Original Article

Estimation of DNA Damages, Cytotoxicity and Antioxidant Status of Heavy Metals and Benzene among Petrol Workers in Baghdad-Iraq

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Abstract

This study was conducted to estimate the genetic damages and cytotoxicity of heavy metals (Zn, Cu, Pb) and benzene on the Al-Dorah refinery and petrol stations workers in Baghdad/Iraq. The results showed no significant differences (P 0.01) between the levels of these heavy metals in the serum of both groups and control. Lead (Pb) showed a significant level in the refinery workers serum (0.92±0.043 ppm). The toxicity of lead and benzene on workers was clearly seen in the genetic parameters used in this study. The mitotic index (MI), nuclear division cytotoxicity index (NDCI) and micronuclei (MN) were significantly high (8.02±0.32, 4.1±0.67, 10.63±2.63, respectively) in the exposed workers in the refinery workers and workers with a long exposed period (12.23±0.29, 6.2±0.95, 12.5±1.75, respectively). The results also showed a correlated increase between NDCI and binucleated ratio and between NDCI and MN where the NDCI increased with the increasing of binucleated cells percentage and mitotic index in the exposed groups indicating a double activity in the lymphocytes cell division and increasing in DNA damages. This made, these parameters are as a good indicators to estimate the genotoxicity in lymphocytes. Apoptotic and necrotic cells were also recorded in high numbers in the exposed groups. LDH and ALP enzymes were also detected in a high levels (P=0.01) in the exposed groups (387.73±34.00, 20.92±7.03, respectively). We conclude from these results that heavy metals and benzene have toxic and mutagenic effects upon petrol workers especially those with long period exposure.

Keywords: Cytotoxicity, Benzene, Micronucleus, LDH, ALP, Mitotic index

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Introduction

Heavy metals are chemical elements that have a specific gravity at least five times that of water (Abdull-Wahab, 2004). Some heavy metals, such as zinc, copper, iron and manganese, are required by the body in small amounts, but these elements can be toxic in larger quantities. The main threats to human health from heavy metals are associated with exposure to lead, copper, zinc, cadmium, mercury and arsenic (Lidsky and Schneider, 2003). These metals have been extensively studied and their effects on human health regularly reviewed by international bodies such as the WHO (WHO, 2001). Heavy metals toxicity is well documented and is recognized as a major environmental health risk through out the world (Fairhurst, 2003). They affect humans and animals of all ages, but the effects of lead are most serious in young children. Their poisoning results from the interaction of the metal with biological electron-donor groups and with essential cations, particularly calcium, iron, and zinc (Abdull-Wahab, 2004) or with anions (i.e., carbonate, hydroxide and oxalate) negatively charged moieties on macromolecules such as proteins (Bachanek et al., 2000). Up to 50% of inhaled inorganic heavy metals may be absorbed in the lungs (Anetor et al., 2003). Adults take up 10-15% of them in food (Bachanek et al., 2000). They bound to erythrocytes, and elimination is slow and principally via urine and some of them like lead accumulated in the skeleton, and is only slowly released from this body compartment (Guallar et al., 2002). Workers may come in contact with heavy metals in a number of occupational settings such as petrochemical plants, petroleum refineries, benzene stations and many others. Approximately 1% of benzene is absorbed from skin contact (Bloemen et al., 2004). However dermal absorption is enhanced and may approach 5% when skin is cracked, blistered or abraded as in rubber workers engaged in tire building (Kalnas and Teitelbaum, 2000). Benzene itself is not toxic. It must be broken down by enzymes in the liver into metabolites that are potentially toxic. The major toxicity observed has been on the blood-forming cells of the bone marrow (Hayes et al., 2001). Occupational exposure to heavy metals associated with petrol industries can lead to chromosome abnormalities, the formation of micronuclei, sister chromosome exchanges and enzymes disturbance (Eastmond et al., 2001). The purpose of this study is to investigate the effect of zinc, copper, lead and benzene exposure on chromosomes, formation of micronuclei, some blood parameters and the level of lactate dehydrogenase (LDH) and alkaline phosphatase (ALP) in Aldorah refinery and some benzene stations.

