL of 50 mM sodium carbonate, 0.4 mL of 24 µM Nitro Blue Tetrazolium (NBT), and 0.2 mL of 0.1 mM EDTA were added to 0.5 mL of kidney homogenate. The reaction was initiated by adding 0.4 mL of 1 mM hydroxylamine hydrochloride and absorbance was measured at 560 nm. Units of SOD activity were expressed as the amount of enzyme required to inhibit 50% NBT. Using the formula, the specific activity was shown as a proportion of suppressed SOD activity:

% of inhibition=(Absorbance of Control-Absorbance of Treated/ Absorbance of Control) X 100

Estimation of Catalyse (CAT) Activity

0.5 mL of the kidney homogenate was added to 1.95 mL of 10 mM H_2O_2 in 60 mM phosphate buffer (pH=7.0), and the rate of H_2O_2 degradation was measured at 240 nm. The CAT level expressed in terms of U/mg of protein from the rate of H_2O_2 oxidation.

CAT unit=Mean value of absorbance/co-efficient value

Co-efficient value=43.6/M/cm; U/mg of protein=CAT unit value/protein 50 µL value

Histopathological Studies

Kidney tissues were fixed in 10% v/v buffered formalin solution. Paraffin wax was used to embed tissue samples. Cryostat was used to take several serial slices, each measuring 4-5 µm thick. Sections were stained with Hematoxylin and Eosin stain. Sections mounted on glass slides were viewed under a compound light microscope at 100X, 400X magnification. Histological details were analysed and interpreted. The six coded slides from each group were examined by a pathologist in a blinded manner.

Statistical Analysis

Data were presented as the mean SEM. Utilizing Graph Pad Prism version 7.0 USA, statistical comparisons were carried out using one-way analysis of variance (ANOVA) and Tukey's *post-hoc* test. When compared to the control, a *p* value of 0.05 or less was considered statistically significant.

RESULTS

Effect of Ethanolic Extract of *Centella asiatica* (EECA) on Percentage Body Weight Gain

Gentamicin (100 mg/kg) administered rats lost weight gradually as compared to the control group (***p<0.001; $F_{5,30}$=7.414: Figure 1). When EECA was administered concurrently for eight days in doses of 200 mg and 400 mg/kg to the gentamicin group, their body weight gain was noticeably enhanced (**p<0.01). Standard cystone treatment increased body weight (***p<0.001).

Centella asiatica Treatment Normalised Serum Urea Levels in Diseased Control Animals

When compared to the normal group, animals from the disease control group had a significantly higher amount of serum urea (***p<0.001; $F_{5,30}$=18.05: Figure 2). In the gentamicin group, simultaneous administration of low and high doses of EECA for 8 days substantially lowered serum urea levels (***p<0.001). The serum urea levels in the gentamicin group were reduced by standard cystone (5 mL/kg) given for 8 days (***p<0.001). In normal rats, oral administration of EECA (400 mg/kg) for 8 days had no effect on the serum urea levels.

EECA Administration Restored Blood Urea Nitrogen (BUN)

In the gentamicin group, the BUN level was considerably greater than in the control group (***p<0.001; $F_{5,30}$=7.41: Figure 3). *Centella asiatica* (low and high doses) and standard drug cystone restored BUN levels in disease control animals (*** p<0.001).

Serum Creatinine is Restored by *Centella asiatica* Extract

A gentamicin injection (100 mg/kg, for8 days) significantly increased serum creatinine levels in comparison to the control (***p<0.001; $F_{5,30}$=11.66: Figure 4). Treatment with EECA (low and high doses) and cystone for 8 days resulted in significantly lower serum creatinine levels in the gentamicin group (***p<0.001). The EECA groups' restoration of serum creatinine levels was on line with that of the standard cystone.

Effect of EECA on Urine Urea in Gentamicin Administered Animals

Gentamicin administration caused a noticeably decreased level of urine urea when compared to the control group (**p<0.01; $F_{5,30}$=6.662: Figure 5). *Centella asiatica* restored urine urea levels, but it was not statistically significant. The cystone treatment (5 mL/kg) significantly raised the urine urea levels in gentamicin group (*p<0.05).

