Effect of Benzene emission exhaust on some physiological parameters of albino male rats

N. F. Hassan
College of Veterinary Medicine/ Al-Qadisiya University

Abstract

Benzene is one of the most environmental contaminants, absorbed by inhalation, oral and dermal exposure. Forty adult male rats (*Rattus norvigicas*) were divided into two equal groups: control and treated group, the treated group were housed near the source of exhaust, 2 litters of benzene were used in generator (23 Am) daily for 4 hours for 30 days, then rats were sacrificed, body weight was measured and blood samples collected for Hb, RBC, WBC, Blood Urea, Cholesterol, Creatinine, Protein and Albumin assessment as well as histological study for liver and kidneys. The results showed decrease in body weight. Hb, RBC, WBC and blood urea were significantly increased, whereas serum cholesterol, creatinine, protein and albumin concentrations showed slight increase as compare to treated group, as well as congestion in liver and kidney. In conclusion, the present study demonstrated that benzene emission exhaust resulted in sever change to the liver, nephropathy and blood picture.

تأثير عادم انبعاث البانزين في بعض المعايير الفسلجية لذكور الجرذان البيضاء

نيران فليح حسن
كلية الطب البيطري/ جامعة القادسية

الخلاصة

البانزين هو احد أهم الملوثات الجوية، يتم امتصاصه عن طريق الاستنشاق، الفم والتعرض عن طريق الجلد. تضمنت التجربة استخدام 40 جرذاً بالغاً قسمت إلى مجموعتين متساويتين: مجموعة السيطرة ومجموعة المعاملة، تم وضع مجموعة المعاملة قرب مصدر العادم ،لترين من البانزين تم استخدامهم في مولد 23 أمبير يومياً أربع ساعات لمدة ثلاثون يوماً، ثم تم تشريح الحيوانات وقياس أوزانها واخذ نماذج دموية لإجراء الفحوصات التالية الهيموغلوبين، كريات الدم الحمراء والبيضاء، الكولسترول، اليوريا، الكرياتينين، البروتين والألبومين بالإضافة إلى دراسة نسيجية للكبد والكلية. أظهرت النتائج انخفاض وزن الجسم وتركيز الهيموغلوبين وعدد كريات الدم الحمر والبيض معنوياً وزيادة نسبة اليوريا، الكولسترول، الكرياتينين، البروتين الكلي والألبومين معنوياً. مع احتقان الكبد والكلية. يستنتج من الدراسة الحالية ان عوادم انبعاث البانزين ينتج عنه تغيرات حادة في الكبد وتأثير سام للكلية ويسبب تغير بالصورة الدموية.

Introduction

A number of environmental contaminants including certain chemicals, drugs and various organic solvents alter the structure and functions of various organs including: intestine, liver, heart and kidney and produce multiple adverse effects (1,2). Benzene also known as benzole, the physical and chemical properties of benzene include:Hazardous substance data bank No: 2554 (3). Synonyms: annulene, benzene (Dutch), benzene (Polish), benzene, benzol, benzole, benzolo (Italian), bicarbonate of hydrogen, coal naphtha, cyclohjexatriene, frenzen (Czech), بنزين (Arabic).Registered trade name: Polystream. molecular formula: C_6H_6, Molecular weight: 78.11 (4). Vapor

pressure: 75 mm Hg at 20 C°, water solubility: 1750 mg/L at 25 C°(5). miscible with ethanol, ethylether, acetone and chloroform. Melting point: 5.5 C° ,Boiling point: 80.1 C° at 760 mm Hg, Oder: aromatic, Test threshold: 0.5 to 4.5 mg/L (3). Benzene is readily absorbed from inhalation, oral and dermal exposure (6). The metabolism of benzene is still not thoroughly understood. It is generally accepted that benzene itself is not directly responsible for causing the toxic effects; However, the metabolic product or products responsible for the noncinogenic and carcinogenic effects of benzene exposure have not clearly defined (7,8). The products of the phenol pathway (Catechol, hydroquinone and p – benzoquinone), MA and benzene epoxide have been proposed as benzene metabolites that may have toxic effects of benzene exposure(9). Potential mechanisms of benzene toxicity have been investigated primarily through their effects on metabolism in liver and the formation of epoxide, benzene oxide, catalayzed by cytochrome P450 2E1 (CYP2E1) (10). and transportation to the bone marrow for secondary metabolism Myeloperoxidade, quinine oxidoreductase (MPO,NQO1) (11,12), oxidative stress from reactive oxygen species generated by redox cycling (13), chromosome alterations including translocations, deletions and aneuploidy (14), protein damage to tubulin, histone proteins, topoisomerase II (10) and immune system dysfunction (TNF– α, INF– γ, AhR) (15 - 17), benzene induces chromosomal alterations similar to those found in therapy related MDS and AML and in denovo leukemia (18,19). Therefore this study was designed to show the effect of benzene emission exhaust on some blood parameters.

