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Research Article

Studies on Hexachlorobenzene (HCB) Induced Toxicity and Oxidative Damage in the Kidney and Other Rat Tissues -

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ABSTRACT

Hexachlorobenzene (HCB), one of the most environmental pollutants induces porphyria and causes toxic effects in the intestine, liver, kidney, skin and brain both in humans and experimental animals. The present study examined the effect of HCB on the enzymes of carbohydrate metabolism, Brush Border Membrane (BBM), lysosomes and oxidative stress to understand the mechanism of HCB induced toxicity in various rat tissues. Male Wister rats were given HCB (15mg/ kg) in corn oil orally by gavages for 25 d. Blood and tissues were collected and analyzed for various biochemical and enzymatic parameters. HCB administration significantly increased blood urea nitrogen, serum creatinine, cholesterol and phospholipids indicating HCB induced nephrotoxicity and hepatotoxicity. The activity of BBM enzymes significantly decreased in the renal cortex and medulla, liver and brain but increased in the intestine. The activity of lactate dehydrogenase (LDH) decreased but that of Malate Dehydrogenase (MDH) increased in the renal tissues and liver. In contrast, LDH increased whereas MDH decreased in the intestine and brain. HCB significantly decreased the activity of glucose – 6 - phosphatase and fructose -1, 6 - bisphosphatase but increased glucose – 6 - phosphate dehydrogenase in all the tissues. NADP - malic enzyme decreased in the kidney and brain whereas increased in the liver and intestine. The activity of acid phosphatase significantly decreased in the cortex, medulla, intestine and brain but increased in the liver. We conclude that HCB administration caused severe damage to the kidney, liver, intestine and brain albeit differentially and altered energy metabolism by inducing oxidative stress.

Keywords: Hexachlorobenzene; Carbohydrate metabolism; Oxidative stress; Tissue; Toxicity

HIGHLIGHTS

- Hexachlorobenzene (HCB) administration causes nephrotoxicity and hepatotoxicity.
- The renal proximal tubule, particularly its BBM and liver seem to be the major HCB targets.
- HCB produces multiple adverse effects in the liver, kidney, intestine and brain and alters metabolic functions.
- HCB appeared to shift energy dependence from aerobic to anaerobic glycolysis by causing damage to mitochondria.
- HCB caused nephrotoxicity and other deleterious effects by increasing ROS generations.

INTRODUCTION

We are exposed daily to numerous environmental toxins and pollutants including industrial solvents that pose serious health problems [1-8]. Hexachlorobenzene (HCB) is one of the most persistent environmental and bio-accumulative pollutants that have been used as pesticide and fungicide in agriculture [9], and also in some industries [10-12]. Due to wide spread toxic effects of HCB, its production and use was discontinued in 1970s in many countries. However, it is still released into the environment from the manufacturing of various chlorinated compounds as a by-product and / or impurity [13,14]. HCB-contaminated food is the principal source of environmental exposure, exposure can also occur through the inhalation of HCB-contaminated air, by dermal contact, or through in utero exposure and breast milk [15-17]. Long term HCB exposure caused toxic effects in the liver, thyroid, nervous system, bones, kidneys, skin, lung, blood and immune, endocrine and reproductive systems [9,10,17-22]. Porphyria was one of the most notable toxic manifestations of HCB observed in large human populations in 1950s [23]. HCB affected neurological and behavioral development in young children [24-26]. HCB was also found to cause cancer of the liver, kidney, and thyroid in animals but there is no evidence of it causing cancer in humans [27-28].

HCB has been shown to cause ultra-structural effects in several tissues including liver, kidney, thyroid, spleen and ovary [21,29-31]. It produced severe morphological alterations in the kidney and liver leading to their enlargements and necrosis. The proximal tubules, distal tubules and glomeruli in the kidney and hepatocytes in the liver were specific HCB targets. The mitochondria, lysosomes, microvilli, smooth endoplasmic reticulum with increased vacuole mass in these tissues were particularly affected [30,31]. Despite the accumulations of large body of data on the toxic, carcinogenic and porphyrogenic effects of HCB, detailed biochemical effects and/ or mechanism involved in the cellular response to tissue injury are not completely elucidated neither those participating in inflammation, necrosis and oxidative stress or energy yielding metabolic activities.