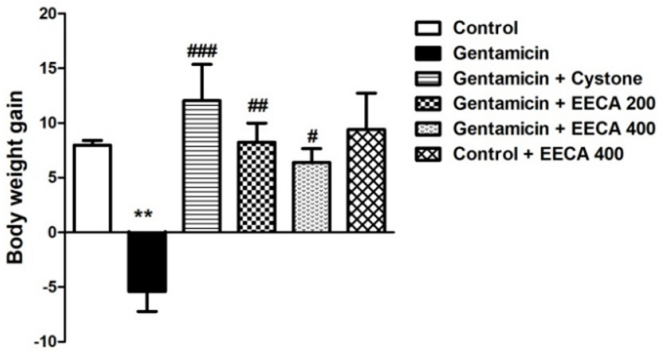

Figure 1: Effect of EECA on body weight gain in gentamicin-induced nephrotoxicity in rats. Control: Animals received only distilled water. Gentamicin: Animal was administered with single dose of gentamicin injection (100 mg/kg) respectively for 8 days. Gentamicin+EECA 200: Animals were administered with low dose (200 mg/kg, p.o.) of ethanolic leaf extract of *Centella asiatica*+gentamicin (100 mg/kg, i.p.), respectively for 8 days. Gentamicin+EECA 400: Animals were administered with high dose (400 mg/kg, p.o.) of ethanolic leaf extract of *Centella asiatica*+gentamicin (100 mg/kg, i.p.), respectively for 8 days. Gentamicin+Cystone: Animals were administered with cystone (5 mL/kg b.w.)+gentamicin (100 mg/kg, i.p.), respectively for 8 days. Control+EECA 400: Animals were administered with high dose (400 mg/kg, p.o.) of ethanolic leaf extract of *Centella asiatica* respectively for 8 days in normal animals. Data expressed as Mean±SEM. One-way ANOVA followed by Tukey's *post-hoc* test for relative body weight gain. **p<0.01 vs. control. ###p<0.001, ##p<0.01, #p<0.05 vs. gentamicin.

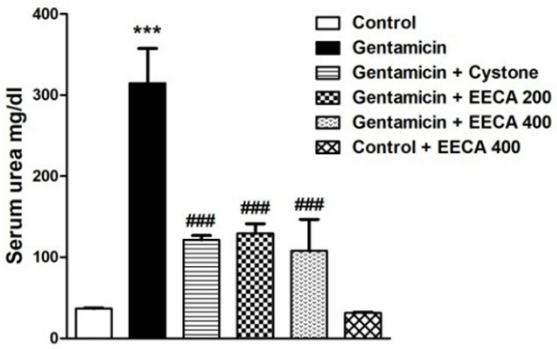

Figure 2: Effect of EECA on serum urea in gentamicin induced nephrotoxicity in rats. Control: Animals received only distilled water. Gentamicin: Animal was administered with single dose of gentamicin injection (100 mg/kg) respectively for 8 days. Gentamicin+EECA 200: Animals were administered with low dose (200 mg/kg, p.o.) of ethanolic leaf extract of *Centella asiatica*+gentamicin (100 mg/kg, i.p.), respectively for 8 days. Gentamicin+EECA 400: Animals were administered with high dose (400 mg/kg, p.o.) of ethanolic leaf extract of *Centella asiatica*+gentamicin (100 mg/kg, i.p.), respectively for 8 days. Gentamicin+Cystone: Animals were administered with cystone (5 mL/kg b.w.)+gentamicin (100 mg/kg, i.p.), respectively for 8 days. Control+EECA 400: Animals were administered with high dose (400 mg/kg, p.o.) of ethanolic leaf extract of *Centella asiatica* respectively for 8 days in normal animals. Data expressed as Mean±SEM. One-way ANOVA followed by Tukey's *post-hoc* test for relative serum urea level. ***p<0.001 vs. control. ###p<0.001 vs. gentamicin.

EECA Normalised Urine Creatinine Levels in Disease Control Group

The gentamicin group exhibited considerably lower urine creatinine than the normal group (*p<0.05; $F_{5,30}$=2.650: Figure 6). Treatment with EECA low dose, high dose, and standard cystone for 8 days resulted in higher urine creatinine levels in the gentamicin group, although this increase was not statistically significant.

