Effect of benzene on oxidative stress and the functions of liver and kidney in rats

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ABSTRACT

Aim: Benzene is a volatile organic compound known to be carcinogenic to humans. The present study was conducted to determine the effects of exposure to average indoor concentrations of benzene on liver and kidney of rats. Methods: Adult male albino Wistar rats were exposed to 10, 30 and 50 ppm of benzene for 14 days. The effect on lipid peroxidation (LPO), nitric oxide (NO) and glutathione (GSH) as oxidative markers, in addition, liver and kidney function levels were determined in adult male rats. Results: Benzene intoxication increased the activities of liver enzymes (alanine aminotransferase, aspartate aminotransferase and alkaline phosphatase) and the bilirubin level, in addition to the levels of uric acid, urea and creatinine were increased in the serum. Moreover, benzene inhalation at 10, 30 and 50 ppm for 14 days in adult male rats enhanced LPO and NO production in both serum and liver with a concomitant reduction in GSH content. Conclusions: On the basis of the above results, it can hypothesis that benzene inhalation caused hepatic and renal damages even at low concentration.

INTRODUCTION

Volatile organic compounds (VOCs) are pollutants that are often associated with human activities. They are widely encountered in our environment at low levels in the air, water, and even soil [1]. Indoor air pollution is usually first to come to mind when discussing VOCs, because People spent up to 90% of their time indoors [2,3]. Indeed, VOCs concentrations measured in the indoor air often exceed (up to 10 times) concentrations in the outdoor air [1,4].

Benzene is a VOC known to be carcinogenic to humans and is classified as a Group 1 carcinogen [5,6]. Benzene is a ubiquitous pollutant and known human leukemogen. The WHO has estimated that a lifetime exposure of 1 μg/m³ of benzene leads to about six cases of leukemia per million inhabitants [7]. Benzene is volatile, well absorbed, extensively metabolized, and does not persist in the body for long periods of time. Benzene can produce neurological impairment and can cause hematological effects including a plastic anemia and acute myelogenous leukemia [1]. Benzene can be enzymatically bioactivated to reactive intermediates that can lead to increased formation of reactive oxygen species (ROS) [8].

Inhalation accounts for more than 95-99% of the benzene exposure of the general population, whereas intake from food and water consumption is minimal [9]. Indoor benzene is associated with human activities such as cleaning, painting, the use of consumer products and mosquito repellents, photocopying and printing, the storage and use of solvents and smoking tobacco [7,10-12]. Therefore, the present study was undertaken to evaluate the oxidative stress induction and tissues injury in liver of adult male rats followed exposure to benzene at different concentrations.

MATERIALS AND METHODS

Animals

Adult male Wistar albino rats weighing 160-200 g (8-10 weeks) were obtained from The Holding Company for Biological Products and Vaccines (VACSERA, Cairo, Egypt). The animals were kept in wire bottomed cages in a room under standard condition of illumination with a 12 h light-dark cycle at 25 ± 2°C for 1 week until the beginning of treatment. They were provided with tap water and balanced diet ad libitum. All animals have received human care in compliance with the state authorities following the Egyptian rules of animal protection.
Benzene Exposure System

Rat whole body inhalation exposure was conducted in a chamber (dark plastic box; 50 cm long, 42 cm wide and 40 cm deep; volume = 0.084 m³) in an experimental room. Chamber airflow entered through two rubbers tubing (5 m length; 1 cm diameter) into two sides of the chamber to allow continuous air revival during the benzene exposure period [13]. Pure benzene was allowed to pass within the airflow with different concentration at each cage (concentration were 10, 30 and 50 ppm).

Rats (in their rearing cages) were placed in the inhalation chamber to benzene (C₆H₆; molecular weight = 78.11; sigma) for 5 h/day (from 9 a.m. to 1 p.m.), 5 days/week (no weekend exposures) for 2 weeks. This experiment was conducted in June 2013.

