



RESEARCH ARTICLE

# *Spilanthes acmella* extract alleviates cisplatin-induced nephrotoxicity and DNA damage in human embryonic kidney cells

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## Abstract

Kidney dysfunction has a devastating effect on health and quality of life, as it is one of the vital organs responsible for maintaining body homeostasis. Chemotherapeutic agents, especially anticancer medications, induce significant toxicity, resulting in renal dysfunction and irreversible kidney damage. This has led to a renewed focus on finding safer, more effective alternatives, such as pharmaceuticals derived from natural sources. The present study investigates the protective effects of ethyl acetate extract of *Spilanthes acmella* (EASA) against cisplatin-induced nephrotoxicity in human embryonic kidney (HEK293) cells. Preliminary phytochemical screening revealed Alkaloids and phenols as the predominant constituents. A neutral red uptake assay determined the non-toxic concentration of *S. acmella* and the combinatorial treatment with EASA restored cell viability to 72 %, compared to 47 % in cisplatin treated cells. The antioxidant enzymes SOD and catalase were shown to be diminished by cisplatin, indicating oxidative damage, accompanied by an increase in lipid peroxidation products. EASA significantly elevated enzyme levels and reduced membrane peroxides. Reactive oxygen species production was shown to be reduced in groups co-treated with EASA, as assessed by DCFDA fluorimetry studies. The comet assay also showed a reduction in cisplatin-induced DNA damage following EASA co-treatment. These findings demonstrate that EASA may mitigate the oxidative stress generated by cisplatin, thereby preventing oxidative renal injury.

**Keywords:** cisplatin; comet assay; HEK293 cell lines; nephrotoxicity; *Spilanthes acmella*

## Introduction

Nephrotoxicity induced by anticancer drugs is a significant and growing adverse event that limits the efficacy of cancer treatment. Because of glomerular filtration and tubular secretion, the kidney performs a crucial role in the elimination of several antineoplastic medications and their metabolites (1). Any part of the nephron, including its microvasculature, may be affected by chemotherapy medications, including conventional, cytotoxic and molecularly targeted therapies, leading to a range of clinical symptoms (2). Cisplatin is a widely used chemotherapy drug that is significantly linked to nephrotoxicity (3). It produces acute renal damage as well as chronic renal disease (4). The pathophysiological characteristics of acute renal injury induced by cisplatin are complex, involving cellular absorption and elimination. They also include apoptosis, vascular damage, oxidative and endoplasmic reticulum stress, as well as inflammation (5, 6). No dependable and consistent pharmacological therapy exists to mitigate renal injury in patients administered cisplatin,

despite decades of extensive research, pharmaceutical interventions and clinical trials.

Medicinal plants serve as vital suppliers of therapeutically active ingredients that provide opportunities for the development of innovative pharmaceuticals. Over 50 % of compounds produced from naturally occurring substances were approved by the US Food and Drug Administration from 1981 to 2014 (7). Phytochemicals like alkaloids, phenols, etc., are plant-derived substances that frequently possess therapeutic qualities. They possess a wide range of bioactive properties (8–10). Historically, plants have been used to address many ailments, including problems associated with pre, intra and post-renal conditions (10). The genus *Spilanthes* has 60 species, spread over tropical and subtropical climates globally (11). Among these, *S. acmella* is the most studied species due to its traditional uses and biological activity (12). *S. acmella* (Fig. 1) is a member of the Asteraceae family, originating from the genus *Aster*. 'Compositae' is the antiquated and acceptable term for describing the distinctive



**Fig. 1.** *Spilanthes acmella* plant.

inflorescence (13). The plant is indigenous to Brazil, also known as the 'toothache plant' since it is traditionally used in relieving dental ailments. The plant is grown all year for decorative or therapeutic uses (14). *S. acmella* is traditionally utilized as a medicine to cure many ailments, largely attributable to its secondary bioactive metabolites (15). The phytochemical analysis of *S. acmella* revealed flavonoids, alkaloids, tannins, phenols, polysaccharides, as well as saponins. The existence of these significant secondary metabolites indicates that the plant has substantial antioxidant activity and contributes to anti-inflammatory, antiseptic, anti-nephrotoxic and anesthetic bioactivities (16, 17).

The impact of renoprotective measures on the effectiveness of cisplatin induced nephrotoxicity requires comprehensive evaluation. This investigation intends to evaluate the impact of the medicinal plant *S. acmella* on toxicity induced by cisplatin in human embryonic kidney (HEK293) cells.

