Immunohistopathology-its correlation in differentconcentaration of chemical treatment

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Abstract:

There are in vitro cultures of cells obtained directly from the organ of interest.Some cell lines become immortal through a process called transformation, which can occur spontaneously or can be chemically or virally induced. In the cytoplasm by modulation of proteins via interaction of their thiol groups with cisplatin The Avidin-Biotin complex method is a multiple-layer method that relies on the extremely high affinity between Avidin, a glycoprotein from egg white, and biotin, a vitamin.Immunohistochemistry (IHC) is the process used to localize specific antigens in tissue sections with labelled antibodies based on antigen-antibody interactions. imminohistological approach. These all figures from 3-11 shows clear relations ship between chemical modification at several level by Cisplatin and effects on apoptosis. Cells treated with 5µg Drug Cisplatin. The result was that due to the effect of drug the cells showed rounding off and apoptosis.

Keywords : Apoptosis, Avidin-Biotin complex (AHC), Immunohistochemistry (IHC),Chemical modifications.

Introduction

Mammals are comprised of a large variety of cell types with specialized functions, including: -providing structure, scaffolding and support, Signalling and sensing, Digestion and absorption of food and water, The transfer of molecular oxygen, Immunity, Motor function and Reproduction. Cell culture refers to the removal of cells from an animal or plant and their subsequent growth in a favourable artificial environment. The cells may be removed from the tissue directly and disaggregated by enzymatic or mechanical means before cultivation, or they may be derived from a cell line or cell strain that has already been already established.

There are in vitro cultures of cells obtained directly from the organ of interest. Cultures are considered primary until the first sub-culture (where cells are taken into a new bioreactor or flask with fresh medium, often at a lower concentration). Cell cultures may be initiated from normal, embryonic or malignant tissue by aseptic collection from the animal host (NELSON, 1940)

Normal cells usually divide only a limited number of times before losing their ability to proliferate, which is a genetically determined event known as senescence; these cell lines are known as finite. Some cell lines become immortal through a process called transformation, which can occur spontaneously or can be chemically or virally induced. There are three main methods of initiating a culture (Das,2014)

A number of additional properties of cisplatin are now emerging including activation of signal transduction pathways leading to apoptosis. Firing of such pathways may originate at the level of the cell membrane after damage of receptor or lipid molecules by cisplatin, in the cytoplasm by modulation of proteins via interaction of their thiol groups with cisplatin, for example involving kinases, and other enzymes or finally from DNA damage via activation of the DNA repair pathways.

The period from 1880 to the early 1900s saw the first development technique to study the behaviour of cells in vitro. Although Harrison is normally accredited with the development of cell culture as a scientific tool, he described his own work as an extension of Wilhelm Roux.Disaggregation of explanted cells and subsequent plating out of dispersed cells was first demonstrated by Rous.

Most cells from solid tissues grow as adherent monolayer, and unless they have transformed and become anchorage independent, after tissue disaggregation or subculture they will need to attach and spread out on the substrate before they will start to proliferate.

Three major classes of transmembrane proteins have been shown to be involved in cell-cell and cell-substrate adhesion.In a diagnostic laboratory when working with formalin-fixed, paraffin-embedded sections.