Benzene Concentration and Gas Chromatographic Analyses

The benzene concentration in the inhalation chamber was measured in a pre-study by gas chromatography. Samples of chamber’s air, taken at 30-min intervals during 4 h (exposure period) were injected and analyzed in a gas chromatograph Hewlett-Packard gas chromatography (GC) (Model HP6890), fitted with a flame ionization detector. An HP-5 (30 m ×320 μm) capillary column was used with hydrogen as a carrier gas and temperature programming from 30°C (5 min) to 250°C at 10°C min⁻¹. The instrument was checked on a daily basis based on the drift in retention times and responses of selected compounds in the standard calibration injection. The concentrations of benzene were quantified by an external standard calibration.

Experimental Design

Animals were randomly divided into four groups (6 rats/group) as follows:
1) The first group served as control and did not expose to any concentration of benzene.
2) The second group exposed to 10 ppm of benzene vapor.
3) The third group exposed daily to 30 ppm of benzene vapor.
4) The fourth group exposed to 50 ppm of benzene vapor.

The ratio was calculated according to a long-term measurement of the maximum monthly concentration of benzene in the indoor air (10, 30 and 50 ppm are the means of about half year, 2 and 3 years, respectively) [14].

After 24 h of the last exposure, the animals of all groups were excised, weighed and homogenized immediately to give 50% (w/v) homogenate in ice-cold medium containing 50 mM Tris-HCl, pH, 7.4. The homogenate was centrifuged at 3000 rpm for 10 min at 4°C. The supernatant (10%) was used for the various biochemical determinations. Total protein concentration was measured to express the concentration of different homogenate parameters as mg⁻¹ protein according to the method of Lowry et al. [15] using bovine serum albumin as a standard.

Liver and Kidney Parameters

Serum levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP), total bilirubin (TB), urea, creatinine and uric acid were determined using Kits purchased from Bio-diagnostic Co., Egypt.

Oxidative Stress

Nitrite/nitrate (NO) and lipid peroxidation (LPO) were assayed colorimetrically in liver homogenates according to the methods of Green et al. [16] and Ohkawa et al. [17], respectively, where LPO was determined by using 1 ml of trichloroacetic acid 10% and 1 ml of thiobarbituric acid 0.67% which were then heated in a boiling water bath for 30 min. Thiobarbituric acid reactive substances were determined by the absorbance at 535 nm and expressed as malondialdehyde (MDA) formed. NO was determined by optimized acid reduction method at 540 nm. In addition, the hepatic glutathione (GSH) was determined by the methods of Ellman [18]. This method is based on the reduction of Elman’s reagent (5,5’- dithiobis (2-nitrobenzoic acid) “DTNB”) with GSH to produce a yellow compound. The reduced chromogen was directly proportional to GSH concentration, and its absorbance can be measured at 405 nm.

Statistical Analysis

Results were expressed as the mean ± standard error of the mean (SEM). Data for multiple variable comparisons were analyzed by one-way Analysis of Variance. For a comparison of significance between groups, Duncan’s test was used as a post hoc test according to the Statistical Package Program (SPSS version 17.0) and figures were drawn with Origin (version 8).

RESULTS

Liver Function Levels In Serum of the Different Exposed Groups

Table 1 shows the effect of benzene inhalation at different concentrations (10, 30 and 50 ppm) on liver function levels in serum of rats. From the present data, it can be noticed that ALT and ALP levels in serum is significantly (P < 0.001 and P < 0.05, respectively) affected with increasing the concentration of benzene. The highest ALT level in serum was recorded at 50 ppm of benzene. Moreover, AST level in serum was significantly (P < 0.001) affected with benzene exposure [Table 1]. The maximum AST level in serum was recorded at benzene concentration 10 ppm. Whereas, TB level in serum was decreased significantly at 10 ppm (P < 0.001) and 50 ppm (P < 0.05) when compared to control rats.

