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Renal and hepatotoxic alterations in adult mice on inhalation of specific mixture of organic solvents

Vaghasia K. Ketan[1], Kalariya Bhavyata[1], George Linzbuoy[1] and Highland N. Hyacinth[1]

Abstract

This study was aimed at investigating alterations in renal and hepatic toxicity induced by exposing to a combination of three solvents, namely, benzene, toluene and xylene in adult mice. The mice were divided into three groups (control, low-dose-treated (450 ppm) and high-dose (675 ppm) groups) using randomization methods. The treated groups were exposed to vapours of a mixture of benzene, toluene and xylene at doses of 450 and 675 ppm, for 6 h day^{-1} for a short-term of 7-day exposure period. The study revealed that the solvent exposure resulted in an increase in the weight of liver and kidney as compared to the control. Biochemical analyses indicated a significant decline in the activities of superoxide dismutase and catalase in both the treated groups, with concomitant increase in lipid peroxidation. Liver aminotransferases (alanine aminotransferase and aspartate aminotransferase) were elevated with significant alterations in the levels of protein, creatinine and cholesterol in these tissues upon solvent exposure. Correlated with these changes, serum thyroid hormones T_3 and T_4 were also significantly altered. This study, therefore, demonstrates that inhalation of vapours from the solvent mixture resulted in significant dose-dependent biochemical and functional changes in the vital tissues (liver and kidney) studied. The study has specific relevance since humans are increasingly being exposed to such solvents due to increased industrial use in such combinations.

Keywords

Inhalation, liver, kidney, solvents mixture, oxidative stress

Introduction

Currently, organic solvents like benzene, toluene and xylene pose increasing potential health hazard because of their wide industrial use in the production of plastics, paints, glues, solvents and also as intermediates in the production of other chemicals substances.

The term solvent refers to a class of liquid organic chemicals of variable lipophilicity and volatility. These properties, coupled with small molecular size and lack of charge, make inhalation as the major route of solvent exposure and provide way for ready absorption across the lungs, gastrointestinal (GI) tract and skin, eventually affecting vital organs.

Almost everyone is exposed to these solvents daily, albeit in minute amounts (Ashley et al., 1994), particularly when used in products like aerosol propellants, paint thinners, cleaners and soil fumigants. High concentrations of certain solvents (e.g. 10–520 $\mu g\ m^{-3}$ or 3–163 ppb of benzene) have been measured in urban areas, around petrochemical plants and in the immediate vicinity of hazardous waste sites (Bennett, 1987; Kelly et al., 1993). This increased exposure to solvents has manifold effects on diverse tissues and organs.

Most of the research has been centred around investigating organic solvents individually. Exhaustive studies have been carried out demonstrating inhalation and absorption of benzene (Sabourin et al., 1987). Similarly, Rickert et al. (1979) have reported high benzene concentrations in several tissues, including fat, bone

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marrow, kidney, liver and spleen, suggesting vital organ toxicity. In separate studies, toluene has also been shown to accumulate in vital tissues, with an age-dependent oral toxicity (U.S. EPA, 1990). High-dose exposure (2500 mg kg^{-1}) of toluene was also reported to cause cancer, hypoactivity, ataxia, lacrimation and body tremors in rats. Separate experiments have revealed that xylene metabolites affect blood, lung and intestine (Bergman, 1983).

Hepatotoxicity is a striking feature of exposure to such solvents. Moreover, it has been suspected that other solvents that are less commonly used today may also be hepatotoxic (Lundberg et al., 1994). In addition, the liver toxicity of these solvents may be due to the metabolic activation with the formation of reactive metabolites.

There are certain publications that indicate endocrine alterations on exposure to industrial chemicals, vapours and solvents (Verma and Rana, 2009). In addition, Zaidi et al. (2006) have demonstrated subchemical hypothyroidism in spray painters. These reports therefore reflect the possible endocrine alterations due to solvent exposure. In addition, there are evidences to suggest that significant metabolic alterations result from the influence of solvents on the liver. Bearing these facts in mind, in the present study, the levels of serum thyroid hormones T_3 and T_4 were assayed to confirm the role of the thyroid and its impact on metabolism, under conditions of solvent exposure.

