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Pathological and Cytogenetically Study on the Toxic Effect of Inhaled Benzene among Male Rats

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Abstract

Thirty six Swiss albino male rats, in 12 weeks study exposure whole body (inhaled) to benzene at concentration of 0 , 400 ppm , 800 ppm = (0 , 672 mg / m 3 , 1.343 mg / m^3) respectively, 5 hr. / day for 4 days / week for 12 weeks. Cytogenetic examination by micronuclei (MN) assay and chromosomal aberrations (CA) of the three groups at 6 and 12 weeks of exposure, were significantly increased $P<0.01$ specially in 800 ppm group at 6 weeks of exposure, Exposure rats to benzene reported clinical signs characterized by hypoactivity and convulsion .Histopathological lesions revealed neoplastic lesion in bronchi lung with liver adenocarcinoma than those observed in historical control.

Keywords: Benzene, Cytogenetic, chromosomal aberrations, micronuclei assay, inhalation, Histopathological lesions

Introduction

Benzene is a representative single-ring aromatic compound which ubiquitously distributes in our environment due to its high volatility and extensive emission sources. Benzene occurs naturally in crude oil and gas emission from volcanoes or forest fires(Zhang *at el*.,2017).It is a colorless, flammable liquid with a sweet odor and it evaporates quickly while exposed to air (WHO,2020). There is a chance for occupational exposure since the continuing presence of benzene in the industry as solvent s and glues (Dimitriou , 2019). It can be absorbed in the body through inhalation, ingestion, or skin contact; however, is an important route of exposure (Bahadar *et al*., 2014).According to the International Program on Chemical Safety(IPCS), benzene is one of the ten most azardous substances for public health and is one of the most common environmental pollutants (Smith, 2020) At the same time, the vast majority of studies assess the negative impact of benzene on human health in terms of its hemotoxicity and carcinogenicity (Snyder,2002) Acute inhalation of benzene occurs rarely and may cause toxicity which further leads to lethal reactions if untreated. Its results in serious organ system dysfunction. Most solvents are easily absorbed from the blood into lipid-rich tissues and can cause widespread damage. (Sharapova *et al.,* 2020).Inhalation of benzene vapor in rats led to neurotoxicity represented by behavioral changes, weight loss, and low levels of dopamine. Furthermore, renal and liver toxicity, changes in the blood picture, and

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increased signs of harmful lipids were evidenced. The study recommends conducting further studies on experimental animals to know the detailed mechanisms of the effects of benzene and try to find ways to prevent them**.**(Alshareef and Ibrahim,2020)
Micronucleated and chromosomal aberrations assay can indicate the clastogenic activity and it is mutation assays in both in vivo and in vitro in chemical poisoning indicated in benzene toxicity present of pathological lesion mostly foreign body granuloma in hepatocyte and kidney in exposed rat. (Okuno , 2005) .Respiratory toxicity and asphyxiation have been observed following acute inhalation of benzene by man (Kuznetsova *et al*.,2019). Benzene exposure may lead to subclinical and prepathologic early hepato and nephro malfunction in humans (Neghab *et al*., 2015).
Stott *et al*.,2003 suggesting that significant changes may appear in mouse liver, region specific hepatocellular hypertrophy, and tissue parenchyma including liver cirrhosis and fibrosis. Increases in mitotic figures and S-phase synthesis, and metabolic adaptation indicate formation of a toxic metabolite and subsequent regenerative cell proliferation. In mouse lungs, increases in S-phase DNA synthesis and loss renewal of metabolic capacity in bronchiolar epithelium indicate a population shift, likely in Clara cells, again suggesting formation of a toxic metabolite and regenerative cell proliferation. (Schlaich and Weber,2004)

Materials and Methods

Animals groups: 36 Swiss albino male rats in 12 weeks study exposed whole body (inhaled) for two concentration of benzene as following:
1st group (control group): consist of 12 rat's diet on normal rat pellets.
2nd group: 12 rats exposed whole body to 400 ppm benzene = (1.28 0.672 mg /m^3) benzene in blocked cage for 5 hrs./day for 4 days/week with whole body exposure for 12 weeks.
3rd group: 12 rats exposed whole body to 800 ppm benzene = (1.34 mg /m^3) benzene in blocked cage for 5 hrs./ day for 4 days /week with whole body exposure for 12 weeks .
The animal groups above tested at 6 weeks and 12 weeks of experiment using the following parameters:

1. Cytogenetic examination :

A. Micronuclei (MN): Blood samples were collected from tail vein of animal groups at weeks 6 and 12 .The blood was taken in heparinised tubes. Venous blood was cultured in RPM/ 1640 medium with 15 % fetal calf serum .PHA and Leukocytes from " buffy coat " layer were treated with colcemid (0.6 µg / ml) before incubation .Vincristine (0.01 µg / ml) was added 24 hr. after beginning of incubation period 96 h, the smear of cells on slides dried with air and stained with Giemsa. (Balasem *et al.*,1990)

B. Chromosomal aberrations (CA): Cells were treated with calcemid (Sigma) at the final concentration of 0.05 µg / ml, 4 hr. before fixation. Then the cells were collected by trypsinization , washed and hypotonized with 0.075 M KCL solution for 8-12 min. at 37Cº. (Balasem ,1991)

2. Histopathological examination (HE)

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Bronchi, Lung and Liver tissue were taken at 12 weeks of experiment for HE. The tissue were fixed in Bounis solution (75 ml picric acid , 25 ml (40 %) formalin , 5 ml glacial acetic acid) (Luna ,1968) ,The slides were prepared by classic histokinate technique. , then stained with haematoxylin and eosin stain.

3. Clinical signs: clinical signs were observed in animal groups' every day for 12 weeks.
4. Statistical analysis: least squares method was used for analysis of data program based on the u- test, at $P<0.01$.

Results

1. Cytogenetic examination

The 3rd group which exposure to 800 ppm showed an increase in the number of MN in binucleated Lymphocyte compared to 2nd group which exposure to 400 ppm at 6 and 12 weeks (figure 1) and the increase was highly significant $P<0.01$ at week 6 while control group didn't showed significant increase. Table (1)

Table (1) : Numbers of MN in binucleated lymphocytic cells of rats exposure to (400 and 800) ppm benzene

Weeks of exposure	Group	No. of MN cells	No. of MN	MN/cell
6 weeks	1st group control	14	1	0.07
	2nd:400ppm Benzene	19	16	0.82
	3rd:800ppm benzene	25	37	1.48
12 weeks	1st group control	23	3	0.13
	2nd:400ppm Benzene	19	14	0.67
	3rd:800ppm benzene	27	18	0.74

Number of animal: 12 rats, $P<0.01$

2.Chromosomal aberrations:

The both inhaled groups induced chromosomal and chromatid aberrations mostly breaks which were more significant in 3rd group (800 ppm exposure) after 6 weeks of exposure figure (2) , Table (2) :

Table (2) :Chromosomal aberrations in exposure rats / weeks

Weeks of exposure	Groups	Cell with CA		No. of cells		Per/ 100 chromosome
		No.	%	total	%	
6 weeks	1st group control	7	7	7	0.07	0.0035

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	2nd:400ppm Benzene	22	22*	109	1.09*	0.054*
	3rd:800ppm benzene	48	24*	76	0.38*	0.018.9*
12 weeks	1st group control	13	6.5	19	0.095	0.0047
	2nd:400ppm Benzene	28	28*	49	0.49*	0.0225*
	3rd:800ppm benzene	28	28*	73	0.73*	0.0343*

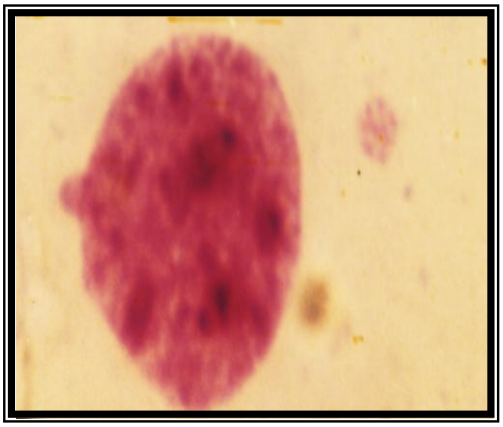
Number of animals: 12 , * = P<0.01

3.Clinical signs: clinical signs in inhaled groups included hypoactivity ruff , ataxia , convulsion with a neuron pale animal at week 12 .

