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RESEARCH ARTICLE

Aerva lanata extract restores cellular viability and mitochondrial function against gentamicin induced nephrotoxicity on human embryonic kidney cells

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Abstract

Aerva lanata, commonly used in traditional medicine systems such as Ayurveda and Siddha, is well known for its diuretic and lithotriptic properties in the management of urolithiasis and other urinary tract disorders. However, despite its widespread traditional use, comprehensive studies elucidating the nephroprotective mechanisms of *A. lanata* under conditions of chemically induced renal damage remain limited, warranting further investigation. The current study investigated the nephroprotective properties of ethyl acetate extract of *Aerva lanata* (EA-AL) against gentamicin induced nephrotoxicity in human embryonic kidney (HEK293) cells. The initial phytochemical evaluation of EA-AL indicated the presence of tannins, which were later quantified. The antioxidant activity of EA-AL was evaluated using the 2,2-Diphenyl-1-picrylhydrazyl (DPPH) assay, while its nephroprotective activity was evaluated using the 2,3-Bis-(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide (XTT) assay. The results demonstrated that EA-AL significantly restored cell viability in gentamicin-exposed cells. Gentamicin exposure induced oxidative stress, evidenced by increased lipid peroxidation products and diminished antioxidant enzyme levels. The levels of antioxidant enzymes were effectively elevated and membrane peroxidation was diminished through co-treatment with EA-AL. Moreover, 2', 7'-Dichlorofluorescin diacetate (DCFDA) staining demonstrated that gentamicin exposure led to increased Reactive Oxygen Species ROS production, which was significantly reduced by EA-AL treatment. Exposure to gentamicin led to an elevation in depolarized viable and non-viable cells, indicating a disruption in mitochondrial membrane potential and overall mitochondrial integrity. The co-treatment with EA-AL successfully restored the population of viable cells. The reinstatement of cellular viability and mitochondrial function highlights the nephroprotective potential of EA-AL against imposed toxicity in HEK293cells.

Keywords: cell viability; gentamicin; HEK293; nephrotoxicity; nephroprotection

Introduction

Renal disorders, encompassing chronic kidney disease (CKD) and acute kidney injury (AKI), are among the most significant non-communicable diseases globally, with a continuously rising prevalence and incidence. These conditions affect over 850 million individuals worldwide, leading to substantial morbidity, economic burden and mortality (1, 2). The kidneys, with their high metabolic activity and role in xenobiotic filtration, are particularly susceptible to various insults, including ischemia, toxins and drug-induced damage (3). Among these, drug-induced nephrotoxicity is a pressing clinical challenge, with agents such as aminoglycoside antibiotics like gentamicin, cisplatin and non-steroidal anti-inflammatory drugs frequently implicated in causing renal injury. Gentamicin, despite its potent antibacterial efficacy, is notably nephrotoxic, primarily through oxidative stress, mitochondrial dysfunction and inflammation leading to proximal tubular damage (4).

Almost every known culture has utilized plant extracts as effective remedies for the prevention and treatment of a variety of health issues for centuries. Ayurveda, the traditional medicinal system of India, has been practiced for over 5000 years and decoctions and extracts from medicinal plants continue to be a crucial aspect of its approach (5, 6). In the quest to minimize nephrotoxicity, natural products have emerged as promising candidates due to their broad bioactivity and relatively favorable safety profile. Among these, *Aerva lanata*, is widely recognized in traditional medicine systems such as Ayurveda and Siddha for treating renal ailments (7).

The genus *Aerva*, a member of the Amaranthaceae family, comprises approximately 10 to 12 recognized species, along with several varieties and subspecies, primarily distributed across tropical and subtropical regions of Asia and Africa. Among them, *Aerva lanata* (L.), commonly known as mountain knotgrass, is the most widely studied and utilized species. It is an erect, diffusely branched herb that grows abundantly in India, Sri Lanka and Southeast Asia, often in open grasslands and wastelands. *A. lanata* is extensively cultivated and traditionally used in Ayurvedic and Siddha medicine for its diuretic, lithotriptic, anti-inflammatory and antioxidant properties, particularly in the management of renal disorders, urinary tract infections and urolithiasis. Scientific investigations have validated some of these traditional claims and demonstrated its hepatoprotective, anti-diabetic and antimicrobial effects, supporting its diverse therapeutic potential. Furthermore, its antioxidant properties suggest a possible role in neutralizing reactive oxygen species (ROS), which are central to the pathogenesis of nephrotoxicity (8-10).

However, despite its long-standing ethnomedicinal relevance, limited experimental evidence exists to substantiate its nephroprotective efficacy, particularly against drug-induced renal toxicity. In this context, the present study aims to evaluate the nephroprotective effect of ethyl acetate extract of *Aerva lanata* (AL-EA) against gentamicin-induced nephrotoxicity *in vitro*, focusing on oxidative and inflammatory markers. This investigation is anticipated to provide novel insights into the plant's renoprotective mechanisms and support its development as a potential adjunct in managing nephrotoxicity.

