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ATTENUATION OF NEPHROTOXICITY INDUCED WITH CISPLATIN IN HEK 293 CELL LINES BY *SAGADEVI NEI*

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ABSTRACT: *Sagadevi nei* is a Siddha polyherbal formulation indicated for *Neerkattu* and *Neersurukku*. The main ingredient of the formulation is *Vernonia cinerea,* belonging to the family Fabaceae, which has been proven scientifically to exhibit renal tubular regrowth. Based on Literature, each ingredient of the mixture has nephroprotective, diuretic, antioxidant, and anti-inflammatory properties. Cisplatin is a metallic compound antineoplastic drug with a known side effect of renal damage. The present study aims to elucidate the attenuative effect of Sagadevi Nei against Nephrotoxicity. MTT assay is a sensitive and reliable indicator of cellular metabolic activity. This study is done *in-vitro* by MTT assay in Human Embryonic Kidney (HEK) 293 cell lines. The toxicity was induced by cisplatin and the amount of cell damage was calculated. The Nephroprotective activity assessed by MTT Assay against cisplatin-treated HEK293 Cell lines co-treated with Sagadevi Nei showed an increase in the percentage of cell viability from 49.82% to 67.87% in a dose-dependent manner.

INTRODUCTION: *Sagadevi Nei* is a commonly prescribed Siddha formulation, for anuria and oliguria. There are 11 ingredients in this formulation, and it majorly consists of leaf juice of *Neichitti* (*Vernonia cinerea*) [1]. Oliguria is common in renal disorders like Vasculitis, glomerulonephritis, scleroderma, malignant hypertension, or interstitial nephritis [2]. Anuria is present in extreme dehydration, Anaemia, Severe infection, shock, heart failure, lung failure and renal failure [3]. All the ingredients of the formulation have nephroprotective, diuretic, antioxidant, and anti-inflammatory activity [4]. The compound formulation itself proved to have antimicrobial activity with *E. coli* biofilm prevention [5].

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Since, there is a vast usage and lack of scientific evidence for the effectiveness of the formulation, the present study aims to explicit the nephroprotective activity of *Sagadevi Nei* in Cisplatin-induced renal damage of HEK 293 Cell lines. HEK cell lines are time-honoured cell lines as expression tools for recombinant proteins. Even though it is of epithelial origin, the post-translational folding and processing necessary to produce functional, mature protein from a variety of mammalian and non-mammalian nucleic acids by the biochemical machinery of this cell [6].

These cell lines are a particular immortalized cell line created primarily in 1973 using tissue-cultured human embryonic kidney cells from a female foetus or spontaneously miscarried or aborted foetus. Cisplatin (Cis-Diammine Dichloroplatinum) is a widely used metallic compound anti-cancerous drug, which has a notable side effect of renal failure and renal tubular damage. Earlier studies on side effects of cisplatin reported Nephrotoxicity as a major limiter in 25-30% cause of epithelial cell

damage in renal tubules [7]. HEK cell lines are often used as expression platforms for recombinant proteins [8]. According to Siddha literature, the main ingredient *Sagadevi* indicated for the treatment of metallic poison like mercuric chloride, since cisplatin is a metallic compound, this is used to induce toxicity in the HEK cell line. There is lack of scientific evidence for the effectiveness of *Sagadevi Nei*, the present study aims to explicit the nephroprotective activity of *Sagadevi Nei* in Cisplatin-induced renal damage of HEK 293 Cell lines.

MATERIALS AND METHODS:

Plant Material Identification and Authentication: A mature plant of *Vernonia cinerea* was collected from the herbal garden at the National Institute of Siddha, Tambaram, Chennai. The raw drugs were procured from a raw drug shop in Tambaram, Chennai. The plants and herbal raw drugs were identified through morphological characteristics assessment and authenticated by the Botanist at the National Institute of Siddha, Tambaram, Chennai. The Authentication number is NISMB6382023. The freshly gathered plant materials were cleansed to eliminate attached dust and ground to obtain juice. The raw drugs were purified, powdered, and sieved.

Ingredients: All the ingredients and drug profile are represented in **Table 1** [1, 4, 8, 9, 10].

Shelf life: 6 months

Dosage: 16ml BD.

TABLE 1: REPRESENTATION OF DRUG PROFILE OF THE STUDY DRUG

S. no.	Botanical Name	Tamil Name	Quantity Required
1	Root of *Piper longum*	Thippilimoolam	4.5g
2	*Picrorhizakurroa*	Katugurohini	4.5g
3	*Glycyrrhiza glabra*	Ati madhuram	4.5g
4	*Plectranthus vettiveroides*	Vilamichamver	4.5g
5	*Vettiveria zizanoides*	Vetiver	4.5g
6	*Nymphea pubescens*	Alli thandu	4.5g
7	*Syzygium aromaticum*	Lavangam	4.5g
8	*Santalum album*	Santhanam	4.5g
9	*Hemidesmus indicus*	Nannari	4.5g
10	*Vernonia cinerea* leaf juice	Neichitti keerai	750ml
11	Ghee	Pasu Nei	750ml

Preparation of Cell Line: Initially, the cell lines were obtained from the National Centre for Cell Sciences, Pune (NCCS), India, and kept in Dulbecco's Modified Eagles Medium (DMEM).

