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Effect of benzene on the enzymes of carbohydrate metabolism, brush border membrane (BBM) and oxidative stress in kidney and other rat tissues

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Abstract
Benzene found all over our environment and are toxic to general population especially children. This aromatic hydrocarbon being used in making of rubbers, lubricants, drugs, dyes and used as intermediate to make other chemicals and thus cause occupational hazard. Studies were carried out exposing the adult Wistar rats of 175-200gm to benzene (800mg/ kg body weight) via gavaging in corn oil for a period of 30 days and control rats received only vehicle for same period. The aim of the present work was to study the effect of benzene on the enzyme of carbohydrate metabolism, BBM and antioxidant defense parameters in different rat tissues. The nephrotoxic effect of benzene was manifested by increase blood urea nitrogen, serum creatinine and cholesterol levels. The activity of lactate dehydrogenase (LDH) increases whereas malate dehydrogenase (MDH) was decreased by benzene. The biomarker of brush border membrane alkaline phosphatase, gamma-glutamyl tranferase and leucine amino peptidase decreased in BBM of rat tissues. The activity of gluconeogenic enzymes G6Pase and FBPase declined by benzene exposure. In addition, the activities of superoxide dismutase and catalase significantly decrease with associated increase in lipid peroxidation. The results indicate that benzene induced nephrotoxicity and lowered the enzymes of carbohydrate metabolism and BBM most likely by inducing oxidative stress.

Keywords: Benzene; toxicity; carbohydrate metabolism; oxidative stress.

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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 (Ozturk et al., 1997; Fatima et al., 2004, 05; Kohn et al., 2005; Ruder., 2006; Farooq et al., 2007; Banday et al., 2008). Benzene is a ubiquitous environmental pollutant being used for industrial purposes. It is used in manufacturing products such as rubber, lubricants, detergents, drugs and pesticides. It is a clastogenic and carcinogenic agent. The most common exposures occur through auto-exhaust, industrial emissions, cigarette and smoke (ASTDR-2007). In addition to this, it originates from the following sources: exhaust emission of motor vehicles, oil refineries, shoes industry and waste products of timber industry (Andrews et al., 1997; Sammett et al., 1985; Anonymous, 1993). Benzene exposure suppresses bone marrow function, causing blood changes and CNS depression (U.S EPA). In addition to this, it has been shown benzene exposure affect the variety of organs such as kidney, liver, and brain (Dundaroz et al., 2003).

Numerous studies indicate that the metabolism of benzene is required for its toxicity (Irons, 1985; Eastmond et al., 1987; Barale et al., 1990). The metabolism of benzene is thought to be qualitatively the same for both animal and humans. The metabolism of benzene involves a series of oxidation of the benzene ring by the Cyt-P450, which is found through out the body but is concentrated in liver. However, benzene can stimulate its own metabolism, and can therefore increase the rate of formation of toxic metabolites (ASTDR, 1992). Since benzene is able to conjugate, the elevation of metabolites interactions may increases its toxicity (Arfellini et al., 1985). Benzene toxicity is related to the ability of its reactive intermediates bind to cellular macromolecules to induce damage (Levay and Bodell., 1992; Synder and Hedli, 1996). Earlier reports have shown, people, who work on sites where benzene concentration are high, may have physical discomfort, changes in their blood glucose level and energy metabolism dysfunction (Raumio et

al., 1988; Powley and Carlson, 1999; Dere et al., 2003).

In view of benzene toxicity in various tissues, the present investigation was undertaken to examine the effect of benzene on the enzymes of carbohydrate metabolism, brush border membrane and antioxidant defense mechanism in different rat tissues to understand putative and biochemical mechanism of benzene induced toxic insult. The results demonstrate that oral exposure of benzene resulted in increased blood urea nitrogen (BUN), serum creatinine, cholesterol, phospholipids and inorganic phosphate increases. The enzymes of carbohydrate metabolism selectively altered and the activities of BBM enzymes; ALP and GGT significantly decreased in all tissues studied. Lipid peroxidation (LPO), an indicator of tissue injury significantly enhanced whereas the activity of SOD and catalase decreased in rat intestine, liver, brain and renal cortex and medulla although to different extent. The results indicate that benzene exposure produced significant biochemical and metabolic alterations in different rat tissues albeit differentially most likely by inducing oxidative stress.

