Research Article

An Exploratory Study to Evaluate the Nephro-protective Effect of *Scopariadulcis*. Linn. Ethanol Extracts in Gentamycin Induced Toxicity in HEK 293 Cells.

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ABSTRACT

Among various organs in human being kidney occupies a cardinal position, since it being discharging a plethora of functions, the major being elimination of unwanted metabolites and other substances. Apart from being and organ for excretion, it discharges other functions which contribute to the maintenance of body homeostasis too. It also assists the functioning of other organs and influence and interferes indirectly with other bodily activities-to exemplify, the vasoconstriction envisaged through the rennin-angiotensin- aldosteron axis. In this study, we evaluated the nepro-protective activity of ethanol extract of *Scorpiadulcis*. Linn (whole plant) by MTT assay. It was found that the extract showed significant increase ($p<0.001$) in cell viability in cultured HEK 293 cell lines as compared to gentamycin and control. The observed % of viability at concentrations of 6.25, 12.5, 25, 50 and 100 µg/100µ l concentrations were (Mean ±SE) 58.72 ± 0.0598, 59.80± 0.0323, 65.25 ± 0.2150, 78.83± 0.1074 and 82.61± 0.3269.respectively, while that of gentamycin, the antibiotic was 48.58 ± 0.5985%.

Keywords: Scorpiadulcis, Nephroprotective activity, Renal damage.

INTRODUCTION

Scopariadulcis. Linn; family scrophulariaceae, also known as sweet broom wort or sweet broom weed grows in wild and shrubberies of India and Bangladesh. The plant is purporting to have many medicinal activities including anti-diabetic, antipyretic and analgesic activities. It is also used traditionally in diarrhea, jaundice and externally in warts. Some phytochemical and pharmacologic screening work has been done in this palnt. A preliminary investigation and TLC profiling for identification of phytochemical entities have been conducted by Chetia et al.[1]Many work exploring the antidiabetic and antioxidant property of S.dulcis has been published.[2, 3]. It is said to be used traditionally for haemorrhoids[4] and in sickle cell anemia [5].

The normal function of kidneys is compromised due to many reasons. Among various factors, drugs and xenobiotics are a major cause for acute renal failure. To exemplify, aminoglycosides induced nephoropathy poses a major threat to the geriatric patients. Many plants and plant based medicines is said to be effective in acute renal failure. [6,7].

MATERIALS AND METHODS

Preparation of Plant Extract

Biologically active compounds are present in the medicinal plant, *Scopariadulcis, Linn*. as primary and secondary metabolites and these are responsible for medicinal activity of the plant also. For this study, the chemical substances, reagents and ethyl alcohol were purchased from approved companies. The plant was collected from southern part of Tamilnadu district. Whole part of the plant material was thoroughly washed with water and then placed two weeks under sunshade for air drying. The dried material was cut in to small pieces for extraction. Ethanolic extract of the dried material was prepared by maceration technique and the resulted product is a greenish black stick material.

Determination of Nephroprotective Activity, *Invitro study* by MTT Assay.[8, 9]

Cell line, Human embryonic kidney cells (HEK 293), from National Centre for Cell Sciences (NCCS), Pune, India. The cells were maintained in Dulbecos modified Eagles medium (Gibco, Invitrogen). In a 25 cm^2 tissue culture flask, the cell line was cultured with DMEM solution containing 10% FBS, L-glutamine, sodium bicarbonate and antibiotic solution. Then the culture was incubated at 37ºC in a humidified 5% CO_2 incubator (NBS Eppendorf, Germany).

By the use of Inverted phase contrast microscope, viability of the cells was evaluated by direct observation and then carried out. the MTT Assay.

Cells seeding processes

Two days old confluent monolayer of cells was trypsinized. The cells were suspended in 10% growth medium. In a 96 well tissue culture plate, 100µl cell suspension ($5x10^4$ cells/well) was seeded and incubated at 37ºC in a humidified 5% CO_2 incubator.

Preparation of plant extract

1mg/ml of plant extract solution was prepared in DMEM by the use of acyclomixer and filtered through 0.22 µm Millipore syringe to ensure the sterility.

Cytotoxicity Evaluation

After attaining sufficient growth, 50mM gentamycin solution was added to induce toxicity and incubated for one hour. The plant extract in a dilution of 100µg, 50µg, 25µg, 12.5µg, 6.25µg in 100µl were prepared in 5% DMEM. The concentrations in 100µl were added in triplicates to the respective wells and incubated for 24 hours at 37ºC in a humidified 5% CO_2 incubator.

Cytotoxicity Assay by Direct Microscopic observation

After incubation period, the plates were observed through an inverted phase contrast tissue culture microscope (Olympus CKX41 with Optika Pro5 CCD camera). The microscopic observation was recorded as images. The indications for cytotoxicity are any detectable changes in the morphology of the cells, such as rounding or shrinking of cells, granulation and vacuolization in the cytoplasm of the cells

Cytotoxicity Assay by MTT Method[13]

MTT (Sigma, M-5655), 15mg was reconstituted and dissolved completely in 3 ml PBS, then sterilized by filtration sterilization.