Kidney Function Levels in Serum of the Different Exposed Groups

Exposure to benzene (10 ppm) caused a significant increase in serum urea and creatinine levels when compared to the control values [Table2]. Moreover, inhalation of 50 ppm of benzene...
was caused significant ($P < 0.001$) increase in serum urea, uric acid, and creatinine. At the same manner, exposure to 50 ppm of benzene caused a significant increase in urea and uric acid at $P < 0.001$. The maximum effect in the kidney function parameters was seen in 30 ppm exposed rats.

**LPO Levels in Serum and Liver of the Different Exposed Groups**

Figure 1 shows the effect of inhalation of benzene concentrations 10, 30 and 50 ppm on LPO levels in serum and liver tissue of male rats. From the present data, it can be noticed that benzene inhalation has a significant effect on the levels of serum and hepatic LPO formation on rats. The maximum elevation in LPO levels in both serum and liver were occurred at benzene concentration 30 ppm.

**Nitric Levels in Serum and Liver of the Different Exposed Groups**

There was a significant decrease in hepatic NO level with increasing the concentration of benzene [Figure 2]. The maximum elevation in NO level was recorded at benzene concentration 50 ppm. On the other hand, exposure to benzene (10 and 30 ppm) was caused a significant increase in NO production in serum of rats. However, there is no significant difference in NO level in serum between control rats and rats exposed to 50 ppm benzene.

**Glutathione Levels in Serum and Liver of the Different Exposed Groups**

The toxicity of benzene on serum and liver is represented in Figure 3. The exposure of rats to benzene (30 and 50 ppm) caused a significant decrease in GSH levels in serum and liver. GSH contents in the serum and hepatic tissue decreased significantly compared to the controls in a dose-dependent manner. The maximum decrease in GSH levels in both serum and hepatic homogenates were recorded at benzene concentration equal 50 ppm.

**DISCUSSION**

**Benzene and the Redox Status**

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. The current results reveal that benzene inhalation at different concentrations (for 10, 30, and 50 ppm) 14 days causes significant increase in serum and liver LPO, this effect increased with increasing the concentration of benzene. Our results are in good agreement with Rana et al. [19] who found that administration of benzene to rats induces LPO in liver and bone marrow, increased LPO has been attributed to low GSH. Increased level of LPO was also noticed by Sharma and Sangha [20]. LPO is known to disturb the integrity of cellular membranes, has been implicated in the pathogenesis of various liver and kidney injuries [21]. ROS and LPO have been supposed to serve as common mediators of apoptosis in response to many toxicants and pathological conditions [22].

**Table 1: The effect of benzene inhalation at different concentrations (10, 30 and 50 ppm) on liver function levels in serum of adult male albino rats**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Liver function</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALT (IU/L)</td>
<td>AST (IU/L)</td>
</tr>
<tr>
<td>Control</td>
<td>72.5±2.23</td>
</tr>
<tr>
<td>10 ppm</td>
<td>94.7±1.56**</td>
</tr>
<tr>
<td>30 ppm</td>
<td>100.9±1.35**</td>
</tr>
<tr>
<td>50 ppm</td>
<td>102.4±1.09**</td>
</tr>
</tbody>
</table>

Values are means±SEM ($n=7$), *$P<0.05$, significant change with respect to control group, ** changes between $P<0.01$ and $P<0.001$, SEM: Standard error of mean, ALT: Alanine aminotransferase, AST: Aspartate aminotransferase, ALP: Alkaline phosphatase, TB: Total billirubin.

**Table 2: The effect of inhalation of benzene concentrations 10, 30 and 50 ppm on kidney functions level ($\mu$mol/mg protein) in serum of adult male albino rats**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Kidney functions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urea (mg/dL)</td>
<td>Uric acid (mg/dL)</td>
</tr>
<tr>
<td>Control</td>
<td>31.3±0.71</td>
</tr>
<tr>
<td>10 ppm</td>
<td>36.99±1.39*</td>
</tr>
<tr>
<td>30 ppm</td>
<td>39.80±1.37***</td>
</tr>
<tr>
<td>50 ppm</td>
<td>40.11±0.86***</td>
</tr>
</tbody>
</table>

Values are means±SEM ($n=7$), *$P<0.05$, significant change with respect to control group, ** changes between $P<0.01$ and $P<0.001$, SEM: Standard error of mean.