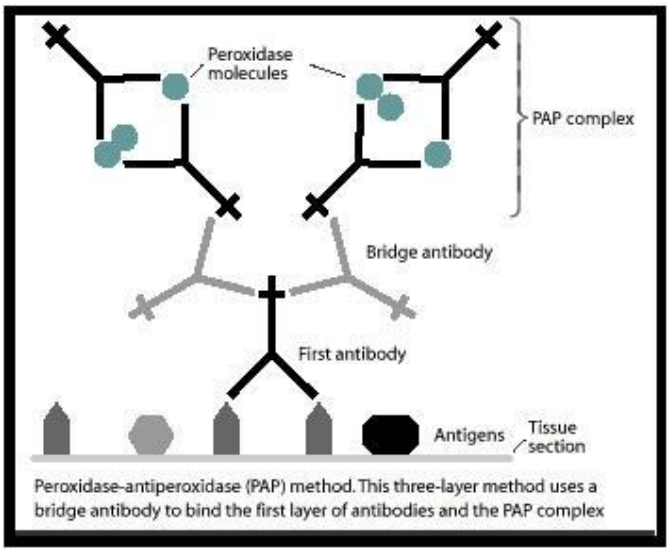

Figure 1: Peroxidase-ant peroxidase (PAP) method

With the PAP method, two first layers of antibodies are similar to the indirect method but are not labelled. The third layer consists of immunoglobulins that recognize (bind) the peroxidase molecules (PAP complex) and they are raised in the same species as for the first antibody layer. Therefore, the second antibody layer will act as a "bridge" between the first and the third layer of antibodies. This method is 100-1000 times more sensitive than the indirect method (due to the increased number of signal-generating molecules per molecule of antigen) without loss of specificity.

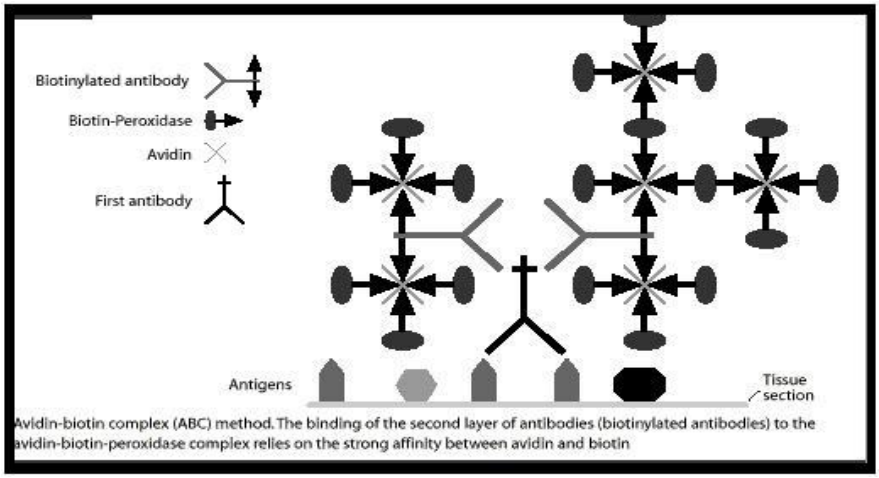

Figure 2: Avidin-Biotin complex (ABC) method

The Avidin-Biotin complex (ABC) method is a multiple-layer method that relies on the extremely high affinity between Avidin, a glycoprotein from egg white, and biotin, a vitamin. In this method, the second antibody is biotinylated and the third layer is a complex of Avidin mixed with biotin that is labelled with a marker.

Materials and methods :

Immunohistochemical staining:(Vincent,2011;
Prescott, 2002)

Immunohistochemistry (IHC) is the process whereby antibodies are used to detect proteins (antigens) in cells within a tissue section (for instance liver, pancreas or the heart). This tool is used to localize specific antigens in tissue sections with labelled antibodies based on antigen-antibody interactions. The immune reactive products can be visualized by a marker including fluorescent dyes, enzymes in general; radioactive elements or colloidal gold.

Immunohistochemistry (IHC) is a simple, yet a highly essential, research and diagnostic tool in the field of molecular biology and medical science. It is a procedure of antigen detection in a tissue by using the principle of antigen antibody reaction. This is the technique by which different tumor markers are detected in tumor samples. The method
of staining tissue sections and detecting antigen in thetissue is calledimmunohistochemistry. Similar process to detect antigen in a cell is called immunocytochemistry.

IHC was performed to locate the protein within a cell for instance in the nucleus, cytoplasm or membrane.

The protocol is as follows:

1. The Slides were appropriately cleaned with 70% Isopropyl Alcohol.
2. Slides were then labelled properly with the cell line name- panc-1, the culture date, with respective time intervals and respective concentration and a slide labelled as control into which no drug was added.
3. After plate had been incubated for 24hrs the Sampling- observation was carried out.
4. The plate was observed under the microscope, following that the sampling was done.
5. Some amount of cell suspension was taken onto the slide.
6. The slides were then observed under the microscope.
7. The Slides were then allowed to dry.
8. After the slides had been properly dried the stained surface was marked with Dako pen.
9. Chilled methanol was then added as a fixative.
10. Slides were then washed with DPBS.
11. Slides were incubated for 1 hour with Blocking Solution.
12. Following this the incubation was carried out with Primary Antibody for 1 hour.
13. The slides were then washed with DPBS.
14. The slides were incubated with Secondary Antibody (Enzyme labelled) for about 1 hour.
15. Then, DAB substrate was added and incubated for 30minutes.
16. The slides were then washed with water.
17. Counter stain Methyl green was then applied onto the slides for 3-4 minutes.