The National Institute of Occupational Safety and Health (1977) has emphasized major health impacts with organic solvent exposure, which includes nervous, reproductive, skin, kidney and liver damages. However, most of these pioneering studies focus on the effects of benzene, toluene or xylene alone; very few investigations indicate the impact of combinations of such solvents. Chen et al. (1991) have described hepatotoxic effects of solvent mixtures among paint workers. Wang and Chen (1993) earlier reported acute and neurological symptoms among those workers who were occupationally exposed to mixtures of organic solvents. Effects of xylene and formaldehyde inhalations were also shown to have severe toxic effects on vital functions.

In highly industrialized zones, workers in paint and thinner units are constantly being exposed to varied mixtures of benzene, xylene and toluene vapours. However, there are very few basic research reports documenting the hazardous impact of mixtures of these three noxious solvents. Hence, research directed towards the possible synergistic toxicity or augmented mode of these solvents in combinations and mixtures is now imperative.

Materials and methods

Healthy adult male albino mice, *Mus musculus* of Swiss strain, weighing between 25and 39 g were obtained from a recognized supplier. Prior to the commencement of treatment, all the animals were acclimatized for 7 days and were maintained under controlled conditions with 12-h light and 12-h dark cycles at temperature of 26 $\pm$ 2°C and relative humidity of 30–70%. Standard chow (obtained from Amrut Laboratory, Baroda, India) and water were provided *ad libitum.* Experiments were conducted in accordance with the guidelines set by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), India, and experimental protocols were approved by the institutional animal ethics committee (167/1999/CPCSEA).

Experimental design

Inhalation procedures were followed in accordance with those described by Valentine and Kennedy (2001), with minor modifications based on the method employed by Uboh et al. (2005). Mice were subjected to whole body exposure of solvents, the details of which have been mentioned in the following paragraph. Each cage had a separate supply of test solvents. To acclimatize the mice, they were housed in closed chambers/mice cages, made of stainless steel, and kept in sets of six animals per cage under standard laboratory conditions (light period 6 a.m.–7 p.m. at a temperature of 26 $\pm$ 2°C; water and standard pellet diets were given *ad libitum*) for 1 week. At the beginning of the experiment, all the mice were divided into three groups (control, low-dose-treated (450 ppm) and high-dose (675 ppm) groups) using randomization. The treated animal groups were exposed to vapours of a solvent mixture of benzene, toluene and xylene in the ratio of 1:1:1 at the doses of 450 and 675 ppm for 6 h day^{-1} (from 9 a.m. to 3 p.m.) for 1 week. The mixture was placed in a slot provided in a specially designed exposure cage with dimensions (49 $\times$ 27 $\times$ 29 cm^3) correlated with which the required solvent volume was calculated, in accordance with the standard specifications given by Uboh et al.(2005). After the exposure period, the animals were autopsied according to the CPCSEA specification. Control animals were placed in a cage without the exposure chamber. All the chemicals used in the experiment were of analytical grade.

Tissue collection

After the exposure period, the mice were anaesthetized and killed as per CPCSEA specifications. Liver and kidney were dissected out carefully, blotted free of blood and weighed. Tissue was processed and the homogenate was prepared.

Superoxide dismutase activity

Superoxide dismutase (SOD) activity in liver and kidney of mice were estimated using the technique of Kakkar et al. (1984). In this method, the formazan formed at the end of the reaction indicates the presence of the enzyme. One unit of the enzyme activity is defined as the enzyme concentration required to inhibit 50% of the absorbance of chromogen formed in 1 min at 560 nm under the assay condition. Results were expressed as unit of SOD per minute per milligram protein.

Determination of thiobarbituric acid reactive substances

The thiobarbituric acid reactive species (TBARS) levels in liver and kidney of control and treated animals were determined by the method of Ohkawa et al. (1979).This method is based on the formation of a red chromophore that absorbs at 532 nm following the reaction of TBA with malonyl dialdehyde (MDA) and other breakdown products of peroxidized lipids collectively called as TBARS. Lipid peroxidation (LPO) was expressed in terms of nano moles of MDA formed per milligram tissue using an extinction coefficient of $1.56 \times 10^5\ M^{-1}\ cm^{-1}$.

Catalase

The catalase (CAT) activity in the liver and kidney of control and treated animals was assayed by the modified method of Sinha (1972).

Protein

Protein levels in liver and kidney of control and treated groups of animals were estimated using the method of Lowry et al. (1951). Protein-containing preparation when treated with phenol reagent of Folin–Ciocalteau developed blue colouration and was read at 540 nm.