**Figure 1: The effect of benzene inhalation at different concentrations (10, 30 and 50 ppm) on lipid peroxidation levels in serum and liver tissue of adult male albino rats**
The present study reveals that benzene exposure caused a significant increase in serum and hepatic NO level. The elevated NO levels observed may have contributed to the development of protein damage, since NO reacts directly with the tyrosyl radical to form 3-nitrotyrosine [23,24]. Moreover, in high concentrations, NO together with superoxide anion (O2•−) give rise to peroxynitrite (ONOO−), which is a major nitration agent [23,25]. In addition to protein nitration, peroxynitrite could also induce protein carbonylation, common oxidative protein damage [25]. Both forms of protein damage can lead to changes in protein function and subsequent alteration in biological responses [25].

Liver and serum GSH shows a significant decrease in the animals exposed to benzene. GSH is an important intracellular radical scavenger, which regulates the redox status of many other cellular substances, thus playing an essential role in the detoxication processes [26,27], especially those involved in the biotransformation of benzene [28]. Previous studies indicated that GSH depletion is one of the important consequences of toxic injury and established the critical role of GSH in protecting tissues from toxic effects of accumulating reactive intermediates [22].

These results are supported by the previous study of Raza et al. [29] who found a decrease in GSH with a concomitant increase in LPO levels after benzene exposure in rats. GSH is involved in benzene biotransformation in the liver. In the first step, benzene is oxidized to benzene oxide in a reaction catalyzed by cytochrome P450 2E1; rearrangement generates phenol; reaction with GSH catalyzed by GST produces less toxic metabolites, such as S-phenylmercapturic acid, which is excreted in the urine [27,30,31].

The activation of benzene and its metabolites culminates in damage to lipids, proteins, and carbohydrates by various chemical reactions such as oxidation, nitration, and halogenation leading to functional alterations in different tissues [26,28]. Furthermore, chronic benzene exposure compromises antioxidant capacity, which also contributes to the development of oxidative damage in exposed subjects [26]. Measurement of enzyme activity in serum is of importance since it helps to assess the state of the liver and other organs [32].

**Benzene and the Cellular Integrity**

Liver function levels in serum were significantly affected with exposure to benzene, level of ALT, AST, ALP and TB increased with increasing the concentration of benzene.

The liver has been shown to be one of the target organs of benzene vapors toxicity [33]. The relationship between the hepatic oxidative damage and increase in the activities of such serum enzymes as ALT, AST, ALP and TB has been well documented [33,34]. Exposure to benzene vapors stimulates cellular MDA, a product of LPO [35], which affect the
permeability barrier of the plasma membrane. The result of this study gives an indication that the hydrocarbons and other chemical constituents of the benzene vapors are likely metabolized in the liver, among others, to reactive species which interact with the tissues to cause LPO, thereby exhibiting their toxic or hazardous effects. The observed increase in the activities of plasma ALT, AST, ALP and TB level, is likely to be due to LPO of biomembranes that causes leakage of cellular components [32]. Therefore, it is likely that the increase in the liver enzymes reported in this present study may be due to the accumulation of benzene constituents and their reactive metabolites in hepatic tissues which enhance formation of LPO.

There was a significant effect of inhalation of benzene on kidney functions level in serum of adult male albino rats. The concentrations of urea, uric acid and creatinine in serum were significantly increased with increasing the concentration of benzene. This is in accordance with Jia et al. [36]. The kidneys play a special role in concentrating toxic substances within its tubules and excreting them. These functions render it susceptible to damage by certain chemical substances. The kidney is the major organ of excretion of metabolites of benzene components [37].

CONCLUSION

From this study, we conclude the dangerous effects of benzene, especially due to their wide spread: in persons who directly exposed to these substances at work chronically. Exposure to benzene is associated with adverse health effects as toxicity to liver and kidney.

REFERENCES

33. Uboh FE, Akpanabi MI, Eteng MU, Ebong PE, Umoh IB.