In view of widespread HCB induced toxicity, the present study investigated the effects of HCB on the enzymes of carbohydrate metabolism, plasma membrane and oxidative stress in the kidney, intestine, liver and brain to understand the mechanism and cellular response of HCB induced toxic and other adverse effects in rat kidney, liver, intestine and brain. The results obtained show that oral HCB exposure caused nephrotoxicity and hepatotoxicity and caused specific alterations in the activities of metabolic, membrane and lysosomal enzymes most likely by inducing oxidative stress although differentially in different rat tissues.

MATERIALS AND METHODS

Hexachlorobenzene (HCB) was purchased from Sisco Research Laboratory (Mumbai, India). All other chemicals used were of analytical grade and were purchased either from Sigma Chemical Co. (St Louis, MO, USA) 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 (CPCSEA), Ministry of Environment and Forests, Government of India. Adult male Wistar rats (10 rats/ group), weighing 150 – 200 g were acclimatized to the animal facility for one week on a standard rat diet (Aashirwad Industries, Chandigarh, India) and water ad libitum. HCB treated rats were given HCB (15mg/ kg body weight/ d) in corn oil by gavages whereas the control rats received same amount of corn oil daily for 25 days. This dose was chosen by preliminary experiments that have minimal death occurring in experimental animals. Similar doses (10 - 20 mg/ kg body weight/ d) were also frequently used in the study of liver, renal, cardiac, respiratory, endocrine and reproductive effects in short-term and long- term experimental conditions in the rat model [32-34]. Bourque et al. [35] also administered similar doses in monkeys and resulted in mitochondrial degeneration.

After 25 days of HCB administration, the rats were sacrificed under light ether aesthesia and blood samples were collected 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 mMTris - 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 as described earlier [4]. Body weights of the rats were recorded at the start and at the completion of the experimental procedure.

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 50 mM mannitol, Ph - 7.0, in a glass / Teflon homogenizer with five complete strokes [4,36]. The homogenate was then subjected to high speed homogenization in an Ultra Turex homogenizer (Type T - 25, Janke & Kunkel GMBH & Co. KG. Staufen) for three strokes of 30 s each with an interval of 30 s. The kidneys were decapsulated and kept in ice-cold 154 mM NaCl and 5 mM Tris- HEPES buffer, pH - 7.5. The cortical and medullary regions were carefully separated and homogenized in 50mM mannitol buffer to obtain 10% (w/ v) homogenate. The 10% liver and brain homogenates were similarly prepared in 10 mM Tris-HCl buffer, pH 7.5. The homogenates were centrifuged at 3000 g at 4˚C for 15 min to remove cell debris and the supernatant was saved in aliquots and stored at -20˚C for assaying the enzymes of carbohydrate metabolism, free-radical scavenging enzymes and for estimation of total -SH and lipid per oxidation.

Preparation of Brush Border Membrane Vesicles (BBMV)

Intestinal BBM was prepared at 4˚ C using the $CaCl_2$ precipitation method [36]. 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 10 mM and the mixture was stirred for 20 min on ice. The final membrane preparations were suspended in 50 mM sodium maleate buffer, pH - 6.8, with four passes by a loose fitting Dounce homogenizer (Thomson PA, Wheaton IL, USA) in a 15 ml Corex glass tube and centrifuged at 35000 g 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 –20˚ C until further analysis.

Kidney BBM was prepared from whole cortex homogenate using the $MgCl_2$ precipitation method as described by Yusufi, et al. [37]. The final preparations were suspended in 300 mM mannitol, pH - 7.4. Aliquots of homogenates and the BBMV 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 chemistries

Serum parameters- Serum samples were deproteinated with 3% trichloro acetic acid in a ratio 1:3, left for 10 minutes and then centrifuged at 2000 x g for 10 minutes. The protein free supernatant was used to determine inorganic phosphate and creatinine. The precipitate was used to quantities of total phospholipids. Blood Urea Nitrogen (BUN) and cholesterol levels were determined directly in serum samples. Glucose was estimated by o-toluidenemethod using kit from Span diagnostics (Mumbai, India). These parameters were determined by standard procedures as mentioned in a previous study [4,38].

