

Biotoxic and Haemotoxic Effects of Air Pollutants at a Benzene Station on Albino Rats

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Abstract: This study was directed to explore the hazardous effect of occupational exposure to air pollutants arising from benzene station. A total of 48 albino rats were classified into three groups each of sixteen rats. Groups one and two were kept at a benzene station for 60 and 120 days, respectively and the third group was kept as a control under laboratory conditions. After termination of the experiment, animals were sacrificed and blood samples were collected for hematological and biochemical investigations. Sera were separated and used to check the effects of air pollutants arising at a benzene station on the liver and kidney functions as well as on the immune status of the body. Results indicated a pronounced time-dependent reductions in RBCs, Hb, PCV, total and differential (neutrophil and lymphocyte) leucocytic counts. Total protein, albumin, globulin and immunoglobulins IgG, IgA and IgM showed marked lower levels in animals exposed to air pollutants in the benzene station. Organ function tests revealed elevations of the levels of AST, ALT, ALP and GGT that indicate impaired liver function compared to those of the corresponding control. Similar results were recorded for creatinine kinase, urea and creatinine indicating toxic effects on the heart and kidneys.

Keywords: Benzene, blood, kidney, liver, pollutants, toxic

INTRODUCTION

Occupational toxicity due to the effect of pollutants at work place are of basic importance because of the time factor, lasting about 8 h daily.

Benzene is a heavily used industrial chemical, a petroleum-by-product, an additive in unleaded gas, and an ubiquitous environmental pollutant. It is classified as a "Known" carcinogen "Category A" under the risk assessment Guidelines of 1986. Many experimental animal studies, both inhalation and oral, also support the evidence that exposure to benzene increases the risk of cancer in multiple organ systems including the haemopoietic system, oral and nasal cavities, liver, fore stomach, preputial gland, lung, ovary and mammary gland (USEPA, 1998).

Cody *et al.* (1993) investigated the haematological effect of Benzene caused by job exposure in rubber workers. Significant lower average of white and red blood cell counts had been recorded at each month during the first year of work in workers exposed to the above median benzene exposure when compared with workers exposed below the median.

Jacobs *et al.* (1993) recorded that during a 3 year period 229 patients in U.K. with haematological disorders thought to be associated with occupational hazards were notified to the British Society for Haematological/Health and Safety Executive Office. Most were suffering from malignant, premalignant or aplastic anaemia. Benzene and ionizing radiation were the most common agent recorded.

Ruiz *et al.* (1993) found that macrocytosis and lymphopenia are the earliest haematological signs of benzene toxicity and Ruiz *et al.* (1994) recorded alteration in bone marrow and neutropenia in patients due to chronic exposure to organic solvents (Benzene).

Burns *et al.* (1994) found a decrease in erythrocyte number with a concomitant increase in mean corpuscular Hb and mean corpuscular volume due to exposure of female mice to nitrobenzene.

Immunologically, many workers exposed to benzene were studied, the results showed that serum complement levels, IgG and IgA were depressed but IgM levels were slightly higher. These observations taken together with well known ability of benzene to depress leukocyte counts, may explain why benzene-intoxicated individuals are readily succumb to infection (IARC, 1980).

Eyong *et al.* (2004) suggested that ingestion of shellfish exposed to crude oil-polluted water or the polluted perse to rats resulted in haematotoxicity in the form of significant changes in blood parameters.

Uboh *et al.* (2008) reported that gasoline exposure caused weight loss, growth depression and haemotoxicity in the exposed rats. They added that administration of vitamin A to the affected rats significantly regained these toxic effects especially in female rats.

Uboh *et al.* (2012) reported significant decrements in haematological parameters including RBCs count, HB, PCV, MCV, MCH, MCHC and neutrophils to rats administered nitrocellulose thinner orally as a single daily dose for 30 days. On the other hand, they found significant increments in other parameters including leucocyte count, platelets and lymphocytes.

The present study was designed to investigate the possible alterations in biochemical and haematological parameters in workers who are exposed daily to benzene and other gasses arising at a gasoline station using albino rats as experimental model.

MATERIALS AND METHODS

Animals, grouping and exposure: The study was carried out on 48 mature wister rats of both sexes ranged between 120 and 140 g body weight. These animals were classified into three groups each of 16 rats. The first and the second groups were kept in a benzene station for 60 and 120 days, respectively, while the third group was kept as a control in our laboratory animal house. At the benzene station, animals were exposed to some air pollutants particularly volatile organic compounds, e.g., Benzene, kerosin, different oils...etc. All animals were offered balanced diet and water *ad libitum*.