These cell lines were cultured in a $25cm^2$ tissue culture flask with DMEM supplemented with 10% Foetal Bovine Serum (FBS), L-glutamine, and sodium bicarbonate, along with an antibiotic solution containing penicillin (100 U/ml), streptomycin (100 µg/ml), and amphotericin B 2.5 µg/ml). These cultured cells were incubated at 37°C in a humidified 5% CO_2 incubator.[11, 12]

Cells Seeding in 96 well Plate: After tryptinizing a confluent monolayer of cells that were two days old, the cells were suspended in 10% growth media. A 100µl cell suspension (5× 103 cells/Well) was then seeded in 96-well tissue culture plates and cultured at 37° C on an incubator with 5% CO_2 humidity.

Preparation of the Compound Stock: A cyclomixer was used to weigh and dissolve 1mg of the substance in 1 mL of 0.1% DMSO. A 0.22 µm Millipore syringe filter had been used to filter the sample solution to ensure sterility. To create toxicity, cisplatin (50 µg/ml) was added [11, 12].

Nephroprotective Activity by MTT Assay: A 100µl cell suspension (5 x 103 cells/well) was trypsinized and suspended in a 10% growth medium and was seeded into 96 well tissue culture plates. The plates were then placed in a humidified 5% CO_2 incubator and heated to 37°C for 24hrs. Each freshly synthesized compound in DMEM was diluted successively five times by two-fold dilution (100 µg, 50 µg, 25 µg, 12.5 µg, and 6.25 µg. In 500ml of DMEM and then each concentration of 100µl was put in triplicate to the appropriate wells. This was done after 24 hours. The wells were then incubated at 37 °C in a 5%

CO_2 humidified atmosphere. Additionally, normal control cells were retained. After 48 hrs Cisplatin 50g/ml is added to each well except for the control cells and incubated for 24 hrs. Olympus CKX41 with an Optika Pro5 CCD camera, which is an inverted phase contrast tissue culture microscope was used to view the entire plate after 24 hours after treatment for observing the morphological changes, and microscopic observations were captured as photographs. Cell morphological alterations such as rounding or shrinking, granulation, and vacuolization in the cytoplasm were all considered indications of cytotoxicity. Finally, 15 mg of MTT (Sigma, M-5655) was prepared, completely dissolved, thoroughly filtered, and sterilized in 3 ml of PBS.A 24-hour incubation period was followed by the removal of the sample from the wells, the addition of 30 µl of reconstituted MTT solution to each test and control cell well, a gentle shaking of the plate, and an additional 4 hours of incubation at 37 °C in an incubator with humidified 5% CO_2. After incubation, the formazan crystals were gently broken down by pipetting uphill and downward while adding 100 µl of MTT Solubilization Solution (DMSO, Sigma Aldrich, USA) to the supernatant. The absorbance readings were determined using a microplate reader at a wavelength of 540 nm. The following formula was used to compute the percentage of growth inhibition [11, 12].

% of viability = Mean OD Samples x 100 / Mean OD of control group

RESULTS:

Nephroprotective Activity by MTT Assay: HEK 293 cell lines are normal embryonic kidney cell lines that are induced toxicity with cisplatin of 50g/mL. The untreated cells were 99.99% viable. The vitality of HEK 293 cell lines after the treatment of cisplatin was 48.1% i.e., the viability was reduced by 52%. When the test drug Sagadevi nei was treated to the cisplatin-exposed cells in an increasing concentration ranging from 6.25, 12.5, 25, 50, and 100µg/ ml, the viability of the cells was 49.28%, 54.32%, 58.49%, 60.65%, and 67.87% respectively i.e., the viability of the cells increased from 48.1% to 67.87%. The P-value was found by ANOVA Dunnett's test, and a comparison was made between control and cisplatin which was significant having ap-value<0.001 and the comparison of cisplatin-treated with SN-treated cisplatin exposed groups the p-value was more significant showing <0.01. Thus, the drug will effectively act as a nephroprotective agent.