Enzyme assays

The activities of BBM biomarkers enzymes, alkaline phosphatase (ALP), leucine amino peptidase (LAP), and γ - glutamyl transferase (GGTase) in the homogenates and BBM preparations and lysosomes marker enzyme, acid phosphatase (ACPase) in the homogenates were determined as described earlier [39]. The enzymes of carbohydrate metabolism, e.g., lactate (LDH), malate (MDH), glucose – 6 - phosphate (G6PDH) dehydrogenase and NADP-Malic Enzyme (ME), involved in oxidation of NADH or reduction of NADP were determined by measuring the extinction changes at 340 nm in a spectrophotometer (Cintra 5; GBC Scientific Equipment, Pty., Victoria Australia) as described elsewhere [40]. The other enzymes, glucose – 6 - phosphatase (G6Pase), fructose - 1, 6 - bisphospatase (FBPase) and hexokinase (HK) were determined as described in our previous studies [3]. The activities of superoxide dismutase (SOD), catalase and glutathione peroxidase (GSH-Px) were determined as described by Priyamvada, et al. [6]. Lipid peroxidation (LPO) and total SH-groups were estimated as described earlier [6]. Protein concentration was determined by the modified method of Lowry, et al. [40] as described by Yusufi, et al. [37].

Statistical analyses

All data are expressed as Mean ± SEM for at least 4 - 5 different preparations. Statistical evaluation was conducted by one-way ANOVA and by unpaired student's t test using SPSS 7.5 software. A probability level of $p < 0.05$ was selected as indicating statistical significance. All the changes were compared with control values for better understanding and clarity.

RESULTS

Apparently, there was no significant difference in daily food and water intake between control and HCB treated rats and they remained clinically well throughout the study. However, HCB treatment caused a significant decrease (-22%) in the body weight (Table 1) compared to control rats (Control = 172 ± 10; HCB = 128 ± 9).

Effect of HCB on serum parameters

HCB exposure resulted in significant increase in serum creatinine (+ 18%) and BUN (+ 46%), serum cholesterol (+ 20%) and phospholipids (+ 34%) whereas Pi (- 46%) was significantly decreased indicating HCB-induced nephrotoxicity and hepatotoxicity (Table 2).

Effect of HCB on enzymes of brush border membrane (BBM) and lysosomes in different rat tissues

To assess the integrity of certain membranes/organelles, the effect of HCB was determined on BBM and lysosomal marker enzymes in the liver, brain, intestinal, renal cortical and medullary homogenates and in the isolated BBM vesicles from renal cortex and intestinal

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

Groups	Before treatment	After treatment	% Change
Control	160.00 ± 12.02	172.00 ± 10.06	+ 8%
HCB	165.00 ± 8.56	128.00 ± 9.20*	- 22%

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

mucosa. The results summarized in Table 3 show that HCB decreased the activities of ALP (- 14 to - 20%) in all tissues including brain albeit differentially in different tissues. The activity of GGT significantly decreased in the cortex (- 34%), medulla (- 24%), liver (- 44%) and brain (- 52%) by HCB exposure to much greater extent compared to ALP activity in these tissues. Similarly, HCB exposure also resulted in marked decrease in the activity of LAP in almost all tissues. However, in contrast to others tissues, the activities of ALP (+ 31%), GGT (+ 27%) and LAP (+ 23%) markedly enhanced in the mucosal homogenate whereas the activity of sucrase (-36%), an intestinal BBM marker was significantly lowered by HCB administration.

HCB also altered lysosomal enzymes activity, Acid Phosphatase (ACP) although differentially in different tissues. The activity of ACP decreased in the intestine (- 19%), renal cortex (- 24%) and medulla (- 13%) and brain (- 22%) but slightly increased (+ 17%) in liver homogenates.

