

Biochemical Changes Followed Experimental Respiratory Distress by Benzene Vapours

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<http://dx.doi.org/10.13005/bpj/1654>

(Received: 09 October 2018; accepted: 24 November 2018)

Monitoring of exposure to chemical matters is seriously needed for evaluating health hazards resulted from its inhalation. The present study was carried out to determine the biochemical, immunological and oxidative stress parameters as well as the possible histological effects of exposure to benzene vapours in male albino rats. Results indicated that; Benzene vapours exposure induced significant increasing in Myeloperoxidase (MPO) enzyme levels. This goes with marked immunologic changes presented by decreases in immunoglobulins; IgA and IgG, along with increases in levels of IgM and IgE. Also, Malondialdehyde (MDA) levels were significantly increased. Meanwhile, reduction in different biochemical parameters including; Superoxide Dismutase (SOD), Catalase (CAT) levels and Glutathione (GSH) content. Lung sections taken showed; Thickening of alveolar septa with chronic inflammation and/or fibrosis, Congested vessels/thick walled vessels and Peri-bronchiolar fibrosis. Hence, the study concluded that; prolonged benzene (BNZ) vapours exposure lead to biochemical, immune disturbance and his to pathological changes probably through potentiating oxidative stress and inflammation pathways.

Keywords: Benzene exposure, Inflammatory pathways, Oxidative stress.

Benzene is an aromatic hydrocarbon; toxic to the blood forming organs. The severity of the damage depends on the level, timing and pattern of exposure. This volatile compound in urban air pollution induces DNA oxidation (Uzma *et al.*, 2010). Chronic benzene vapours exposure leads to decrease in antioxidant enzymes activity and hematologic disorders. Oxidative stress produces reactive oxygen species (ROS) in excess of the available antioxidant buffering capacity (Adly., 2010). Moreover, iron and other transition metals leaching from particles or by their presence on particle surfaces play a role in the generation of ROS in biological systems (Ghio *et al.*, 2000).

Metals and other inorganic elements play important roles in a wide variety of biological processes of living systems. Several essential transition elements, such as zinc, magnesium, iron, copper, cobalt and manganese participate in the control of various metabolic and signaling pathways. However, their rich coordination chemistry and redox properties are such that they are capable of escaping out of the control mechanisms, such as homeostasis, transport, compartmentalization, and binding to the designated tissue and cell constituents. Breakdown of these mechanisms, caused by stimuli such as benzene exposure has been involved in a large variety of diseases

(Jomova and Valko,2011). Disruption of metal homeostasis is known to modulate gene expression by interfering with signal transduction pathways. This action may lead to uncontrolled metal-mediated formation of free radicals participating in the modification of DNA bases, enhanced lipid peroxidation and altered sulfhydryl homeostasis (Valko *et al.*, 2007).

Repeated exposure to kerosene and petrol fumes causes degenerative changes in the ultra structural integrity of the hepatic cells which may impair the normal liver functions(Uboh *et al.*, 2005).

MATERIALS AND METHODS

Forty male albino rats, 12 weeksold were taken, and divided into three main groups;the first kept as a negative control group (Un-exposed), the second consisted of 15 rats subjected to benzene vapors for 8 hours daily for 3 months and the third contained also 15 rats exposed to benzene vapoursfor 8 hours daily for 6 months. All rats were sacrificed and their lungs were immediately collected for analysis and placed in formalin solution 10.0 % to reserve organ cells for histopathological examination. All animal experiments were approved by the egyptian research institute.

Data Collection and Estimated Parameters

After 90 days of exposure to benzene vapours, blood samples will be collected from rats of each group. The samples will be taken in the early morning after overnight fasting from the medial conthus of the eyes by using the heparinized microhematocrite tubes to heparin and plain centrifuge tubes, then animals sacrificed. Plain tubes then allowed to be coagulate at room temperature for 30 minutes . The serum will be separated in dry sterile tube then kept in deep freeze until using for subsequent biochemical assays.

The sera were subjected to estimation of copper, zinc, iron, manganese, and lead byatomic absorption spectra-photometry (PyeUnicum), according the methods, described byWilse, (1960)and Bauer(1982) as well as total protein immunoglobulines (IgM, IgG, IgA)(Whicker *et al.*, 1984), IgE(Plebani *et al.*,1998) and nitric oxide.(Montgomery and Dymock, 1961)as well

Table 1. Biochemical effect of benzene vapor on some serum biochemical parameters in male rats after 3 and 6 months of exposure

