In vitro genotoxicity of pyridine in human lymphocytes

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Abstract: This work was carried out to study the genotoxicity of pyridine *in vitro* on human leucocyte culture. Cyclophosphamide, a well-known carcinogen was used as positive control. The four different concentrations of pyridine and cyclophosphamide showed breaks and pulverization of chromosomes in dose dependent manner. Higher number of pulverization was observed with higher concentration of pyridine (3.25µg/mL). Based on this data, our results confirm that both pyridine and its precursor showed genotoxicity against human lymphocytes.

Keywords: Pyridine, Human leucocytes, Genotoxicity, Chromosome damage.

INTRODUCTION

The increase in population in recent years has called for an increase in productivity. This, in turn, requires more industrialization, and it has resulted in a major leap towards progress. However, one unfortunate impact of this massive industrialization process is the deleterious effect it has created on the environment, and thereby, it is threatening the very existence of mankind on earth. Most of the raw materials that are being used in industries are toxic or hazardous in nature (Adeola 2011). Organic chemicals varying from simple hydrocarbons aliphatic and aromatic to those containing heteroatom, like O, N, S, P, etc and polycyclic ring compounds that are widely used in various industries have toxicological manifestations (Zeliger 2008). Heterocyclic compounds have been estimated to represent more than one third of all organic compounds on the earth, and pyridine and its derivatives account for a sizeable fraction of all heterocyclic compounds (Thummel 2008). The gasification of coal and oil shale results in the production of pyridine in the environment (Stuermer *et al*., 1982; Sims and O'Loughlin 1989). The compound is mobile in soil and persists in ground water (Stuermer *et. al*., 1982; Sims and Sommers 1985; Sims *et al*., 1986; Sims and O' Loughlin 1989). Due to its acute toxicity and apparently teratogenicity, it is possibly dangerous to health (PHE 2015). Therefore, thoughtful consideration of its environmental fate is vital. Its related compounds are anthropogenic in nature, once they enter the environment, they may remain for long periods, and impart an undesirable odor and taste and reduce the potability of ground water. Due to its teratogenic nature, pyridine is listed as one of the major organic pollutants by United States Environmental Protection Agency (USEPA) (Richard & Shieth 1986) and its removal from waste water prior to disposal is essential.

The exposure of humans to pyridine could be through skin or eye contact or by inhaling or ingesting it (PHE 2015). One of the many serious health issues cause by pyridine exposure is liver damage. In addition, skin and eye irritations, kidney damage and nervous system depression, are other health concerns associated with pyridine exposure. Therefore, the current study was undertaken in order to investigate the *in vitro* genotoxic effects of pyridine using peripheral blood lymphocytes.

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MATERIALS AND METHODS

Non-citrated blood from healthy AB donors was obtained and allowed to clot. The serum was separated and inactivated at 56^{o}C for 15mins. It was then filtered through a Seitz filter and stored at -20^{o}C in small aliquots for further use.

Preparation of Human Lymphocytes

For *in vitro* testing, human peripheral blood was collected from five healthy individuals (male and female), about twenty years of age. The individuals were not associated with any disease and had not taken any medicine in the last six months. The blood and the chromosomal medium B, which contains Phyto-hemagglutinin, antibodies, FBS, and heparin, were incubated at 37^{o}C.

Chromosome preparation

The lymphocyte cultures were incubated at 37^{o}C for seventy-two hours. Pyridine and cyclophosphamide were added in different concentrations forty-eight hours after initiating the culture. Prior to two hours of cell harvesting, 0.06µg/mL of colchicine was added to each set in order to arrest cells in a metaphase stage. Further, cells were subjected to hypotonic treatment using 0.4% KCl solution for 15 minutes at 37^{o}C followed by fixing two to three times in cold methanol: Glacial acetic acid (3:1v/v) solution at room temperature. The cells were centrifuged and spotted onto clean slides for visualization. The slides of positive and negative control were coded independently before analysis under a microscope. A minimum of two-hundred well-spread metaphases were scored per concentration and control, equally divided among duplicates, if applicable. If a high number of