Material and Methods

- **Experimental design:** The present study has been performed on 40 adult male rats (Rattus norvigicas), weighted (150– 175)g, rats were well housed indoor and allowed diet and water adlibitum, conditioned for 1 week before the start of experiment, and divided into 2 equal groups: control and treated group (20 rats/ group). Benzene was purchased from the main station in Al-diwanyia city center. 2 litters of benzene were used in a generator (23Am) and the treated group were housed near the source of benzene emission exhaust 4 hours daily for 30 days, male rats were sacrificed under ether anesthesia. Body weight was measured and blood samples were collected in anticoagulant tubes for the whole blood tests (Hb, WBCs and RBCs count), and other part of the blood leaved to clot and centrifuged for serum biochemical parameters (Blood urea, Cholesterol, Creatinine, Protein and Albumin), For histopathology, pieces of (1) cm^3 were taken from liver and kidney, they kept in 10% neutral buffered formalin for fixation, processed routinely in Histokinette, cut at 5 μm thickness by microtome (Jung4291, West Germany) and stained with Haematoxylin and Eosin stain then examined under light microscope (20).
- **Statistical Analysis:** All data of the experiment were analyzed statistically using the student *t*- test. The significance were considered at 5% level (21).

Results

- **Effect of benzene emission exhaust on body weight:** Body weight of male rats of benzene emission exhaust group (T) tend to decline as compared with controls rat which did not reach the significant level (Table 1).

Table (1) Effect of benzene emission exhaust on body weight of mature male rats (g)

Group	Before treatment	After treatment
Control	165.3 ± 3.70	177.1 ± 9.12
Treated	166.8 ± 7.20	160.6 ± 4.80

Values represent Mean ± S.E

- **Effect of benzene emission exhaust on some serum biochemical constituent:** Benzene emission exhaust caused significant elevation (P≥ 0.5) in blood urea mg/dl, Cholesterol mg/dl, Creatinine mg/dl, total protein mg/dl and Albumin g/dl in treated group compared with that of control (Table 2).

Table (2) Effect of benzene emission exhaust on some serum biochemical constituent

Group	Blood Urea mg/dl	Cholesterol mg/dl	Creatinine mg/dl	Protein mg/dl	Albumin g/dl
Control	27.67 ± 3.62	1.71 ± 0.08	14.79 ± 0.62	7.8 ± 0.9	4.8 ± 0.6
Treated	38.27 ± 3.01*	2.03 ± 0.09*	20.32 ± 1.15*	5.2 ± 0.7*	5.6 ± 0.9*

Values represent M ± S.E

*** There was a significant difference (P ≥ 0.5)**

- **Effect of benzene emission exhaust on some blood parameters:** Benzene emission exhaust caused a significant decrease (P≥ 0.5) in WBCs count in treated group in comparison with that of control group, while Hb g/100ml, RBCs count decreased insignificantly (P≥ 0.5) in treated group as compared with control group (Table 3).

Table (3) Effect of benzene of some blood parameters

Group	Hb g/100ml	WBCs	RBCs
Control	10.2 ± 0.9	$5.4 \times 10^3 \pm 27.9$	$4.5 \times 10^6 \pm 12.43$
Treated	9.6 ± 0.7	$3.2 \times 10^3 \pm 48.3^*$	$4.1 \times 10^6 \pm 10.86$

Values represent M ± S.E

*** There was a significant difference (P ≥ 0.5)**

- **Histological study:** The histological study for liver showed a severely congested and hepatocellular hypertrophy and hepatocytes damage. there is sever congestion of kidneys, cortical areas of dilated tubules, vasoconstriction which may increase the renal damage, congested glumeruli, infiltration of lymphocyte and nephritis in treated group in comparison to the control group.