Parameters Groups	Cu µg/dl	Zn µg/dl	Fe µg/dl	Pb µg/dl	Cd µg/dl	NO µmol/l	MPO ng/ml	IgM mg/dl	IgG mg/dl	IgA mg/dl	IgE IU/ml
Control normal	58.82 ± 1.77 ^a	42.25 ± 1.19 ^a	92.34 ± 1.79 ^a	6.58 ± 0.38 ^c	1.61 ± 0.12 ^c	4.43 ± 0.21 ^a	7.72 ± 0.49 ^c	54.62 ± 1.85 ^c	494.30 ± 3.35 ^a	27.07 ± 0.91 ^a	2.99 ± 0.07 ^c
Exposed After 3 months	44.89 ± 1.16 ^b	31.73 ± 0.97 ^b	53.97 ± 2.16 ^c	8.97 ± 0.30 ^b	1.90 ± 0.10 ^b	2.59 ± 0.16 ^b	10.44 ± 0.46 ^b	76.72 ± 2.69 ^b	425.20 ± 3.52 ^b	18.03 ± 0.94 ^b	8.32 ± 0.35 ^b
group After 6 months	36.27 ± 1.01 ^c	24.88 ± 1.06 ^c	59.92 ± 2.08 ^b	10.61 ± 0.34 ^a	2.57 ± 0.15 ^a	2.07 ± 0.12 ^c	15.62 ± 0.38 ^a	95.56 ± 2.83 ^a	398.40 ± 3.61 ^c	10.88 ± 0.77 ^c	14.43 ± 0.54 ^a

Data shown are mean ± standard deviation of number of observations within each group. Mean values with different superscript letters in the same column are significantly different at (P<0.05).Small letters are used for comparison between the means within the column

as Myeloperoxidase Enzyme activity (ELISA kit -Kamiya Biomedical Co.).

The remaining amount of blood were taken into clean dry tube containing heparin 0.5% and used for preparation of hemolysate by Digitonine after washing erythrocytes by physiological saline described by Kornburg and Korecker (1955). This hemolysate was subjected for quantitative determination of erythrocyte (CAT)(Aebi, 1984) and (Fossatiet *al.*, 1980), (SOD)(Nishikimi *et al.*, 1972), (GSH)(Beutler *et al.*, 1963), (GP_x)(Paglia and Valentine, 1967) and (MDA)(Satoh, 1978).

Statistical analysis: Data analysis was expressed as mean \pm S.E. and analyzed for statistical significance by one-way ANOVA followed by Tukey's post-hoc test for multiple

comparisons, using SPSS program for Windows version 22.0 (SPSS Inc., Chicago, USA). Values were considered statistically significant at $P < 0.05$ correlations between the measured variables were assessed by linear regression analysis by the least squares method.

RESULTS

Results obtained in table (1) showed a significant decrease ($P < 0.05$) in serum Copper, Zinc and iron levels in rats exposed to benzene vapours for three months and highly significant decrease ($P < 0.05$) in Cu, Zn after six months of exposure when compared to control. Also, a highly significant decrease was noticed in serum immunoglobulins IgG and IgA levels at ($P < 0.05$)

Table 2. Biochemical effect of benzene vapor on erythrocyte MDA, GSH, SOD, CAT and GPx in male rats after 3 and 6 months of exposure

Parameter Groups	MDA nmol/g. Hb	GSH mg/dl	SOD U/g. Hb	CAT U/L	GPxm U/ml
Control normal	8.22 \pm 0.41 ^c	29.61 \pm 0.75 ^a	16.36 \pm 0.81 ^a	494.40 \pm 3.63 ^a	40.55 \pm 1.07 ^a
Exposed groups					
After 3 - months	14.40 \pm 0.76 ^b	16.71 \pm 0.56 ^b	11.14 \pm 0.86 ^b	446.90 \pm 2.52 ^b	28.92 \pm 0.91 ^b
After 6 - months	20.95 \pm 1.06 ^a	11.19 \pm 0.63 ^c	6.98 \pm 0.72 ^c	407.60 \pm 2.45 ^c	20.53 \pm 0.83 ^c

Data shown are mean \pm standard deviation of number of observations within each group. Mean values with different superscript letters in the same column are significantly different at ($P < 0.05$). Small letters are used for comparison between the means within the column.

Histopathological Results of lung section

Patterns of injury	Score			
Lung architecture	0 (Preserved) 1 (Disturbed)			
Thickening of alveolar septa with chronic inflammation and /or fibrosis	0 (None) 1 (Mild)			
Congested/thickened wall vessels	2 (Moderate) 3 (Severe)			
Peri-bronchiolar inflammation and fibrosis				

Parameter Groups	Lung architecture	Thickening of alveolar septa with chronic inflammation and /or fibrosis	Congested vessels/ Thick walled vessels	Peri-bronchiolar fibrosis
Control	0	0	0	0
3 months exposure	0	2	2	0
6 months exposure	1	3	2	3

in spite of increasing of serum lead and cadmium metals, IgM and IgE in rats exposed to benzene vapours for three months and highly significant increase after six months of exposure when compared to control. Moreover, serum Nitric Oxide showed a significant decrease at ($P < 0.05$) in rats exposed to benzene vapours for three months and highly significant decrease after six months of exposure in comparison with control. Serum MPO levels showed a significant increasing in rats exposed to benzene vapours for three months and highly significant increasing ($P < 0.05$) after six months of exposure when compared to control.