18. Again, the slides were washed with water.

19. The slides were then allowed to dry.

Mounting: The steps are as follows: -(Prescott, 2002; Kuberan,2011)

1. The Slides were dipped into Xylene Solution
2. A drop of DPX Mountant was then put onto the cover slips.
3. The Cover slips were then fixed onto the slides.

Results and Discussion:

The aim of the present research work was to study the effect of Drug on Cell Adhesion molecule and its detection by immunohistochemistry (IHC) technique. For this the cells were treated with the drug at different concentrations and observed at different time intervals.
The cell count obtained was as follows:

1	0	4	0	=	5
1	2	0	0	=	3
0	0	0	1	=	1
2	0	0	0	=	2

Thus the total numbers of cells obtained were 11. So the calculation was as follows:

$$11\times 2500 \times 4 \times 2 = 2.2 \times 10^5$$

The effect of drug at different time intervals and at different concentrations

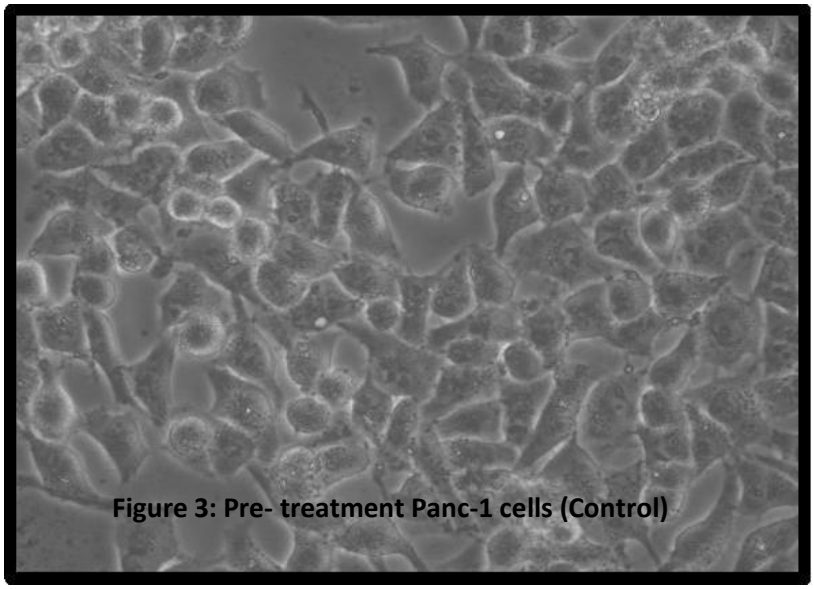

Figure 3: Pre- treatment Panc-1 cells (Control)

Control depicts attached panc-1 cells. The morphology is epithelial-like.

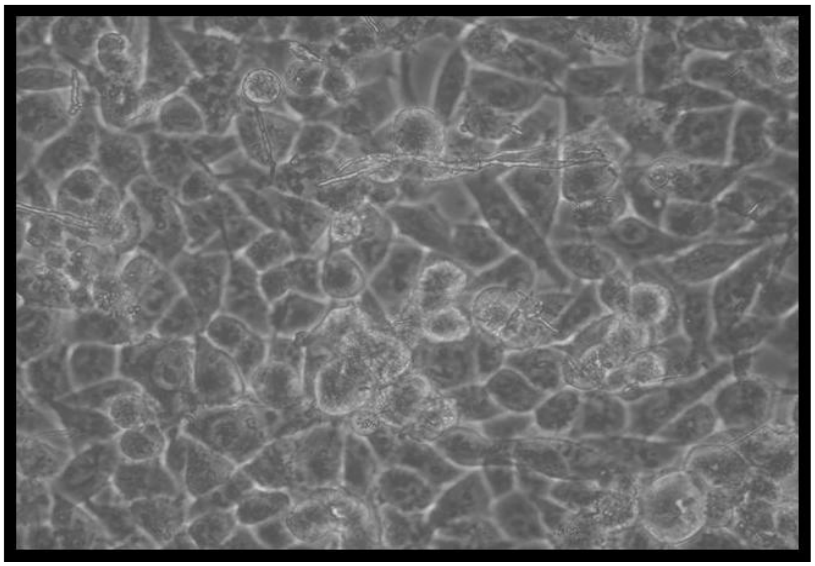

Figure 4: Post treatment Panc-1 cells Cisplatin-1µg

Panc-1 cells treated with 1µg Drug Cisplatin. The result was that most of the cells showed attachment.