Materials and Methods

Benzene was purchased from Sisco Research Laboratory (Mumbai, India). Sucrose, p-nitrophenyl phosphate, NADH and NADP+ were purchased from Sigma Chemical Co. (St Louis, MO, USA). All other chemicals used were of analytical grade and were purchased from Sigma Chemical Co. or Sisco Research Laboratory (Mumbai, India).

Experimental design

The animal experiments were conducted according to the guidelines of the committee for Purpose of Control and Supervision of Experiments on Animals, Ministry of Environment and Forests, Government of India. All experimental animals were kept under conditions that prevented them from experiencing unnecessary pain and discomfort according to guidelines approved by the ethical committee. Adult male Wistar rats, weighing 150–175 g, fed with a standard pellet diet (Aashirwad Industries, Chandigarh, India) and allowed water *ad libitum*, were conditioned for 1 week before the start of the experiment. Two groups of rats (eight to ten rats/ group) were studied.

Benzene treated rats were given (800mg/ kg body weight/ d) in corn oil by gavages whereas the control rats received same amount of corn oil over 30 days. After 30 days of benzene administration, the rats were sacrificed under light ether anesthesia. Blood samples were collected from non-fasted rats and the liver, kidney, brain and intestine (starting from the ligament of Trietz to the end of the ileum) were extracted and kept in buffered saline. The intestines were washed by flushing them with ice-cold buffered saline (1 mM Tris-HCl, 9 g/L of NaCl, pH 7.4). All the preparations and analyses were carried out simultaneously under similar experimental conditions to avoid any day-to-day variations.

Preparation of homogenates

The washed intestines were slit in the middle and the entire mucosa was gently scraped with a glass slide and weighed. A 6.5% homogenate of this mucosa was prepared in 50mM mannitol, pH-7.0, in a glass Teflon homogenizer (Remi Motors, Mumbai, India) with five complete strokes. The homogenate was then subjected to a high-speed Ultra-Turrex homogenizer (Type T-25, Janke & Kunkel GMBH & Co. KG., Staufen) for three pulses of 30s each with an interval of 30 s between each stroke.

The kidneys were decapsulated and kept in ice-cold 154 mM NaCl and 5mM Tris-HEPES buffer, pH-7.5. The cortical and medullary regions were carefully separated and homogenized (as mentioned above) in 50mM mannitol buffer to obtain 10% (w/v) homogenate. The 10% liver and brain homogenate were similarly prepared in 10mM Tris-HCl buffer pH 7.5. One part of the homogenates (of intestine, kidney, liver and brain) was centrifuged at 2000g for 10min at 4°C and the supernatant was saved for assaying the enzymes of carbohydrate metabolism; the second part was centrifuged at 3000g for 15min at 4°C and the supernatant was used for assay of free radical scavenging enzymes; and the third part was used for the estimation of LPO.

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Preparation of BBM

Intestinal BBM was prepared at 4°C using differential precipitation by $CaCl_2$ (Kessler et al., 1978). Mucosa scraped from four to five washed intestines was used for each BBM preparation. $CaCl_2$ was added to the homogenate to the final concentration of 10mM and the mixture was stirred for 20 min on ice. The final membrane preparations were suspended in 50mM sodium maleate buffer, pH-6.8, with four passes by a loose fitting Dounce homogenizer (Thomson PA, Wheatson IL, USA) in a 15ml corex tube and centrifuged at 35000g for 20 min. The outer white fluffy layer of pellet is resuspended in sodium maleate buffer. Aliquots of homogenates (after high speed homogenization) and BBM thus prepared were saved and stored at –20oC until further analysis. Kidney BBM was prepared from whole cortex homogenate using the $MgCl_2$ precipitations method as described by (Yusufi et al., 1994). The final preparations were suspended in 300mM mannitol, pH-7.4, and the BBM thus prepared were saved and stored at –20°C until further analysis for BBM enzymes. Each sample of BBM was prepared by pooling tissues from two to three rats.

