

Cytogenetic Effects of Benzene on Human Blood Cells

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Abstract

The study aims to investigate the cytogenetic effect of benzene on human blood cells in vitro using chromosomes abnormalities and mitotic index test. Different concentrations of benzene were added to human blood culture at 24 hour, then cells were arrested at metaphase to detect chromosomes malformations and its proliferation, the result show that benzene causes increased in mitotic index level and different aberrations in chromosomes which increased with benzene concentrations.

Keywords: Benzene, Chromosomes abnormalities, Mitotic index.

1. Introduction

Benzene is an important pollutant compound, present in both occupational and general environment. Chronic exposure to high concentrations of benzene in human is associated with an increased incidence of myelodysplastic syndrome (MDS) and acute myelogenous leukemia (AML) (Baudouin *etal.*,2002). It is well known that individuals occupationally exposed to benzene are at a much higher risk of developing leukemia than the normal population (Carere *etal.*, 1998). The absorption, distribution, metabolism and excretion of benzene have been intensively investigated in several experimental animal species and in humans. Benzene is readily absorbed from oral and inhalation exposures. Dermal absorption is also rapid; however, quantitatively, dermal absorption is very low due to rapid evaporation from skin. Benzene is rapidly distributed throughout the body after exposure by all routes, and accumulation in fatty tissues is observed (ATSDR. 2007).

In studies of occupational exposure, benzene was found to cause chromosome changes at concentrations that induced blood dyscrasias (Weisel,2010). At concentrations below (31 ppm), workers exposed for 10 to 26 years had significantly more chromosome breaks and gaps in peripheral lymphocytes than found in controls, and 31 of the 33 workers had no other evidence of clinical or hematological effects (Rappaport,*etal.*,2009).

At exposure levels of less than (10 ppm) over one month to 26 years, workers also had a significantly higher number of chromosomal aberrations in peripheral lymphocytes than controls (Khalade,*etal.*,2010). Benzene has been also implicated as an environmental risk factor in leukemia and other hematological diseases. The main sources of environmental exposure to benzene are road traffic exhaust (Zhang,*etal.*,2006) and volatile organic compounds; this means urban air pollution in general (Bi,Y. *et al.*,2009). Lifestyle factors, such as cigarette smoking, can contribute to exposure (Eastmond *etal.*,1994). The soil obtained from oil production facilities and coastal refineries is also highly contaminated by benzene (Eastmond *etal.*,2001).

2. Materials and methods

2.1. Blood samples: Blood samples were collected from 25 healthy (male and female) in Age (20±5 years), that was not directly exposed to benzene and they are smoker.

2.2. Blood culture: Blood planting according to (Chen *etal.*,1994), then plant of blood divided in to five gropes every gropes had 5 replicates.

2.3. Treatment: after 24 hours from incubating, 300 µl of different benzene Concentrations was added to blood culture.

1- First group (2.5×10^{-5}) molar

2-Second group (5×10^{-5}) molar

3-Third group (10×10^{-5}) molar

4-Fourth group (20×10^{-5}) molar

5-Fifth group without any addition of benzene and this group is the negative control. Then cytogenetic tests were performed according to (Clare *etal.*,1984).

3. Results

Treatment human blood cells with different concentrations of benzene causes increased in mitotic index as shown in table (1), also causes different chromosomal abnormalities as shown in table (2) and figure (1, 2).

4. Discussion

The association between benzene exposure and the appearance of structural and numerical chromosomal aberrations in human lymphocytes suggests that benzene may be considered as a human clastogen. In animal studies, benzene induced cytogenetic effects, including chromosome and chromatid aberrations, sister chromatid exchanges, and micronuclei (Clare *et al.*, 1984, Liu *et al.*, 2000, Liu *et al.*, 2003) There is some evidence that benzene can induce chromosomal abnormalities in mammalian cell cultures (Marcon *et al.*, 1999). Metabolites of benzene (hydroquinone, catechol, diol epoxides and trihydroxybenzene) induced sister chromatid exchanges in V79 cells (Zhang, L. *et al.*, 2010) several metabolites, including muconaldehyde, have induced micronuclei in cell cultures (Ji, Z. *et al.*, 2010).