Methods

Subjects

The study was conducted on Al-Dorah refinery and different area of Baghdad city petrol stations from July 2008 to March 2009. The study included 60 workers (30 from the refinery and 30 from the benzene stations) who exposed to benzene for 1-37 years and 20 healthy volunteers from Baghdad university students serve as control group. None of them had clinical evidence of disease that would affect the parameter to be measured.

Measurement of heavy metals

The levels of serum zinc-Zn, copper-Cu and lead-Pb for all subjects were measured in the

biology department of the Science College-Baghdad University by using atomic absorption spectrophotometer-Perkln Elmer-USA.

Blood culture

Peripheral blood (5mL) using heparinised syringes were collected from workers and unexposed men. Blood (0.5 mL) was added to 5 mL of standard supplemented RPMI 1640 medium containing 20% fetal calf serum and 2% of phytohemagglutinin (PHA). Two cultural tubes were used for each sample, one for micronuclei assay and the other for cytogenetic purposes. The tubes were cultured at 37 °C and after 44 hrs, 4 ug of cytochalasinB/mlL (Sigma Chemical Co.) was added to culture tubes of micronuclei experiment (Fenech, 2000). 100 microliters of colchicines (0.45 mg/mL) was added to the cytogenetic culture tubes just before 2 hrs of cell harvesting. Cells from all culture tubes were harvested after 72 hrs by centrifugation and after a mild hypotonic treatment with 3 mL 0.075 M KCl at 4 °C .The cells were precipitated by another centrifugation. The supernatant was discarded, the cells re-dissolved with remaining hypotonic solution and fixed with 5 ml fixative solution (Methanol: Glacial acetic acid, 3:1). Centrifugation and fixation were repeated four times at intervals of 20 min. Slides were prepared by the air-drying method. The slides were stained the following day for 10 min in 10 mL 5% buffered Giemsa solution, pH 6.8. Two slides were prepared for each sample. Abnormalies were determined in 500-1000 cells per individuals. The MI, NDCI values and binucleated cells ratio were calculated according to (Fenech, 2000; Carere et al., 1995; Yadav and Seth, 2001). Differences between groups were assessed using a t-test.

Measurement of the levels of LDH and ALP

The serum which was collected from each blood samples were used for estimation of serum LDH and ALP level. The level of LDH was estimated according to Decker and Lohmann-Matthes (Decker and Lohmann-Matths,1988) using LDH kit while ALP level was estimated according to Hope (Hope, 1966) by using ALP kit.

Blood picture

RBCs, WBCs and platelets were measured in using standard laboratory method. Blood film was also prepared for every samples,stained with leshmina stain,dried and blood cells abnormalities were examined under microscope (100X).

Results

Heavy metals levels

Zn, Cu and Pb levels in the exposed workers and unexposed control were shown in Table 1. There are no significant differences between the level of these heavy metals in the serum of petrol station workers and control while lead showed a significant level in blood serum of the Al-Dorah refinery workers (0.92±0.043 ppm). This elevation was higher in the serum of the workers with exposure period more than ten years.

Genotoxicity of heavy metals and benzene

The toxicity of heavy metals and benzene on workers was clearly seen in the mitotic index (MI) was shown in Table 3, nuclear division cytotoxicity (NDCI) was shown in Table 4, and micronuclei (MN) was shown in Table 5. The MI, NDCI and MN in exposed workers were significantly higher ($P<0.01$) than those in control. It was maximum level in Al-Dorah refinery workers and in the workers with a high exposure period (more than