Serum biochemical parameters

Serum samples were deproteinized with 3% tricholoroacetic acid at a ratio of 1:3, left for 10 min, and centrifuged at 2000g for 10 min. The protein –free supernatant was used to determine Pi. The precipitate was used to quantitate total phospholipids. Serum urea nitrogen and cholesterol levels were determined directly in serum samples. Glucose was estimated by an o-toluidine method using kit from Span Diagnostics (Mumbai, India). These parameters were determined by standard procedures as mentioned in a previous study (Farooq et al., 2006).

Assay of carbohydrate metabolism enzymes

The activities of the enzymes involving oxidation of reduced nicotinamide adenine dinucleotide or reduction of nicotinamide adenine dinucleotide phosphate were determined spectrophotometrically on a Cintra 5 fixed for 340nm using 3 ml of assays in a 1-cm cuvette at room temperature (28-30°C). The enzymes assays of lactate dehydrogenase (LDH; EC 1.1.1.27), malate dehydrogenase (EC.1.1.1.37), malic enzyme (ME; E.C.1.1.1.40), glucose-6-phosphate dehydrogenase (G6PDH; E.C.1.1.1.49), glucose-6-phosphatase (G6Pase; E.C.3.1.3.3), and fructose 1,6-biphosphatase (FBPase; E.C.3.1.3.11) activities were studied as described by (Khundmiri et al., 2004). Hexokinase was estimated by the method of Crane and Sols (1953) and the remaining glucose was measured by method of Nelson (1944).

Assay of BBM marker enzymes and lysosomal marker enzymes

The activities of alkaline phosphatase (ALP), leucine amino peptidase (LAP), γ-glutamyl transferase (GGT), sucrase and acid phosphatase (ACP) were determined as described by Farooq et al (2004).

Assay of enzymes involved in free radical scavenging

Superoxide dismutase (E.C.1.15.1.1) was assayed by method of (Marklund et al., 1974). Catalase (E.C.1.11.1.6) activity was assayed by the method of (Giri et al., 1996).

LPO estimation

LPO was estimated by the method of (Ohkawa et al., 1979).

Definition of unit

One unit of enzyme activity is the amount of enzyme required for the formation of 1 μmole of product per hour under specified experimental conditions. Specific activity is enzyme units per milligram of protein.

Statistical analysis

All results are expressed as mean ± SEM for at least three to four separate preparations. The data were analyzed for statistical significance by Student's t test for group comparisons or by analysis of variance. $P < 0.05$ was considered statistically significant.

Results

Effect of benzene on body weight and serum parameters

The effect of benzene was determined on body weight and various serum parameters and on the enzymes of carbohydrate

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metabolism, brush border membrane (BBM) and oxidative stress in different rat tissues. Benzene was given orally by gavage with the dose of 800mg/kg body weight for 30 days. This dose was chosen for preliminary experiments which has minimal death occurring in experimental animals. Apparently, there was no significant difference in food and water intake between control and benzene rats. However, benzene caused a small decline in the body weight in benzene compared to control rats (Table-1).

Benzene exposure resulted in significant increase in serum creatinine (+30%) and blood urea nitrogen (BUN; +33%) indicating benzene induced nephrotoxicity. Serum cholesterol (+15%), phospholipids (+18%) and inorganic phosphate Pi (+75%) significantly increased by benzene administration (Table-2).

Table 1: Effect of benzene consumption on body weight (grams) of rats.

Groups	Before Treatment	After Treatment	% change
Control	156.7±2.30	166.7±8.20	(+6%)
Benzene	170.8±4.20	158.6±3.60	(-7%)

Results are mean ± SEM of eight different preparations.
Values in parenthesis represent percentage change from control.
* Significantly different at $P< 0.05$ from controls.

Table 2: Effect of benzene consumption on serum parameters.

Groups	BUN (mg/dL)	Creatinine (mg/dL)	Cholesterol (mg/dL)	PL (µg/mL)	Inorganic PO_4 (µmol/mL)
Control	28.24±3.56	15.23±0.41	1.61±0.07	1.14±0.12	0.73±0.07
Benzene	37.49±2.04*	19.81±1.05*	1.86±0.07*	1.34±0.07	1.28±0.03*
	(+33%)	(+30%)	(+15%)	(+18%)	(+75%)

Results are mean ± SEM of eight different preparations.
Values in parenthesis represent percentage change from control.
* Significantly different at $P< 0.05$ from controls.