## Materials and Methods

### Reagents and chemicals

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

### Collection and extraction of plant extract

The *S. acmella* plant was identified and obtained from the local regions of Thiruvananthapuram. The leaves were collected, surface sterilized and shade dried. After drying, it was chopped into fine pieces. 10 g of dried leaves were added to 200 mL of 70 % ethyl acetate. 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 *S. acmella* were removed from the petri plate and preserved in an Eppendorf tube for further study.



**Fig. 2.** Ethyl acetate extract of *S. acmella* (EASA).

### Test for alkaloids

#### Dragendorff test

Dragendorff reagent consists of  $\text{Bi}(\text{NO}_3)_3 \cdot 5\text{H}_2\text{O}$  in  $\text{HNO}_3$  and KI, which reacts with alkaloids to form a reddish-orange precipitate. 2 mL of HCl was added to 0.5 mL of the extract. Subsequently, 1 mL of reagent was introduced to this acidic medium. The presence of alkaloid was indicated by the instantaneous formation of an orange-red precipitate (18).

### Detection of phenols

#### Ferric chloride test

To 2 mL of distilled water, 2 mL of the extract and ten drops of 10 % aqueous  $\text{FeCl}_3$  solution were added. The emergence of green or blue colour indicates the existence of phenols.

### Test for flavonoids

To 0.5 mL of alcoholic extract, 5-10 drops of dilute HCl and a small piece of  $\text{ZnCl}_2$  or Mg were added and the solution was heated for a few minutes. Flavonoids produced a reddish-pink or dirty brown appearance.

### Preparation of compound stock and administration of ethyl acetate extract of *Spilanthes acmella* (EASA)

A cyclomixer was employed to dissolve 1 mg of EASA in 1 mL of DMSO. To ensure sterility, the test solution was filtered using a 0.22  $\mu\text{m}$  Millipore syringe filter. New media with varying concentrations of EASA were introduced to the cells, incubated at 37 °C with 5 %  $\text{CO}_2$  for 24 hr.

### Cell lines

HEK 293 cell lines were initially obtained from National Centre for Cell Sciences (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 at 37 °C in a humidified 5 %  $\text{CO}_2$  incubator. The cells were trypsinized two days prior to seeding them. A 96-well tissue culture plate was transplanted with a 100  $\mu\text{L}$  cell suspension ( $5 \times 10^4$  cells/well), maintained at 37 °C incubator in humid atmosphere with 5 %  $\text{CO}_2$ .

### Determination of *in vitro* cytotoxic effect of EASA on HEK293 cells by neutral red assay

Neutral red is exclusively absorbed and bound by live cells. This ability declines in injured or necrotic cells. The amount of

accumulated neutral red was proportional to the number of viable cells. Adjust pH of neutral red to 6.35 by using 1 M  $\text{KH}_2\text{PO}_4$ . After attaining sufficient confluence, the cells treated with samples of different concentrations (100, 50, 25, 12.5, 6.25, 3.1, 1.5 and 0.75  $\mu\text{g/mL}$ ) were incubated for 24 h before the staining procedure.

Treat the culture plate with the sample and add 20  $\mu\text{L}$  neutral red, incubate at 37 °C for 3 hr. Fix with 50 % ethanol and 1 % acetic acid after rinsing with PBS, kept for 1 min. Add 200  $\mu\text{L}$  extraction buffer and incubate at room temperature for 20 min. The absorbance was measured at 540 nm using a microplate reader and the percentage viability was calculated.

$$\text{Percentage viability} = \frac{\text{Mean OD samples}}{\text{Mean OD control}} \times 100 \quad (\text{Eqn. 1})$$

#### **In vitro nephroprotective activity of EASA on HEK293 cells treated with cisplatin by neutral red assay**

##### **Induction of cisplatin toxicity**

HEK293 cells were incubated with 50  $\mu\text{M}$  cisplatin for 1 hr to induce toxicity.

##### **EASA co-administration and detection of nephroprotective effect**

After the stimulated toxicity induced by cisplatin was achieved, the cells were subjected to the addition of new medium along with different doses of EASA, kept at 37 °C and 5 %  $\text{CO}_2$ . After incubation for 24 hr the nephroprotective effect of EASA was assessed by neutral red assay.