10 years). The only exception of this correlation is the high level of MN recorded in the Al-Dorah refinery workers with exposed period 1 to 5 years. The frequency of cells with more than one nucleus in both exposed groups showed a significant increase in the NDCI and in binucleated cells ratio compared to control (Table 4). NDCI result in Table 4 and MN values in Table 5 showed also a correlated significantly increase in both exposed groups. The correlated increased between NDCI and binucleated ratio and between NDCI and MN indicating a double activity in the lymphocytes cells division and increasing in DNA damages which represent a good value to measure the genotoxicity of the heavy metals and benzene. This DNA damage can be seen in the value of the necrotic and apoptotic cells recorded in both workers groups where high numbers of these cells were detected (22 and 14; 8 and 12, respectively) (Figure 1). The increasing levels of the genetic parameters detected previously in both exposed groups showed to not correlated with blood parameters used in this study where normal values were seen in all exposed groups and control (Table 6) except that abnormal blood cells were seen in two cases from the refinery (one case with eosinophilia and the second case was with target cells). But the serum LDH and ALP levels have a significant increase (38.7±6.01 U/ml and 20.92 ±1.87, respectively) ($p<0.001$) in both exposed groups compared with control (204.95±14.90 and 12.5±0.27, respectively).

Table 1 The levels of Zn, Cu and Pb in the serum of Al-Dorah refinery workers, petrol station workers and unexposed control

Exposure time (yrs)	Number of samples	Range and mean ± SD(ppm) levels of		
		Zn	Cu	Pb
Al-Dorah refinery workers				
Control / Unexposed	20	0.61-1.01	0.31-0.56	0.01-0.13
		0.84 ± 0.04	0.27 ± 0.03	0.071 ± 0.01
Exposed	30	0.64-0.96	0.37-0.58	0.07-0.21
		0.79 ± 0.02	0.44 ± 0.13	0.92* ± 0.043
>5	10	0.69-0.76	0.39-0.42	0.09-0.121
6-10	6	0.64-0.71	0.37-0.39	0.08-0.13
11.20	6	0.72-0.82	0.43-0.79	0.12-0.16
21-30	2	0.79-0.96	0.46-0.51	0.16-0.20
30<	6	0.84-0.89	0.49-0.58	0.12-0.21
Al-Dorah refinery workers				
Control / Unexposed	20	0.61-1.01	0.31-0.56	0.01-0.13
		0.84 ± 0.04	0.27 ± 0.03	0.071 ± 0.01
Exposed	30	0.64-0.92	0.32-0.39	0.06-0.08
		0.83 ± 0.12	0.33 ± 0.1	0.07 ± 0.01
0-3	26	0.69-1.09	0.32-0.36	0.06-0.07
4-7	4	0.78-2.13	0.34-0.39	0.07-0.08

* $p < 0.01$

Table 2 Mitotic Index (MI) in lymphocytes of Al-Dorah refinery, petrol station workers and unexposed control

Al-Dorah refinery workers				
Exposure time (yrs)	**Number of samples**	**Number of examined cells (n1)**	**Number of metaphases (n2)**	**mean ± SD n2/n1x100**
Control / Unexposed	20	22700	960	4.23 ± 0.41
Exposed	30	3880	3100	8.02* ± 0.32
<5	10	1838	1210	6.58 ± 0.51
6-10	6	6010	670	8.17 ± 0.23
11.20	6	5600	685	12.23 ± 0.29
21-30	2	3200	219	6.84 ± 0.56
>30	6	7150	631	8.82 ± 0.62
Al-Dorah refinery workers				
Control / Unexposed	20	22700	960	4.23 ± 0.41
Exposed	30	36183	2040	5.63* ± 0.62
0-3	26	30	1850	5.57 ± 0.32
4-7	4	2983	190	6.36 ± 0.31

* $p < 0.01$

Table 3 The frequency of cells with 1,2,3,4 nuclei, necrotic, apoptotic cells and nuclear division cytotoxicity index (NDCI) in Al-Dorah refinery, petrol station workers and unexposed control