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Table 3: Effect of benzene on metabolic enzymes of cortex and medullary homogenates.

Tissues	Hexokinase (µmol/mg prot/h)	LDH (µmol/mg prot/h)	MDH (µmol/mg prot/h)
Cortex			
Control	44.00±1.00	7.33±0.97	8.70±0.40
Benzene	36.62±0.76	10.14±0.87*	3.70±0.16
	(-17%)	(+38%)	(-57%)
Medulla			
Control	35.44±1.60	7.71±0.50	9.91±0.20
Benzene	33.30±0.51	9.45±1.59	7.10±0.05*
	(-6%)	(+23%)	(-28%)
Intestine			
Control	63.10±3.33	5.35±0.86	11.20±0.13
Benzene	70.10±1.28	15.93±2.94*	5.89±0.42
	(+11%)	(+197%)	(-47%)
Liver			
Control	10.21±0.16	32.85±1.05	3.82±0.20
Benzene	11.43±0.24	47.00±2.40*	2.20±0.24
	(+12%)	(+43%)	(-42%)
Brain			
Control	19.48±0.95	3.40±0.10	7.20±0.32
Benzene	16.67±0.11	5.20±0.24*	5.26±0.26*
	(-14%)	(+53%)	(-27%)

Results are mean ± SEM of eight different preparations.

Values in parenthesis represent percentage change from control.

* Significantly different at $P < 0.05$ from controls.

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Table 4: Effect of benzene on gluconeogenic enzymes of cortex and medullary homogenates.

Tissues	G6Pase (µmol/mg prot/h)	FBPase (µmol/mg prot/h)
Cortex		
Control	0.34±0.02	2.23±0.05
Benzene	0.27±0.01	1.83±0.02
	(-21%)	(-18%)
Medulla		
Control	0.06±0.01	2.43±0.03
Benzene	0.05±0.004*	1.53±0.05*
	(-17%)	(-37%)
Intestine		
Control	1.61±0.13	4.02±0.01
Benzene	1.46±0.23*	3.24±0.20
	(-9%)	(-19%)
Liver		
Control	0.17±0.02	0.82±0.01
Benzene	0.03±0.01*	0.70±0.02
	(-82%)	(-15%)
Brain		
Control	0.19±0.03	0.32±0.02
Benzene	0.11±0.001*	0.25±0.01*
	(-42%)	(-22%)

Results are mean ± SEM of eight different preparations.

Values in parenthesis represent percentage change from control.

* Significantly different at $P < 0.05$ from controls.

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Table 5: Effect of benzene consumption on brush border membrane enzymes in cortex and medullary homogenates.

Tissues	ALP (μmol/mg prot/hr)	GGT (μmol/mg prot/hr)	LAP (μmol/mg prot/hr)	ACP (μmol/mg prot/hr)	Sucrase (μmol/mg prot/hr)
Cortex					
Control	33.68±1.64	39.54±2.68	4.31±0.51	1.95±0.18	
Benzene	23.49±0.96*	33.14±2.70	3.91±0.09	2.36±0.09*	
	(-30%)	(-16%)	(-9%)	(+21%)	
Medulla					
Control	26.71±2.79	55.67±0.79	3.08±0.09	2.98±0.11	
Benzene	21.33±0.40*	45.40±0.65	2.69±0.03	4.01±0.09*	
	(-20%)	(-18%)	(-13%)	(+35%)	
Intestine					
Control	3.40±0.085	32.36±1.63	5.31±0.21	2.18±0.12	21.2±1.80
Benzene	2.26±0.11*	19.42±0.59*	3.73±0.26*	1.48±0.06	13.1±1.30*
	(-34%)	(-40%)	(-30%)	(-32%)	(-38%)
Liver					
Control	1.23±0.05	20.68±1.83	0.82±0.02	1.07±0.02	
Benzene	0.99±0.03*	13.75±0.41	0.45±0.02*	0.89±0.008	
	(-19%)	(-34%)	(-45%)	(-17%)	
Brain					
Control	1.36±0.06	6.00±0.32	0.17±0.01	0.41±0.04	
Benzene	1.21±0.08*	4.80±0.10*	0.14±0.02	0.34±0.02	
	(-11%)	(-20%)	(-18%)	(-17%)	

Results are mean ± SEM of eight different preparations.