Materials and Methods

Cell lines

HEK 293 cell lines were initially obtained from National Centre for Cell Science (NCCS) in Pune, India. Cells were cultured in Sigma-Aldrich USA's Dulbecco's Modified Eagle's Medium (DMEM) enriched in Fetal Bovine Serum (FBS), Penicillin and Streptomycin. Propagated the cell line in 24 well plates using DMEM enriched by 10 % FBS, sodium bicarbonate, L-glutamine, Amphotericin B, Streptomycin and Penicillin. Cells were maintained amphotericin B, streptomycin and penicillin at 37 °C in a humidified 5 % CO_2 incubator and trypsinized two days prior to seeding them. A 96-well tissue culture plate was transplanted with a 100 µL cell suspension ($5x10^4$cells/well), maintained at 37 °C incubator in humid atmosphere with 5 % CO_2

Reagents and chemicals

Sigma-Aldrich USA, Merck, Germany or Hi Media Laboratories Private Limited supplied all reagents and chemicals used and had analytical quality.

Collection and extraction of plant extract

The *A. lanata* plant was identified and obtained from the local regions of Thiruvananthapuram (Fig. 1). The leaves were collected, surface sterilized and shade dried. After drying it was chopped into fine pieces. Add 100 mL of 70 % ethyl acetate to 20 g of dried leaves. It was maintained at room temperature in a shaker overnight at 100 rpm. The liquid was obtained by squeezing the material using a muslin cloth. It was placed in a petri dish and kept for desiccation. The evaporated powdered particles (Fig. 2) of *A. lanata* (EA-AL) were removed from the petri plate and preserved in an eppendorf tube for further study. Compound stock was prepared by weighing 1 mg EA-AL and dissolving in 1mL DMSO (0.01 %). To ensure sterility, filter the solution through 0.22 µm Millipore syringe filter.

Fig. 1. *Aerva lanata* plant.

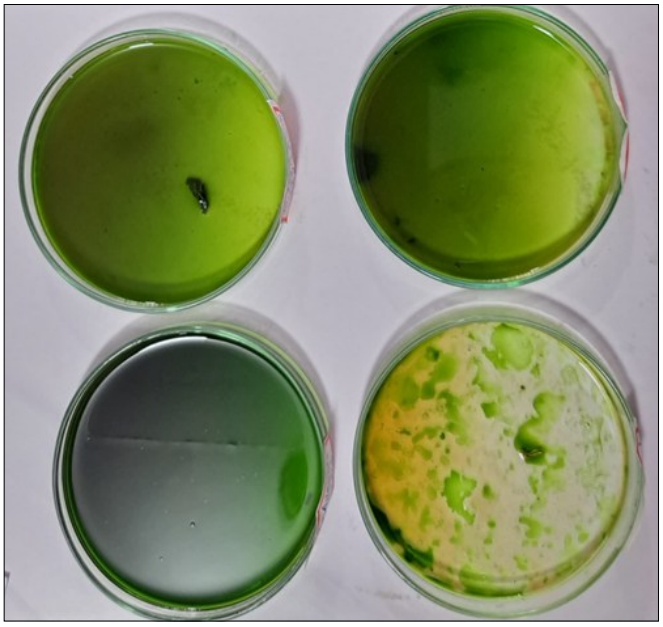

Fig. 2. Ethyl acetate extract of *Aerva lanata* (EA-AL).

Phytochemical screening of *Aerva lanata* (EA-AL)

The phytochemical analysis of EA-AL was conducted using the methodology outlined by Harborne, Onwukaeme and coworkers (11). Transfer 1 mL of test sample to a test tube. Add 0.5 mL Folin-Denis reagent and 1 mL 20 % sodium carbonate. Make upto 10 mL with distilled water. Mix well and let stand 30-60 min at room temperature to develop colour. Read the absorbance at 700-nm. Create a standard curve (absorptance vs. concentration) with tannic acid solutions. Compare absorbance values to the standard curve to determine sample extract tannin content.

Radical scavenging assay using 2,2-Diphenyl-1-picrylhydrazyl (DPPH)

The DPPH assay was employed to evaluate the extracts' radical scavenging activity in accordance with the method outlined by Chang et al. (12). A 0.1 mM DPPH solution was formulated by

dissolving 4 mg of DPPH in 100 mL of methanol. The final volume of 20 µL was achieved by combining various quantities of extracts from the stock solution, ranging from 1.25 µL to 20 µL, with DMSO, followed by the addition of 1.48 mL of DPPH (0.1 mM). Set up a control using an equivalent volume of distilled water, excluding the test compound. After 20 min of dark, room temperature incubation measured the absorbance of the mixture at 517 nm.

Induction of nephrotoxicity

Cells were pre-treated with 50 mM of gentamicin in a 96-well culture plate ($5x10^3$cells/well) and were incubated at a temperature of 37 ºC within a humidified incubator supplemented with 5 % CO_2 for a period of 1 hr (13).