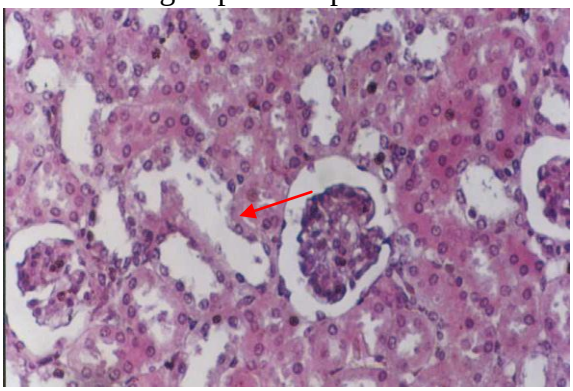

Fig. (1) Kidney of treatedt group shows areas of dilated renal cortical tubules (X 50 H & E)

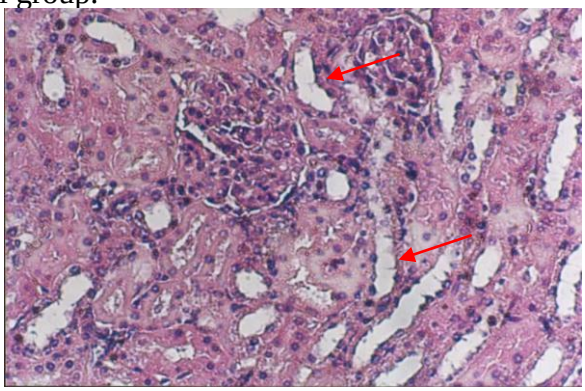

Fig. (2) Kidney of treated group shows areas of dilated renal cortical tubules (X 50 H& E)

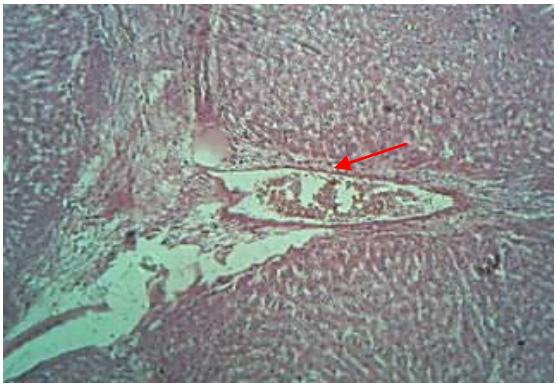

Fig. (3) Liver of treated group shows hyperplasia of bile duct and congestion of central vein (X 50 H & E)

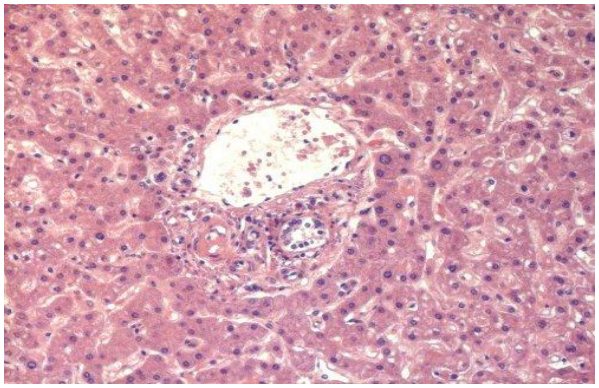

Fig. (4) Liver of treated group shows congestion of hepato – portal vein congestion of (X 50 H & E)