Sampling: At the end of the experimental periods, animals were sacrificed, and immediately the blood was

collected for the haematological investigation, sera were separated and kept frozen at -20°C till the biochemical investigations indicating liver and kidney functions and immune status.

Methods: Haematological investigation in this study was carried out according to Kelly (1984). Serum total protein, albumin and globulins were estimated according to Weichselbaum (1946), Daumas *et al.* (1971) and Coles (1986), respectively. Serum urea and creatinine were determined according to Husdan and Rapoport (1968) and Chaney and Morbach (1963), respectively. Serum immunoglobulins (IgG, IgA and IgM) were measured using single radial immunodiffusion kits (Kallested Chaska, USA) according to Pfeiffer *et al.* (1977). Serum Gamma glutamyl transferase (GGT and serum creatine kinase was carried out according to Szasz (1969) and Miller *et al.* (1984), respectively.

RESULTS

Table 1 shows the haematological picture in albino rats exposed to air pollutants arising from benzene station. Significant or highly significant decrease was recorded for RBCs, Hb, PCV and total leucocytic count. Differential leucocytic counts revealed significant reduction in neutrophil and lymphocyte counts.

Table 2 shows pronounced reduction in total protein, albumin and globulins. Immunoglobulins IgG, IgA and IgM showed marked lower levels in animals exposed to air pollutants in benzene station.

The effect of air pollutants of benzene station on some organ functions was recorded in Table 3 where the levels of AST, ALT, ALP and GGT that indicate liver function showed significant increases when compared to the those of the corresponding control ones. Similar results were recorded for creatinine kinase, urea and creatinine.

Table 1: Haemogram of albino rats exposed to air pollutants arising from benzene station for 60 and 120 days versus control

	Group 1 (unexposed group)	Group 2 (animals exposed for 60 days)	Group 3 (animals exposed for 120 days)
RBCs×10 ⁶	9.75±0.76	8.69±0.90 n.s	4.28±0.66n.s
Hb (gm%)	13.15±0.86	9.55±0.72*	6.23±0.64**
PCV (%)	41.25±1.12	35.22±1.10*	28.31±0.89**
WBCs×10 ³	12.12±2.31	10.15±2.12 n.s	8.21±1.89n.s
Neotrophils×10 ³	8.62±1.12	7.23±1.09 n.s	6.12±1.06*
Eosinophils×10 ³	0.46±0.19	0.41±0.17 n.s	0.45±0.17n.s
Lymphocytes×10 ³	2.45±1.18	1.97±1.12 n.s	1.12±1.09*
Monocytes×10 ³	0.59±0.18	0.54±0.16 n.s	0.52±0.017n.s

*: Significant, p<0.05; **: Highly significant, p<0.01; n.s.: Nonsignificant

Table 2: Effect of air pollutants arising from benzene station on some biochemical parameters indicating immune status of albino rats after 60 and 120 days of exposure versus control

	Group 1 (unexposed group)	Group 2 (animals exposed for 60 days)	Group 3 (animals exposed for 120 days)
Total protein (gm%)	8.78±0.31	6.20±0.11**	5.98±0.11**
Albumin (gm%)	4.84±0.34	3.29±0.26*	2.69±0.51*
Globulins (gm%)	3.95±0.15	3.52±0.40 n.s	2.69±0.16**
IgG (mg/mL)	379.70±4.37	373.09±10.43n.s	347.68±11.55*
IgA (mg/mL)	70.33±0.82	67.31±0.82*	60.31±2.38*
IgM (mg/mL)	52.06±0.63	48.68±0.58*	41.30±0.58**

*: Significant p<0.05; **: Highly significant p<0.01; n.s.: Nonsignificant

Table 3: Effect of air pollutants arising from benzene station on some biochemical parameters indicative for some organ functions of albino rats after 60 and 120 days of exposure versus control