EA-AL co-administration

Following the induction of toxicity by gentamicin, treat the cells with a new medium having varying amounts of EA-AL (1.5, 3.1, 6.25, 12.5 and 25 µg/mL) and subsequently incubated for 24 hrs at 37 ºC with 5 % CO_2

In vitro nephroprotective effect of EA-AL on gentamicin exposed HEK293 cells using XTT assay

The XTT assay is a colorimetric method that is used to assess the proliferation of cells and viability by quantifying cellular metabolic activity (14), which entails metabolically active cells reducing an yellow tetrazolium salt (XTT) to an orange formazan dye. Supplement the confluent cell layer with varying amounts of EA-AL and allow overnight incubation. Freshly solubilize 1 mg XTT in 1 mL DMEM. To this, 2.5 µL of PMS was incorporated, from which 50 µL of the solution was added to each well and incubate for 2-3 hrs. After incubation, 100 µL sample was then placed on a separate ELISA plate, analyzed with an ELISA plate reader at a wavelength of 450 nm. Calculate percentage by,

$$\text{Percentage viability (\%)} = \frac{\text{Absorbance of Test}}{\text{Absorbance of Control}} * 100 \quad \text{(Eqn.1)}$$

viability

Apoptosis study by double staining method using acridine orange (AO) - ethidium bromide (EtBr)

Different groups comprising untreated control cells and cells treated with sample were incubated for 24 hrs and proceeded with fluorescent staining as per methods described by Ramachandran and Mini, 2014 (15). Following incubation, the cells were rinsed with cold PBS and left to stain for 10 min at room temperature using a combination of AO, 100 µg/mL and EtBr, 100 µg/mL. Following staining, rinsed twice with 1X PBS and subsequently imaged using a fluorescent microscope. (Olympus CK X 41) with a blue filter. Living cells (green nucleus), early apoptotic (bright green nucleus with condensed or fragmented chromatin), late apoptotic (orange-stained nuclei with condensed or fragmented chromatin) and necrotic cells (orange nuclei) were detected and classified.

Effect of EA-AL co-administration on antioxidant markers of gentamicin exposed HEK293

Preparation of cell lysate

Trypsinize the cells that had been treated with HiMedia Trypsin-EDTA Solution. Cells were, collected in eppendorf tubes. Discarded the supernatant after 5 min spinning in a centrifuge at 5000 rpm, reconstituted using 200 µL lysis buffer containing 0.1M Tris, 0.2M EDTA, 2M NaCl and 0.5 % Triton. Subjected to 20 min incubation at 40 °C. Further investigations were carried out in cell lysate.

Estimation of lipid peroxidation

Activity of malondialdehyde (MDA) was detected since poly unsaturated fatty acid peroxides generate MDA upon decomposition. 50 µL sample, 1 mL 1 % TBA and 500 µL 70 % alcohol were taken and boiled for 20 min. 50 µL of acetone was added to quantify MDA activity. Read the absorbance at 535 nm after cooling to room temperature (16).

MDA Units = Absorbance X 7.57 (Eqn. 2)

Estimation of catalase activity

The quantity of hydrogen peroxide dissociation is in direct proportion to activity of catalase.

Mix 1.2 mL Phosphate buffer and 50 µL sample with 1 mL of hydrogen peroxide. Subsequent to the introduction of the reagents, measure absorbance at 234 nm at 0 minute and 10 minute (17)

Activity of catalase = Δ OD/0.1116 (Eqn. 3)

In vitro ROS measurement using DCFDA

2, 7-Dichloroflurescein diacetate (DCFDA) is a fluorogenic dye that evaluates intracellular ROS activity. Treat the cell culture in 48 well plates with gentamicin and compound at final concentrations described previously. Introduce 50 µL DCFDA after washing with PBS. Keep in an incubator for 30 min. After washing excess dye with PBS measure the fluorescence at 470 nm excitation and emission at 635 nm.

Reduced glutathione (GSH) activity

The accumulation of oxidized glutathione (GSSG) due to continuous ROS production, including lipid peroxides, H_2O_2 and OH, leads to decreased GSH levels, serving as a precise indicator of the cell's oxidative state. To measure GSH levels, 0.5 mL of phosphate buffer, 1.3 mL of distilled water, 1 mL of cell lysate and 0.2 mL of 5,5'-dithiobis (2-nitrobenzoic acid) (DTNB) were combined, thoroughly mixed. Absorbance was measured at 420 nm. GSH levels were evaluated by comparing them to a standard curve of reduced glutathione (GSH) (18).

Determination of mitochondrial membrane potential by Fluorescence-Activated Cell Sorting (FACS)

The cells were cultured; treatments were administered accordingly and incubated for 24 hrs. Following incubation, cells were trypsinized and subjected to flow cytometry. The Muse™ MitoPotential Dye was diluted in 1X assay buffer at a ratio of 1:1000 to create a working solution. The cells were resuspended in 1X assay buffer, supplemented with 95 µL of the mito potential working solution after centrifugation. Mix well and incubate at 37 °C in a CO_2 incubator for 20 min. Following incubation, add 5µL of Muse MitoPotential 7-AAD to the wells and incubated for an additional 5 min. The samples were introduced into a flow

cytometer (Millipore, USA) and collected the events following gating and compare with controls.

Statistical analysis

Statistical analysis was performed using GraphPad Prism Software 5.01 (GraphPad Software, Inc., San Diego, CA). The data were represented as mean ± SD (n = 3) and analyzed by one-way analysis of variance (ANOVA) and $P < 0.05$ was considered significant.

