Effect of Vitamin E on benzene induced alterations of some enzymes of carbohydrate metabolism and oxidative stress in rat liver

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Abstract -The effect of Vitamin E (1 mmol/kg body weight/day given intraperitoneally for 7 days before the administration of benzene) on benzene (500 mg/kg body weight/day in corn oil) induced alterations of some enzymes of carbohydrate metabolism and oxidative stress in rat liver was investigated. Control group was give only corn oil. Each treatment was carried out for 20 days. Results revealed that administration of benzene caused a significant increase in the levels of lactate dehydrogenase (LDH), Lipid peroxidation and malondialdehyde (MDA). Whereas the levels of glucose–6 phosphatase, reduced glutathione (GSH) superoxide dismutase (SOD) and Catalase were significantly decreased compared to control animals. But in those animals which received Vitamin E therapy 7 days before being challenged with benzene, the changes in those parameters were almost statistically non-significant. This study indicated that, Vitamin E, a well known antioxidant can protect the major profiles of Carbohydrate metabolism and normal oxidative state of liver from the detrimental effects of benzene at this concentration.

Keywords: Benzene, Vitamin E, Carbohydrate metabolism, Lipid peroxidation, antioxidant

INTRODUCTION

Benzene, a toxic aromatic hydrocarbon can alter the microanatomy and physiology of different organs including liver [1]. Benzene is widely used in different industries like rubber, drugs, detergents etc. [2]. It can creates cancer also [3]. However, we expose to benzene through auto-exhaust and other smokes [4]. Metabolism of benzene includes a series of enzymatic reactions including Cyt P_{450}, a hepatic microsomal enzyme, which generates toxic metabolic intermediates and by products by forming different conjugates [5]. Benzene can also able to generate reactive intermediates, mainly reactive oxygen species (ROS) that can cause cellular damage [6]. People working in those areas where benzene is present in high concentration may suffer from some metabolic disorders. Thus, it can creates occupational hazards. Vitamin E, a chain-breaking antioxidant eliminates lipid peroxyl and alkoxyl radicals and therefore suppresses the chain reaction of lipid peroxidation and promotes the production of scavenger-antioxidant enzymes [7]. In our present study, we would like to investigate whether or not Vitamin E play some protective role on benzene induced alterations of some enzymes of carbohydrate metabolism and oxidative stress in rat liver.

MATERIAL AND METHODS

Experimental Animals

Eight week old male sprague-Dawley rats weighing between 170-190 gm. were used in the experiments. All experimental animals were kept under the conditions according to the guidelines as provided by the Indian National Science Academy for the care and use of laboratory animals. Three groups of rats [twelve in each group] were selected for our study. Twelve male rats were randomly selected to receive benzene in corn oil at a dose of 500 mg/kg body weight/day [Group-A]. Another twelve rats were given same dose of benzene after 7 days intraperitoneal therapy of Vitamin E [1 mmol/kg body weight/day]. Each benzene administration was carried out for 20 days. Rest of the group was marked as control and received only corn oil during the experimental study.

Removal and presentation of liver

The experimental animals were anesthetized by intraperitoneal injection of pentabarbitone Sodium [0.4 mmol/kg]. The anesthetic was injected 10 minutes before the removal of liver. The livers of the animals were rapidly excised and immediately frozen in Freon 12 [CCl_2F_2] which was chilled to the freezing point with liquid nitrogen. Each of the experimental and control livers was then separately studied. The frozen livers were stored at -75^{0}C.

Some Biochemical assay of liver

Approximately 100 mg. of liver tissue was weighed and extracted with $HClO_4$. The enzymes lactate dehydrogenase [LDH], malate dehydrogenase [MDH], glucose-6 phosphatase, Fructose 1, 6 bisphosphatase activities were examined by the method of Khundmiri [8] The activity of hexokinase was studied by the method of Crane and Sole [9]. Reduced glutathione [GSH] level of the liver homogenate was measured by the method of Ellman [10]. Superoxide dismutase [SOD] was assayed as described by Marklund [11]. Catalase activity was studied by Aebi's method [12]. Colorimetric estimation of malondialdehyde [MDA] was done by Kei Satoh method [13]. Lipid peroixdation was

examined by the method of Rehncrona [14]. All the chemicals including benzene were purchased from B.C. Chatterjee and Co. [Kolkata]. Chemicals used were of analytical grade.

Statistical analysis of data

All data are expressed as mean ± SEM. Student t-test was done for statistical evaluation of the data and for the determination of level of significance in various groups of animals. The values were considered significant at the level $p<0.05$.