Al-Dorah refinery workers									
Exposure time (yrs)	**Number of samples**	**Mono**	**bi**	**Tri**	**Tetra**	**Binucleated cells ratio**	**Necrotic cells**	**Apoptotic cells**	**NDCI Mean ± SD**
Control/ Unexposed	20	1150	287	44	63	18.58	00	00	2.39 ± 0.36
Exposed	30	1296	1098	413	318	35.13	22	14	4.1* ± 0.67
<5	10	439	235	58	81	28.9	3	5	4.8 ± 0.69
6-10	6	261	183	68	78	31.55	2	1	6.2 ± 0.95
11-20	6	253	251	130	58	36.27	2	2	4.2 ± 0.76
21-30	2	82	195	61	29	40.68	5	3	5.2 ± 0.46
>30	6	271	311	96	72	41.46	8	6	5.4 ± 0.58
petrol station workers									
Control/ Unexposed	20	1150	287	44	63	18.58	0	0	2.39 ± 0.36
Exposed	30	1319	899	290	299	32.02	8	12	3.38* ± 0.54
0-3	26	998	682	234	246	31.31	8	11	3.54 ± 0.62
4-7	4	321	217	56	53	33.33	0	1	2.9 ± 0.81

* $p < 0.01$

Table 4 The distribution and number of micronuclei (MN) in lymphocytes of Al-Dorah refinery, petrol station workers and unexposed control

Exposure time (yrs)	Number of samples	Distribution of micronucleus 1MN	2MN	3MN	Total	Main micronuclei per 1000 binucleated Mean ± SD
Al-Dorah refinery workers						
Control / Unexposed	20	169	2	1	172	8.6 ± 1.21
Exposed	30	263	50	6	319	10.63* ± 2.63
<5	10	92	11	4	107	17.7 ± 1.48
6-10	6	63	6	1	70	11.6 ± 2.89
11-20	6	41	8	1	70	8.33 ± 1.45
21-30	2	16	9	0	25	12.5 ± 1.75
>30	6	51	16	0	67	11.16 ± 2.68
petrol station workers						
Control/ Unexposed	20	169	2	1	172	8.6 ± 1.21
Exposed	30	288	17	3	308	10.26 ± 0.50
0-3	26	248	8	2	258	9.92 ± 0.34
4-7	4	40	9	1	50	12.5 ± 0.43

* $p < 0.01$

Table 5 Blood parameters of Al-Dorah refinery, petrol station workers and unexposed control

Exposure time (yrs)	Number of samples	Counts of blood cells/ range-ccm/ mean RBC-x10^{12}	RBC-x10^{2}	Platelet x10^{9}	Observations
Control / Unexposed	20	3.9-6.5	42-108	150-380	
			82	280	
Exposed	55	2.9-5.3	48-106	165-350	
		4.2	84	290	
<5	20	2.96-4.98	52-98	175-402	Eosinophilia count=
		3.67	89	360	443 in one case
6-10	16	3.23-4.97	48-102	156-368	
		3.87	95	345	
11-20	12	4.2-6.1	56-105	201-402	
		4.8	96	389	
21-30	3	4.8-5.7	64-97	189-390	+Target cell in one case
		4.37	89	286	
>30	4	3.2-4.1	45-108	157-268	
		3.7	86	245	

Normal values were seen in all exposed and control.