Values in parenthesis represent percentage change from control.

* Significantly different at $P < 0.05$ from controls.

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Table 6 and Table 7 are at the end of this document.

Effect of TCE on enzymes of carbohydrate metabolism

The effect of benzene was determined on the enzymes involved in various pathways of carbohydrate metabolism in different rat tissues (Table-3). Benzene administration cause increase in hexokinase (glycolysis) activity in the intestine (+11%) followed by liver (+12%) but the activity decline in the brain (-14%), renal cortex (-17%) and medulla (-6%). However LDH activity, a marker of anaerobic glycolysis profoundly increased in the intestine (+197%), liver (+53%), brain (+53%), renal cortex (+38%) and medulla (+23%). In contrast, TCE significantly inhibited the activity of MDH, a TCA cycle enzyme in the intestine (-47%), liver (-42%), brain (-27%), renal medulla (-28%) and the activity markedly decreased in renal cortex (-57%).

The effect of benzene was also determined on the activities of enzymes involved in gluconeogenesis (Table-4). The activity of both G6Pase and FBPase declined in all tissues. The activity of G6Pase maximally declined in the liver (-82%) whereas the FBPase activity maximally decreased in renal medulla (-37%).

Effect of TCE on enzymes of brush border membrane and lysosome in different tissues

The effect of benzene was determined on BBM and lysosomal enzymes in the liver, intestine, brain and renal cortical and medullary homogenates and in isolated BBM vesicles from renal cortex and intestinal mucosa. The results summarize in (Table 5) show that benzene differentially altered the activities of various enzymes in the homogenates of different rat tissues. The activity of ALP markedly decreased in all tissues (-19% to -34%) except in brain where the activity was only slightly declined (-11%). The activity of GGT significantly lowered in intestine (-40%), liver (-34%), and brain (-20%), but to a lesser extent in renal cortex (-16%) and medulla (-18%). Moreover, benzene exposure resulted in marked decrease of LAP in the liver (-45%) and intestine (-30%) as compared to brain (-18%), renal cortex (-9%) and medulla (-13%). Benzene also altered lysosomal enzyme, acid phosphatase (ACP) differentially in different tissues. The activity of ACP increased in the renal cortex (+21%) and medulla (+35%) whereas significantly decreased in the brain (-17%), liver (-17%) and intestine (-32%). Similar to homogenates, the activities of ALP, GGT, LAP and sucrase decreased in intestinal BBM by benzene exposure (Table-6). In accordance with the alterations in cortical homogenate, benzene caused significant decline of ALP, GGT and LAP activities in the BBM, isolated from renal cortex (Table-6).

Effect of TCE on the antioxidant parameters

It is evident that reactive oxygen species (ROS) generated by various toxins are important mediators of cell injury and pathogenesis of various diseases especially in the kidney and other tissues (Walker et al., 1999). A major cellular defense against ROS is provided by superoxide dismutase (SOD) catalase and some other enzymes. To ascertain the role of antioxidant system in TCE induced toxicity, the effect of benzene was examined on certain parameters of oxidative stress. Benzene administration caused significant decrease in SOD and catalase activities in almost all tissues (Table 7). However, the decrease in SOD and/ or catalase activity was different in different tissues. The decrease in antioxidant enzyme activities was associated with significant elevated lipid peroxidation (LPO) measured in terms of malondialdehyde (MDA) and slightly lower total thiol (SH) levels.

Discussion

Benzene is an aromatic hydrocarbon used for industrial purposes. It can cause serious, negative health effects in humans depending upon both the amount and duration of the exposure (Toxprobe). Benzene being lipid soluble is transported in the blood and absorbed by red cell membrane. It tends 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, primarily by Cyt-P450 systems (Hannumantharao et al., 2001). Acute and chronic exposure of benzene suppresses bone marrow function, causing blood changes, leukemia, CNS depression, headaches, sleepiness, loss of consciousness, Defatting dermatitis. Despite extensive knowledge of TCE toxicology and

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accumulation of large body of data in last few decades, its effect on tissue metabolism, energy yielding reactions and oxidative stress has not been examined in detail.