Effect of HCB on enzymes of Brush Border Membrane (BBM) in isolated BBM preparations from renal cortex and intestinal mucosa

The effect of HCB on BBM marker enzymes was further analyzed in BBM preparations isolated from renal cortex and intestinal mucosa. The data summarized in Table 4 show a similar activity pattern of BBM enzymes as observed in the homogenates, however, the magnitude of HCB effect was more pronounced especially in the mucosal BBM. HCB caused profound increase in the activity of ALP (+ 73%), GGT (+ 174%) and LAP (+ 64%) but significantly lowered the sucrase activity in mucosal BBM. Similar to cortical homogenates, the activities of ALP, GGT and LAP significantly decreased in the BBM, isolated from renal cortex.

Table 2: Effect of Hexachlorobenzene (HCB) on various serum parameters.

Groups	BUN (mg / dL)	Creatinine (mg / dL)	Cholesterol (mg / dL)	Phospholipid (µgs / mL)	Inorganic PO_4 (µmol / mL)
Control	38.00 ± 1.80	0.73 ± 0.01	75.19 ± 1.57	64.16 ± 2.30	5.70 ± 0.12
HCB	55.61 ± 1.13*	0.86 ± 0.03*	90.25 ± 3.18*	86.17 ± 3.80*	3.10 ± 0.25*
	(+ 46%)	(+18%)	(+ 20%)	(+ 34%)	(- 46%)

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

Effect of HCB on enzymes of carbohydrate metabolism

The main function of kidney i.e. reabsorption of various ions and solutes depends on the continuous energy supply as ATP which is generated by various metabolic pathways including glycolysis and oxidative metabolism. The acute renal failure produced by toxic insult resulted in reduced oxygen consumption due to damage caused to mitochondria and other organelles by toxic insult. The effect of HCB was examined on certain enzymes of carbohydrate metabolic pathways. The activities of hexokinase and LDH (glycolysis), MDH (TCA cycle), G6Pase and FBPase (gluconeogenesis) were determined in the homogenates of various rat tissues. HCB treatment caused profound increase in LDH activity in the intestine (+110%) and slight increase in brain (+17%) whereas the activity markedly decreased in liver (-64%), renal medulla (-67%) and to some extent in renal cortex (-13%) compared to control rats. However, the activity of other glycolytic enzyme was differentially altered (Table 5). The activity of hexokinase significantly increased in the cortex (+24%), liver (+41%) but decreased in renal medulla (-31%), intestine (-33%) and brain (-26%). In contrast, HCB caused marked increase in MDH activity in all tissues except in brain (-42%) and intestine (-19%) where the activity was decreased.

The effect of Hexachlorobenzene was also determined on the activities of enzymes involved in gluconeogenesis and hexose monophosphate (HMP) shunt pathway (Table 6). The enzymes of these pathways were differentially altered by Hexachlorobenzene

Table 3: Effect of Hexachlorobenzene (HCB) on brush border membrane enzymes in different tissue homogenates.

Tissues	Groups	ALP (µmol / mg protein / h.)	GGT (µmol / mg protein / h.)	LAP (µmol / mg protein / h.)	ACP (µmol / mg protein / h.)	Sucrase (µmol / mg protein / h.)
Cortex	**Control**	12.55 ± 0.57	43.38 ± 2.96	5.27 ± 0.14	3.35 ± 0.22	
	HCB	10.32 ± 0.57	28.43 ± 1.52*	4.25 ± 0.51	2.55 ± 0.35*	
		(- 18%)	(- 34%)	(- 19%)	(- 24%)	
Medulla	**Control**	29.68 ± 0.72	27.75 ± 1.35	6.95 ± 0.31	2.31 ± 0.27	
	HCB	25.40 ± 0.41	21.00 ± 0.99*	4.87 ± 0.11*	2.02 ± 0.12	
		(- 14%)	(- 24%)	(- 30%)	(- 13%)	
Intestine	**Control**	5.85 ± 0.38	9.20 ± 0.94	29.21 ± 2.16	2.15 ± 0.15	42.20 ± 5.25
	HCB	7.69 ± 0.59*	11.72 ± 0.90*	36.00 ± 2.20*	1.75 ± 0.12	27.26 ± 1.10*
		(+ 31%)	(+ 27%)	(+ 23%)	(- 19%)	(- 36%)
Liver	**Control**	0.95 ± 0.05	5 .04 ± 0.40	6.67 ± 0.37	0.58 ± 0.10	
	HCB	0.76 ± 0.35*	2.80 ± 0.50*	5.18 ± 0.50	0.68 ± 0.06	
		(- 20%)	(- 44%)	(-22%)	(+17%)	
Brain	**Control**	1.38 ± 0.02	6.05 ± 0.43	0.26 ± 0.15	1.25 ± 0.12	
	HCB	1.13 ± 0.01	2.93 ± 0.86*	0.15 ± 0.12*	0.98 ± 0.05*	
		(- 18%)	(- 52%)	(- 42%)	(- 22%)	