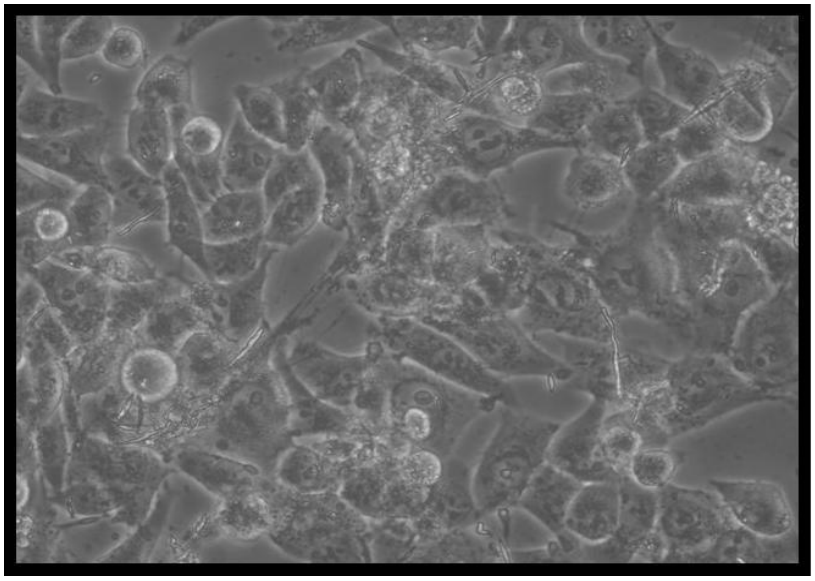

Figure 5 : Post treatment Panc-1 cells Cisplatin-2.5µg

Panc-1 cells treated with 2.5µg Drug Cisplatin. The result was that due to the effect of drug most of the cells showed attachment and rounding off.

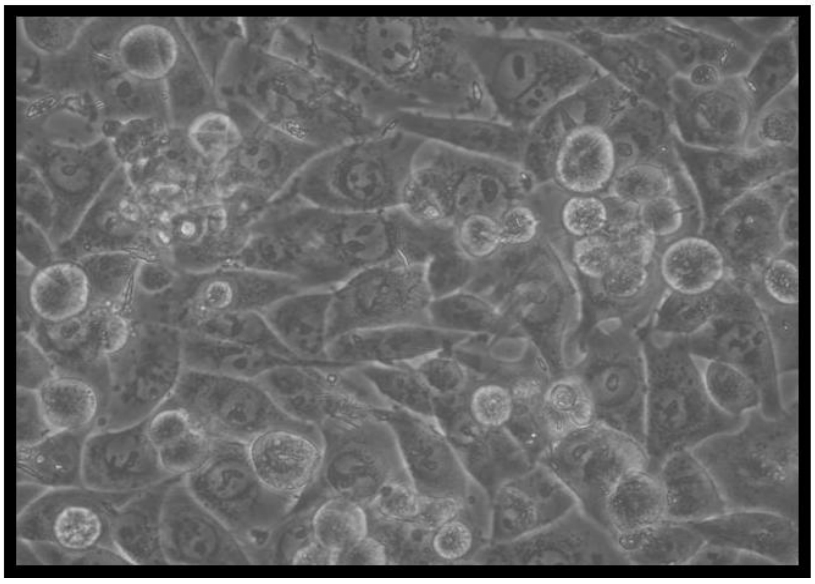

Figure 6: Post treatment Panc-1 cells Cisplatin-5µg

Panc-1 cells treated with 5µg Drug Cisplatin. The result was that due to the effect of drug the cells showed rounding off and apoptosis.