Catalase (CAT) Activity in Kidney Was Restored by EECA

Rats given gentamicin showed higher inhibition of CAT activity compared to the control group. Compared to the gentamicin group, treatment with EECA and standard cystone for 8 days resulted in less suppression of CAT activity (p<0.001; $F_{5,30}$=5.897: Figure 7).

Effect of EECA of % Inhibition SOD Activity on Gentamicin Induced Nephrotoxicity in Rats

Animals in the gentamicin group had significantly reduced SOD activity than those in the control group (p<0.01; $F_{5,30}$=4.838: Figure 8). It is noteworthy that EECA and cystone therapy markedly increased the enzyme's activity (p<0.01; $F_{5,30}$=4.838:

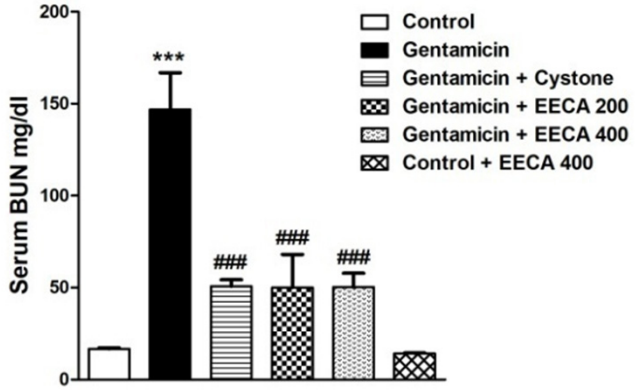

Figure 3: Effect of EECA on BUN in gentamicin induced nephrotoxicity in rats: Control: Animals received only distilled water. Gentamicin: Animal was administered with single dose of gentamicin injection (100 mg/kg) respectively for 8 days. Gentamicin+EECA 200: Animals were administered with low dose (200 mg/kg, p.o.) of ethanolic leaf extract of *Centella asiatica*+gentamicin (100 mg/kg, i.p.), respectively for 8 days. Gentamicin+EECA 400: Animals were administered with high dose (400 mg/kg, p.o.) of ethanolic leaf extract of *Centella asiatica*+gentamicin (100 mg/kg, i.p.), respectively for 8 days. Gentamicin+Cystone: Animals were administered with cystone (5 mL/kg b.w.)+gentamicin (100 mg/kg, i.p.), respectively for 8 days. Control+EECA 400: Animals were administered with high dose (400 mg/kg, p.o.) of ethanolic leaf extract of *Centella asiatica* respectively for 8 days in normal animals. Data expressed as Mean±SEM. One-way ANOVA followed by Tukey's *post-hoc* test for relative BUN. ***p<0.001 vs. control, ###p<0.001 vs. gentamicin.

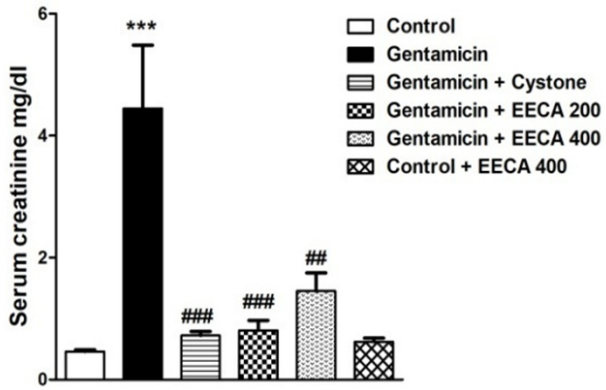

Figure 4: Effect of EECA on serum creatinine in gentamicin induced nephrotoxicity in rats. Control: Animals received only distilled water. Gentamicin: Animal was administered with single dose of gentamicin injection (100 mg/kg) respectively for 8 days. Gentamicin+EECA 200: Animals were administered with low dose (200 mg/kg, p.o.) of ethanolic leaf extract of *Centella asiatica*+gentamicin (100 mg/kg, i.p.), respectively for 8 days. Gentamicin+EECA 400: Animals were administered with high dose (400 mg/kg, p.o.) of ethanolic leaf extract of *Centella asiatica*+gentamicin (100 mg/kg, i.p.), respectively for 8 days. Gentamicin+Cystone: Animals were administered with cystone (5 mL/kg b.w.)+gentamicin (100 mg/kg, i.p.), respectively for 8 days. Control+EECA 400: Animals were administered with high dose (400 mg/kg, p.o.) of ethanolic leaf extract of *Centella asiatica* respectively for 8 days in normal animals. Data expressed as Mean±SEM. One-way ANOVA followed by Tukey's *post-hoc* test for serum creatinine level. ***p<0.001 vs. control. ## p<0.01, ### p<0.001, ## p<0.01 vs. gentamicin.