#### **Determination of effects of EASA on antioxidant markers**

##### **Preparation of cell lysate**

Trypsinize the cells that had been treated with HiMedia trypsin-EDTA Solution. Cells are collected in Eppendorf tubes. Spun in a centrifuge at 5000 rpm for 5 min. Discarded the supernatant and reconstituted in lysis buffer (200  $\mu\text{L}$ ) containing 0.1 M Tris, 0.2 M EDTA, 2 M NaCl and 0.5 % Triton. Subjected to 20 min of incubation at 40 °C. Further investigations were carried out in the cell lysate.

##### **Estimation of SOD activity**

Mix 50  $\mu\text{L}$  sample with 50 mM phosphate buffer (pH 7.8), 45  $\mu\text{M}$  methionine, 5.3 mM riboflavin and 84  $\mu\text{M}$  potassium ferricyanide. After 10 min of incubation at 25 °C measure the absorbance using a spectrophotometer at 600 nm to quantify enzyme activity (19).

##### **Estimation of lipid peroxidation**

Activity of malondialdehyde (MDA) was detected since polyunsaturated fatty acid peroxides generate MDA upon decomposition.

50  $\mu\text{L}$  sample, 1 mL 1 % TBA and 500  $\mu\text{L}$  70 % alcohol were taken and boiled for 20 min. 50  $\mu\text{L}$  of acetone was added to quantify MDA activity. The absorbance was read at 535 nm using a spectrophotometer after cooling to room temperature (20).

$$\text{MDA Units} = \text{Absorbance} \times 7.57 \quad (\text{Eqn. 2})$$

##### **Estimation of catalase activity**

Catalase activity is directly proportional to the amount of

dissociation of hydrogen peroxide.

Mix 1.2 mL of Phosphate buffer and 50  $\mu\text{L}$  sample with 1 mL of hydrogen peroxide. Subsequent to the introduction of the reagents, measure absorbance at 234 nm at 0 min and 10 min (21).

$$\text{Activity of catalase} = \Delta D / 0.1116 \quad (\text{Eqn. 3})$$

#### **Determination of collagen deposition by Sirius red method**

The cells were treated with cisplatin as stated previously, then the medium was withdrawn and stained for 1 hr using 0.1 % solution of Sirius Red in saturated picric acid. Washing thoroughly with 0.01 M HCl removed the unreacted stain, making it colorless. The leftover stained collagen was dissolved in 0.1 M NaOH. Spun at 700 rpm for 5 min and read the supernatant OD at 530 nm. Control cells also received the same treatment.

#### **In vitro ROS measurement using DCFDA**

2,7-Dichlorofluorescein diacetate (DCFDA) is a fluorogenic dye that evaluates intracellular ROS activity. Treat the cell culture in 48 well plates with cisplatin and compound at final concentrations described previously. Introduce 50  $\mu\text{L}$  DCFDA after washing with phosphate buffered saline (PBS). Kept in an incubator for 30 min. After washing excess dye with PBS, measure the fluorescence at 470 nm excitation and emission at 635 nm (Qubit 3.0, Life Technologies USA) and expressed in arbitrary units.

#### **Comet assay**

The comet assay is a simple way to measure eukaryotic cell DNA strand breakage. High pH electrophoresis produces a comet-like structure, visible via fluorescence microscopy. The comet tail's intensity compared to the head indicates DNA breakage. In this study, it was used to assess the DNA repair activity induced by *S. acmella* extract. Cells were cultured and treated with cisplatin, cisplatin + sample and control was also maintained. Incubate the cells for 24 hr. After trypsinization rinsed using fresh medium and used for the comet assay.

1 mL each of 1 % normal melting agarose (NMA, Invitrogen, USA) dissolved in PBS was used to precoat frosted microscopic slides. Stored at 4 °C for 20 min. Cells were added to slides covered with a cover slip. Removed the cover slips, recoated with 0.75 % low-melting agarose (LMA). Incubated the slides for 10 min at 4 °C to settle agarose after each step. Then slides are placed in cold lysis buffer for 1 hr. Subsequently, maintained in electrophoresis solution for 20 min to facilitate DNA unwinding. Performed electrophoresis for 20 min using 0.8 V/cm. Finally, washed the slides with neutralization buffer three times for 5 min and stained with 20  $\mu\text{g/mL}$  of ethidium bromide for 5 min in order to detect the oxidative damage, followed by washing with PBS. Detect the DNA damage using a fluorescent microscope (Olympus CKX41 with Optika pro5 CCD camera). Perform comet scoring by TRITEK software and correlate statistically.