Biomarker assessment assay (IHC) for Panc-1 cells:

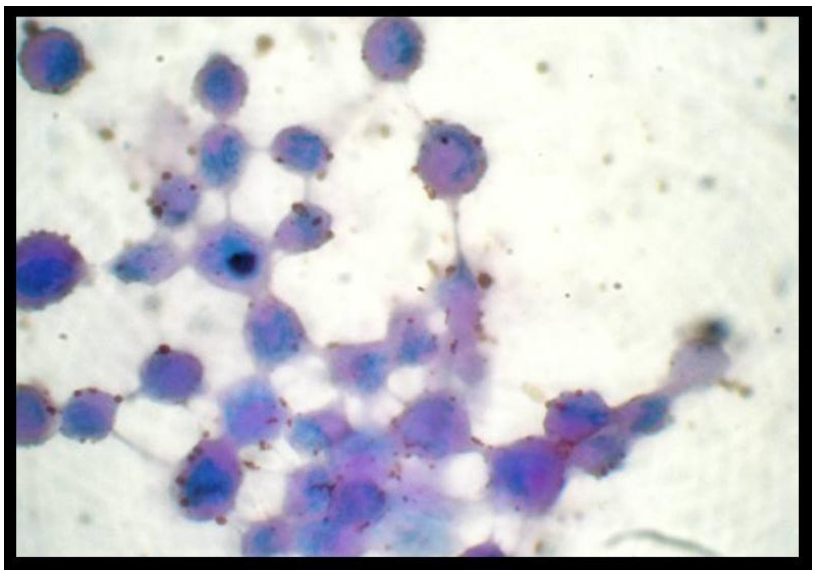

Figure 7 :- Pre treatment Panc-1 cells (Control)

Figure 7depicts the Panc-1 cells without treatment of Drug Cisplatin. Nucleus, Cytoplasmand Membrane appears blue.

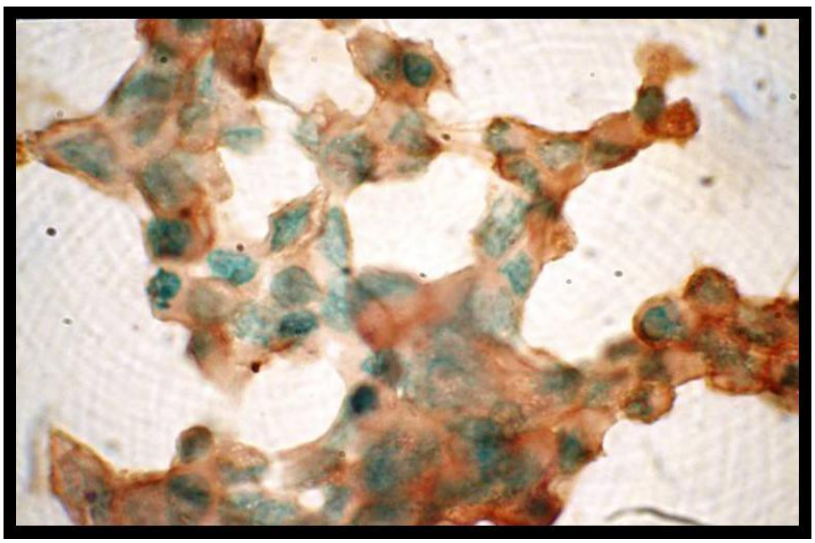

Figure 8:-Post treatment Panc-1 cells Cisplatin-1µg (48 Hours)

Figure 8depicts the presence of expression of the Cell Adhesion Molecule on Membraneand Cytoplasm after 48 hrs due to the effect of Drug (Cisplatin-1µg). Membrane and cytoplasm are stained brown by DAB treatment. The Nucleus appears Blue because of the counter stain Methyl Green.