The present investigation was undertaken to determine multifaceted effects of long term benzene administration on various enzymes involved in carbohydrate metabolism, brush border membranes and oxidative stress in the kidney, intestine, liver and brain to understand the mechanism of benzene induced toxicity in these tissues involving various cellular organelles. Benzene administration resulted in significant increase in serum creatinine, BUN, serum cholesterol indicating significant damage to the kidney accompanied by increase in serum phospholipids and inorganic phosphate.

The effect of benzene administration was examined on enzymes of carbohydrate metabolism in various rat tissues to assess its effect on energy yielding reactions. The activities of various enzymes involved in glycolysis, TCA cycle and gluconeogenesis were selectively altered by benzene exposure. TCE administration markedly enhanced the activity of LDH (glycolysis) whereas decreased MDH activity (TCA cycle) in the liver, intestine, brain and/ or renal tissues. Although the actual rate of glycolysis or TCA cycle was not determined, marked decrease in MDH activity appears to be due to benzene induced damage to mitochondria as suggested by Andrew C et al (2006). Moreover, the significant increase in LDH activity appears to be an adaptive cellular effect in energy dependence from aerobic metabolism alternatively to anaerobic glycolysis also due to benzene induced mitochondrial toxicity. As shown in the "Results", significant decrease in the activities of G6Pase and FBPase, enzymes of gluconeogenesis in all the tissues including kidney, intestine, liver and brain suggest that the production of glucose by gluconeogenesis was also greatly hampered by benzene administration as shown by histological studies (Kaminski et al., 1985; Ozdikicioglu and Dere, 2004) The decrease of gluconeogenic enzymes may be the result of decrease in TCA cycle enzymes. This can be explained by the fact that the reduced activities of TCA cycle enzymes especially that of MDH will result in lower oxaloacetate production from malate which is not only required for the continuation of TCA cycle but also for gluconeogenesis.

In general, mitochondria, plasma membrane, microsomes and in some cases lysosomes are major targets of toxic insults (Sanchez et al., 2001; Taulan et al., 2004; Fatima et al., 2004, 05; Banday et al., 2008). Benzene is rapidly metabolized in liver producing benzene oxide and various phenolics products. These metabolites may cause toxic effects in liver and other tissues. Andrew C et al. (2006) investigated benzene metabolism in liver and kidney, therefore it was proposed that benzene oxide is rapidly formed and is carried by blood circulation to other tissues, where it causes toxicity. It is also known to damage mitochondria and inhibit respiration (Andrew and James, 2006). Since brush border membrane (BBM) and other intracellular organelles such as mitochondria and lysosome are known targets of toxic insult. The structural and functional integrity was assessed by the status of their respective marker enzymes. The activity of BBM enzymes, ALP, GGT and LAP variably declined in the homogenates of all the tissues by benzene administration. The activity of lysosomal enzyme, acid phosphatase (ACP) increased in the renal cortex and medulla but increased in intestine, liver and brain. The activities of BBM enzymes were similarly altered in isolated cortical and mucosal BBM preparation as observed in their respective homogenates. The data clearly demonstrate that the plasma membranes and lysosomes were selectively damaged by benzene although to different extents in different tissues. The differential effect of benzene on various BBM enzymes in the intestine and kidney can be attributed to their differential locations in the thickness of the membranes (Yusufi et al., 1994) and/ or due to the differential accessibility/ accumulation of benzene in these tissues.