Results and Discussion

The increased relative blood flow to the kidneys makes them especially vulnerable to drug-induced damage (19). Gentamicin, an aminoglycoside antibiotic, significantly contributes to drug-induced nephrotoxicity. However, gentamicin is extensively employed because of its exceptional efficacy in treating infections (20). Therefore, it is essential to formulate strategies to mitigate its nephrotoxic effects. In this context we investigated the impact of EA-AL on gentamycin-induced nephrotoxicity in HEK-293 cells using an *in vitro* model. Among the bioactive components, we concentrated on tannins, as they are the least investigated constituents. The extract yielded 9.6 % and exhibited a brownish green hue.

Phytochemical screening of *A. lanata*

A. lanata is a well-known plant traditionally employed in medicine to address various ailments, including inflammation and diabetes. The chemical composition of EA-AL has been examined, with initial phytochemical analysis verifying the existence of tannins. The tannins were quantitatively assessed by creating a standard curve by taking absorptance vs. concentration with tannic acid solutions. Compare absorbance values to the standard curve to determine sample extract tannin content. This quantitative analysis indicated that tannin content of EA-AL was 21.8 mg / g of the extract.

Free radical scavenging activity of EA-AL by DPPH free radical scavenging assay

The DPPH assay for free radical scavenging is a recognized technique for evaluating antioxidant activity (21). Violet hued DPPH solution is reduced to yellow diphenyl picryl hydrazine in a concentration-dependent manner upon interaction with the extract. This study analyzed the *in vitro* free radical scavenging efficacy of EA-AL against DPPH radicals. The findings indicated that EA-AL displayed considerable scavenging ability that is concentration-dependent (Table 1), with an IC50 value of 119.13 µg/mL, as determined using ED50PlusV0.1 software. These findings indicate that EA-AL is a promising source of antioxidants and a potential therapeutic agent for conditions related to free radical damage.

Table 1. DPPH radical scavenging assay

Concentration of EA-AL (µg/mL)	Percentage inhibition (%)
Control	0.00
12.5	19.06
25	30.99
50	41.59
100	56.43
200	60.31

Cell viability determination by XTT assay

This study involved exposing HEK293 cells to gentamycin and co-treating them with EA-AL to assess its nephroprotective effects via the XTT assay. Gentamicin exposure markedly diminished cell viability to 47.52 %. Co-treatment with EA-AL at a concentration of 25 µg/mL enhanced cell viability to 87.26 % (Table 2 & Fig. 3). The findings demonstrated that EA-AL co-treatment enhanced cell viability by as much as 40 % relative to cells exposed to gentamycin. The findings confirmed the protective effect of EA-AL, prompting further experiments at a concentration of 25 µg/mL.

Table 2. *In vitro* nephroprotective effect of EA-AL in terms of percentage viability of HEK293 cells exposed to gentamicin with varied concentrations

Concentration of EA-AL (µg/mL)	Percentage viability
Control	100
Gentamicin	47.52
1.5	52.84
3.1	56.26
6.25	63.06
12.5	70.53
25	87.26

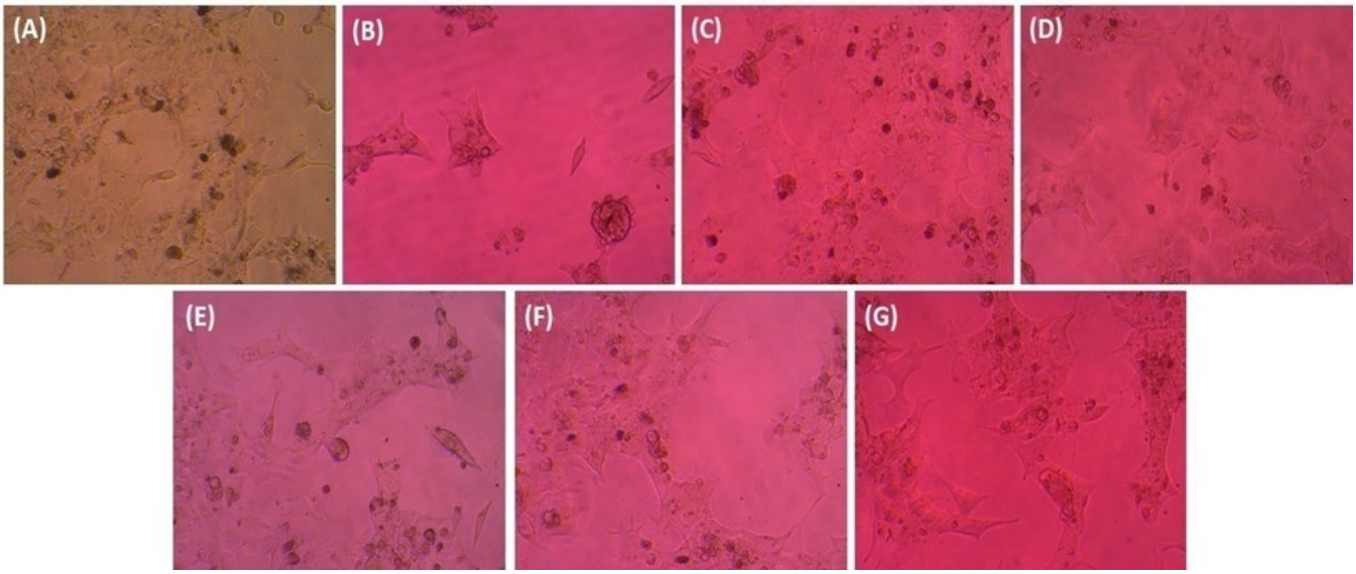

Fig. 3. Photomicrographs depicting the effect of EA-AL co-administration on viability of cultured HEK293 cells exposed to gentamicin (A) Untreated control cells (B) Gentamicin exposed cells and gentamicin exposed cells co-administrated with varied concentrations of EA-AL (C) 1.5 (D) 3.1 (E) 6.25 (F) 12.5 and (G) 25 µg/mL.