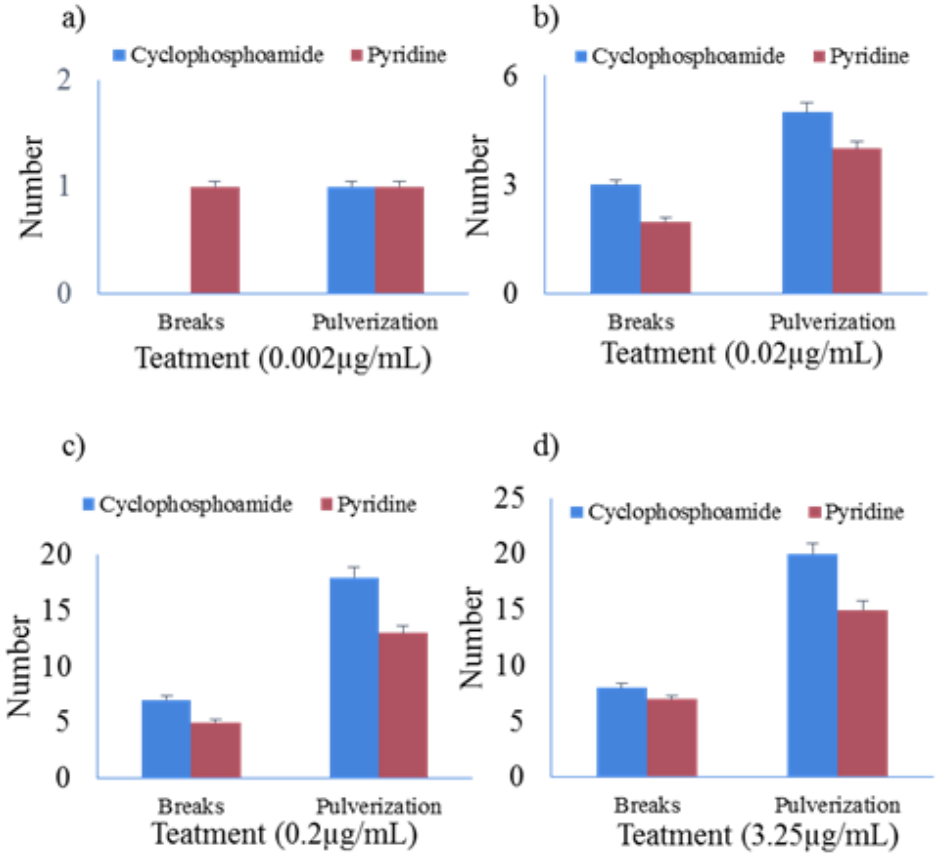

Fig. 1: Effect of cyclophosphamide and pyridine on chromosomal breaks and pulverization.

aberrations were observed, then the number could be reduced. However, the purpose of test was to determine structural chromosomal aberration. Moorhead *et al*., (1960) and Preston *et al*., (1987a; 1987b) described methods were used to prepare macro cultures of peripheral human blood lymphocytes. In a blind test, from five individuals, over one-hundred metaphases were analyzed from each control and treatment culture.

RESULTS

The genotoxicity of the pyridine was determined by studying its effects on human leucocyte culture. In the present investigation, the pyridine was introduced to a human leucocyte culture. Cyclophosphamide a proven mutagen was used as a positive control. Genotoxicity screening of the chemicals was employed in order to check its effects on chromosomal aberrations *in vitro*. The negative effects predict the potential carcinogenicity for humans. In this work, both non-carcinogenic and carcinogenic compounds were tested. The protocol for the two end-points was developed using the carcinogen cyclophosphamide. By using human leucocyte cultures, both the cyclophosphamide and pyridine showed chromosomal aberration, few breaks, and pulverization. Rossner *et al*., (2005) reported the chromosome breaks and pulverization with cyclophosphamide and pyridine, because both have similar toxicity against the normal chromosome.

The results obtained with the infusion of a human blood sample with pyridine shows the metaphase cells with the positive control. Fig. 1 shows the effect of various concentrations of pyridine and cyclophosphamide on chromosomal aberrations. Fig. 1a shows that no breaks were obtained in cyclophosphamide with 0.002µg/ml of concentration. Fig. 1b shows that a similar number of breaks and pulverization were obtained with 0.02µg/ml of concentration of both pyridine and cyclophosphamide. Fig. 1c shows that there was a greater number of pulverization in both the positive and negative controls when 3.25µg/ml of concentration was used.

Based on the current results, both pyridine and cyclophosphamide have similar breaks and pulverization with various concentrations. At lower concentrations, less number of breaks and pulverization were observed; however, greater number of breaks and pulverization were observed with higher concentration. This indicates that high concentrations of pyridine compound are toxic to human chromosomes.