## **Results**

Cisplatin is often used in the treatment of several types of cancer. One significant adverse effect of cisplatin is its capacity to induce renal impairment as it is absorbed by renal cells,



undergoing a sequence of chemical processes that produce toxic metabolites capable of damaging the renal tubular cells, resulting in compromised renal function (2-5). Here, we set out to measure the *in vitro* effects of *S. acmella* ethyl acetate extract on cisplatin induced nephrotoxicity in HEK-293 cells. The dried EASA has been produced as detailed in the methodology section.

**Table 1.** Phytochemical screening of *S. acmella*

Phytochemicals	Present/ Absent
Alkaloids	+++
Phenol	+
Flavonoids	-

### Phytochemical screening of EASA

Alkaloids and phenols are the predominant phytochemicals identified by preliminary examination of EASA (Table 1).

### *In vitro* cytotoxic effect of EASA on cultured HEK293 cells by neutral red assay

HEK293 cells were treated with EASA at multiple doses (0.75 to 100 µg/mL of EASA) and a neutral red assay was conducted to evaluate the cytotoxic effects of EASA. Our findings indicated that exposure of the cell line to 100 µg/mL EASA exhibited a 32.07 % decrease in cell viability compared to control. The ED50Plus V0.1 software was employed to ascertain the IC50 value, which was found to be 53.22 µg/mL. The results obtained indicate that the EASA has a minor cytotoxic effect towards HEK293 cell lines. Hence, for the protective activity, a lower concentration of EASA (3.1 µg/mL) was chosen (Table 2).

### *In vitro* nephroprotective efficacy of EASA on cultured HEK293 cells subjected to cisplatin, evaluated using neutral red test

**Table 2.** *In vitro* cytotoxic effect of EASA in terms of percentage viability on HEK293 cells with varied concentrations

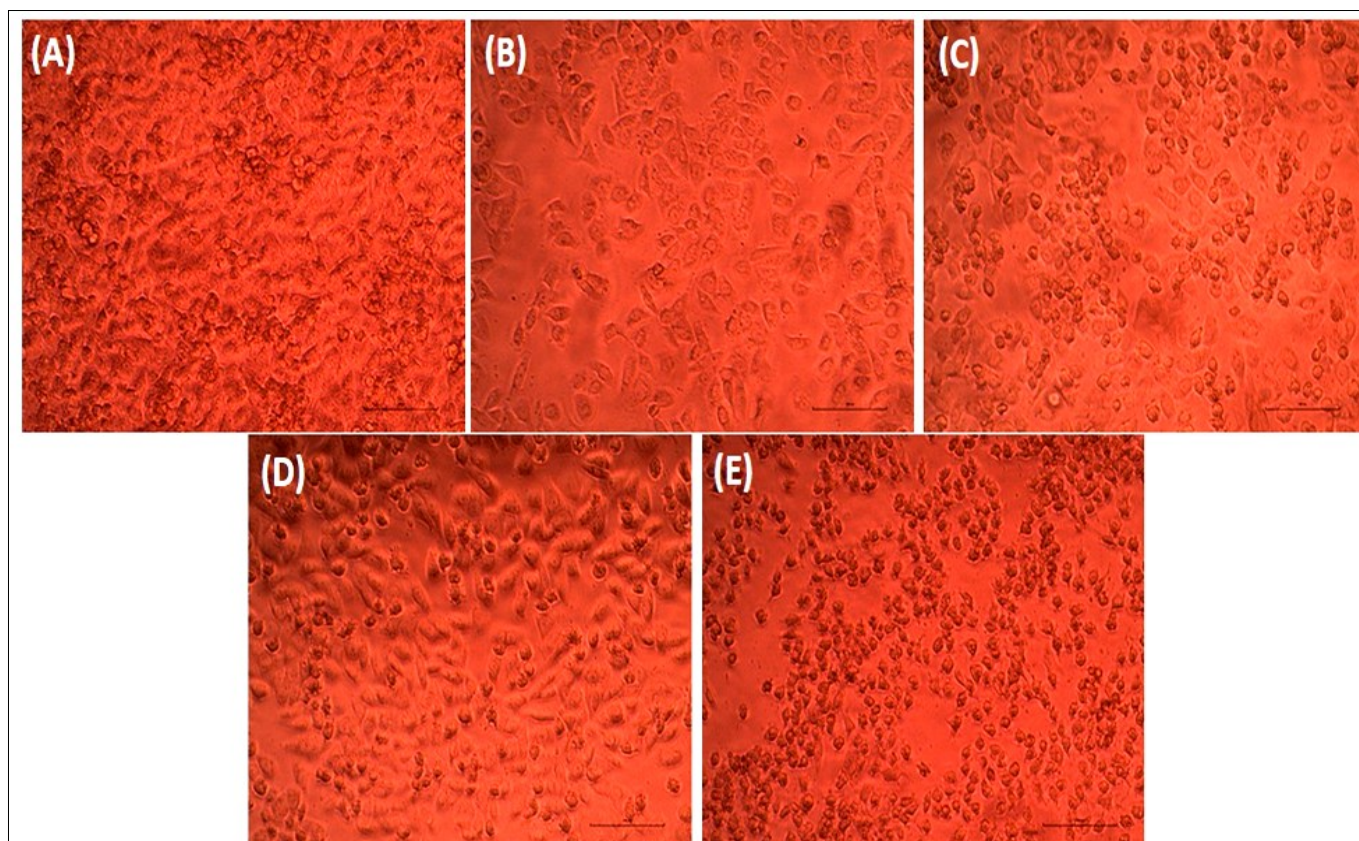
Concentration of EASA (µg/mL)	Percentage viability
Control	100
0.75	80.21
1.5	85.67
3.1	98.21
6.25	79.66
12.5	64.57
25	54.01
50	50.71
100	32.07