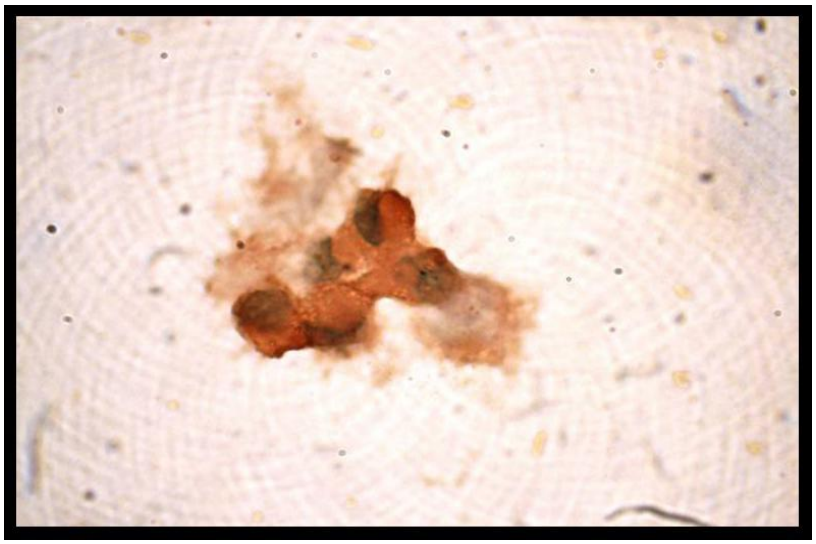

Figure 9:- Post treatment Panc-1 cells Cisplatin-2.5µg (48 Hours)

Figure 9depicts the presence of expression of the Cell Adhesion Molecule on Membraneand Cytoplasm after 48 hrs due to the effect of Drug (Cisplatin-2.5µg). Membrane and cytoplasm are stained brown by DAB treatment. The Nucleus appears light brown.

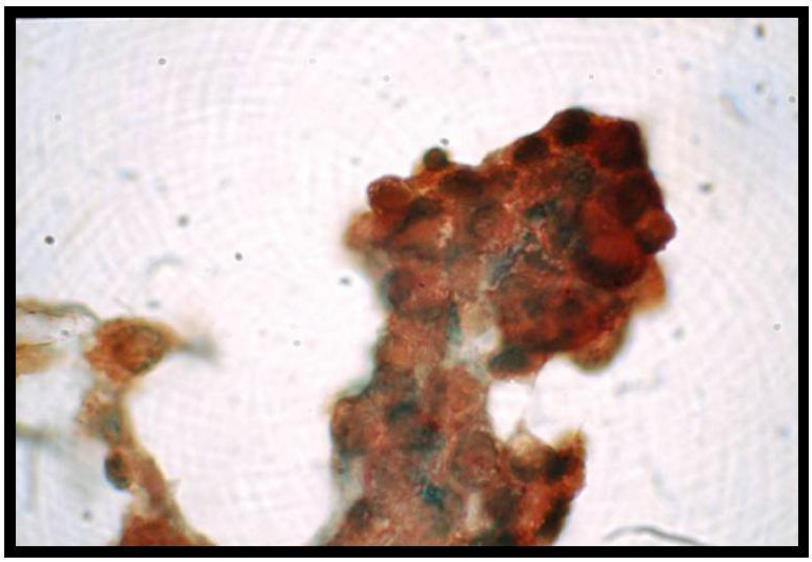

Figure 10:- Post treatment Panc-1 cells Cisplatin-1µg (72 Hours)

Figure 10depicts the presence of expression of the Cell Adhesion Molecule on Membraneand Cytoplasm at time interval of 72 Hours due to the effect of Drug (Cisplatin-1µg). Membrane and cytoplasm are stained brown by DAB treatment. The Nucleus appears Brown.