Apoptosis by double staining with AO / Et-Br

The AO/Et-Br dual staining technique is employed to assess programmed cell death (PCD) via fluorescence microscopy. This technique differentiates viable from non-viable cells through their unique absorption of the two fluorescent dyes, AO and Et-Br (22). Qualitative analysis utilizing the AO/Et-Br method demonstrated a substantial increase in apoptosis in HEK293 cells exposed to gentamicin. Two cell types were identified, with treated cells displaying orange-red fluorescence, signifying the degree of apoptosis relative to untreated control cells. Co-treatment with EA-AL markedly diminished the apoptosis levels induced by gentamicin, underscoring its nephroprotective properties (Fig. 4).

Effect of EA-AL on antioxidant markers in gentamicin exposed HEK293 cells

Lipid peroxidation

The findings of our study demonstrated a marked elevation in lipid peroxidation levels in treated cells compared to control, signifying oxidative stress induced by gentamicin exposure. Treatment with EA-AL markedly diminished lipid peroxidation levels (Table 3).

Catalase activity

Exposure to gentamicin can result in variations of catalase levels in cells, especially as component of the cellular reaction to drug-induced oxidative stress. Catalase is an essential antioxidant enzyme that deactivates H_2O_2, a detrimental ROS, transforming it into water and oxygen (23). The findings of the study indicated that gentamycin exposure markedly diminished catalase levels relative to untreated HEK293 cells. Co-treatment with EA-AL, however, led to an elevation in catalase levels, thereby further substantiating the nephroprotective effect of EA-AL (Table 3).

Reduced glutathione

Reduced glutathione functions as a marker for cellular oxidative health and a substantial decline in its levels may result in membrane peroxidation and subsequent cell death (24). Our study revealed that the concentration of reduced glutathione was markedly diminished in gentamicin-exposed HEK293 cells relative to control. Co-administration of EA-AL significantly elevated reduced glutathione relative to the untreated HEK293 cells (Table 3).

Determination of ROS generation using DCFDA staining method

Oxidative stress occurs when the generation of ROS exceeds the capability of endogenous antioxidant defense. Oxidative damage and ROS are associated with numerous human diseases like neurodegenerative disorders, atherosclerosis, cancer and diabetes. Consequently, precise quantification of ROS as well as oxidative damage is crucial for comprehending their involvement in disease progression (25). This study employed the DCFDA staining technique to assess the impact of EA-AL co-treatment on ROS production. The findings indicated that ROS production was markedly elevated in HEK-293 cells subjected to gentamicin relative to the untreated control cells. Co-treatment with EA-AL substantially neutralizes excess ROS, illustrating its antioxidant capability (Table 3 & Fig. 5).

Assessment of mitochondrial membrane potential (MMP)

Healthy mitochondria typically sustain a negative membrane potential of approximately -180 mV, which can be visualized with cationic dyes such as TMRE (26). Tetra methyl rhodamine ethyl ester emits red fluorescence, quantifiable via flow cytometry to evaluate the $\Delta\Psi m$ status in cells. Our findings indicated that gentamicin treatment led to heightened mitochondrial damage. Co-treatment with EA-AL, however, markedly diminished the mitochondrial damage caused by gentamicin (Table 4 & Fig. 6).

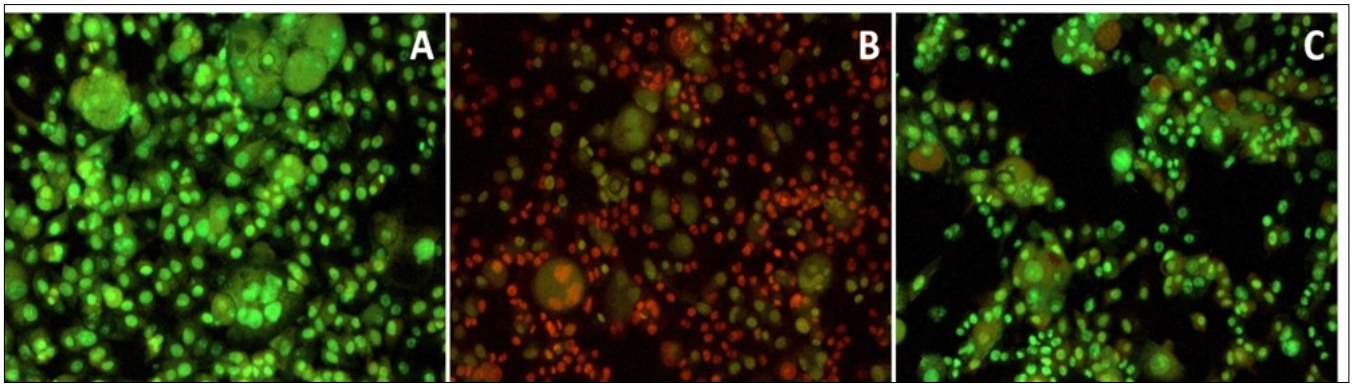

Fig. 4. Assessment of apoptosis by double staining technique by AO/Et-Br; (A) Untreated control cells (B) Gentamicin exposed cells (C) Gentamicin exposed cells co-administrated with EA-AL.