HEK293 cells were subjected to cisplatin exposure and concurrently administered EASA, with its nephroprotective impact evaluated by the neutral red assay. Exposure of the cell lines to cisplatin resulted in a substantial drop in viability to 47.3 %. Our findings indicated that the cell line treated with cisplatin in conjunction with EASA exhibited a viability of 72.07 % at a dose of 3.1 µg. The results demonstrate that the co-administration of EASA with cisplatin-exposed cells improved vitality by as much as 25 %. The results validate the protective efficacy of EASA and further studies were performed at a dosage of 3.1 µg. Table 3 depicts the percentage of cells that are viable. Phase contrast images depicted membrane blebbing, shrinkage and apoptotic bodies in groups treated with cisplatin, whereas co-administrated with EASA shows reversal of morphological changes (Fig. 3).

### Effect of EASA co-administration on cisplatin exposed HEK293 on antioxidant markers

#### Superoxide dismutase

The data indicate a considerable drop in SOD levels after cisplatin exposure, perhaps arising from oxidative stress and



**Fig. 3.** Photomicrographs depicting the effect of EASA co administration on cultured HEK293 cells exposed to cisplatin. (A) Untreated control cells; (B) Cisplatin exposed cells and cisplatin exposed cells co administrated with varied concentrations of EASA; (C) 0.75; (D) 1.5 and (E) 3.1 µg/mL.

**Table 3.** *In vitro* nephroprotective effect of EASA in terms of percentage viability

Treatment	Percentage viability
Control	100
Cisplatin	47.30
0.75 µg/mL	51.60
1.5 µg/mL	60.68
3.1 µg/mL	72.07

ensuing cellular death. The co-administration with EASA has elevated SOD levels and confirms the safeguarding efficacy of EASA against nephrotoxicity caused by cisplatin (Table 4).

#### Lipid peroxidation

Malondialdehyde is employed as a biomarker to evaluate the degree of lipid peroxidation. The findings indicate a considerable rise in lipid peroxidation levels as compared to the untreated control cells. This signifies that the oxidative stress caused by cisplatin exposure and EASA exposure has markedly reduced the degree of lipid peroxidation, retaining membrane stability (Table 4).

#### Catalase activity

The cellular antioxidant defense systems, which include catalase, can be overpowered by oxidative stress induced by cisplatin. Cellular components, particularly enzymes such as

**Table 4.** Effect of EASA co-administration on cisplatin-exposed HEK-293 on antioxidant markers

Sample	SOD	MDA	Catalase
Control	1.79±0.02	1.007±0.08	1.29±0.1
Cis	1.36±0.007	4.761±0.52	0.55±0.03
Cis + EASA	1.63±0.01	2.564±0.34	0.89±0.06

catalase, are susceptible to damage by prolonged exposure to high levels of ROS. As a result, catalase activity may decrease due to cellular injury or enzyme inactivation or depletion in an effort to adapt to the stimulus. The results obtained indicate that the catalase level has been substantially reduced compared to the untreated control HEK293 cells as a consequence of the cisplatin exposure. The co-administration of EASA has resulted in an increase in catalase levels, which supports the nephroprotective properties of EASA (Table 4).