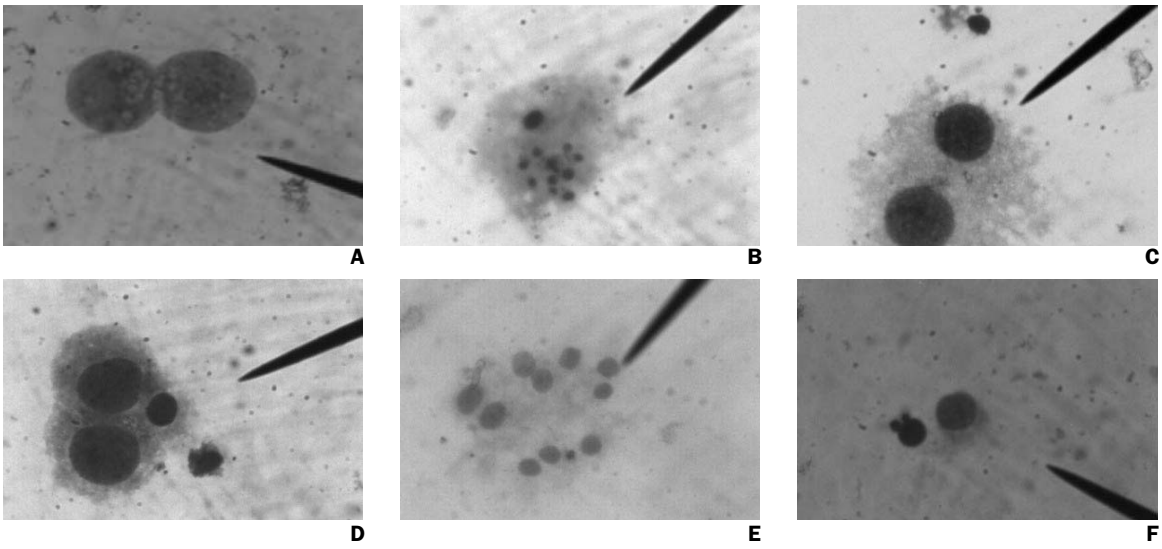

Figure 1 Hemopoietic pictures showing binucleated (A, C), Micronuclei (D, F), necrotic (B), and apoptotic (E) cell (100X).

Table 6 Serum LDH and ALP levels in Al-Dorah refinery, petrol station workers and unexposed control

Groups	Levels of (U/mL)*	
	LDH	ALP
unexposed	204.95, 14.90, 42.15	12.5, 0.26, 0.75
Exposed	387.73, 6.01, 34.00	20.92, 1.87, 7.03

$p < 0.01$ *mean ± S.E.;S.D.

Discussion and Conclusion

Many heavy metals such as lead, copper and zinc were often implicated in human poisoning (Lidsky and Schneider, 2003). Small amounts of these elements are common in our environment and diet and are actually necessary for good health, but large amounts of any of them may cause acute or chronic toxicity (poisoning). Heavy metals become toxic when they are not metabolized by the body and accumulate in the soft tissues. Their toxicity can result in damage central nervous, blood composition and many organs (Bachanek et al., 2000; Mortada et al., 2001). For some heavy metals, toxic levels can be just above the background concentrations naturally found in nature. As a rule, acute poisoning is more likely to result from inhalation or skin contact of dust, fumes or vapors, or materials in the workplace. In the current study, we try to assess the genotoxicity of lead, copper, zinc and benzene on the petrol station and Al-Dorah refinery workers by detecting several bio-parameters. All genotoxicity parameters used in