Table 3. Effect of EA-AL on antioxidant markers

Parameter assessed	Control	Gentamicin treatment	Gentamicin + EA-AL treatment
MDA (µM / mg protein)	0.1626 ± 0.001*	0.8034± 0.061*	0.5391 ± 0.004*
Catalase (Enzyme activity in U/mL)	0.5487 ± 0.031*	0.3772 ± 0.002 **	.4728 ± 0.027 *
GSH (µM / mg protein)	10.339 ± 2.064*	4.0931 ±0.580*	6.8991 ± 0.496**
ROS generation (Fluorescence intensity)	2487.25 ± 78.49**	15458.37 ± 104.57**	6991.78 ± 127.82*

Values are expressed as mean ± SD (n = 3). Statistical analysis was performed using GraphPad Prism Software 5.01. Data were analyzed by one-way ANOVA. *P < 0.05, **P < 0.01 compared to control

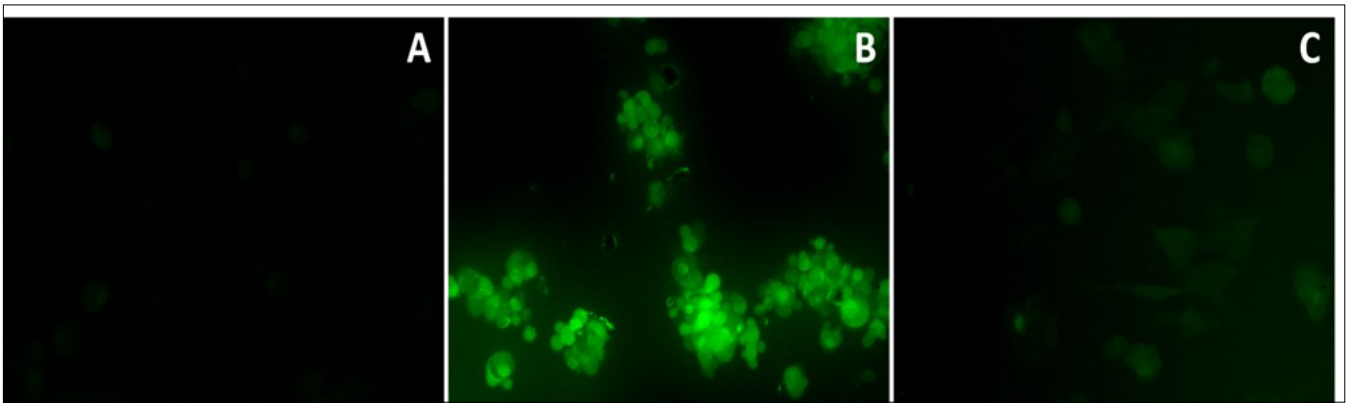

Fig. 5. Determination of ROS generation by DCFDA staining method; (A) Untreated control cells (B) Gentamicin exposed HEK-293 cells (C) Gentamicin exposed HEK-293 cells co-administrated with EA-AL.

Table 4. Cell health profile MMP - FACS

	Cells gated (%)		
Cell health profile	**Control**	**Gentamicin**	**Gentamicin + EA-AL**
Live (LR)	91.65	9.61	86.60
Depolarized live (LL)	7.50	72.86	10.20
Depolarized dead (UL)	0.00	0.60	0.00
Dead (UR)	0.85	16.93	0.20

Discussion

Drug-induced nephrotoxicity continues to pose a significant clinical challenge, especially regarding aminoglycoside antibiotics like gentamicin, which are recognized for causing considerable renal tubular injury via oxidative and mitochondrial mechanisms (27, 28). The EA-AL exhibited notable antioxidant and nephroprotective properties *in vitro*, confirming its potential as a protective agent against gentamicin-induced renal damage. EA-AL exhibited a concentration-dependent antioxidant activity in the DPPH radical scavenging assay, with an IC_{50} value of 119.13µg/mL. This confirms the previous findings that *A. lanata* has a high bioactive component, which contributes to the neutralization of free radicals (29, 30). Antioxidants are essential in safeguarding renal cells from oxidative stress, a primary factor in nephrotoxicity, by neutralizing ROS prior to the onset of lipid peroxidation and mitochondrial dysfunction (31). Gentamicin markedly diminished the viability of HEK293 cells to approximately 47 %, aligning with prior research indicating its cytotoxic effects via ROS overproduction and apoptosis (32, 33). Cell viability was significantly increased to 87.26 % following co-treatment with EA-AL at 25µg/mL, indicating a strong cytoprotective effect. These results were additionally verified by AO/EtBr dual staining, which revealed a decrease in gentamicin-induced apoptosis following EA-AL treatment. The extract's capacity to maintain membrane integrity and mitigate apoptotic cell death may be attributed to its membrane-stabilizing effects and antioxidant constituents. The protective effect of EA-AL was further substantiated by oxidative stress markers. Typical symptoms of oxidative renal injury include increased lipid peroxidation (as shown by increased MDA levels), decreased catalase activity and reduced glutathione levels; these were all brought about by gentamicin exposure (34). The reversal of these changes, brought about by EA-AL treatment, suggests that the cellular redox balance has been restored. Restoring both glutathione and catalase levels following EA-AL treatment demonstrates the extract's potential in reducing oxidative damage. Glutathione is the principal non-enzymatic antioxidant and catalase is the principal enzymatic protector against oxidative stress (35, 36). These results were further validated by quantifying ROS using DCFDA staining. The cells that were exposed to gentamicin showed an excess of ROS buildup, while EA-AL greatly reduced ROS production, highlighting its antioxidant capabilities. This aligns with prior studies demonstrating the antioxidant properties of *A. lanata* (37). Mitochondrial membrane potential ($\Delta\Psi m$) is a critical parameter for mitochondrial health, regulating ATP synthesis, ion homeostasis and cell survival. Loss of $\Delta\Psi m$ is a hallmark of mitochondrial dysfunction and early apoptosis, particularly relevant in nephrotoxicity induced by aminoglycosides such as gentamicin (38). The co-treatment with Aerva lanata ethyl acetate extract (EA-AL) substantially reduced mitochondrial damage. The significant restoration of mitochondrial potential underscores the mitoprotective properties of EA-AL by stabilising mitochondrial membranes. Consequently, EA-AL exhibits significant therapeutic potential in safeguarding renal epithelial cells against drug-induced mitochondrial dysfunction.

