3.4: Different Cytotoxicity Assays

Learning Objectives

- 1: Know different types of cytotoxicity assays.
- 2: Know how different cytotoxicity assays are used when cells are exposed to toxicants or mutagens.

The goal of cytotoxicity assay is to determine whether any chemicals or drugs will do any toxic effect or load on milieu or genetic material of the cells caused for lethality of the cells or caused for different diseases.

The following are the different types of cytotoxicity assays:

- DNA fragmentation/ladder assay
- Comet assay
- Necrosis assay
- Enzyme assay
- Proteomics assay
- Expression array assay

4.1: DNA Fragmentation/Ladder Assay

DNA fragmentation or ladder assay are used to know the fragmented DNA of the cells caused by chemicals or drugs. Fragmented DNA can be separated by agarose gel electrophoresis and can be visualized as “ladder” by ethidium bromide staining. The evaluation of cytotoxicity through cell death is an acceptable common assessment.

Ladder assays are performed for the following reasons:

1. to simply characterize the toxicity of the chemicals or drugs in cells, or
2. to determine the maximum doses of the test chemicals or drugs that can be used for cells without causing too much cell death.

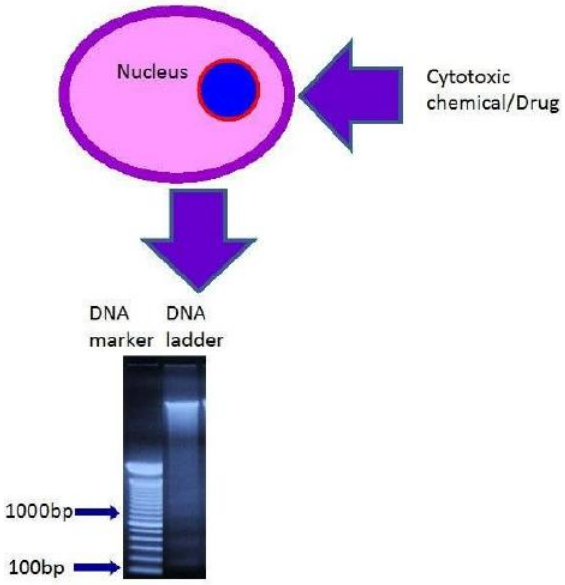

Figure 3.4.1: DNA ladder. Nucleotide base pair mentioned as,“bp”.

4.2: Comet Assay

The Comet Assay (single cell gel electrophoresis /SCGE) is used to detect DNA damage by using a micro gel electrophoresis. The image of the damaged DNA shows a comet with head and tail. The analysis of image for comet assay is calculated for the “tail length” of the comet which is the measurement from the point of highest intensity within the comet head as well as the “tail moment” which is the product of the tail length and the fraction of total DNA present within the tail.

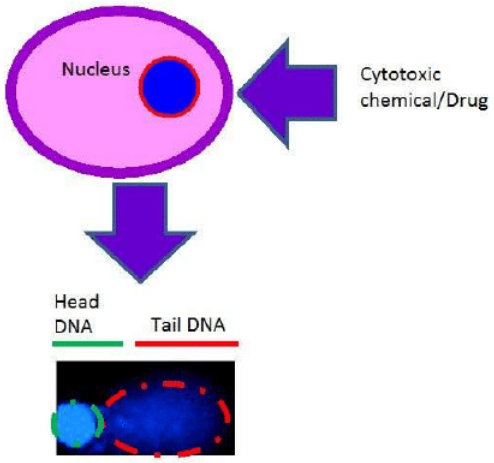

Figure 3.4.2: Comet assay showed amount of damaged DNA by comet tail moment.

4.3: Necrosis Assay

The necrosis assay is performed by flow cytometry analysis with staining of Annexin V and propidium iodide (PI) in the cells. The cells are considered in the stage of necrosis if the cells lose membrane integrity and die promptly due to cell lysis when exposed to chemicals, drugs, toxins or foreign antigens. In necrosis, cells show swelling, loss of membrane integrity and disruption of metabolism. Cells with necrosis do not go to stage of apoptosis, apoptotic cell may undergo secondary necrosis. These necrotic cells will shut down metabolism, lose membrane integrity, lyse and formed cell injury autolysis.