Cholesterol

The levels of cholesterol in the liver and kidney of control and treated groups of mice were estimated by the method of Zlatkis et al. (1953). In the presence of concentrated sulfuric acid and glacial acetic acid, cholesterol forms a coloured complex with ferric chloride, which was measured using a Systronics Digital Spectrophotometer 167 (Systronics (India) Limited, Ahmedabad), against blank.

Creatinine

The activity of creatinine was estimated in the kidney of control and treated-animal groups according to the method given by Merck (1974). Creatinine is present in the tissue homogenate that reacts with picric acid in an alkaline medium to form orange–red coloured complex, which is measured spectrophotometrically at 520 nm.

Alanine aminotransferases and aspartate aminotransferases

Serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels are elevated in viral and other forms of liver diseases associated with hepatic necrosis. ALT is present in liver cells in much higher concentrations than in any other organs. In serum, ALT and AST activities were determined using commercially available kits (Span Diagnostics Ltd, Surat, Gujarat, India, – code no. 76 MB101-50) and the absorbance was read at 505 nm using spectrophotometer (Systronics (India) Limited, Ahmedabad, visiscan 167).

Serum triiodothyronine and thyroxine hormones

Serum triiodothyronine (T_3) and thyroxine (T_4) hormone levels were assayed by direct competitive enzyme immunoassay using horseradish peroxidase – thyroxine and tetramethyl benzidine substrate. The test was carried out using specific enzyme-linked immunosorbent assay kits from Labora diagnostics Nord GmbH and Co., KG, Nordhorn, Germany.

Data analyses

All the data are presented as mean $\pm$ standard error. Statistical analysis was carried out using the SPSS software package version 16.0 (USA). Student's t test was carried out taking significance at the 5% confidence limit ($^{**}p < 0.001$ and $^{*}p < 0.01$)

Table 1. Body weight and organ weight of control and exposed animals.[a]

Groups	Treatment	Body weight	Organ weight	
			Liver	Kidney
I	Control	39.00 ± 0.570	2.24 ± 0.015	0.455 ± 0.022
II	450 ppm	39.80 ± 0.057[b]	2.77 ± 0.225[b]	0.529 ± 0.031[b]
III	675 ppm	40.23 ± 0.145[b]	2.83 ± 0.010[c]	0.535 ± 0.013[d]

[a]Values are mean ± standard error.
[b]Nonsignificant.
[c]$p < 0.001$.
[d]$p < 0.01$.

Table 2. Activities of SOD, CAT and TBARS in liver of control and treated animals.[a]

Liver				
Groups	Treatment	SOD	CAT	TBARS
I	Control	0.111 ± 0.003	2.88 ± 0.087	53.00 ± 2.00
II	450 ppm	0.069 ± 0.001[b]	1.67 ± 0.174[c]	82.67 ± 3.75[b]
III	675 ppm	0.069 ± 0.0008[b]	0.99 ± 0.078[b]	89.33 ± 3.75[b]

SOD: superoxide dismutase; CAT: catalase; TBARS: thiobarbituric acid reactive substance; MDA: malonyl dialdehyde; H_2O_2: hydrogen peroxide.
[a]Values are mean ± standard error. SOD activity is expressed in units per milligram protein. TBARS is expressed in nano moles of MDA per 100 mg tissue weight. CAT is expressed in micro moles of H_2O_2 consumed per minute per milligram protein.
[b]$p < 0.001$.
[c]Nonsignificant.

Table 3. Activities of SOD, CAT and TBARS in kidney of control and treated animals.[a]

Kidney				
Groups	Treatment	SOD	CAT	TBARS
I	Control	0.143 ± 0.005	1.85 ± 0.042	47.67 ± 2.02
II	450 ppm	0.098 ± 0.002[b]	1.44 ± 0.084[b]	77.33 ± 2.96[b]
III	675 ppm	0.078 ± 0.002[b]	0.78 ± 0.029[b]	79.67 ± 2.02[b]

SOD: superoxide dismutase; CAT: catalase; TBARS: thiobarbituric acid reactive substance; MDA: malonyl dialdehyde; H_2O_2: hydrogen peroxide.
[a]Values are mean ± standard error. SOD activity is expressed in units per milligram protein. TBARS is expressed in nanomoles of MDA per 100 mg tissue weight. CAT is expressed in micromoles of H_2O_2 consumed per minute per milligram protein.
[b]$p < 0.001$

Results

An insignificant increase in body weight was observed in both low-dose and high-dose groups of solvent-exposed mice as compared to control mice (Table 1). An insignificant increase was observed in organ weights after low-dose solvent exposure. Mice exposed to a high-dose level of 675 ppm showed that the organ weights of liver and kidney were significantly increased ($p < 0.001$ and $p < 0.01$, respectively) as compared to control mice.