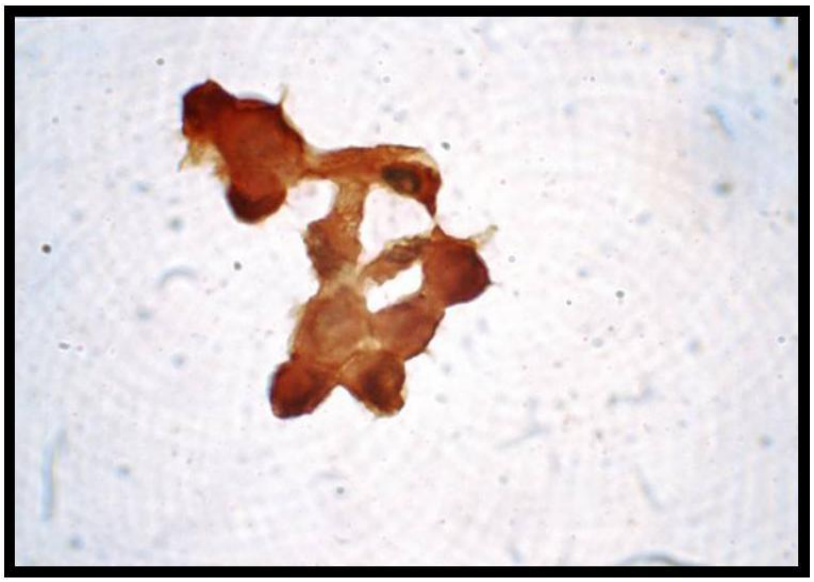

Figure 11:- Post treatment Panc-1 cells Cisplatin-2.5µg (72 Hours)

Figure 11depicts the presence of expression of the Cell Adhesion Molecule on Membraneand Cytoplasm at time interval of 72 Hours due to the effect of Drug (Cisplatin-2.5µg). Membrane, cytoplasm and Nucleus are stained brown by DAB treatment.

Cell culture. HeLa strain human epidermoid carcinoma cells (Scherer et al., 1953) were grown on flying cover slips in Leighton tissue culture tubes. They were cultivated in a nutrient medium consisting of 5 per cent human serum, 20 per cent filtered chick embryo extract (Bryant et al., 1953) and 75 per cent Earle's balanced salt solution (Earle, 1943). These were classical and innovative methods whose fundamentals were used in this study with imminohistological approach. These all figures from 3-11 shows clear relations ship between chemical modification at several level by Cisplatin and effects on apoptosis.

Cell line	Staining	Control	Cisplatin (1 µg)		Cisplatin (2.5 µg)	
Panc-1 (48 hrs)	Cytoplasmic	- ve	Cytoplasmic	+ ve	Cytoplasmic	+ ve
	Membrane	- ve	Membrane	+ ve	Membrane	+ ve
	Nuclear	- ve	Nuclear	- ve	Nuclear	Mild + ve
Panc-1 (72 hrs)	Cytoplasmic	- ve	Cytoplasmic	+ ve	Cytoplasmic	+ ve
	Membrane	- ve	Membrane	+ ve	Membrane	+ ve
	Nuclear	- ve	Nuclear	Mild + ve	Nuclear	+ ve

Conclusion:

Control cells are attached, elongated and shows Cytoplasmic, membrane and nuclear negative for biomarker.Membrane and Cytoplasmic presence of biomarker is gained in presence of DNA damaging agent in dose dependent manner.

References :

- ***BRYANT, J. C***., EARLE, W. R., AND PEPPERS, E. V. 1953 The effect of ultracentrifugation and hyaluronidase on the filtrability of chickembryo extract for tissue culture. J. Nat. Cancer Inst., 14, 189-225.
- ***Das, T.T.,*** Gohel, H.R., Panchal, M.R., Ghosh, S.K., Braganza, V.J. 2014. Determination of crude oil degradation efficiency of glass biofilm of isolated bacterium and fungus. *Int. Res. J. Biol.Sci.,* 3(2): 67 69.
- ***EARLE, W. R.*** 1943 Production of malignancy in vitro. IV. The mouse fibroblast cultures and changes seen in the living cells. J. Nat. Cancer Inst., 4, 165-212.
- ***Kuberan T.,*** Degradation and quantification of polyaromatic hydrocarbon by Pseudomonas sp., *Environ.Sci.,* **6, (2011)**
- NELSON, J. B. 1940 Infectious catarrh of the albino rat. II. The casual relation of coccobacilliform bodies. J. Exptl. Med., 72, 655-662.
- ***Prescott,*** Harley. 2002. Communications Laboratoy exercises in microbiology.

- ***SCHERER, W. F***., SYVERTON, J. T., AND GEY, G. 0. 1953 Studies on the propagation in vitro of poliomyelitis viruses. J. Exptl. Med., 97, 695-710.
- ***Vincent,***Akpoveta, O., Egharevba Felix, Medjor, O. Weltime, Osaro, K. Ize- Iyamu, Enyemike, E. Daniel, 2011. Microbial degradation and its kinetics on crude oil polluted soil. *Res. J.Chem. Sci.,* 1(6): 8 14.