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

Table 4: Effect of Hexachlorobenzene (HCB) on BBM Vesicles isolated from small intestine and renal cortex.

Groups	Small Intestine				Cortex		
	ALP (µmol / mg protein / h.)	GGT (µmol / mg protein / h.)	LAP (µmol / mg protein / h.)	Sucrase (µmol / mg protein / h.)	ALP (µmol / mg protein / h.)	GGT (µmol / mg protein / h.)	LAP (µmol / mg protein / h.)
Control	59.56 ± 3.69	50.60 ± 4.51	25.41 ± 1.16	141.74 ± 23.12	48.50 ± 3.69	88.90 ± 6.49	54.48 ± 2.64
HCB	103.26 ± 6.48*	138.89 ± 14.95*	41.65 ± 3.17*	85.30 ± 13.38*	35.42 ± 0.93*	43.95 ± 3.03*	40.04 ± 3.14*
	(+ 73%)	(+ 174%)	(+ 64%)	(- 40%)	(- 27%)	(- 51%)	(- 27%)

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

Table 5: Effect of Hexachlorobenzene (HCB) on metabolic enzymes in different tissue homogenates.

Tissues	Groups	Hexokinase (µmol / mg protein / h.)	LDH (µmol / mg protein / h.)	MDH (µmol / mg protein / h.)
Cortex	**Control**	30.88 ± 1.18	13.16 ± 1.02	16.51 ± 2.01
	HCB	38.34 ± 2.10*	11.51 ± 1.02	20.32 ± 2.05*
		(+ 24%)	(- 13%)	(+ 23%)
Medulla	**Control**	36.17 ± 1.50	11.08 ± 1.04	0.27 ± 0.02
	HCB	24.89 ± 0.87*	3.63 ± 0.01*	0.41 ± 0.14*
		(- 31%)	(- 67%)	(+ 52%)
Intestine	**Control**	54.54 ± 1.73	26.71 ± 2.20	4.05 ± 0.32
	HCB	36.55 ± 4.15*	56.01 ± 2.25*	3.28 ± 0.22*
		(- 33%)	(+ 110%)	(- 19%)
Liver	**Control**	11.35 ± 1.09	22.00 ± 3.02	6.64 ± 0.04
	HCB	15.96 ± 1.18*	8.00 ± 2.02*	15.61 ± 1.03*
		(+ 41%)	(- 64%)	(+ 135%)
Brain	**Control**	31.22 ± 0.77	6.66 ± 1.02	16.77 ± 1.03
	HCB	23.13 ± 2.55*	7.80 ± 1.82	9.66 ± 0.04*
		(- 26%)	(+ 17%)	(- 42%)

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

in different tissues. The activities of both G6Pase and FBPase significantly declined in all tissues albeit differentially. The activities of G6Pase (-86%) and FBPase (-77%) were maximally decreased in the liver (the major site of gluconeogenesis). However, they were also decreased in the renal tissues followed by the intestine and brain. The activity of G6PDH was increased in all tissues with marked elevation in liver (+156%) whereas ME decreased in all tissues except intestine (+35%) and liver (+143%).