Figure 8). SOD activity between the control and control+EECA groups did not differ significantly ($p<0.01$; $F_{5,30}=4.838$: Figure 8).

Histopathological Assessment

The kidney tissue from the EECA *per se* group, the EECA-treated groups, the gentamicin-given group, and the control group are shown in the histological photographs (Figure 9). There were no indications of necrosis or degeneration in either the control or EECA-treated groups. Gentamicin administration resulted in cytoplasmic inclusions and tubular necrosis with vacuoles. These necrotic and degenerative modifications were less severe in groups that underwent EECA treatment (Figure 9). When rats received EECA alone, there was no intertubular response. The glomerulus and cortex were unaltered.

DISCUSSION

Gentamicin is typically used to treat infections caused by gram-negative bacteria.[16,17] Nephrotoxicity is a result of structural modifications and functional deficits in the epithelial membrane, mitochondria, and lysosomes.[17] In the current study, nephrotoxicity caused by gentamicin (100 mg/kg; i.p. for 8 days) led to higher levels of serum urea, serum creatinine, blood urea nitrogen, and urine uric acid as well as decreased levels of urinary urea and urinary creatinine. Additionally, gentamicin led to renal tubular necrosis. The interesting thing is that *Centella asiatica* reduced nephrotoxicity by lowering oxidative stress, increasing antioxidants, and correcting morphological abnormalities in nephron.

The majority of the chemical constituents of *Centella asiatica* (CA) are triterpenes, mainly pentacyclic triterpenic acids and their related glycosides. These include asiatic acid, asiaticoside, madecassic acid, madecassoside, brahmoside, brahmic acid, brahminoside, thankuniside, isothankuniside, centelloside, madasiatic acid, centic acid, and cenellic acid. Numerous flavonoids, such as quercetin, kaempferol, catechin, rutin, and naringin, are present and may have a significant role in the antioxidant activity of *Centella asiatica*.[18] Previous research indicates that CA has anti-lipoperoxidative and free radical scavenging abilities.[19,20]

The results of the current study are consistent with those of earlier ones. By lowering glomerulus damage and proteinuria, CA therapy for two months entirely prevented kidney damage in streptozotocin-induced diabetic mice. CA reduced vascular remodelling, glomeruli injury by increasing antifibrotic factor

