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Short Communication
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MTT Cytotoxicity Study of Extracts of *Meyna laxiflora* Seeds and *Tectona grandis* Bark using Cell Lines LLC PK1

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Abstract

The LLC-PK1 (*Sus scrofa* kidney epithelial cell line) is purchased from ATCC, USA. The cells were maintained in DMEM high glucose media supplemented with 10% FBS along with the 1% antibiotic-antimycotic solution in the atmosphere of 5% CO_2, 18 to 20% O_2 at 37°C temperature in the CO_2 incubator and sub cultured for every 2 days.

Keywords: Cytotoxicity; Cell lines; DMEM; Lactate dehydrogenase; Diaphorase; Tetrazolium salt

Introduction

Lactate Dehydrogenase (LDH) is a cytosolic enzyme present in many different cell types. Plasma membrane damage releases LDH into the cell culture media. Extracellular LDH in the media can be quantified by a coupled enzymatic reaction in which LDH catalyses the conversion of lactate to pyruvate *via* NAD+ reduction to NADH. Diaphorase then uses NADH to reduce a tetrazolium salt (INT) to a red formazan product that can be measured at 490 nm. The level of formazan formation is directly proportional to the amount of LDH released into the medium, which is indicative of cytotoxicity [1].

Materials and Methods

1. Cell lines [2,3]:

a) LLC-PK1-Porcine Kidney Proximal tubular epithelial cell line (From ATCC, USA)

2. Cell culture medium: DMEM-High glucose media - (Cat No:2120785, Gibco)
3. Adjustable multichannel pipettes and a pipettor (Benchtop, USA)
4. Fetal Bovine Serum (#RM10432, Himedia)
5. EZ Count LDH Assay Kit (Cat No: CCK-036, HiMedia)
6. DMSO (#PHR1309, Sigma)
7. D-PBS (#TL1006, Himedia)
8. 96-well plate for culturing the cells (From Corning, USA)
9. T25 flask (# 12556009, Biolite - Thermos)
10. 50 ml centrifuge tubes (# 546043 TORSON)
11. 1.5 ml centrifuge tubes (TORSON)
12. 10 ml serological pipettes (TORSON)
13. 10 to 1000 µl tips (TORSON) [4,5].

Equipments

1. Centrifuge (Remi: R-8°C).
2. Pipettes: 2 µl to 10 µl, 10 µl to 100 µl, and 100 µl to 1000 µl.
3. Inverted microscope (Biolink)
4. 37°C incubator with humidified atmosphere of 5% CO_2 (Healforce, China)

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Assay controls

(i) Medium control (medium without cells)

(ii) Negative control (medium with cells containing acclimation medium but without the experimental drug/compound)

(iii) Positive control (cells treated with 10 µl of lysis buffer provided in kit)

Steps followed

1. Seed 100 µl cell suspension in a 96-well plate at required cell density (20,000 cells per well), without the test agent. Allow the cells to grow for about 24 h.

2. Add fitting convergences of the test specialist (Mentioned in the results - Excel sheet). 10 µl of lysis buffer provided by kit is considered as positive control.

3. Brood the plate for 24 h at 37°C in a 5% CO_2 environment.

4. After the incubation period, takeout the plates from incubator and centrifuge the plate at 1500 rpm for 5 min to settle the cell debris at the lower part of wells.

5. Aseptically transfer 50 µl supernatant from each well to a new 96 well plate.

6. Add 50 µl of CV solution into each well. Hatch the plate at room temperature for 15 min to 30 min.

7. Add 50 µl of stop solution and of the gems. 13. Understand the absorbance on a spectrophotometer or an ELISA reader at 570 nm with a reference frequency

8. % LDH leakage is calculated using the below formula [5-7].

% LDH leakage = A-C/B-C × 100

Here,

A: Avg absorbance of test -blank

B: Avg absorbance of most extreme LDH control Avg absorbance

C: Avg absorbance of untreated control

Results

See Figures 1-2 and Tables 1-2.

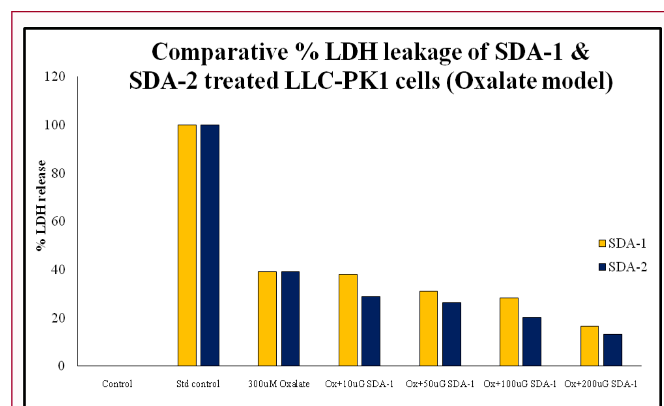

Figure 1: Overlaid bar graph showing the % of LDH leakage observed in Untreated, Lysis solution, Oxalate and Test compounds conjugated with Oxalate with various concentrations of 10 uG, 50 uG, 100 uG and 200 uG/mL treated LLC-PK1 cell lines by LDH study.