Effect of HCB 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 disease. Glutathione (GSH) and its redox cycle enzymes e.g. SOD, GSH-PX along with lipid per oxidation are components of oxidant/ antioxidant system. To ascertain, the role of oxidative stress in HCB induced toxicity, the effect of HCB was examined on certain enzymatic and nonenzymatic parameters. HCB administration caused significant increase in LPO measured as Malondialdehyde (MDA) in almost all tissues except medulla (Table 7). It appears that HCB exposure caused severe tissue injury as indicated by profound increase in LPO content in the intestine (+114%), liver (+75%), brain (+65%) and renal cortex (+54%). HCB also markedly decreased the activity of SOD (-22% to -74%) and catalase (-33% to -71%) although to different extent in different tissues. However, HCB caused profound increase in SOD (+125%) and catalase (+178%) activities in medulla that was associated with marked decrease in LPO (-75%).

DISCUSSION

HCB is a well-known toxic environmental pollutant. Here we determined the effect of HCB administration on certain serum parameters and on the enzymes of carbohydrate metabolism, plasma membrane, lysosomes and oxidative stress in different rat tissues. A number of investigators have shown the toxic, carcinogenic and porphyrogenic effects of HCB in several tissues and organs [19,41-43].The most notable targets of HCB toxicity are the liver, kidney and central nervous system [11,24,25,27]. HCB has been shown to cause marked morphological changes in liver and kidney leading to their enlargement and necrosis [30-32]. Since the plasma membranes (BBM), mitochondria, endoplasmic reticulum and lysosomes in the renal tubules and hepatocytes were critical targets of HCB-induced toxicity [30,31] the present investigation was undertaken to determine the effect of HCB on the enzymes of carbohydrate metabolism, brush border membrane, mitochondria, lysosomes and oxidative stress in the kidney, liver along with intestine and brain for understanding its mechanism of action.

Repeated oral administration of HCB significantly increased serum creatinine, BUN, serum cholesterol, and phospholipids but decreased inorganic phosphate (Pi) indicating HCB induced nephrotoxicity and hepatotoxicity in agreement with earlier morphological and some biochemical studies [18,30-34,43-45].

The structural and functional integrity of plasma membrane / brush border membrane BBM) as assessed by the status of biomarkers enzymes showed marked reductions in the activities of ALP, GGT, LAP by HCB administration in the homogenates of renal cortex, medulla and in the liver and brain. In contrast, the activities of ALP, GGT and LAP significantly increased but sucrase, a specific marker of mucosal BBM decreased in the intestine by HCB exposure. Further, the effect of HCB compared to homogenates was much more pronounced in isolated BBM preparations from renal cortex/ intestine. These hydrolytic enzymes are components of the plasma membranes of the hepatocytes, neuronal cells, and renal proximal tubular and mucosal BBM and are involved in terminal digestion and absorption/ reabsorption and other cellular functions [37,46]. The significant decrease in membrane enzymes might be due to the loss of active enzyme molecules from damaged membranes [18,30] and excreted in the urine [10,11] as also observed with other toxicants [1-4,7,38]. The differences in the effect of HCB on BBM enzymes in different tissues can be attributed to differential bioactivities and

Table 6: Effect of Hexachlorobenzene (HCB) on metabolic enzymes in different tissue homogenates.

Tissues	Groups	G6Pase (µmol / mg protein / h.)	FBPase (µmol / mg protein / h.)	G6PDH (µmol / mg protein / h.)	ME (µmol / mg protein / h.)
Cortex	**Control**	0.38 ± 0.03	0.60 ± 0.02	0.42 ± 0.02	1.34 ± 0.68
	HCB	0.30 ± 0.02*	0.36 ± 0.05*	0.52 ± 0.02*	0.84 ± 0.04*
		(- 21%)	(- 40%)	(+ 24%)	(- 37%)
Medulla	**Control**	0.42 ± 0.06	0.35 ± 0.02	0.32 ± 0.03	0.94 ± 0.21
	HCB	0.17 ± 0.04*	0.23 ± 0.03*	0.38 ± 0.02*	0.79 ± 0.01
		(- 60%)	(- 34%)	(+ 19%)	(- 16%)
Intestine	**Control**	1.84 ± 0.08	0.68 ± 0.09	0.55 ± 0.02	0.43 ± 0.02
	HCB	1.24 ± 0.20*	0.48 ± 0.03*	0.72 ± 0.09*	0.58 ± 0.11*
		(- 33%)	(- 29%)	(+ 31%)	(+ 35%)
Liver	**Control**	0.14 ± 0.03	0.69 ± 0.03	0.87 ± 0.11	0.70 ± 0.07
	HCB	0.02 ± 0.09*	0.16 ± 0.02*	2.23 ± 0.21*	1.70 ± 0.02*
		(- 86%)	(- 77%)	(+ 156%)	(+ 143%)
Brain	**Control**	0.29 ± 0.03	0.32 ± 0.02	1.96 ± 0.05	0.15 ± 0.002
	HCB	0.21 ± 0.06*	0.26 ± 0.01	2.62 ± 0.13*	0.12 ± 0.003*
		(- 28%)	(- 19%)	(+ 34%)	(- 20%)