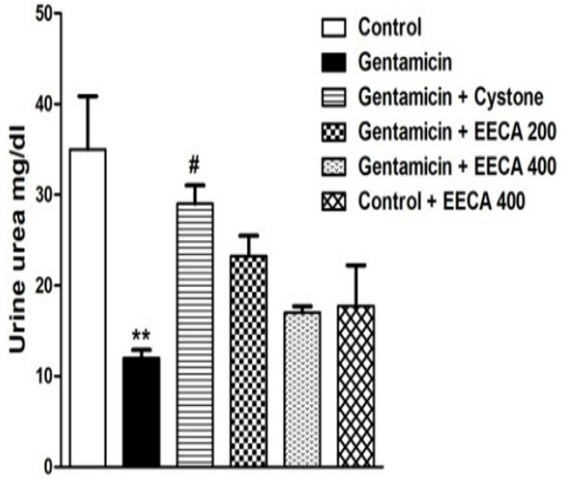

Figure 5: Effect of EECA on urine urea in gentamicin induced nephrotoxicity in rats. Control: Animals received only distilled water. Gentamicin: Animal was administered with single dose of gentamicin injection (100 mg/kg) respectively for 8 days. Gentamicin+EECA 200: Animals were administered with low dose (200 mg/kg, p.o.) of ethanolic leaf extract of *Centella asiatica*+gentamicin (100 mg/kg, i.p.), respectively for 8 days. Gentamicin+EECA 400: Animals were administered with high dose (400 mg/kg, p.o.) of ethanolic leaf extract of *Centella asiatica*+gentamicin (100 mg/kg, i.p.), respectively for 8 days. Gentamicin+Cystone: Animals were administered with cystone (5 mL/kg b.w.)+gentamicin (100 mg/kg, i.p.), respectively for 8 days. Control+EECA 400: Animals were administered with high dose (400 mg/kg, p.o.) of ethanolic leaf extract of *Centella asiatica* respectively for 8 days in normal animals. Data expressed as Mean±SEM. One-way ANOVA followed by Tukey's *post-hoc* test ($F_{5,18=}$5.56) for urine urea level. $^{**}p<0.01$ vs. control. $^{\#}p<0.05$ vs. gentamicin.

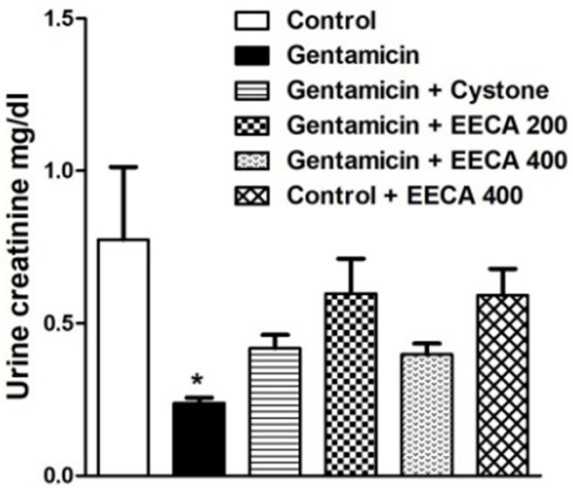

Figure 6: Effect of EECA on urine creatinine in gentamicin induced nephrotoxicity in rats. Control: Animals received only distilled water. Gentamicin: Animal was administered with single dose of gentamicin injection (100 mg/kg) respectively for 8 days. Gentamicin+EECA 200: Animals were administered with low dose (200 mg/kg, p.o.) of ethanolic leaf extract of *Centella asiatica*+gentamicin (100 mg/kg, i.p.), respectively for 8 days. Gentamicin+EECA 400: Animals were administered with high dose (400 mg/kg, p.o.) of ethanolic leaf extract of *Centella asiatica*+gentamicin (100 mg/kg, i.p.), respectively for 8 days. Gentamicin+Cystone: Animals were administered with cystone (5 mL/kg b.w.)+gentamicin (100 mg/kg, i.p.), respectively for 8 days. Control+EECA 400: Animals were administered with high dose (400 mg/kg, p.o.) of ethanolic leaf extract of *Centella asiatica* respectively for 8 days in normal animals. Data expressed as Mean±SEM. One-way ANOVA followed by Tukey's *post-hoc* test for urine creatinine level. $^{*}p<0.05$ vs. control.