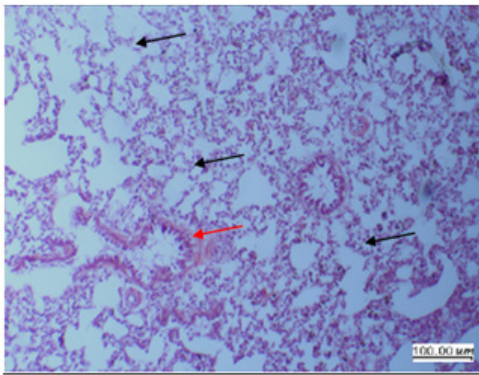
The data recorded in table (2) showed; a significant reductions in RBCs SOD, CAT, GPx and GSH levels ($P < 0.05$) in rats exposed to benzene vapours for three months and highly significant

reduction in RBCs SOD, CAT, GPx and GSH levels after six months of exposure when compared to control. in spite of increasing of Lipid peroxidation, MDA levels in rats exposed to benzene vapours for three months and highly significant increase after six months of exposure when compared to control.

DISCUSSION

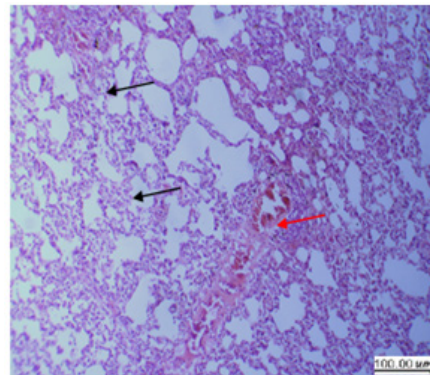
Monitoring of exposure to chemical matters is seriously needed for evaluating health hazards and providing suitable strategies for making safe work environment. Among the most toxic chemicals is gasoline and its most constituent, benzene that is strongly and causally related to wide spread of health problems (Ibitoroko *et al.*, 2011).

Normal

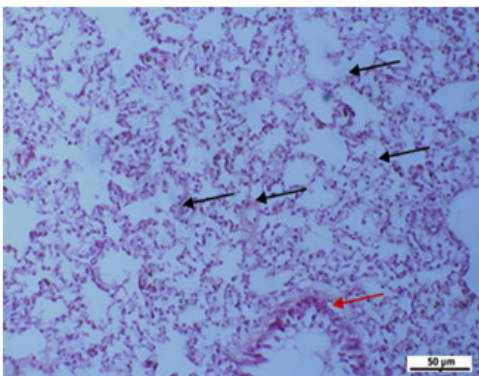


Normal alveolar spaces and thin alveolar septum (black arrows). Uniform bronchiolar wall is also seen (Red arrow) (H&E, 10x)

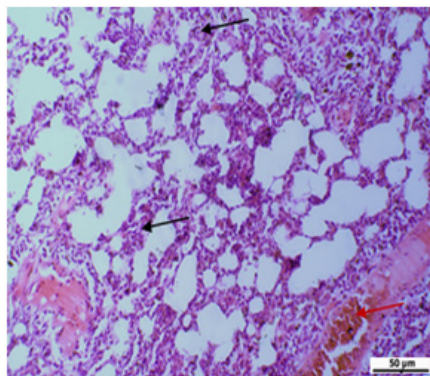
3 months exposure



There is preserved lung architecture with moderate thickening of alveolar septa (black arrows). There is congested blood vessel (Red arrow) (H&E, 10x)



Normal alveolar spaces and thin alveolar septum (black arrows). Uniform bronchiolar wall is also seen (Red arrow) (H&E, 20x)



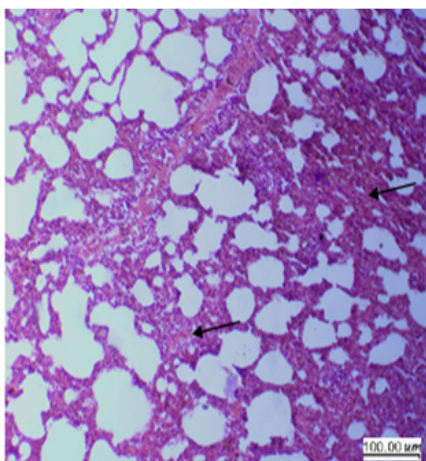
There is moderate thickening of alveolar septa with chronic inflammatory cells (black arrows). There is congested blood vessel (Red arrow) (H&E, 20x)