Collectively, these findings affirm the protective efficacy of EA-AL against gentamicin-induced nephrotoxicity through multiple mechanisms by ROS scavenging, inhibition of lipid peroxidation, preservation of antioxidant enzyme levels, stabilization of mitochondrial function and attenuation of apoptosis. Despite these promising results, further studies are warranted to *in vivo* isolate the bioactive constituents responsible for the observed effects and to validate these findings in nephrotoxicity models. Additionally, molecular-level investigations into the modulation of apoptotic and oxidative signaling pathways would provide deeper insight into the nephroprotective mechanisms of EA-AL.

Conclusion

The kidney, an essential organ for sustaining the body's overall balance, is crucial for health and well-being. As a result of the substantial toxicity caused by antibiotics and anticancer medications, researchers are diligently exploring alternatives,

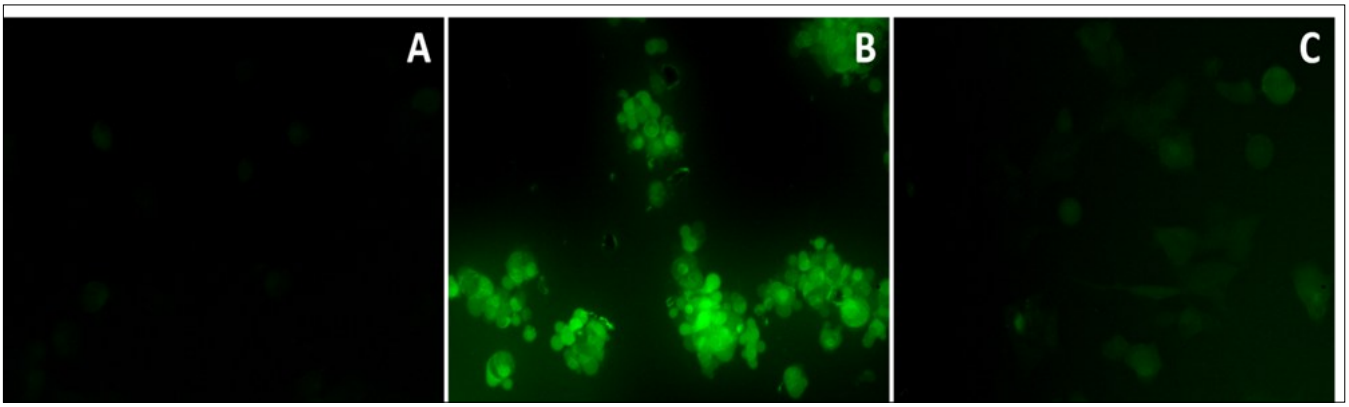

Fig. 5. Determination of ROS generation by DCFDHA staining method; (A) Untreated control cells (B) Gentamicin exposed HEK-293 cells (C) Gentamicin exposed HEK-293 cells co-administrated with EA-AL.

Table 4. Cell health profile MMP - FACS

		Cells gated (%)	
Cell health profile	**Control**	**Gen**	**Gen + EA-AL**
Live (LR)	91.65	9.61	86.60
Depolarized live (LL)	7.50	72.86	10.20
Depolarized dead (UL)	0.00	0.60	0.00
Dead (UR)	0.85	16.93	0.20