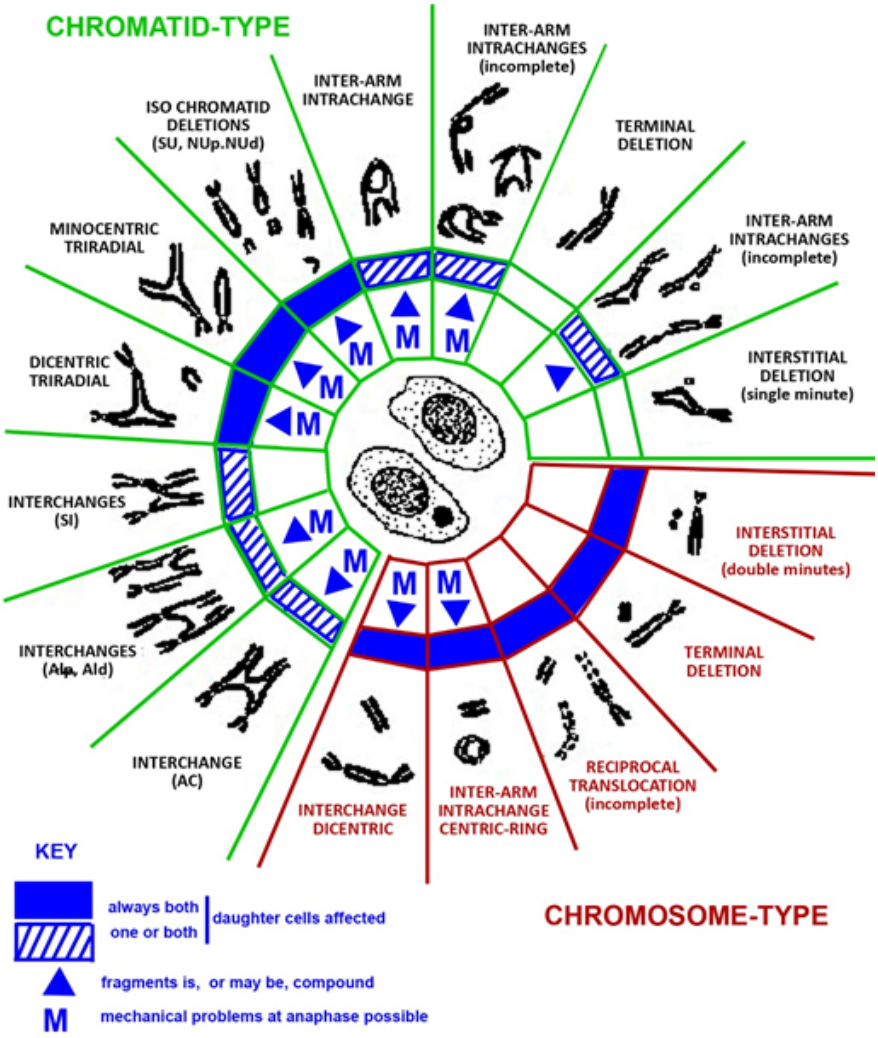

Fig. 2: Proposed chromosomal aberrations that contributes acentric fragments (Savage JRK, 2000).

The results of this study shows that all concentrations of pyridine and cyclophosphamide showed chromosome breaks and pulverization. Higher concentration of pyridine (3.25µg/ml) causes relatively more pulverization. This shows that both have similar toxic effects on normal human chromosomes. Therefore, an occupational limit of exposure to pyridine was set up by the Occupational Safety and Health Administration (OSHA) as 5ppm in workplace air for an eight-hour workday over a forty-hour workweek.

DISCUSSION

The positive results of the *in vitro* chromosomal aberration test indicates that the substance used causes the structural chromosome aberration in cultured cells. However, the negative results indicate that the induced substance does not induce chromosome aberration in cultured cells (Gerald and Edward, 1989). There are many reports, which indicate that pyridine damages the liver, and pyridine ranks eleventh out of 1,416 on the national priorities list of the U.S. Environmental Protection Agency (EPA). Hence, exposure to pyridine compounds should be controlled. It seems inevitable to avoid the risks associated with this class of compound to the humans who are under its constant exposure. Fig. 2 depicts the principal structural chromosol aberrations that subsidize acentric fragments to form micronuclei (Savage, 2000). Fragment loss causes genetic imbalance and further leads to ultimate cell death. This may affect either both or one daughter cell. Due to frequent aberration per cell, the probability of both daughter cells being affected increases. A vast majority of chemical clastogens produce chromatid-type structural changes.

There is evidence, which suggests that the increase in the frequency of chromosomal aberration in peripheral blood lymphocytes could predict cancer. Metaphase cells have been routinely used for decades as an approach to screen occupational and environmental acquaintances to genotoxic carcinogens.

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