4.Histopathological examination:

The histopathological lesion showed sever change at 800 ppm exposure rats characterized by lung focal emphysema and diffuse proliferation of alveolar septa were exhibited , figure (3) .

Bronchi and bronchioli adenoma were presented with multifocci granuloma figure (4) : Livers in both doses of exposure showed hemosidrosis fig(5)with liver carcinoma characterized by increase mitotic figures , fibrotic stroma with presence of basophilic cells different in shape cuboidal to oval shaped kind of cells were present in bile ducts which indicated liver adenocarcinoma figure 6 :Figure 7 .



Figure(1) Rat lymphocyti Micronuclei 800 ppm exposure at 12 week (X 400 :Giemsa stain)

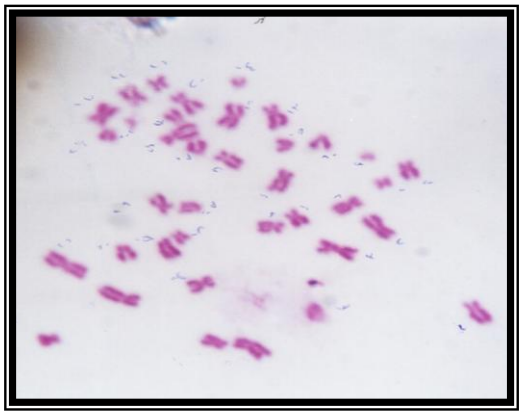


Figure (2) Rat chromosomal aberrations (breaks) 400 ppm exposure at 6 week (X100 :Gimesa stain)

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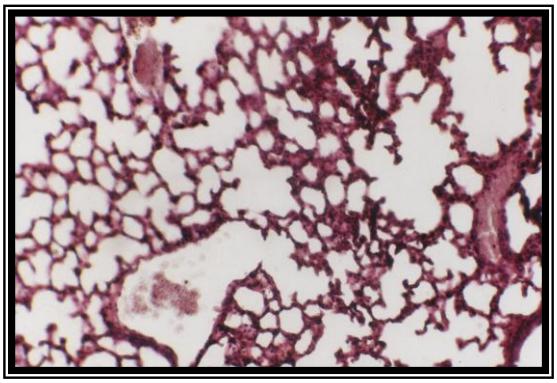

Figure (3) :Lung of rat 800ppm benzene exposure focal emphysema with diffuse proliferation of alveolar septa (X40:H & E stain)

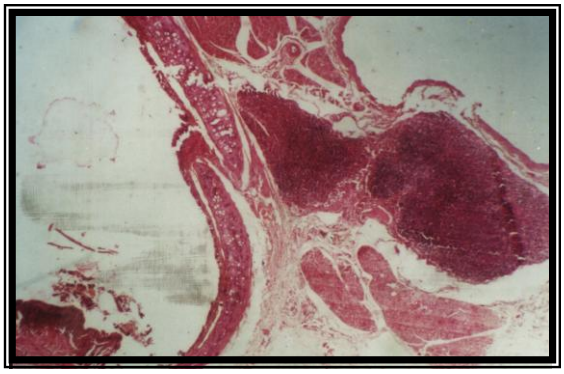

Figure (4): Lung of Rat 400 ppm benzene exposure bronchi and bronchioli presented multifocal granuloma (X40 :H & E stain)

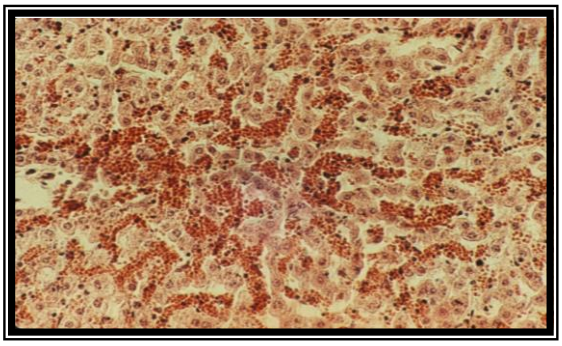

Figure (5) : Liver of rat 800 ppm benzene exposure showed liver hemosidrosis (X40 :H & E stain)