#### Effect of EASA on collagen level

Cisplatin-induced oxidative stress may cause cellular damage and disrupt cellular processes. In certain instances, exposure to cisplatin can lead to reduced collagen synthesis or enhanced collagen degradation in HEK293 cells. This may result from cisplatin's effect on the cellular machinery responsible for collagen production, including transcription

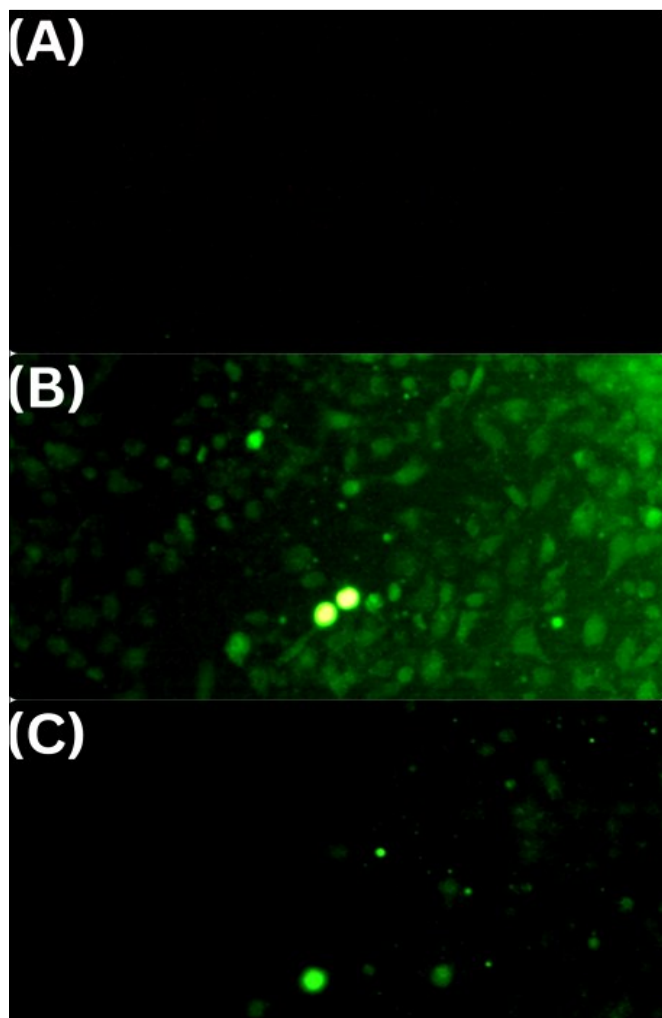
**Table 5.** Effect of EASA co-administration on cisplatin-exposed HEK-293 on collagen level

Sample	Optical density at 530 nm
Control	0.2600 ± 0.005
Cis	0.1336 ± 0.021
Cis + EASA	0.3850 ± 0.009

factors or enzymes involved in collagen synthesis. The results indicate a substantial decrease in collagen in comparison to untreated control cells, whereas collagen levels were restored with EASA co administration (Table 5).

#### Determination of ROS generation by DCFDA staining

To ascertain the impact of EASA on ROS, we used the DCFDA

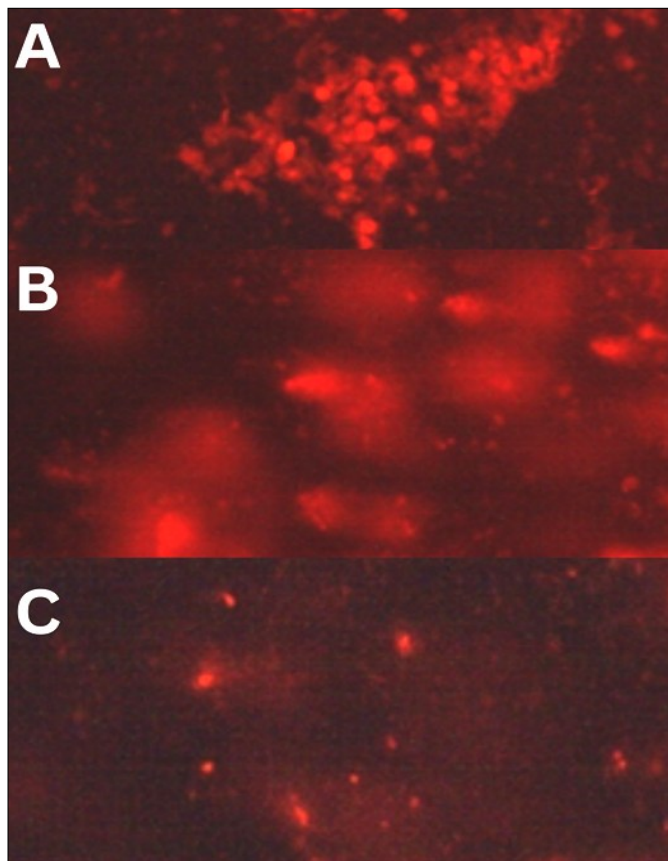
**Fig. 4.** Determination of ROS generation by DCFDA staining method. A) Untreated control cells; B) Cisplatin exposed HEK293 cells; C) Cisplatin exposed HEK293 cells co administrated with EASA.