Free radicals generated by solvent administration caused modulation of SOD activity, the first line of antioxidant defence (Tables 2 and 3). Solvent exposure resulted in decreased activity of hepatic SOD in low-dose ($p < 0.001$) and high-dose ($p < 0.001$) groups as compared to control mice (Tables 2 and 3), thereby lowering the antioxidant protective activity in the tissue. Hepatic LPO was enhanced after solvent administration significantly ($p < 0.001$) in low- and high-dose exposed animals. The liver CAT

Table 4. Levels of protein and cholesterol in control and treated animals.[a]

Liver			
Groups	Treatment	Protein	Cholesterol
I	Control	10.77 ± 0.17	0.85 ± 0.05
II	450 ppm	11.25 ± 0.53[b]	1.38 ± 0.04[c]
III	675 ppm	11.85 ± 0.35[d]	1.86 ± 0.07[c]

[a]Values are mean ± standard error. Protein is expressed in milligram per 100 mg tissue weight. Cholesterol is expressed in milligram per 100 mg tissue weight.
[b]Nonsignificant.
[c]$p < 0.001$.
[d]$p < 0.01$.

activity was decreased more significantly after high-dose solvent exposure ($p < 0.001$), while an insignificant decrease was found in the group exposed to low-dose solvent mixture. In kidney tissues after treatment, a significant decrease ($p < 0.001$) was observed in both low- and high-dose groups.

Tables 4 and 5 show the protein content in the liver and kidney of solvent-treated group and control group, respectively. Solvent treatment brought about an increase in the protein content in the low-and high-dose groups ($p < 0.01$). Protein levels in kidney showed a highly significant increase ($p < 0.001$) after high-dose treatments. Creatinine and cholesterol levels significantly increased ($p < 0.001$) in both the treatment groups studied.

Serum ALT and AST enzyme activity was also significantly increased ($p < 0.01$) in both the treatment groups, as compared to the control group. An insignificant increase in serum T_3 and T_4 levels was observed in both low- and high-dose groups of solvent-exposed mice as compared to control mice (Table 6).

Discussion

The results obtained in the present study indicated that organic solvents in mixtures caused immense toxicity in vital tissues such as liver and kidney. The increase obtained in the weights of liver and kidney suggests hepatocyte swelling and inflammation with possible oedema. Similar observations have been made by Sadiye et al. (2010), who have reported increased organ weights on exposure to a mixture of other solvents, namely xylene and formaldehyde. It was also observed that increase in liver and kidney weight was significantly correlated with the dose of toxic solvent administered at levels between 270 and 600 ppm Sadiye et al. (2010).

Exposure to the mixtures of toluene, xylene and benzene in this investigation also caused an increase in the level of protein, creatinine and cholesterol in the vital tissues studied indicating possible accumulation of the metabolites due to impaired metabolic turnover. In an earlier study, Aiso et al. (2005) also reported elevated protein and cholesterol levels in the liver of animals exposed to 600 ppm of a benzene derivative. In addition, the toxicity of the solvent mixture administered in the present study was evident with the significant increase in the activities of ALT and AST. These results suggest severe hepatotoxicity along with the changes in liver metabolism. Moreover, our findings conform with those of Uboh et al. (2005), who reported 191% increase in ALT and 161% increase in AST activity on exposure to a crude solvent (kerosene).

The findings of this investigation also revealed oxidative stress with increased oxygen toxicity due to reactive oxygen species coupled with impaired protective free radical scavenging enzymes like SOD and CAT. LPO was also found to be enhanced as evidenced by the increased MDA levels in these vital tissues. Contrary to these observations, Kum et al. (2007) have demonstrated that there was no statistically significant alteration in the SOD, CAT and glutathione peroxidase activities in the xylene–formaldehyde exposed groups as compared to the control.