The flow cytometric analysis for necrosis assay showed that the cells stained positive for both FITC Annexin V and PI are in the end stage of apoptosis and are undergoing to the stage of necrosis as dead cells stained PI positive. Cells that stain negative for both FITC Annexin V and PI are alive and not undergoing apoptosis or necrosis.

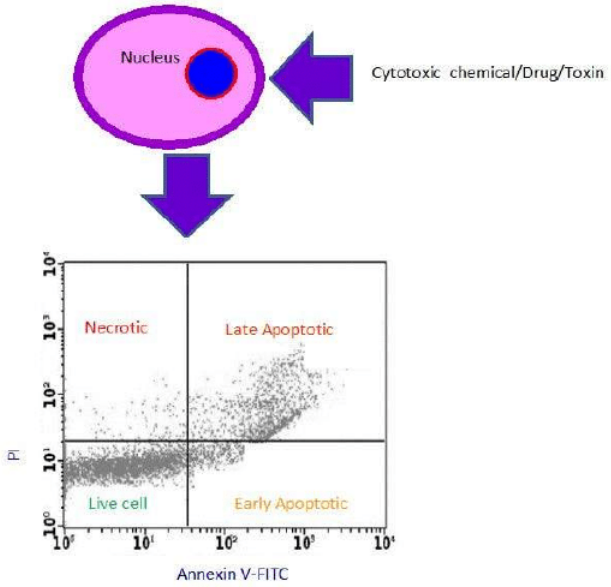

Figure 3.4.3: Flow cytometry analysis of cells showing necrosis stage.

4.4: Enzyme Assay

The enzyme assay is used to monitor passaging of lactate dehydrogenase (LDH), due to loss of cell membrane integrity when cells are exposed to cytotoxic compounds. LDH reduces NAD to NADH which generates a color change by interaction with a specific probe (Figure 4a). In other enzyme assay, Adenosine triphosphate (ATP)-based assay combined with bioluminescent assay are used to measure cytotoxicity of the cells in which ATP is the reagent for the luciferase reaction.

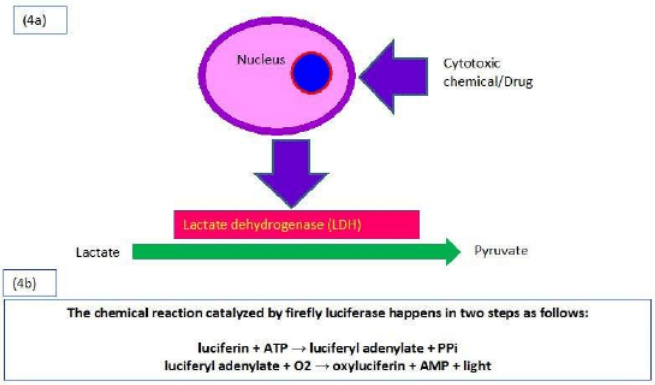

Figure 3.4.4: Schematic presentation of enzyme assay. (a) LDH assay (b) ATP based assay

4.5: Proteomics Assay

The proteomic assay is performed to know the mechanism of cellular toxicity by measuring expression of a specific protein which may consider as a biomarker for particular toxic mechanism or cellular toxicity signaling pathway. Immunofluorescence, immunoprecipitation and immunoblot assay are mainly used to know the effect of toxicants in cellular toxicity signaling pathway or mechanism.