Benzene can induce structural and numerical chromosome aberrations, sister chromatid exchanges and micronuclei by various routes of exposure (North, M. *et al.*, 2009). Most studies were performed with fairly high concentrations, but (Badham *et al.*, 2010) detected sister chromatid exchanges in peripheral lymphocytes and micronuclei in the bone marrow of rats at 9.6 and 3.2 mg/m³, respectively (Badham, H. J. *et al.*, 2010). were able to detect chromosome aberrations in lung macrophages after prolonged exposure (6 weeks) at concentrations as low as 0.32 mg/m³, and in lymphocytes from the spleen of mice at 0.13 mg/m³ (Shuga. *et al.*, 2010). However, there was no dose–response relationship in the latter study, as the highest exposure (3.2 mg/m³) produced fewer aberrations than the middle exposure (32 mg/m³).

The chromosomal effects in these studies are evident at concentrations of around 320 mg/m³ (100 ppm) or higher, but in some studies effects were reported in workers chronically exposed to levels of around 32 mg/m³ (10 ppm) (Gillis, *et al.*, 2007). Sarma *et al* (2011) reported that the frequency of chromosome aberrations decreased when exposure levels decreased from 3–69 mg/m³ to 1–18 mg/m³ (Sarma, *et al.*, 2011). In the study by. Marti'nez-Vela'zquez, M *et al* (2006) a decrease in sister chromatid exchanges but not in chromosomal aberrations was noted in a group of female workers when examined with a 5-year interval during which the mean benzene concentration had decreased from 26 to 16 mg/m³ (Marti'nez-Vela'zquez, M. *et al.*, 2006). Smoking did not influence the results (Sasiadek ., *et al.*, 1998).

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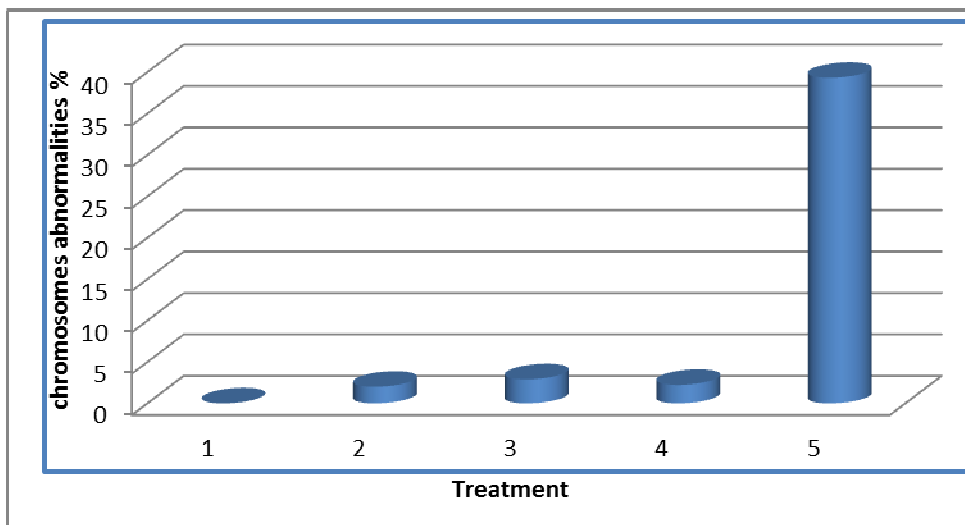


Figure (1): Percentage of chromosomes abnormalities in human blood cells that treated by different concentration of benzene. (1) NC, (2) 2.5×10^{-5} , (3) 5×10^{-5} , (4) 10×10^{-5} , (5) 20×10^{-5}

