EFFECT OF MERCURY ON CULTURED HUMAN LYMPHOCYTES

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ABSTRACT

Primary animal cells can grow in simple plastic or glass containers in synthetic media but only for limited generations, due to cell apoptosis and cell senses . These cultured cells can be used to study and analyze under certain favorable or unfavorable stress conditions, to understand the complex city of animal physiology. Freshly explanted lymphocytes and haemopoietic cells can grow and differentiate to terminally functioning cells in culture. In this present study we had selected lymphocytes from peripheral blood , because they are easy to maintain and easily grow in laboratory conditions. The culture is primary cell culture being inoculated in media with 2-5% serum in it. After incubation of 24 hours cells were treated with concentrations of mercury ions in the solution which was 0.4mg/ml, 0.2mg/ml and 0.05mg/ml.

Key words: Cell Culture, Mercury, Peripheral blood , Lymphocytes.

I. INTRODUCTION

Lymphocyte is a type of white blood cell (leukocyte) that is of fundamental importance in the immune system because lymphocytes are the cells that determine the specificity of the immune response to infectious microorganisms and other foreign substances (1). In human adults lymphocytes make up roughly 20 to 40 percent of the total number of white blood cells. They are found in the circulation and also are concentrated in central lymphoid organs and tissues, such as the spleen, tonsils, and lymph nodes, where the initial immune response is likely to occur.

Mercury: Mercury is one of the heavy metals found on the earth's crust. Certain heavy metals in small quantities are essential for a healthy life. Heavy metals become toxic when they are not metabolized in the body and hence get accumulated in the soft tissues. Acute mercury exposure may occur in the mining industries and in manufacturing of fungicides, thermometers and thermostats. Symptoms of acute exposure are cough, sore throat, shortness of breath, abdominal pain, nausea, vomiting, diarrhoea, headache, weakness, visual disturbances, tachycardia, hypertension, and a metallic taste in mouth. Chronic exposure to mercury may result in permanent damage to central nervous system and kidneys (2).

Thus in this study effects of mercury ions was seen on the human lymphocytes *in vitro* conditions and different concentrations i.e 0.4mg/ml, 0.2mg/ml and 0.05mg/ml were selected .

II. MATERIAL AND METHODS.

Media Preparation:

5.2gm of RPMI 1640 medium powder was dissolved in 250ml double distilled water with constant gentle stirring until the powder was completely dissolved. The pH 7.2 was adjusted. 1.48 gm of HEPES and 0.625gm of Sodium Carbonate were added to form the buffer reaction. Medium was sterilized by 0.22 micron membrane filter with the help of 1 ml syringe. 4ml of Amphotericin (250µg/ml in .75% saline) was added in medium as antibiotic. 2% of human blood serum was added to the medium prior to use.

Preparation of Reagents:

Buffer Solution: phosphate buffer solution was prepared by dissolving 0.144g of di sodium hydrogen phosphate (anhydrous Na_2HPO_4) and 0.24g of potassium di hydrogen phosphate (KH_2PO_4) along with 0.8 gm of sodium chloride and 0.02 gm of potassium chloride in 100 ml distilled water.

Giemsa Stain (Stock Solution): Giemsa stock solution was prepared by adding 0.76 gm od Giemsa powder to 50ml glycerol and 50 ml of methanol. The solution was heat stirred at 60^{o}C for 30 min over a magnetic heat stirrer. The stain was filtered and stored at 4^{o}C in an amber glass bottle.

Giemsa stain (Working solution): 5ml of giemsa stock was added to 40 ml of distilled water followed by addition of 5 ml of HBSS.

High Saline Solution: 10gm of sodium chloride was dissolved in 100 ml of water to prepare 10% high saline solution.

Sample Collection: Sample was collected with the help of lab technician from subject after his/her informed consent as per the ethical norms. 3ml of EDTA treated blood was collected with a disposable syringe.

Setting up of the Lymphocyte Culture:

The washed EDTA treated blood sample were aseptically transferred into a sterile culture vial with 5ml of RPMI 1640 medium each supplemented with 1-2% of human blood serum(3). Culture were incubated for 24 hrs to check the viability and for any contamination. After 24 hrs no contamination was observed, and cells appeared healthy .Culture were then treated with different concentrations of mercury chloride i.e 0.4mg/ml, 0.2mg/ml and 0.05mg/ml . The culture was incubated in a desiccator for 72 hrs following the protocol based on (1 and 4).