Discussion

Benzene is an aromatic hydrocarbon used for industrial purposes, it causes serious negative health effects in humans and animals depending on both the amount and duration of exposure (22). Benzene, is lipid soluble, it is transported in the blood and absorbed by red cell membrane. It tend to accumulate in tissues with high lipid content and about 50% of the absorbed dose may be eliminated unchanged while the remaining is metabolized in liver (23). Acute and chronic exposure of benzene suppresses bone marrow function, causing blood changes, leukemia, CNS depression, headaches, sleepiness, loss of consciousness, defatting dermatitis, it's effect on tissue metabolism, energy yielding reactions and oxidative stress has not been examined in detail (24). Benzene emission exhaust resulted in slight decrease in body weight. This decrease may be due to its effect on CNS level decreasing the appetite and food intake (25). Benzene emission exhaust resulted in significant increase in blood urea, cholesterol, creatinine and albumin and significant decrease in protein which may be considered as a significant indicate of kidney damage (26). Benzene metabolites are known to produce oxidized species and reactive oxygen indicating the increased risk of cell membrane damage which may be considered the causative agent of the slight decrease in hemoglobin concentration and red blood cells count, while it causes a significant decrease in white blood cells count (27). The primary target of benzene exposure in humans are the hematopoietic (blood cell forming) system and the immune system and persistent decreases in the hematopoietic cells (28,29).This study agreed with Zhang (30) who studied the effect of repeated exposure to benzene in rabbits (80 ppm, 175 total exposures), rats (88 ppm, 136 total exposures) and guinea pigs (88 ppm, 193 total exposures), the observed effects indicated leukopenia. Similar observations have been made by Wolf et al. (31) who showed that a 14 days exposure of mice to 50 ppm (162 mg/m^3) benzene resulted in a significantly reduced blood leukocyte count. Epidemiologic studies showed clear evidence association between benzene exposure and certain leukemias (32,33).The data of experimental animal added to the argument that exposure to benzene increases the risk of cancer in multiple species at multiple organ sites (hematopoietic, oral and nasal, liver, forestomach, preputial gland, lung, ovary and mammary gland), it is likely that these responses are due to interactions of the metabolites of benzene with DNA (34 , 35). The pathological study agreed with many workers (36,37), changes that observed in rats with occasional focal lesions in the adrenal cortex, tubular dilatation, interstitial nephritis, foci of regenerative epithelium and congestion of the kidney since it is the main site of excretion accumulation of toxic emission in the nephrous leading to dilatation of nephrous tubules. Liver congestion and hyperplasia of bile duct which might be regarded as compensatory mechanism to distortion and lack of blood supply, accumulation of fat as a result of disturbance of fat metabolism in the liver due to toxic effect of benzene emission exhaust. Congestion of central vein might be due to liver injury (38– 41). In conclusion, the present study demonstrated that benzene emission exhaust resulted in sever damage to various rat tissues, blood cells, hepatotoxic, nephrotoxic effect and renal injury also it is causes leukopenia.

References

1. Ruder, A. M. (2006). Potential health effects of occupational chlorinated solvent exposure. Ann NYA Cad Sci., 1076: 207 – 227.
2. Banday, A. A.; Farooq, N.; Priyamvada, S.; Yusufi, A. N. K. & Khan, F. (2008). Time dependent effects of gentamycine on the enzymes of carbohydrate metabolism, brush border membrane and oxidative stress in rat kidney tissues. Life Sci., 82: 450 – 459.
3. HSDB (Hazardous Substances Data Bank). (1997). National library of medicine, National Toxicology Program (Via Toxnet), Bethesda, MD.
4. Budvari, S. (1989). The Merck Index: An encyclopedia of chemicals, drugs and Biochemical's. 11th ed. Pathways, NJ. Merck and CO.
5. Banerjee, S.; Yalkowsky, S. H. & Valvani, S. C. (1980). Water solubility and Octanol/ Water partition coefficients of organics. Limitations of the