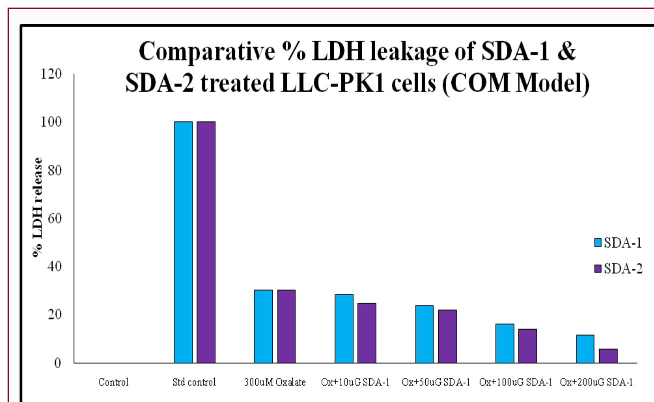

Figure 2: Overlaid bar graph showing the % of LDH leakage observed in untreated, lysis solution, Calcium Oxalate Monohydrate (COM) and test compounds conjugated with COM with various concentrations of 10 uG, 50 uG, 100 uG and 200 uG/mL treated LLC-PK1 cell lines by LDH study.

Table 1: Table showing the % LDH leakage of the test compounds, SDA-1 and SDA-2 conjugated with Oxalate with the concentration of 300 uM treated LLC-PK1 cell lines after the incubation period of 06 hrs.

S. NO	Sample	Oxalate Model	
		SDA-1	SDA-2
1	Control	0	0
2	Std control	100	100
3	Oxalate-300uM	38.94	38.94
4	Oxalate-300uM+Test-10 uG	37.86	28.78
5	Oxalate-300uM+Test-50 uG	30.83	26.2
6	Oxalate-300uM+Test-100 uG	28.18	19.98
7	Oxalate-300uM+Test-200 uG	16.36	13.07

Table 2: Table showing the % LDH leakage of the test compounds, SDA-1 and SDA-2 conjugated with Calcium Oxalate Monohydrate (COM) with the concentration of 133 uG/cm² treated LLC-PK1 cell lines after the incubation period of 6 h.

S. NO	Sample	Calcium Monohydrate Oxalate (COM) Model	
		SDA-1	SDA-2
1	Control	0	0
2	Std control	100	100
3	Oxalate-300uM	30.31	30.31
4	COM-133uG+Test-10 uG	28.47	24.75
5	COM-133uG +Test-50 uG	23.76	22.1
6	COM-133uG +Test-100 uG	16.14	13.99
7	COM-133uG +Test-200 uG	11.63	5.96

Conclusion

- The Observations in statistical data of LDH leakage study suggesting us that against LLC-PK1 cell lines test compounds namely SDA-1 and SDA-2 conjugated with the 300 uM of Oxalate exhibiting cytoprotective potency on dose dependent manner respectively. Standard drug, provided in the kit effectively released LDH to the surrounding medium by causing 100% lysis on cells respectively. Among the given compounds, SDA-2 showing highly significant cytoprotective potency and other compound, SDA-1 showing moderate cytoprotective potency in Oxalate induced LLC-PK1 cells.

- The Observations in statistical data of LDH Leakage study suggesting us that against LLC-PK1cell lines test compounds namely SDA-1 and SDA-2 conjugated with the 133 uG/cm^2 of Calcium Oxalate Monohydrate (COM) exhibiting cytoprotective potency on dose dependent manner respectively. Standard drug, provided in the kit effectively released LDH to the surrounding medium by causing 100% lysis on cells respectively. Among the given compounds, SDA-2 showing highly significant cytoprotective potency and other compound, SDA-1 showing moderate cytoprotective potency in COM induced LLC-PK1 cells.

- A Further studies like Cell Cycle Study by PI staining, Apoptosis concentrate by Annexin V/PI staining, Apoptotic Protein articulations like Caspase 3,7,9, Bcl2, p53 and ROS study to assess the mechanism of activity of test intensifies *viz.*, SDA-1 and SDA-2 behind the anticancer potential in *in vitro* conditions [7,8].

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