The sample content in wells after incubation period were taken and added 30 µl of MTT solution to all test wells and control wells, the plate was gently shaken, then incubated at 37ºC in a humidified 5% CO_2 incubator for 4 hours. After that the supernatant was removed and added 100µl of MTT Solubilization Solution (DMSO).The wells were mixed gently to solubilize theformazan crystal and then measured the absorbance values by using microplate reader at a wavelength of 570 nm (Laura B. Talarico et al., 2004).

The growth inhibition was calculated as % viability by using the formula:

$$\% \text{ of Viability} = \frac{\text{Mean OD of Samples} \times 100}{\text{Mean OD of Control group}}$$

RESULTS AND DISCUSSION

Groups in 100µl	Optical Density (Mean ± SE)	% viability (Mean± SE)	Statistics
Control (A)	0.6202± 0.002	100	
Gentamycin (B)	0.3013± 0.0006	48.58 ± 0.5985***	A & B
Ex- 6.25µg (C)	0.3642± 0.0003	58.72 ± 0.0598***	A & C
Ex- 12.5µg (D)	0.3709± 0.0002	59.80± 0.0323***	A &D
Ex- 25µg (E)	0.4047± 0.002	65.25 ± 0.2150**	A&E
Ex- 50µg (F)	0.4889± 0.0011	78.83± 0.1074***	A&F
Ex- 100µg (G)	0.5123± 0.0011	82.61± 0.3269***	A&G
*** Significant, p<0.001, ANOVA followed by Dunnets multiple comparison, N=3.			

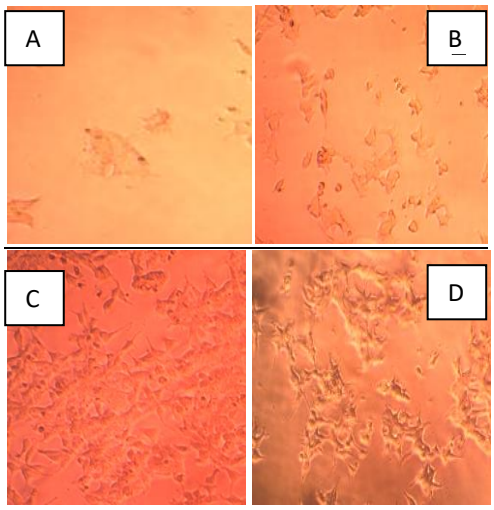

Figure 1: Evaluation of effect of extract of S.dulcis on Gentamycin induced toxicity in HEK293 cell lines. A: Gentamycin control. B: Gentamyciin + Extract 6.25 µg, C: Gentamyciin + Extract 50 µg/100lµl D: Gentamyciin + Extract 100 µg/100µl.

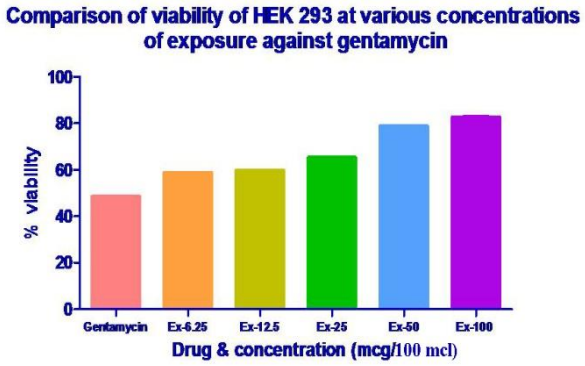

Figure 2: Graph showing comparison of cell viablility of HEK 293 cell lines after exposure at various concentrations of extracts and Gentamycin.

Gentamycin, the medication belongs to a class of drugs known as[10] amino glycoside antibiotics which is used to prevent or treat a large range of bacterial infections. It functions by stopping the growth of bacteria, acts mostly against Gram-negative bacteria including *Escherichia*

coli, Klebsiella, Proteus, Pneumonia, Pseudomonas, Serratia and the Gram-positive Staphylococcus. However studies describe cellular toxicity is a common trait of amino glycosides and kidney damage is a problem in 10-25% of people who were[11] received the drug. Acute kidney injury remains a common and potentially serious clinical problem associated with the use of gentamicin amino glycoside as gentamicin is one of the most nephrotoxic of the class. Hence gentamicin amino glycoside is used to induce toxicity in HEK 293.

Five different concentrations of the drug extract solutions were used to study the nephro protective action after inducing toxicity by gentamicin antibiotic and the results are tabulated as percentage of viability. The maximum percentage of viability was recorded in the 100 µg/100µl concentration and the same strength was observed as the concentration which produces maximum nephro protective activity in gentamicin induced Human embryonic kidney cells.

CONCLUSION

The study nephro protective activity of ethanolic extract of *S.dulcis* in gentamicin induced toxicity in Human embryonic kidney cells, in vitro method has been evaluated the pharmacological action of plant extract aptness for kidney protection and is proved to be safe and valuable. The measured data is found to be precise, accurate and linear over the concentration of range tested. The maximum protective action is obtained in the 100µg quantity.The positive results obtained for the crude extract could be due to the presence of phyto chemical constituents, and a follow up study in animal model must be conducted for validating the activity.

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