Harvesting Of Cultured cells :

After 72hrs of incubation the cell suspensions were transferred into labeled eppendorf tubes and are centrifuged at 1000 rpm for 10 min. The supernatant was discarded and the pellet was treated with warm 0.075M KCl as hypotonic solution by gentle flushing and mixing. It was incubated again for 45 minutes at 37^{o}C. It was centrifuged carefully at 1000rp for 15 minutes. The supernatant was removed and 5ml of fresh cornoy's fixative was added for washing the cells.

Cell Viability Assay: The number of viable cells in a sample is determined by staining the sample with 0.4% tryphan blue stain. The sample and the stain are taken in the ratio 1:1 and mixed gently. Then the sample is loaded to Neubauers chamber and observed and counted under the microscope at 40x resolution as described by (5).

Preparation of Slides:

Slides were prepared by air drop method. Cleaned frosted slides were dipped in methanol and chilled prior to slide preparation. Each slide was marked with identification number. Slides were dried at room temperature(6).

Staining Of Slides:

The nucleus of lymphocytes was stained with giemsa working solution for 5minutes. Extra stain was removed by single rinse with distilled water based on the protocol given by (7).

Observation of Slides:

All the slides were observed under 40x objective lens of microscope to observe the cells and changes in their morphological structures.

III. RESULT AND DISCUSSION:

After 72 hours of incubation cells were harvested and slides were prepared to observe morphological details of cells. It was observed that as the exposure of heavy metal concentration was increased *in vitro*, the cell viability decreased. In the lower concentrations (Fig 3.), it was found that the cell membrane of cultured lymphocytes became irregular, and the nucleus became distorted when compared to control cells . In higher concentration sample (Fig 4.), the cells looses their viability and the nucleus was disrupted which may be due to per-oxidation mechanism which leads to depletion of free radicals hence inhibition of cell activities. Fig 5 represents the cells stained with tryphan blue stain at 24 hrs of incubation.

IV. CONCLUSION

Heavy metals depletes free radicals present in culture media which impairs cellular activities due to per oxidation mechanism and leads to disintegration of cellular membranes.

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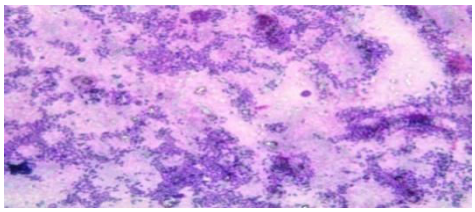

Fig 1.Microphotograph at 40X of Control culture

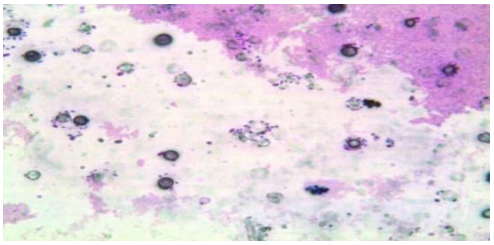

Fig 2. Microphotograph at 40X ofUnstained lymphocytes treated with 0.05mg/ml of mercury

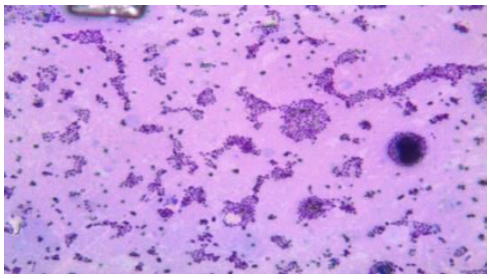

Fig 3. Microphotograph at 40X of Stained cells treated with 0.05mg/ml of mercury

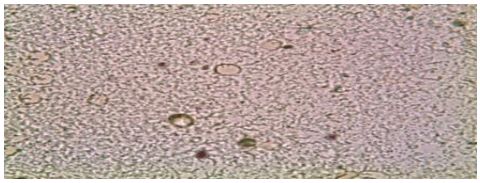

Fig 4. Microphotograph at 40X of dead lymphocytes treated with 0.4mg/ml of mercury

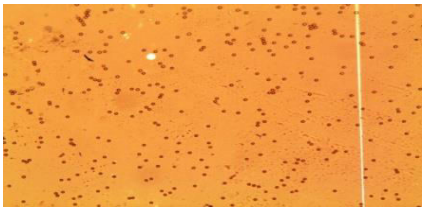

Fig 5. Microphotograph at 40X of Tryphan blue Viability assay