staining technique. The findings demonstrate that ROS production is significantly elevated in cisplatin-exposed HEK293 cells compared to untreated control cells. The co-administration of EASA with cisplatin exposed cells has substantially scavenged the excess ROS produced (Fig. 4).

#### Comet assay

The magnitude of nuclear DNA damage was evaluated by examining tail length after performing the comet test under alkaline conditions, which reflects the level of DNA breakdown, which involves both single strand as well as double strand breaks. The samples were analyzed with a CCD Pro5 camera and an inverted epi-fluorescent microscope (Olympus CK41). The comets observed from the single cell gel electrophoresis (comet assay) were analyzed using Image J Triton software to correlate the comet parameters. Cells exposed to cisplatin exhibited an increased comet length compared to the untreated control cells. However, co-administration of EASA





**Fig. 5.** Photomicrograph depicting comet assay. (A) Untreated control cells; (B) Cisplatin exposed cells; (C) Cisplatin exposed cells co administrated with EASA.

resulted in a significant reduction in comet length (Fig. 5). The results confirm that EASA effectively mitigates the genotoxic effects induced by cisplatin exposure.

## Discussion

The global burden of renal disease is steadily rising, projected to become the fifth most prevalent non-communicable disease (NCD) contributing to mortality by 2040. Recognizing renal disease as a significant contributor to NCD related mortality will result in the implementation of coordinated global initiatives to alleviate the burden of renal disease and save lives (22). Early detection and therapy might potentially impede or halt the development of renal disease (23). A prototype of renal dysfunction, nephrotoxicity is a condition of renal failure resulting from exposure to pharmaceuticals or environmental and industrial pollutants. Modern medicine lacks definitive therapeutic strategies to mitigate the nephrotoxicity induced by medicines such as cisplatin and gentamicin (24). Several malignancies are effectively treated with cisplatin, a chemotherapeutic agent; nevertheless, its application is restricted by its adverse impact on healthy tissues. Cisplatin is toxic to nephrons and has the potential to induce acute as well as chronic renal disorders (4).

The demand for herbal treatments is rapidly increasing worldwide because of the cost-effectiveness as well as low toxicity. Phytochemicals with therapeutic potential for renal diseases may be found and extracted from medicinal plants. Numerous herbal and traditional substances and their origins are being evaluated globally to substantiate their efficacy in

renal disorders (25).

The objective of our investigation was to verify the ameliorative effects of EASA on experimental nephrotoxicity that was induced by cisplatin. The *in vitro* investigations were conducted using human embryonic kidney cells HEK 293. An early screening of phytochemicals in ethyl acetate fractions of *S. acmella* has verified the presence of alkaloids as the predominant secondary metabolite. The results align with the previous study (26). The non-toxic concentration of *S. acmella* extract was ascertained using the neutral red test, which indicated very little toxicity at lower doses, which was employed for evaluating nephroprotective properties. We assessed the protective effects of EASA against cisplatin toxicity by co-treatment. After 24 hr, it was noted that cell viability, originally reduced to 47 % with cisplatin treatment, was restored to 72 % by EASA. The concurrent enhancement of cell morphology indicated that EASA enhanced cell viability.

The impact of EASA on intracellular antioxidant enzyme activity and oxidation product levels has been examined spectrophotometrically. Our data indicate that the levels of catalase and superoxide dismutase were substantially decreased in groups treated alone with cisplatin. EASA co-treatment was observed to elevate levels contributing to cellular homeostasis. The findings indicate a reduction in antioxidant enzyme levels in groups administered cisplatin, which was elevated by EASA, indicating an ameliorative action towards oxidative stress.