TABLE 2: REPRESENTATION OF CELL VIABILITY TREATED WITH CISPLATIN AND SN

Sample Concentration (µg/ml)	Average Absorbance @ 540nm	Percentage Viability
Control cells	0.5068	99.99
Cisplatin	0.2439	48.13
SN 6.25	0.2525	49.82
SN12.5	0.2753	54.32
SN 25	0.2964	58.49
SN 50	0.3074	60.65
SN 100	0.3439	67.86

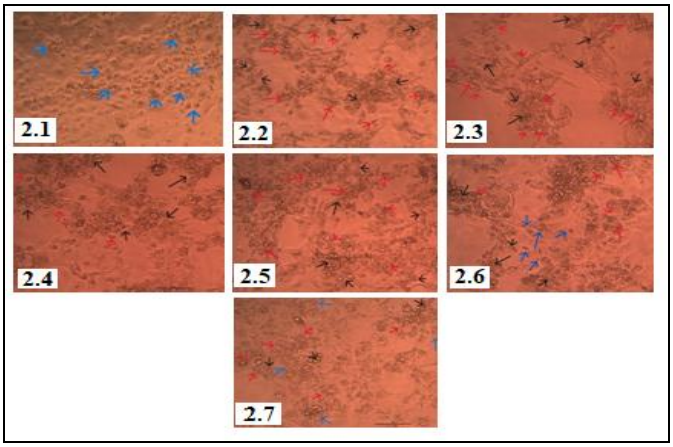

FIG. 1: PHASE CONTRAST MICROSCOPIC IMAGES. (2.1) - control cells: blue arrow represents the normal healthy cell; (2.2) – cisplatin treated cells stating whole distorted architect as compared to control. The red arrow represents the swollen cell and the black arrow represents the degranulated cell. (2.3), (2.4), and (2.5) show significant changes in the architect of the cell, the red arrow represents the rounding, shrunken, and degranulation of cells. (2.6), (2.7) showing normal architects with the least dead cells. The blue arrow denotes normal healthy cells.

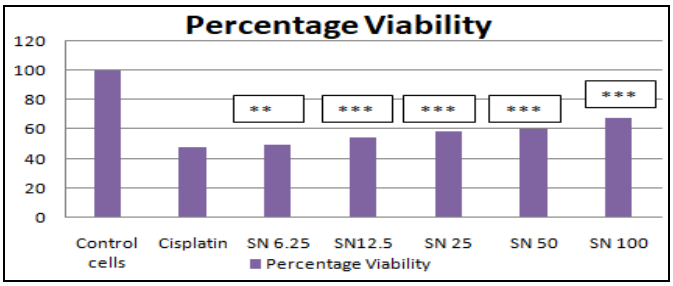

FIG. 2: GRAPHICAL REPRESENTATION OF NEPHROPROTECTIVE ACTIVITY OF SN. The above Graphical representation depicts the nephroprotective effect of SN on cisplatin-exposed HEK 293 cells by MTT assay. Y-axis- Percentage viability, X axis - varied concentration of study drug in µg.

All experiments were done in triplicates and results were represented as Mean+/- SE. One-way ANOVA and Dunnett's test were performed to analyze data. ***p < 0.001 compared to cisplatin exposed groups, **p < 0.01 compared to cisplatin exposed groups.

DISCUSSION: Due to the use of several drugs and lifestyle changes, many people suffer from renal disorders. *Sagadevi Nei* is a Siddha polyherbal formulation prescribed for the treatment of oliguria, yet the ingredients of the formulation have proven as nephroprotective, antioxidant, anti-inflammatory, and diuretic, which helps in treating major symptoms of renal disorders like pitting edema of the lower limb, oliguria, renal damage. The main constituent *Vernonia cinerea* has been proven to regenerate the renal tubules *in-vivo* [13]. Resveratrol from *Piper longum* [14], betulin, and astaxanthine from *Vernonia cinerea* are studied to protect against renal damage. Glabridin helps to maintain residual renal function [15, 16].

Cisplatin induces renal failure, manifested by elevated serum creatinine and urea levels and an increase in reactive oxygen species (ROS) accumulation [17]. This could trigger the synthesis of interleukin 1L-1β, a mediator of inflammation, tumor necrosis factor (TNF-α), and signaling pathways leading to apoptosis. Additionally, the regular pathway may be altered by circumventing the typical process, which may result in cell death and other complications. Thus, one of the main causes of cell death is oxidative stress [18, 19].

The phase contrast images obtained from MTT assay using Olympus CKX41 with an Optika Pro5 CCD camera showed morphological changes like rounding, shrinkage, and degranulation on treatment with cisplatin and on treatment of cisplatin-treated cells with SN on varying concentrations of the number of damaged cells reduced.

Thus, the study drug was chosen to explicitly demonstrate its effectiveness in renal protection. In renal tubules, there is more production of ROS species, which causes damage to the tubules. The study drug has been proven to inhibit the damage caused by the nephrotoxic drug cisplatin by 2% at the minimal dose and by 20% at the maximum dose. The study was carried out in HEK 293 cell lines, and the drug was found to exhibit significant nephroprotective activity in a dose-dependent manner. The cell viability increased from 49.82% to 67.87%.

CONCLUSION: Since, many people ineluctably suffer from renal failure with increased levels of creatinine and serum electrolytes. Thus, this drug can be credibly administered in the required quantity for the management of CKD and other renal disorders. Furthermore, an *in-vivo* study should be conducted to assess the mechanism of action of the study drug and a clinical study should be conducted to explicitly explain the efficacy of the study drug.

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CONFLICT OF INTEREST: Nil.

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