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

Table 7: Effect of Hexachlorobenzene (HCB) on enzymatic and non enzymatic antioxidant parameters in different tissue homogenates.

Tissues	Groups	LPO (nmoles / g tissue.)	SOD (Units / mg protein)	Catalase (µmol / mg protein / min.)
Cortex	**Control**	151.62 ± 10.30	44.30 ± 4.63	53.85 ± 8.52
	HCB	233.83 ± 15.42*	22.45 ± 6.10*	21.24 ± 5.70*
		(+ 54%)	(- 49%)	(- 61%)
Medulla	**Control**	57.49 ± 7.11	35.62 ± 5.25	86.50 ± 8.02
	HCB	14.15 ± 1.79*	80.23 ± 5.23*	240.25 ± 11.00*
		(- 75%)	(+ 125%)	(+ 178%)
Intestine	**Control**	52.40 ± 9.72	3.97 ± 0.80	7.97 ± 0.47
	HCB	111.88 ± 10.36*	3.04 ± 0.29*	3.05 ± 0.65*
		(+ 114%)	(- 23%)	(- 62%)
Liver	**Control**	105.80 ± 5.68	83.64 ± 7.85	13.08 ± 2.11
	HCB	185.14 ± 8.58*	65.16 ± 15.31*	3.82 ± 0.28*
		(+ 75%)	(- 22%)	(- 71%)
Brain	**Control**	74.02 ± 5.33	62.00 ± 7.50	123.38 ± 3.25
	HCB	122.17 ± 6.03*	15.87 ± 4.22*	82.87 ± 1.23*
		(+ 65%)	(- 74%)	(- 33%)

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

accumulation of HCB reactive metabolites [47] and/ or differential localization of ALP, GGT, LAP and sucrase in the thickness of the BBM [37]. Taken together, increased serum creatinine and BUN, decreased BBM enzyme activities along with reported morphological alterations clearly demonstrate that HCB has caused significant structural and functional damage to various rat tissues. The activity of acid phosphatase (ACP, a marker enzyme for lysosomes) also significantly decreased in the kidney, intestine and brain but increased in the liver by HCB. The data clearly indicate that lysosomes along with plasma membranes were selectively damaged by HCB administration.

It is well established that many cellular functions including transport of ions and solutes especially across renal and mucosal BBM required energy as ATP which is provided by various metabolic pathways, the activities of various enzymes involved in glycolysis, TCA cycle, gluconeogenesis and HMP shunt pathway were evaluated in various rat tissues. HCB administration significantly altered the enzymes of glucose metabolism and its production as reflected by the changes in the activities of LDH (glycolysis), MDH (TCA cycle), and G6Pase and FBPase (enzymes of gluconeogenesis). HCB significantly increased the activity of LDH (glycolysis) but decreased

MDH (TCA cycle) in the cortex, medulla and liver. In contrast, LDH activity increased and MDH decreased in the intestine and brain. Although the actual rates of glycolysis and TCA cycle were not determined, marked decrease in LDH activity and profound increase in MDH activity indicate a shift in energy metabolism in the kidney and liver mainly in favour of oxidative metabolism most likely due to mitochondrial enlargement caused by HCB as a compensatory adaptive cellular response. However, the metabolism in the intestine and brain remained predominantly anaerobic in accordance with the fact that anaerobic glycolysis is more prevalent in these tissues to meet energy requirements.