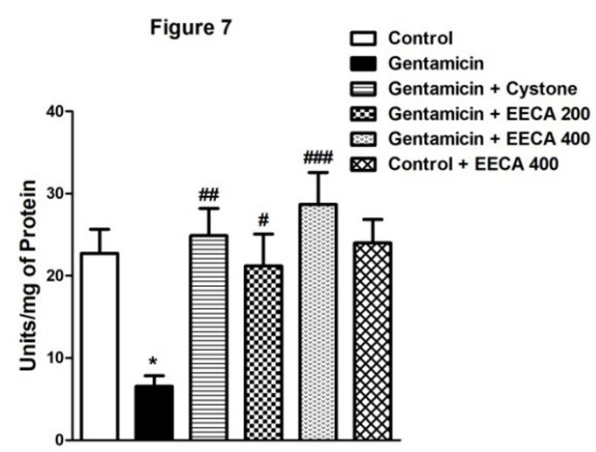

Figure 7: Effect of EECA on catalase activity. Control: Animals received only distilled water. Gentamicin: Animal was administered with single dose of gentamicin injection (100 mg/kg) respectively for 8 days. Gentamicin+EECA 200: Animals were administered with low dose (200 mg/kg, p.o.) of ethanolic leaf extract of *Centella asiatica*+gentamicin (100 mg/kg, i.p.), respectively for 8 days. Gentamicin+EECA 400: Animals were administered with high dose (400 mg/kg, p.o.) of ethanolic leaf extract of *Centella asiatica*+gentamicin (100 mg/kg, i.p.), respectively for 8 days. Gentamicin+Cystone: Animals were administered with cystone (5 mL/kg b.w.)+gentamicin (100 mg/kg, i.p.), respectively for 8 days. Control+EECA 400: Animals were administered with high dose (400 mg/kg, p.o.) of ethanolic leaf extract of *Centella asiatica* respectively for 8 days in normal animals. Data expressed as Mean±SEM. One-way ANOVA followed by Tukey's *post-hoc* test for urine creatinine level. $^{*}p<0.05$ vs. control; $^{\#}p<0.05$, $^{\#\#}p<0.01$, $^{\#\#\#}p<0.001$ vs. gentamicin.

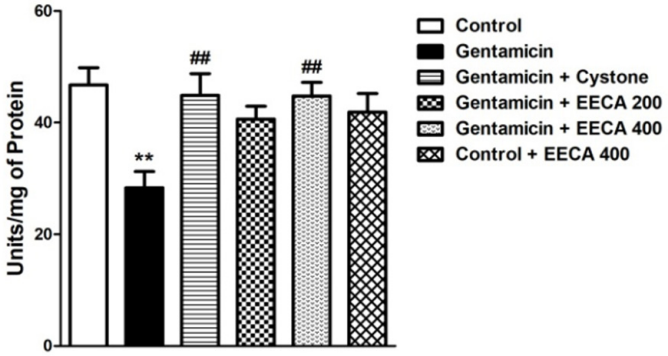

Figure 8: Effect of EECA on SOD activity. Control: Animals received only distilled water. Gentamicin: Animal was administered with single dose of gentamicin injection (100 mg/kg) respectively for 8 days. Gentamicin+EECA 200: Animals were administered with low dose (200 mg/kg, p.o.) of ethanolic leaf extract of *Centella asiatica*+gentamicin (100 mg/kg, i.p.), respectively for 8 days. Gentamicin+EECA 400: Animals were administered with high dose (400 mg/kg, p.o.) of ethanolic leaf extract of *Centella asiatica*+gentamicin (100 mg/kg, i.p.), respectively for 8 days. Gentamicin+Cystone: Animals were administered with cystone (5 mL/kg b.w.)+gentamicin (100 mg/kg, i.p.), respectively for 8 days. Control+EECA 400: Animals were administered with high dose (400 mg/kg, p.o.) of ethanolic leaf extract of *Centella asiatica* respectively for 8 days in normal animals. Data expressed as Mean±SEM. One-way ANOVA followed by Tukey's *post-hoc* test for urine creatinine level. $^{**}p<0.01$ vs. control; $^{\#\#}p<0.01$ vs. gentamicin.