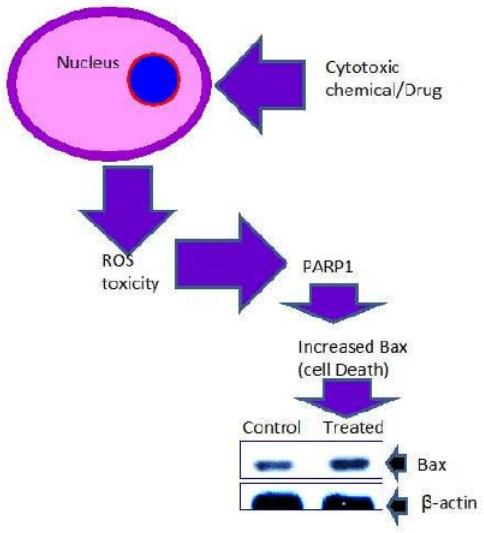

Figure 3.4.5: Schematic presentation of proteomic assay.

4.6: Expression Array Assay

The expression array is the chip based microarray of more gene expressions (finger print of genes) by the effect of cellular toxicants. This is a rapid and sensitive detection method which allows to detect all toxicological end points at wide range of molecular level changes in the cell at single assay. The microarray process can be divided into two main parts. First is the printing of known gene sequences onto glass slides or other solid support followed by hybridization of fluorescently labeled cDNA (containing the unknown sequences to be interrogated) to the known genes immobilized on the glass slide. After hybridization, arrays are scanned using a fluorescent microarray scanner. Analyzing the relative fluorescent intensity of different genes provides a measure of the differences in gene expression.

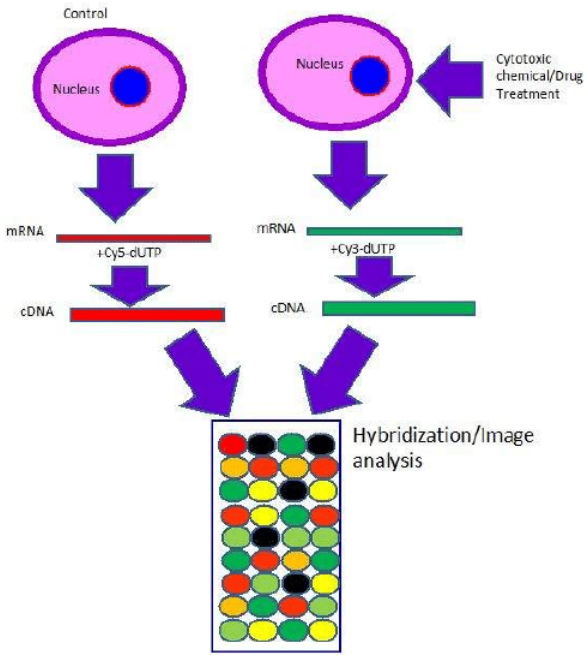

Figure 3.4.6: Schematic representation of gene expression array showed that Cy5(red) and Cy3 (green) labeled cDNA hybridized to a DNA microarray. Yellow spots indicate the genes are expressed in both samples. The intensity and different types of color at each spot indicate the level and presence of genes in samples. Black spots show low level of expression or do not show any expression of genes.

Topic 4: Key Points

In this section, we explored the following main points:

- 1: Different types of cytotoxicity assays.
- 2: How different cytotoxicity assay namely DNA fragmentation/ladder assay, Comet assay, Necrosis assay, Enzyme assay, Proteomics assay, and Expression array assay are used when cells are exposed to cytotoxic agents.

Knowledge Check

1. The cells are considered in the stage of necrosis, if the cells lose membrane integrity and die promptly due to cell lysis when exposed to chemicals, drugs, toxins or foreign antigens.

True

False

Answer

True

2. The Comet Assay is used to detect DNA damage by using a micro gel electrophoresis:

True

False

Answer

True

3. The expression array is the chip based microarray of more gene expressions (finger print of genes) by the effect of cellular toxicants.

True

False

Answer

True

4. Ladder assay are performed for the following reasons: 1) to simply characterize the toxicity of the chemicals or drugs in cells, or 2) to determine the maximum doses of the test chemicals or drugs that can be used for cells without causing too much cell death.

True

False

Answer

True

5. What are the proteomic assays to know the effect of toxicants in cellular toxicity signaling pathway or mechanism?

Immunofluorescence assay

ring

Immunoprecipitation assay

Immunoblot assay

All of the above

Answer

All of the above