The disturbance in many inorganic elements are essential for a multitude of biological processes and their homeostasis, which is maintained within strict limits, is critical for life (Valco, *et al.*, 2005). Disruption of such homeostasis may lead to oxidative stress. The generation of free radicals in living systems is closely linked with the participation of redox-active metals which undergo redox cycling reactions and possess the ability to produce reactive radicals in biological systems. Some redox-active metals including cadmium and lead may increase the susceptibility of membranes

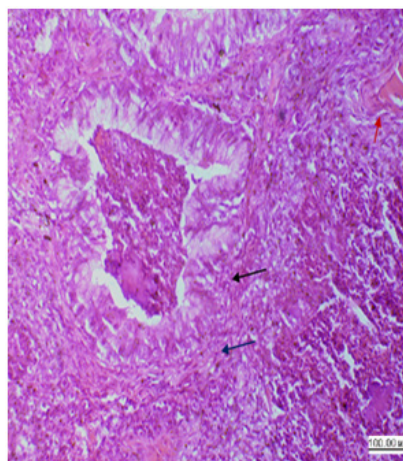
by altering their integrity via causing deterioration of their components (Gurer and Ercal, 2000).

Elevated cadmium level and increased lipid peroxidation represented by increased MDA level in benzene vapour exposed rats were found in this study came in accordance with (Eybl *et al.*, 2006) who reported that, cadmium itself is unable to generate free radicals directly. Copper deficiency alters the role of cellular constituents involved in antioxidant activities, such as iron, selenium and glutathione, so increased cellular susceptibility to oxidative damage and leads to decreased capability

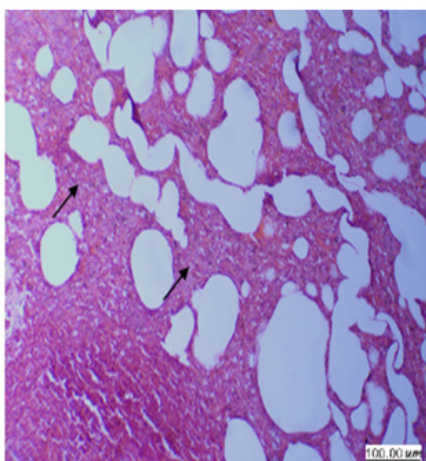
6 months exposure



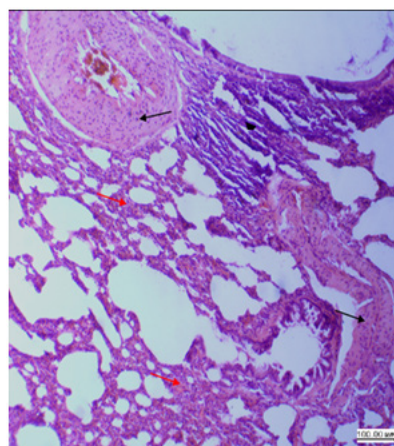
There is marked thickening of alveolar septa with chronic inflammation (black arrows) (H&E, 20x)



There is marked peri-bronchiolar inflammation and fibrosis in wall (Red arrows) (H&E, 10x)



There is focal disturbed lung architecture with marked thickening of alveolar septa with chronic inflammation (Black arrows) (H&E, 10x)



There are thick walled blood vessels (Black arrows). Thickening of alveolar septa is seen (Red arrows), as well as chronic inflammation in bronchiolar wall (Arrow head) (H&E, 10x)

to produce (SOD), thus increasing their propensity to oxidative damage (Pan and Loo, 2000). Mild to moderate zinc deficiency can depress immune function through impairment of macrophage and neutrophil, natural killer cell and complement activity (Wintergerst *et al.*, 2007). This redox-inert metal is an essential component of numerous proteins involved in defense against oxidative stress, as for example (SOD). Besides it possesses neuroprotective properties. Depletion of zinc may enhance DNA damage via impairment of DNA repair mechanisms (Jomova and Valko, 2011).

Our result was in agreement with Meuwese *et al.*, (2007) who stated that; high MPO levels were able to predict increased risk of developing coronary artery diseases (CAD) in healthy individuals. Most of attention was directed toward gasoline related immunotoxicity through decreasing number of immunoglobulins (IgA, IgG) which are often measured to give information about immune system homeostasis (Marques *et al.*, 2016).

CONCLUSION

By the end of this study, we concluded that, the experimental exposure to benzene vapours in male albino rat followed by cascade of Biochemical, immunological and histopathological alteration represented by significant changes in (MPO), immunoglobulins; IgA and IgG, IgM, IgE, (MDA), (SOD), (CAT) and (GSH) content along with significant changes in lung histopathology. These changes might be through initiation of ROS and promotion of oxidative stress and inflammatory pathways.

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