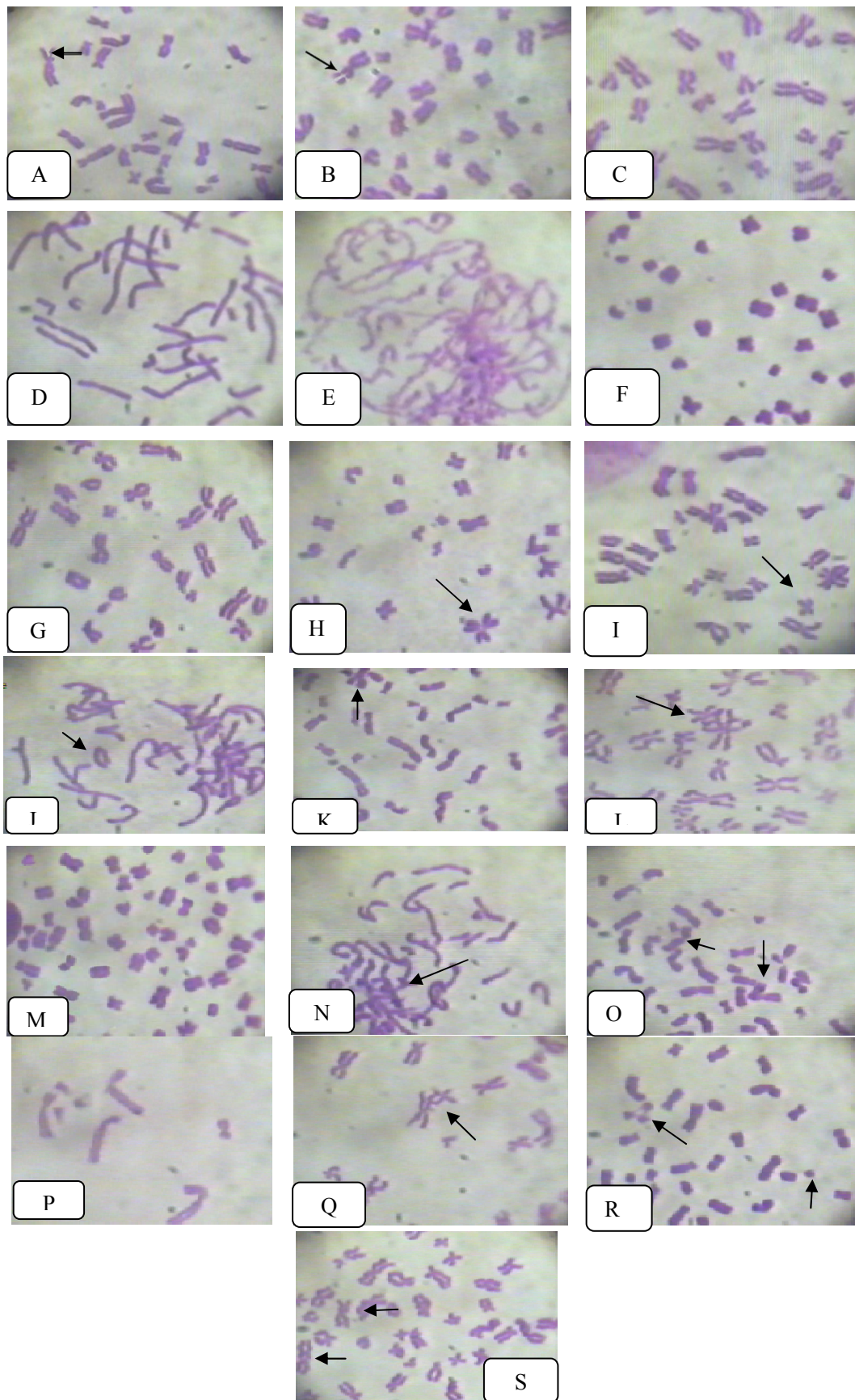


Figure (2) chromosomes abnormalities in human blood cells treated by (10×10^{-5} and 20×10^{-5} molar) of benzene.

(A) Chromatid break. (B) Chromosomal break. (C) Chromatid deletion. (D,E,F) Bizarre configuration. (G,J) Ring chromosome. (H,I) Centromeres stick. (K,L,N) Chromosome stickiness. (M) Hyperploidy. (O,Q) End association. (P) Aneuploidy. (R) Minute chromosome and (S) Polycentric chromosomes.

Table (1) effect of different concentrations of benzene on cell mitotic index

Treatment	MI
Negative control	8.64
2.5×10^{-5}	10.6
5×10^{-5}	10.6
10×10^{-5}	10.6
20×10^{-5}	13.45

Table (2) Differential chromosomal abnormalities in human blood cell that treated by different concentration of benzene.

Treatment	Polycentric chromo	Minute Chromo.	Chromo. stickiness	Ring Chromo.	End to end association	Deletion of chromatid	Bizarre configuration	Deletion	Centromeric association	aneuploidy	Hyperploidy	chromatid break	Chromo. break
N. control	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
2.5×10^{-5}	0	0	0	0	0	0	0	0	0	0	0	0	0
5×10^{-5}	0	0	0	0	0	0	0	0	0	0	0	0.6	1.3
10×10^{-5}	0.1	0.1	4	2	0.5	3.7	5.3	4	2.5	1	0.6	3.2	2
20×10^{-5}	0.1	0.2	2.3	3	0.6	5	4.3	2.3	3	1.3	1.6	7.3	5.6

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