solubility partition coefficient correlation. Environ Sci. Technol., 14: 1227 – 1229.
6. U.S. Environmental Protection Agency. (2003). Integrated risk information system (IRIS): Benzene.
7. ATSDR (Agency for Toxic Substances and Disease Registry). (1997). Toxicological profile for benzene (update). Public health service, U.S. department of health and human services, Atlanta, GA.
8. Lovern, M. R.; Turner, M. J. & Meyer, M. (1997). Identification of benzene oxide as a product of benzene metabolism by mouse, rat and human liver microsomes. Carcinogenesis., 18:1695- 1700.
9. Ross, D.; Siegel, D.; Schattenberg, G.; Sun, X. M. & Moran, J. L. (1996). Cell-specific activation and detoxification of benzene metabolites in mouse and human bone marrow: identification of target cells and a potential role for modulation of apoptosis in benzene toxicity, Environ. Health prespect., 104 (Suppl.6): 1177- 1182.
10. Subrahmanyam, V. V.; Ross, D.; Eastmond, D. A. & Smith, M. T. (1991). Potential role of free radicals in benzene- induced myelotoxicity and leukemia, free radic. Biol. Med., 11: 495- 515.
11. Zhang, L.; Smith, M.; Bandy, B.; Tamaki, S. J. & Davison, A. J. (1994). Role of Quinones, active oxygen species and metals in the genotoxicity of 1,2,4-benzenetriol, a metabolite of benzene, In: H. Nhol, H.; Esterbauer, C. and Rice Evans (Eds.), Free radicals in the environment, medicine and toxicology, Richelieu, London, PP. 521– 562.
12. Ji, Z.; Zhang, L.; Guo, W.; McHale, C. M. & Smith, M. T. (2009). The benzene metabolite, hydroquinone and etoposide both induce indoor duplication in human lymphoblastoid TK6 cells, Mutagenesis, 24: 367 – 372.
13. Cho, J. Y. (2008). Suppressive effect of hydroquinone, benzene metabolite, on in vitro inflammatory responses mediated by macrophages, monocytes and lymphocytes, Mediat. Inflamm., 298010.
14. Ouyang, Y.; Virasch, N. & Hao, P. (2000). Suppression of human IL – 1 beta, IL – 2, TNF gamma – and TNF – alpha production by cigarette smoke extracts, J. Allergy Clin. Immunol., 106: 280 – 287.
15. Yoon, B. I.; Hirabayashi, Y. & Kawasaki, Y. (2002). Aryl hydrocarbon receptor mediates benzene – induced hematoxicity. Toxicol. Sci., 70: 150 – 156.
16. Pederson- Bjergaard, J.; Andersen, M. T. & Christiansen, D. H. (2008). Genetics of therapy- related myelodysplasia and acute myloid leukemia. Leukemia, 22: 240- 248.
17. Hannumantharao, G. R.; Smita, M.; Vrinder, S. P.; Ekta, K.; Yogesh, K. T. & Vishwajeet, R. (2001). Chemoprevention of benzene- induced bone marrow and pulmonary genotoxicity. Tetragen Carcinogen Mutagen, 21: 181- 187.
18. Hedli, C. C.; Rao, N. R. & Reuhh, K. R. (1996). Effects of benzene metabolite treatment on granulocytic differentiation and DNA adduct formation in HL–60 cells. Arch Toxicol., 70: 135-144.
19. Ringer, K. & Addis, P. (1983). Protecting workers from benzene exposure. In: Mehlman M. A., ed, carcinogenicity and toxicity of benzene, princeton, NJ , Princeton Scientific publishing, PP. 77– 89.
20. Luna, L. G. (1968). Processing of tissue. Histologic Staining methods of the armed forces institute of pathology. 3rd ed. McGraw-Hill book comp. London, New York, Toronto, Sydney. PP. 12-31.
21. Al- Rawi, K. M. & Kalaf- Allah, A. M. (1980). Design and Analysis of agriculture experiments. DAR– Al – Kutub, Mosul. Iraq.
22. Holmberg, B. % Lundberg, P. (1985). Benzene standards, occurrence and exposure. Amer. J. of Industrial Med., 7: 373 – 383.
23. Alice, M. D.; Hugh, L. E. & Carroll, A. S. (1984). The temporal relationship between behavioral and hematological effects of inhaled benzene. Toxicol. and Applied Pharmacol., 76 (1):195 – 203.