Likewise, MDA which is produced by membrane oxidation, was seen to be elevated in groups administered cisplatin, but was reduced by EASA co-treatment. These data indicate the protective effects of EASA against cisplatin toxicity.

Fibrosis of renal tubules constitutes a prevalent manifestation of chronic renal disorder, irrespective of the fundamental etiology of the illness. The renal interstitium is primarily composed of collagens and the hallmark of fibrosis is the aberrant synthesis as well as buildup of extracellular matrix. Despite the discovery of many anti-fibrotic targets in preclinical research, no specific treatment has been introduced into the clinical scenario (27). By using Sirius Red staining, we were able to quantify the collagen production in kidney cells. It was discovered that cisplatin altered collagen synthesis and it was shown to be improved with EASA co-therapy.

The nephrotoxicity of platinum-based anticancer agents is believed to be mediated by many processes, including the production of ROS. Several antioxidants administered before or after drug treatment have shown efficacy in alleviating or preventing nephrotoxicity (28). As ROS formation is the primary cause of oxidative damage and renal damage in drug-induced nephrotoxicity, DCFDA was used to validate the antioxidant function of EASA in addition to assessing intracellular enzymes. As ROS production is the main cause of oxidative damage, our results make it clear that cisplatin increased ROS production by almost 94 %, but EASA co-treatment greatly decreased it. These results substantiate that the antioxidant activity of EASA protects cells from oxidative damage induced by cisplatin.

The vulnerability of the kidneys to chemotherapeutic drugs that induce DNA damage is well documented. The kidney's local microenvironment is affected by DNA damage,

which initiates response to damage and stimulation of proliferation of cell to replace damaged ones. Additionally, systemic responses are induced to reduce exposure to genotoxic stress. The renal phenotypes identified in human DNA repair-deficiency conditions and the nephrotoxic consequences resulting from DNA-damaging agents are, therefore, largely determined by the pathological consequences of DNA damage (29). Cisplatin interacts with DNA, combining with purine nucleotides to produce covalent adducts, which is the fundamental mechanism behind its cytotoxic effects (30). The protective effect of EASA was verified through the comet assay, which demonstrated that it can prevent renal damages by mitigating DNA damages. The results indicated that cisplatin caused an increase in DNA damage, which was substantially mitigated by co-treatment with EASA. These results support the idea that *S. acmella* extracts mitigate kidney damage caused by cisplatin by lowering oxidative stress and DNA damage.

This study examined cisplatin's genotoxicity and oxidative damage from multiple basic angles. Additional research is being planned to investigate the pathways involved in cisplatin-induced genotoxicity, the specific repair pathways triggered by the plant extract, the long-term cellular outcomes (such as senescence or apoptosis) and epigenetic changes. Additionally, we are currently strategizing for *in vivo* validation using animal models.

## Conclusion

Deterioration of kidney function has a catastrophic impact on the quality of life and health, as it is one of the essential organs responsible for the body's homeostasis. Renal dysfunction and irreversible kidney injury are the consequences of significant toxicity caused by chemotherapeutic agents, particularly anticancer medications. However, this has resulted in a renewed emphasis on the development of safer, more effective alternatives, including pharmaceuticals that are derived from natural sources. This research investigated the effect of EASA on nephrotoxicity induced by cisplatin in human embryonic kidney cells. The predominant phytochemicals were alkaloids and phenols, as indicated by an initial phytochemical screening. A non-toxic concentration of *S. acmella* was determined using the neutral red uptake assay. The cell viability was restored by the combinatorial treatment with EASA, whereas it had declined to significant level by cisplatin alone. The antioxidant enzymes catalase and SOD were demonstrated to be reduced by cisplatin, which suggests oxidative damage. This was followed by an elevation in lipid peroxidation byproducts. EASA significantly increased enzyme levels and decreased membrane peroxides. DCFDA fluorimetry experiments demonstrated that ROS production was diminished in groups that were co-treated with EASA. Co-treatment with EASA alleviated the increased genotoxicity induced by cisplatin, as evidenced by the comet assay. The results indicate that EASA may mitigate the oxidative stress induced by cisplatin, therefore preventing oxidative kidney damage. The integration of advanced analytical methods and animal model studies will provide a more comprehensive

understanding of the underlying mechanisms and potential for the development of drugs to treat renal injury.

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## Authors' contributions

RR and ML conducted the design of the study, performed formal analysis and revision of the manuscript. AS and VSL performed the investigation, 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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