this investigation showed evaluated values in the exposed workers as compared to control. The MI and NDCI values of the exposed groups were increased significantly. Also the binucleated cell ratio of this group showed double values as compared to control. These three parameters, MI, NDCI and BN ratio gave no dought provment that the benzene contaminated with lead inhaled by the exposed groups have mutagenic activity. This mutagenic activity turned to a great toxicity in the exposed groups with long period exposure where the NDCI value was decreased which indicating cell division disturbances. These findings are in conformity with results obtained by other authors (Fairhurst, 2003; Fenech, 2000; KirschVolders et al., 2003; Kim et al., 2004). Among all parameters used in the cytogenetic approaches, the MN assay using the cytokinesis-block method has shown remarkable and clear results. All exposed groups had a homogenized increase in MN frequency as compared to control. On other hand a great variation was detected in the individual results of each exposed groups unlike the control group, where the individual data were nearly homogeneous. These results shown that the benzene contaminated with lead inhaled by the exposed groups increased the number of BN cells containing more than 1MN/1000BN, while only 3 out of 20 controls showed more than 1MN/1000BN which reflect lead and benzene high genotoxicity. Also the MN frequency results showed a positive association with the exposed periods, where is the highest MN values were a attributable to those with exposed periods ranging from 2 to more years in petrol stations workers and those ranging from 1-5 years and 30 to more years of the Al-Dorah refinery workers. The cytogenetic damage in exposed humans has been reported by many authors and most of published papers are restricted to occupational exposures to benzene (KirschVolders et al., 2003; Kim et al., 2004; Pitarque et al., 2002; Biro et al., 2002). According to the MN results and the NDCI, MI detected in the exposed groups, it could be possible that a correlation found between the elevated values of the MN and the detected NDCI and MI, which would be interesting to confirm. Blood parameters have shown normal values in both tested workers groups except the presence of high count of eosinophils and target cells in two refinery cases. Also a high counts of necrotic and apoptotic cells detected in the refinery workers reflect the higher genotoxicity in Al-Dorah refinery workers than those in petrol stations workers and that may due to the potential toxic saturated environment in the refinery. Whether the genotoxicity detected in the refinery workers due to benzene or lead is not clearly known. However, the genotoxicity detected in petrol stations workers with the presence of normal heavy metals values in their serum refer that the toxicity in both groups may be due to benzene and the higher toxicity in the refinery workers may due to a high exposure not to benzene only but to lead too which make them always at risk. Benzene appears to produce its haematopoietic affects in a number of pathways. Benzene itself not toxic but its toxicity due to its metabolities such as trans-trans mucondialdehyde, phenol, hydroquinone, p-benzoquinone and catechol (Smith et al., 2000; Snyder, 2000; Winn, 2003.; Martyn et al., 2000; Kopstein, 2006). These metabolities showed reduce multipotent haematopoietic stem cells in the bone marrow (Hayes et al., 2001), decrease the ability of stromal cells to support formation of granulocytic/macrophage progenitors (Mehlman,

2004) and cause imbalance in the count of lymphocytes producing leukaemia (Yaris et al., 2004). The present study also showed that heavy metals and benzene affected some enzymes activities in the serum of Al-Dorah refinery and petrol station workers. In general, LDH, ALP and other enzymes activities may show differences depending on the tissues examined (Khanzode et al., 2004). In a study carried out to evaluate the effect of heavy metals and benzene, it was shown that the activity of antioxidant enzyme and LDH enzyme levels were altering in different way (Carletti and Romano, 2002). On other hand, LDH enzyme was significantly increased which agreed with our finding throughout this study. Our finding also suggests that increase of formation of LDH and ALP may be due to increase alteration on hepatic cell. This distraction of liver cells may be caused increase releasing of both enzymes into blood stream. Benzene may directly affect organelles at the cellular level in various tissues, which will indirectly influence enzyme activities such as LDH and ALP. The main hepatic metabolites of benzene are phenol, catechol and hydroquinone. Microsomal metabolism of benzene plays a critical role in benzene toxicity (Lebailly et al., 2002). The genotoxicity detected among petrol workers suggest to apply a special regulation to protect them from the carcinogenicity of the lead and benzene and also to test blood of these workers in regular manner. Our study also proofed the importance of using different parameters to evaluate the role of toxicity among people to achieve correct conclusions.

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References

Abdullull-Wahab SA. Source characterization of atmospheric heavy metals in industrial residential areas: A case study in Oman. *J Air Waste Manag Assoc* 2004. 54(4): 425-31.

Anetor JI, Adelaja O, Adekunle AO. Serum micronutrient levels, nucleic acid metabolism antioxidant defenses in pregnant Nigerians: Implications for fetal and maternal health. 2003. *Afr J Med Med Sci* 32(3): 257-62.

Bachanek T, Staroslawska E, Wolanska E, Jarmolinska K. Heavy metal poisoning in glass worker characterized by severe. *Ann Agric Enviro Med* 2000. 7(1): 51-3.

Bloemen LJ , Youk A, Bradly TD, Bodner KM, Marsh G. Lympho-haematopoietic cancer risk among chemical workers exposed to benzene. *Occup Environ Med* 2004. 61(3): 270-74.

Carletti R, Romano D. Assessing health risk from benzene pollution in an urban area. *Environ Monit Assess* 2002. 80.135-48.