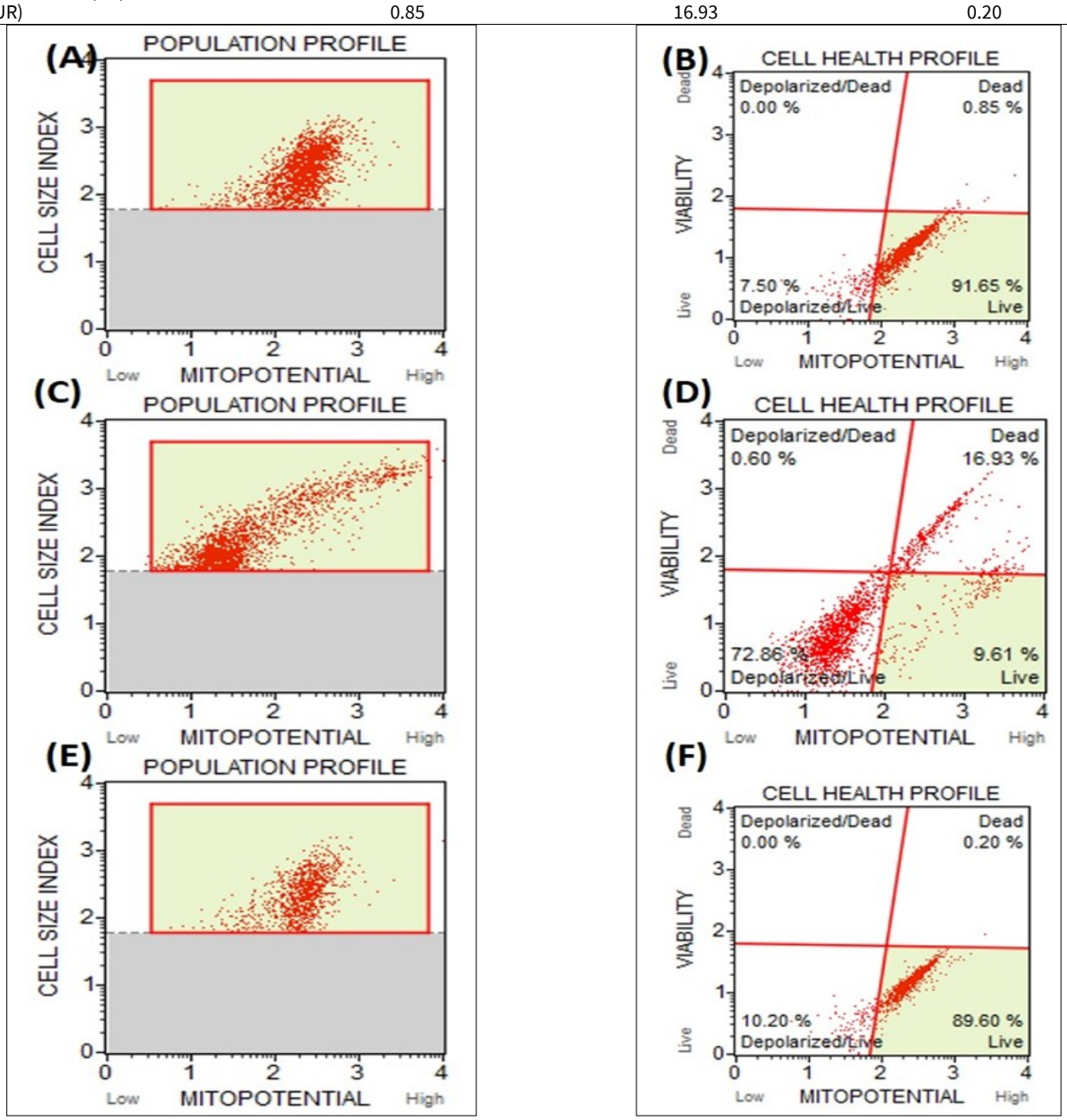

Fig. 6. Determination of MMP using FACS; (A) Population profile: untreated control (B) Cell health profile of untreated control (C) Population profile: gentamicin exposed cells (D) Cell health profile of gentamicin exposed cells (E) Population profile: gentamicin exposed cells co-administrated with EA-AL (F) Cell health profile of gentamicin exposed cells co-administrated with EA-AL.

emphasizing natural compounds that provide efficacy with diminished toxicity. The present study demonstrates that the EA-AL exerts significant antioxidant and nephroprotective effects against gentamicin-induced toxicity in HEK293 cells. The extract effectively scavenged free radicals, restored cell viability, inhibited apoptosis and significantly improved antioxidant defense markers including catalase and reduced glutathione. Moreover, EA-AL reduced lipid peroxidation, mitigated intracellular ROS accumulation and preserved mitochondrial membrane potential, collectively confirming its protective role against oxidative and mitochondrial dysfunction-mediated nephrotoxicity. These findings validate the traditional use of *A. lanata* in renal therapeutics and highlight its potential as a natural nephroprotective agent. However, further bioactive compound isolation, mechanistic pathway elucidation and *in vivo* validation are necessary to advance EA-AL toward clinical application in the prevention or adjunctive treatment of drug induced renal injuries.

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Authors' contributions

RR and ML conducted the design of the study and done formal analysis and revision of manuscript. AS, RCS and VSL performed the investigation and optimization of methodology and performed the statistical analysis, ML and RA participated in the plant collection, data interpretation, draft preparation and adding scientific suggestions. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors declare that there exists no conflict of interest

Ethical issues: None

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work the authors used Quillbot, Grammar and spell checker tools in order to make grammatical changes, language modification and sentence making, not for analysis or interpretation of results. After using this tool/ service, the authors reviewed and edited the content as needed and take(s) full responsibility for the content of the publication.

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