The activities of G6Pase and FBPase (enzymes of gluconeogenesis) significantly decreased in all the tissues studied by HCB exposure indicating an overall suppression of gluconeogenesis in most of the tissues as reported earlier [48,49]. In contrast, HCB caused profound increase in G6PDH (an enzyme of HMP-shunt pathway) activity in the kidney, liver, intestine and brain alike similar to previous studies [52,54]. However, the activity of NADP-Malic Enzyme (ME) significantly decreased in renal tissues and brain but increased in the liver and intestine as shown earlier [50]. It appears that increased production of NADPH by G6PDH and/ or ME may have increased lipid synthesis [52,54] and might be responsible for higher serum cholesterol and phospholipids. Since HCB can diffuse easily into the cells due to its lipophilic nature that might have enhanced lipid metabolism. NADPH also play important role in oxidant/ antioxidant system. The cholesterol and phospholipids are essential membrane components and might be required to facilitate repair and regeneration of various membranes after HCB-induced damage as reported for other toxicants [1-3,6,7]. NADPH also play important role in oxidant/ antioxidant system.

The underlying mechanism by which HCB causes toxic deleterious effects in different tissues is unclear. It is believed that reactive metabolites of HCB might be responsible for most HCB induced toxic and other adverse effects [51]. Recently, we have shown that trichloroethylene (TCE), an aliphatic chlorinated industrial solvent caused nephrotoxicity and produced severe adverse effects in the liver, kidney, intestine and brain by inducing oxidative stress [4]. Fernandez-Tome, et al. [21] and Mazetti, et al. [48] have also suggested induction of oxidative stress as a possible mechanism in HCB induced nephrotoxicity and hepatoxicity. HCB induced decrease in gluconeogenesis and depletion of thyroid hormones have also been suggested as the consequence of HCB induced oxidative stress [48,57]. Recently, it has been shown that HCB caused an increase in LPO accompanied by suppression of SOD, GSH-Px and/ or catalase in brain and liver of fish [58] and female rats [59]. The present results demonstrate that HCB caused perturbation in antioxidant defence parameters as manifested by marked increase in LPO accompanied by decrease in SOD and catalase activities in all the tissues except medulla where LPO markedly lowered and SOD and catalase profoundly increased. It appears that HCB caused perturbation in the antioxidant defence mechanism by decrease in either SOD as in the brain or by catalase as in the liver and in intestine or by both as in renal cortex [55]. The tissue damage caused by toxic insult has been reported to be dependent on prevailing oxidative environment in the tissue [21]. Hence the opposite pattern of HCB effect on oxidative stress parameters in the cortex and medulla might be due to prevailing oxygen tension which is high in the cortex and low in the medulla. This is in agreement of Fernandez-Tome, et al. [21] who have reported higher antioxidant mechanism in the medulla than in any other compartment of the kidney. Thus the effect of HCB on the enzymes of oxidative stress seems to be tissue specific as they are differentially altered in different tissues which can be attributed to differential accumulation of HCB its bioactivities, oxygen tension, and oxidant/antioxidant ratio and/or occurrence of specific metabolic pathways in a particular tissue[56-59].

In summary the present results demonstrate that HCB administration caused specific alterations in the activities of certain enzymes of carbohydrate metabolism, BBM, lysosomes and oxidative stress although differentially in different rat tissues. The plasma membrane, mitochondria and lysosomes appeared to be specific HCB targets as evident by selective alterations in their specific biomarkers. HCB induced nephrotoxic, hepatotoxic and neurotoxic effects appeared to be at least, in part, due to selective alteration in the antioxidant defence parameters. The differential effects of HCB observed in different tissues on oxidative stress, BBM, biochemical and metabolic parameters can be due to its variable accumulations, mode of bio activation and generation of different reactive metabolites. We conclude that HCB caused widespread toxic and other adverse effects in many rat tissues.

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