Decker T, Lohmann-Matthes ML. A quick and simple method for the quantitation of lactate dehydrogenase release in measurements of cellular cytotoxicity and tumor necrosis factor (TNF) activity. *J Immunol Methods* 1988. 115(1): 61-9.

Eastmond DA, Schuler M, Franz CH, et al. Charactrerization and mechanisms of chromosomal aberrations induced by benzene in mice and humans. *Res Rep Health Eff Ints* 2001. 103: 69-80.

Fairhurst S. Hazard and risk assessment of industrial chemicals in the occupational context in Europe. *Food Chem Toxicol* 2003. 41(11): 1453-62.

Fenech M. The in vitro micronucleus technique. *Mutat Res* 2000. 455(1-2): 81-95.

Guallar E, Sanz-Gallardo MI, Van't Veer P, et al. Heavy Metals and Myocardial Infarction Study Group. Mercury, fish oils, and the risk of myocardial infarction. *N Engl J Med* 2002. 347(22): 1747-54.

Hayes RB, Songnian Y, Dosemeci M, Linet M. Benzene and lymphohematopoietic malignancies in humans. *Am J Ind Med* 2001. 40(2): 117-26.

Hope RM. Human serum alkaline phosphatase variants and their association with the ABO blood groups in Australian sample. *Australian J Exper Biol Med Sci* 1966. 44(3): 323-6.

Kalnas J, Teitelbaum D: Dermal absorption of benzene: implications for work practies and regulations. *Int J Occup Environ Health* 2000. 6:(2): 114-21.

Khanzode SS, Muddeshwar MG, Khanzode SD, Dakhale GN. Antioxidant enzymes and lipid peroxidation in different stage of breast cancer. *Free Radic Res* 2004. 38: 81-6.

Kim YJ, ChoY H, Pack D, Chung HW. Determination of chromosome aberrations in workers in a petroleum refining factory. *J Toxicol Environ Health* 2004. 67(A): 1915-22.

KirschVolders M, Sofuni T, Aardema M, et al. Report from the in vitro micronucleus assay working group. *Mutat Res* 2003. 540(2): 153-63.

Kopstein M. Potential Uses of Petrochemical Products Can Result in Significant Benzene Exposures: MSDSs Must List Benzene as an Ingredient. *J Occup Environ Hygiene* 2006. 3(1): 1-8.

Lebailly P, Willett EV, Moorman AV, Roman E, Cartwright R, Morgan GJ, Wild CP. Genetic polymorphisms in microsomal epoxide hydrolase and susceptibility to adult acute myeloid leukaemia with defined cytogenetic abnormalities. *Brit J Haematol* 2002. 116: 587-94.

Lidsky TI, Schneider JS. Lead neurotoxicity in children: basic mechanisms and clinical correlates. *Brain* 2003. 126(1): 5-19.

Mortada WI, Sobh MA, El-Defrawy MM, Farahat SE. Study of lead exposure from automobile exhaust as a risk for nephrotoxicity among traffic policemen. *Am J Nephro* 2001. 21(4): 274-9.

Smith MT, Zhang L, Jeng M, et al. Hydroquinone, a benzene metabolite, increases the level of aneusomy of chromosomes 7 and 8 in human CD34-positive blood progenitor cells. *Carcinogenesis* 2002. 21(8): 1485-90.

WHO. *Arsenic and Arsenic Compounds*. Environmental Health Criteria 224 Geneva: World Health Organization, 2001.

Winn LM. Homologous recombination initiated by benzene metabolites: A potential role of oxidative stress. *Toxicol Sci* 2003. 72(1): 143-9.

Yadav JS, Seth N. Cytogenetical damage in petrol pump workers. *IJHG* 2001. 1(2): 145-50. Yaris F, Dikici M, Akbulut T, Yaris E, Sabuncu H. Story of benzene and leukaemia: Epidemiologic approach of Muzaffer Akosy. *J Occup Health* 2004. 46(3): 244-47.