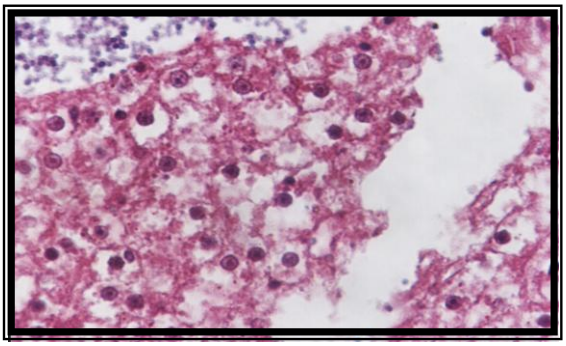

Figure (6) liver of rat 800 ppm benzene exposure Carcinoma charachterized by basophilis masses of cubodiol to avoid shape cells (X40 :H & E stain)

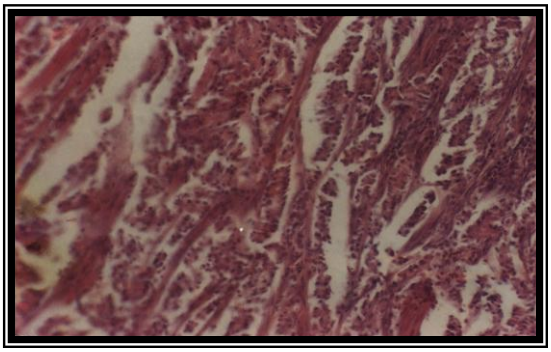

Figure (7): Liver of rat 800 ppm benzene exposure Adenocarcinoma in liver bile duct (X 40 :H & E stain)

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Discussion

Inhalation of benzene results in serious organ system dysfunction. Most solvents are easily absorbed from the blood into lipid-rich tissues and can cause widespread damage (Rana *et al*., 2004) Benzene is well absorbed by inhalation (Sehgal ,1998). Systemic absorption of benzene from the lungs is greatest over the first few minutes of exposure (70 – 80% of inhaled dose) and decreases thereafter to approximately 50% of the inhaled dose after one hour (U.S.EpA, 2003). It has been suggested that MN and CA could be used as biological dosimeter .The number of MN in control group is not far from (Molls, 2004) MN, It's higher in number and frequency in one lymphocyte specially in 800 ppm exposure and in 6 weeks, the overall data indicate that benzene is a point mutagen and interfere with CA (Natarajan and Obe,1984). Micronucleus assays can indicate gene mutation assay in benzene inhalation (Luna, 1968). And they were dose related and may indicated tissue carcinogenesis by presented neoplastic lesion in lung and liver mostly adenocarcinoma, the tumor incidences at 400 and 800 ppm and slightly higher in 800 ppm exposure (Williams *et al*.,2004) workers reported incidence of liver adenoma and fore stomach squamous hyperplasia at 200 ppm in males rat which exposed to benzene for 4 weeks(Sehgal , 1998) . In mice, increased liver weights were accompanied by hepatocellular hypertrophy , mitotic figures, S-phase synthesis, and enzyme activities .S-phase synthesis rates in terminal bronchiolar epithelium were elevated and accompanied by loss of MFO activity., suggest a mode of tumorigenesis dependent upon increased cell proliferation and altered population dynamics in male rat kidney and mouse liver and lungs(Stott *et al*.,2002). Recently, some studies have shown the association between air pollution and lung cancer such as, Khorrami *et al*., (2021) the first to investigate the effect of single and multiple ambient air pollutants on lung cancer in Iran. The findings suggest that ambient air pollutants, especially p-xylene, o-xylene, ethylbenzene, benzene, m-xylene and TBTEX were associated with lung cancer. Previously several studies have also shown a strong association between air pollution and respiratory mortality and respiratory diseases including chronic obstructive pulmonary disease, asthma, bronchitis, and decreased lung function.

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Conclusion

Over all the results of current study consistently appeared that swiss albino male rats inhaled to benzene at (6 and 12) weeks of exposure recording pathological and cytogenetically at 6 weeks of exposure, causing neoplastic lesion in lung bronchi in addition liver adenocarcinoma. It may develop into cancer at less than a month.

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