In the present study, however, the mixture of xylene, benzene and toluene (450 ppm and 675 ppm) resulted in a significant decline in SOD and CAT activities, suggesting poor elimination of oxygen free radicals and consequently increased oxygen toxicity in liver and kidney. It has also been reported (Zaidi et al., 2001) that toxicants, such as solvents, affect hormone production and their action. The thyroid hormones are known to regulate metabolism and their levels vary in relation to liver function. Hence, the thyroid hormones were assayed to evaluate the possible changes in T_3 and T_4 levels and their impact on metabolism after solvent exposure. It was observed in this study that the serum levels of T_3 and T_4 were increased as compared to the control suggesting transient hyperthyroidism, which could be correlated with increased activity of ALT and AST. Our findings are supported with those of Paget (1961), who reported that hyperthyroidism is known to augment hepatic sensitivity to toxicants like chloroform. Also, studies have indicated thyroid gland follicular cell hyperplasia was also observed after exposure to ethyl benzene (National Toxicology Programme, 1999). Thyroid toxicity due to subchronic exposure to a complex mixture of 16 organochlorines has also been

Table 5. Levels of protein, creatinine and cholesterol in the kidney of control and treated animals.[a]

Kidney				
Groups	Treatment	Protein	Creatinine	Cholesterol
I	Control	08.61 ± 0.10	0.65 ± 0.02	0.99 ± 0.04
II	450 ppm	08.75 ± 0.57[b]	0.90 ± 0.02[c]	1.53 ± 0.04[c]
III	675 ppm	10.77 ± 0.37[c]	1.65 ± 0.02[c]	2.76 ± 0.13[c]

[a]Values are mean ± standard error. Protein is expressed in milligram per100 mg tissue weight. Creatinine is expressed in milligram per 100 mg tissue weight. Cholesterol is expressed in milligram per 100 mg tissue weight.
[b]Nonsignificant.
[c]$p < 0.001$.

Table 6. Serum ALT, (AST), T_3, and T_4 levels in control and treated animals.[a]

Serum					
Groups	Treatment	ALT	AST	T_3	T_4
I	Control	1.91 ± 0.04	1.78 ± 0.03	0.76 ± 0.005	0.78 ± 0.005
II	450 ppm	2.41 ± 0.03[b]	2.24 ± 0.03[b]	0.91 ± 0.005[c]	0.96 ± 0.005[c]
III	675 ppm	3.83 ± 0.14[b]	2.80 ± 0.14[b]	0.98 ± 0.005[c]	1.19 ± 0.005[c]

ALT: alanine aminotransferase; AST: aspartate aminotransferase; T_3: triiodothyronine; T_4: thyroxine.
[a]Values are mean ± standard error. ALT and AST enzyme activities are expressed in international units per litre. Serum T_3 and T_4 levels are expressed in micromoles per litre.
[b]$p < 0.001$.
[c]Nonsignificant.

documented. Wade et al. (2002) and, on the other hand, Zaidi et al. (2006), have shown condition of subclinical hypothyroidism in solvent-exposed spray painters, an observation which is contrary to our findings.

Moreover, the toxic changes were manifested in a dose-dependent manner, as it was also observed that the toxicity-induced alterations were more significant in the high-dose-exposed group (675 ppm) as compared with the low-dose group (450 ppm).

Conclusion

The results of this work suggest that solvent exposure at low- and high-dose levels has a definite effect on the organ studies. The body weight is insignificantly increased in both exposure groups as compared to the control group. Moreover, the organ weight of the liver and kidney was significantly increased as compared to control group, suggesting altered tissue metabolism due to exposure.

The data revealed a significantly decrease in the free radical protective enzymes, namely SOD and CAT in liver and kidney, suggesting enhanced oxygen toxicity correlated with increased tissue LPO on exposure to both high-and low-dose solvent mixtures. In addition, the protein and cholesterol in liver and kidney creatinine (kidney) content was significantly increased indicating toxicity induced metabolic changes in the tissues at both doses. Moreover, the elevated activity of serum ALT, AST and T_3 and T_4 levels obtained on exposure to both doses of solvent mixture could be correlated with the induced metabolic alteration and oxygen toxicity observed in liver.

Hence, the data obtained provides evidence of enhanced tissue injury on exposure to a specific mixture of benzene, toluene and xylene at doses of 450 ppm and 675 ppm.

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