24. Andrews, L. S.; Lee, E. W.; Witmer, C. M.; Kocsis, J. J. & Syder, R. (2006). Effect of toluene on the metabolism, disposition and hematopoietic toxicity of (H3)– benzene. Biochem. Pharmacol., 26: 293 – 300.
25. Andrew, C. B. & James, H. (2006). Additive effects and potential inhibitory mechanism of some common aromatic pollutants on in vitro mitochondrial respiration. J. of Biochemical Toxicol., 7(3): 155 – 161.
26. Dundaroz, M. R.; Turkbag, T.; Akay, C.; Sarici, S. U.; Aydin, A.; Denli, M. & Gokcay, E. (2003). Antioxidant enzymes and lipid peroxidation in adolescents with inhalant abuse. The Turkish J. of Pediatrics, 45: 43 – 45.
27. Hayes, R. B. (1997). Benzene and the dose- related incidence of hematologic neoplasms in china. J. of the National Cancer Institute, 89 (14): 1065- 1071.
28. Hayes, R. B. (2000). Benzene and lymphohematopoietic malignancies in china. J. of Toxicol. and Envir. Health. Part A, 61 (5 – 6): 419 – 432.
29. Talamini, R. (2005). Smoking and non – Hodgkin lymphoma: case control study in Italy. Int. J. of Cancer, 115 (4): 606 – 610.
30. Kopstein, M. (2006). Potential uses of petrochemical products can result in significant benzene exposures: MSDSc must list benzene as an ingredient. J. of Occupational and Environment Hygiene, 3 (1): 1 – 8.
31. Zhang, L. (2000). The nature of chromosomal aberrations detected in human exposed to benzene. Critical Review in Toxicol., 32 (1): 1 – 42.
32. Wolf, M. A.; Rowe, V. K. & McCollister, D. D. (1956). Toxicological studies of certain alkylated benzenes and benzene. Arch. Ind. Health, 14: 387 – 398.
33. Aoyama, K. (1986). Effect of benzene inhalation on lymphocyte subpopulations and immune response in mice. Toxicol. Appl. Pharmacol., 85: 92 – 101.
34. Dilley, J. V. (1977). Final report. Toxic evaluation of inhaled chlorobenzene (Monochlorobenzene). Stanford research institute. Contract No. 210 – 76 – 0126. Prepared for: National institute of occupational safety and health division of biochemical and behavioral sciences. Cincinnati, OH: NIOSH. NTIS. No. PB – 276623.
35. Jhon, J. A.; Hayes, W. C.; Hanley, T. R.; Johnson, K. A.; Gushow, T. S. & Raoks, S. (1984). Inhalation teratology study on monochlorobenzene in rats and rabbits. Toxicol. Appl. Pharmacol., 76: 365 – 373.
36. Nair, R. S.; Burter, J. A.; Schrooeder, R. E.; Knezevich, A. & Stack, C. R. (1987). A two – generation reproductionnstudy with monochlorobenzene vapor in rats. Fundam. Appl. Toxicol., 9: 678 – 686.
37. Ogata, M.; Taguchi, T.; Hirota, N.; Shimada, Y. & Nakae, S. (1991). Quantitation of urinary chlorobenzene metabolites by HPLC concentrations of 4 chlorocatechol and chlorophenols in urine and of chlorobenzene in biological specimens of subjects exposed to chlorobenzene. Int. Arch. Occup. Environ. Health, 63: 121 – 128.
38. Huang, J. Y.; Liao, J. W.; Liu, Y. C.; Lu, S. Y.; Chou, C. P.; Chan, W. H.; Chen, S. U. & Ueng, T. H. (2008). Motorcycle exhaust induces reproductive toxicity and testicular interleukine -6 in male rats. Toxicol. Sci., 103(1):137-148.
39. Rae, P. S.; Ansari, M. F.; Gavane, A. G.; Pandit, V. L.; Nema, P. & Devotta, S. (2007). Seasonal variation of toxic benzene emission in petroleume refinery.128(1-3):323-328.
40. Upadhyay, S.; Stoeger, T.; Harder, V.; Thomas, R. F.; Schladweiler, M. C.; SemmlerBehnke, M.; Takenaka, S.; Karq, E.; Reitmeir, P.; Bader, M.; Stampfl, A.; Kodavant, U. P. & Schuiz, H. (2008). Exposure to ultrafine carbon particles at levels below detectable pulmonary inflammation affects cardiovascular performance in spontaneously hypertensive rats. Part Fibre Toxicol., 5:19.
41. Medinsky, M. A.; Wolf, D. C.; Cattley, R. C.; Wong, B.; Janszen, D. B.; Farris, G. M.; Wright, G. A. & Bond, J. A. (2006). Effects of a thirteen-week inhalation exposure to ethyltertiary butyl ether of ficher -344 rats and CD-1 mice. Toxicol. Sci., 5(1):108-118.