INTRODUCTION

Scopariadulcis. Linn; family scrophulariaceae, also known as sweet broom wort or sweet broom weed grows wild in shrubberies of India and Bangladesh. The plant isis purporting to have many medicinal activities including anti-diabetic, anti-inflammatory and analgesic activities. It is also used traditionally in diarrhea, jaundice and externally in warts. Some phytochemical and pharmacological screening work has been done in this plant. A preliminary investigation and TLC profiling for identification of phytochemical entities have been conducted by Chetia et al. Many work exploring the antidiabetic and antioxidant property of S.dulcis has been published. It is said to be used traditionally for haemorrhoids and in sickle cell anemia. 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 nephropathy poses a major threat to the geriatric patients. Many plants and plant based medicines is said to be effective in acute renal failure.

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

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 cm² 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 37°C in a humidified 5% CO₂ 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 (5x10⁴ cells/well) was seeded and incubated at 37°C in a humidified 5% CO₂ 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.

Keywords: Scopariadulcis, Nephroprotective activity, Renal damage.
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% CO₂ 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

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% CO₂ 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 the formazan 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:

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

RESULTS AND DISCUSSION

<table>
<thead>
<tr>
<th>Groups in 100µl</th>
<th>Optical Density (Mean ± SE)</th>
<th>% viability (Mean± SE)</th>
<th>Statistics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (A)</td>
<td>0.620± 0.002</td>
<td>100</td>
<td></td>
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<tr>
<td>Gentamycin (B)</td>
<td>0.301± 0.0006</td>
<td>48.58 ± 0.5985***</td>
<td>A &amp; B</td>
</tr>
<tr>
<td>Ex-6.25µg (C)</td>
<td>0.364± 0.0003</td>
<td>58.72 ± 0.0598***</td>
<td>A &amp; C</td>
</tr>
<tr>
<td>Ex-12.5µg (D)</td>
<td>0.370± 0.0002</td>
<td>59.80± 0.0323***</td>
<td>A&amp;D</td>
</tr>
<tr>
<td>Ex-25µg (E)</td>
<td>0.404± 0.002</td>
<td>65.25 ± 0.2150**</td>
<td>A&amp;E</td>
</tr>
<tr>
<td>Ex-50µg (F)</td>
<td>0.489± 0.0011</td>
<td>78.83± 0.1074***</td>
<td>A&amp;F</td>
</tr>
<tr>
<td>Ex-100µg (G)</td>
<td>0.512± 0.002</td>
<td>82.61± 0.3269***</td>
<td>A&amp;G</td>
</tr>
</tbody>
</table>

*** Significant, p<0.001, ANOVA followed by Dunnett’s 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: Gentamycin + Extract 6.25 µg. C: Gentamycin + Extract 50 µg/100µl D: Gentamycin + Extract 100 µg/100µl.

Figure 2: Graph showing comparison of cell viability 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 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.

REFERENCES