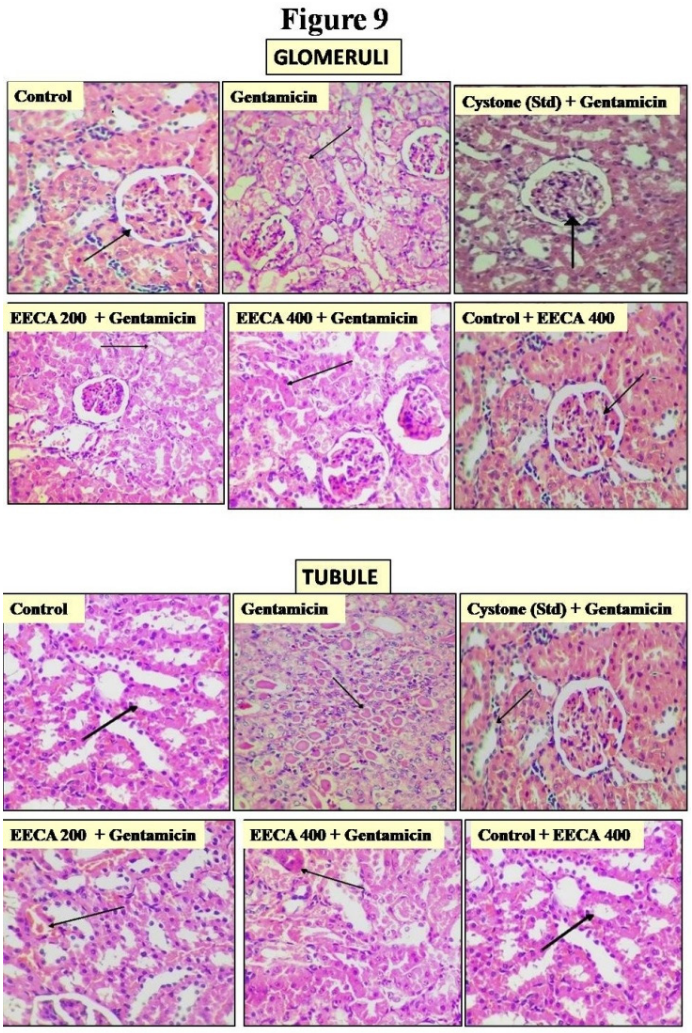

Figure 9: Photomicrograph of rat kidney section (400 X). Control: Animals received only distilled water. Gentamicin: Animal was administered with single dose of gentamicin injection (100 mg/kg) respectively for 8 days. Gentamicin+EECA 200: Animals were administered with low dose (200 mg/kg, p.o.) of ethanolic leaf extract of *Centella asiatica*+gentamicin (100 mg/kg, i.p.), respectively for 8 days. Gentamicin+EECA 400: Animals were administered with high dose (400 mg/kg, p.o.) of ethanolic leaf extract of *Centella asiatica*+gentamicin (100 mg/kg, i.p.), respectively for 8 days. Gentamicin+Cystone: Animals were administered with cystone (5 mL/kg b.w.)+gentamicin (100 mg/kg, i.p.), respectively for 8 days. Control+EECA 400: Animals were administered with high dose (400 mg/kg, p.o.) of ethanolic leaf extract of *Centella asiatica* respectively for 8 days in normal animals.

activity, and inflammatory biomarkers restored renal injury in nephrectomy animals.[21-23]

Different protective mechanisms could be responsible for *Centella asiatica's* nephroprotective effects. Previous research suggested that triterpenoid present in CA increased the activity of antioxidant enzymes and nuclear factor erythroid-2-related factor 2.[24-27] In a recent clinical trial, CA improved dry skin and superoxide dismutase activity in patients with *Diabetes mellitus*.[28]

Additionally, asiatic acid, a component of CA, reduced the damage to the kidneys caused by unilateral ureteral blockage by promoting the generation of an endogenous ligand for the Peroxisome proliferator-activated receptor .[29]

CONCLUSION

In conclusion, the current study looked into the possibility of using an ethanolic extract of *Centella asiatica* (CA) to treat nephrotoxicity brought on by gentamicin. One of the major conclusions of the study was that simultaneous administration of an ethanolic extract of *Centella asiatica* improved kidney function and restored serum urea, creatinine, and blood urea nitrogen levels. The findings of this study point to the potential value of Brahmi as an adjuvant therapy for nephrotoxicity caused by a variety of pharmaceutical treatments as well as a robust free radical scavenger.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTION

The study's inception and design involved input from all authors. Bhagya V, Ramya HB prepared the material and handled the data collection, analysis, and interpretation. Bhagya V wrote the original version of the manuscript, while the other authors provided feedback on previous drafts. The final manuscript was read and approved by all authors.

ABBREVIATIONS

MDA: Malondialdehyde; **SOD:** Superoxide Dismutase; **GSHPx:** Glutathione Peroxidase; **LD_{50}:** Median Lethal Dose; **BUN:** Blood Urea Nitrogen; **CAT:** Catalase; **NBT:** Nitro Blue Tetrazolium; **EDTA:** Ethylenediaminetetraacetic acid; **EECA:** Ethanolic leaf extract of Centella asiatica; **CA:** Centella asiatica.

SUMMARY

The purpose of this study was to comprehend how *Centella asiatica* (CA) leaf extract affected gentamicin-induced nephrotoxicity. In this work, we have shown that Centella asiatica has a protective effect against kidney damage brought on by gentamicin use. CA demonstrated nephroprotective effect, decreased free radical production, and improved antioxidant status. It suggests that CA might be a useful medicinal herb in drug-induced renal injury.

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