	Group 1 (unexposed group)	Group 2 (animals exposed for 60 days)	Group 3 (animals exposed for 120 days)
AST (u/mL)	77.25±2.21	92.53±2.47*	104.00±2.77*
ALT (u/mL)	60.30±2.42	75.41±2.40*	110.63±2.61*
ALP (u/mL)	25.23±1.65	32.16±1.86*	38.23±2.12*
GGT (u/mL)	21.00±3.54	26.67±5.42 n.s	37.33±3.60*
CK (u/mL)	116.00±4.12	334.66±59.70*	348.00±56.10*
Urea (gm/mL)	25.30±1.63	31.92±1.72*	43.50±1.93**
Creatinine (gm/mL)	2.61±0.41	3.62±1 0.72n.s	4.71±1.02*

*: Significant $p < 0.05$; **: Highly significant $p < 0.01$; n.s.: Nonsignificant

DISCUSSION

Human being now is living in an environment in which at least 100,000 chemicals are prevalent and to which approximately, 100 new compounds are added each year (Li, 1993). Benzene is an important industrial solvent and common pollutant which can develop aplastic anaemia and leukemia in exposed individuals.

In our study, Table 1 shows that distinct reductions have been recorded for RBCs, Hb and PCV in animals located at the benzene station. This picture of anaemia was more pronounced in animals of group (3) which were exposed to benzene for longer periods. These haematological disorders may be due to the effect of benzene on the function of bone marrow as described by Ruiz *et al.* (1994) and Plappert *et al.* (1994). Similar results were recorded by Cody *et al.* (1993) and Burns *et al.* (1994). We noticed also that the total leucocytic counts have been reduced significantly in exposed animals and this reduction seems to be mainly due to the decrease in neutrophil and lymphocyte counts. So we expected an alteration in the cellular immune function of exposed animals. The affection of bone marrow may be a possible cause as mentioned before. However, similar results have been recorded in the study of Cody *et al.* (1993), Jacobs *et al.* (1993) and Ruize *et al.* (1993). In an attempt to explore this haematologic change, Berrera *et al.* (1997) stated that Benzene causes irreversible damage to myeloid progenitor cells, causing permanent reduction in concentration of erythrocytes, platelets and neutrophils. On the other hand, data is partially inconsistent with Uboh *et al.* (2012) who reported that administration of nitrocellulose thinner caused significant increase in total leucocytic count in rats.

Table 2 shows that animals exposed to air pollutants arising from a benzene station exhibited reduced levels of total protein, albumin and globulins. Also immunoglobulins (IgA, IgG and IgM) were markedly lowered in exposed animals. These biochemical indices showed that animals exposed to these pollutants have a reduced immune status and consequently become more susceptible to infection. In this direction, we refer to the study of Burns *et al.* (1994) who recorded decreased IgM and phagocytic activity in female mice exposed to nitrobenzene. Our results coincided with the report of IARC (1980) which indicated that a large number of

workers exposed to benzene showed depressed serum complement levels, beside reduction in IgG and IgA. The report suggested that these observations with the ability of benzene to depress leukocytes may explain why benzene-intoxicated individuals are ready succumb to infection.

Concerning the effect of air pollutants at benzene station on the organ functions, Table 3 indicates highly significant alterations in AST, ALT, ALP and GGT. This picture is indicative for the impairment of the liver function.

Our results agree partly with those mentioned by Burns *et al.* (1994) who recorded increased ALT and AST with pathological alterations in liver and spleen of female mice exposed to nitrobenzene.

Our results also revealed disturbed kidney function, and this change was manifested by higher levels of urea and creatinine in serum of exposed animals.

Table 3 also indicates an abnormal increase in creatine kinase in the exposed groups. CK is a cellular enzyme with a wide tissue distribution and found mainly in skeletal and cardiac muscle. Increased CK level in our study indicates impaired cardiac and/or skeletal muscle function in rats exposed to gases arising from a gasoline station. This finding is in partial agreement with EKTACHEM (1995) who mentioned that the rise of the level of CK following acute cardiac infarction, myocarditis and cerebrovascular accidents.

CONCLUSION

It could be concluded that there are many toxic effects due to benzene exposure which included haemotoxicity, lowered immunity, impairment in function of some organs including liver, kidneys and heart. So, in order to minimize the predicted toxic effects of occupational exposure to benzene the following points should be put in considerations:

- Early detection of Benzene haemotoxicity that can be done by continuous health monitoring of exposed workers through preplacement and periodic health examination.
- Regular determination of Benzene concentration in the working atmosphere is of utmost importance. In developed countries, the allowable work place level of benzene is 1 ppm (Bogadi-Sare, 1992).

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