Reactive oxygen species (ROS) are considered to be one of the important mechanisms of toxic insult (Walker et al., 1999; Taulan et al., 2004; Farooq et al., 2007; Banday et al., 2008). ROS are normal by-products of aerobic metabolism produce cellular injury and necrosis via several mechanisms including peroxidation of lipids, protein and DNA (Dean et al, 1991, Szabo et al, 1997). A major cellular defense against

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ROS is provided by SOD and catalase which together convert superoxide radicals first to H_2O_2 and then to water and molecular oxygen (Kaplowitz et al., 1985). Modification in the activities of catalase and SOD in the liver, kidney were recorded in rats and rabbits (Serif et al., 1999). However, the effect of TCE was not observed on antioxidant enzymes associated with alteration in LPO and GSH contents not only in the kidney but also in the other major tissues. The present results show that TCE caused marked but variable reductions in SOD and catalase activities albeit differentially in different tissues. The activity of SOD profoundly decreased in all the tissues, kidney, intestine, liver and brain. In contrast to SOD, catalase activity decreased maximally in the renal medulla, renal cortex and brain but to lesser extent in other tissues, intestine and liver. The decreased in the free radicals scavenging enzymes was associated with increased lipid peroxidation and decreased total SH-content. The imbalance between rate of free radicals and the effect of antioxidants lead to oxidative damage by benzene. Indeed, benzene metabolites are known to produce oxidized species and reactive oxygen indicating the increased risk of cell membrane damage (Dundaroz et al., 2003).

In conclusion, the present results demonstrate that benzene administration resulted in severe damage to various rat tissues. The plasma membrane, mitochondria and lysosomes are specific benzene targets as evident by alterations in their specific biomarkers. Benzene seems to enhance glycolytic enzyme, LDH in order to increase energy dependence on glycolysis rather than on aerobic metabolism mostly likely due to mitochondrial damage and depressed TCA cycle enzyme. The nephrotoxic effect induced by benzene was manifested by decrease in BBM enzymes activities and increase in serum creatinine and BUN. Benzene caused reduction in the activities of antioxidant enzymes with associated increase in LPO. The present results may provide a basis for determination of novel potential biomarkers of toxicity in addition to traditional measures; creatinine and BUN for renal injury.

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Table 6: Effect of benzene on BBM Vesicles isolated from small intestine and renal cortex.

Groups	Small intestine				Cortex		
	ALP (µmol/ mg prot/h)	GGT (µmol/ mg prot/h)	LAP (µmol/ mg prot/h)	Sucrase (µmol/ mg prot/h)	ALP (µmol/ mg prot/h)	GGT (µmol/ mg prot/h)	LAP (µmol/ mg prot/h)
Control	66.14±4.70	104.28±5.23	31.35±0.75	64.70±4.29	133.21±16.48	122.05±15.06	45.16±1.05
Benzene	52.88±1.48*	75.36±2.24*	22.52±1.02*	44.61±1.30*	63.22±2.61*	71.77±3.69*	36.35±1.95*
	(-20%)	(-27%)	(-28%)	(-31%)	(-53%)	(-41%)	(-41%)

Results are mean ± SEM of eight different preparations.

Values in parenthesis represent percentage change from control.

* Significantly different at $P < 0.05$ from controls.

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Table 7: Effect of benzene on enzymic and non-enzymic antioxidant parameters of cortex and medullary homogenates.

Tissues	LPO (nmol/gm/tissue)	CAT (Units/mg protein)	SOD (µmol/mg protein/ min)
Cortex			
Control	117.10±19.55	148.17±8.92	39.09±4.33
Benzene	168.45±20.07*	53.59±6.53*	22.92±1.53*
	(+44%)	(-64%)	(-41%)
Medulla			
Control	89.22±2.30	58.55±2.81	61.39±13.72
Benzene	112.29±4.38	31.78±7.60*	20.21±4.51*
	(+26%)	(-46%)	(-67%)
Intestine			
Control	61.02±2.44	36.27±1.48	82.81±9.82
Benzene	73.00±2.68	24.07±3.49*	45.24±6.35*
	(+20%)	(-34%)	(-45%)
Liver			
Control	62.08±5.87	95.53±13.17	83.27±4.74
Benzene	98.52±2.77*	77.32±11.65	37.58±10.41*
	(+59%)	(-19%)	(-55%)
Brain			
Control	114.27±6.78	16.20±2.70	27.80±7.56
Benzene	150.32±20.72*	7.18±0.26*	10.76±0.72*
	(+32%)	(-56%)	(-61%)

Results are mean ± SEM of eight different preparations.

Values in parenthesis represent percentage change from control.

* Significantly different at $P < 0.05$ from controls.

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