DISCUSSION

Table-1 showed analysis of some enzymes of carbohydrate metabolism like hexokinase and lactate dehydrogenase [LDH], malate dehydrogenase [MDH], glucose 6 phosphatase and fructose 1, 6 biophosphatase levels among the three groups. Changes in the levels of LDH and glucose 6 phosphatase were significant [$p < 0.05$] in Group-A animals when compared with control group. But the result was non-significant statistically when comparison was done between Group-B and control group. Table-2 expressed a significant change in the values of lipid peroxidation, reduced glutathione [GSH], Malondialdehyde [MDA] levels and the levels of two major antioxidant enzymes i.e. superoxide dismutase [SOD] and catalase when compared between control and Group-A animals [$p < 0.05$]. But when comparison was made between control and Group-B animals, the result was non-significant statistically. Previously it was reported that, Benzene damage to the liver via generation of ROS [15]. The enzyme levels involved in major carbohydrate metabolism and antioxidant levels have also been depicted when Benzene induced rats were compared with control group [16]. Significant alterations of LDH, glucose 6 phosphatase, SOD and catalase was observed in Benzene induced rats [17]. Our present study was restricted to assess the protective role of Vitamin E on Benzene induced alterations of rat liver especially the levels of some major enzymes involved in carbohydrate metabolism and antioxidant profile. Previous reports have suggested a close relation between Oxidative damage and Benzene induction. ROS produced during Benzene metabolism responsible for oxidative cellular damage [18]. Benzene impairs both the hepatic carbohydrate metabolism and antioxidant profile [19]. Increased lipid peroxida-tion and oxidative degeneration of poly unsaturated fatty acids of hepatocytic membranes due to generation of free radicals via Benzene toxicity was also reported in previous reviews [20]. Increased level of MDA level is a marker of lipid peroxidation [21] which is seen among Group - A animals in our present study. Thus, in these study, we have considered the levels of some enzymes involved in carbohydrate metabolism like hexokinase, LDH, MDH, glucose-6 phosphatase and fructose 1, 6 bisphosphatase.

We have also investigated the antioxidant profile of hepatocytes considering the levels of lipid peroxidation, GSH, MDA, SOD and catalase. LDH, glucose-6 phosphatase and all of the parameters investigated while considering the antioxidant profile of hepatocytes showed significant changes in Group-A animals when compared with control Group of animals. The oxidative damage mediated through the generation of ROS is considered to be the function of the overall balance between the oxidant and antioxidant levels of a specific tissue [22]. The antioxidants involved both intracellular and extra cellular defense factors where vitamins like Vitamin E, Vitamin A, Vitamin C and some trace elements like Selenium, Zinc etc play major role [7]. In our present study, we had seen that the levels of some enzymes like LDH and glucose 6 phosphatase involved in Carbohydrate metabolism showed significant alterations in Group-A animals when compared with control, and almost non-significant in Group-B animals when compared with control group of animals. Antioxidant profile study also showed similar result in both Group-A and Group-B animals. Thus, our results claim that administration of Vitamin E, a well known antioxidant can able to protect the oxidative damage induced by Benzene at this concentration. But whether or not Vitamin E can protect the liver from Benzene toxicity at higher concentration is not included in this study. It requires further investigations which is under way.

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Table 1- The levels of some hepatic enzymes of Carbohydrate metabolism in control, Group-A and Group-B animals.

Groups	Hexokinase [µmol/mg protein/hour]	LDH [µmol/mg protein/hour]	MDH [µmol/mg protein/hour]	Glucose 6 Phos-phatase [µmol/mg protein/hour]	Fructose 1, 6 Bisphos-phatase [µmol/mg protein /hour]
Control	11.31 ± 0.81	33.17 ± 0.68	4.21 ± 0.31	0.21 ± 0.01	0.93 ± 0.03
Group-A	12.11 ± 0.63 #	44.32 ± 0.57 *	3.13 ± 0.42 #	0.02 ± 0.01 *	0.84 ± 0.01 #
Group-B	11.57 ± 0.61 #	34.82 ± 0.62 #	4.17 ± 0.53 #	0.16 ± 0.01 #	0.89 ± 0.06 #

Values are expressed as Mean ± SEM, n = 12.
* $p < 0.05$, when compared with control.
# non-significant when compared with control.

Table 2- Antioxidant profile of Liver tissues in Control, Group-A and Group-B animals.

Groups	Lipid Peroxidation [nmol/mg tissue]	GSH [nmol/mg tissue]	MDA [nmol/L]	SOD [µmol/mg protein min]	Catalase units/mg protein
Control	69.31 ± 4.31	4.13 ± 0.21	0.65 ± 0.03	86.23 ± 6.17	93.68 ± 7.18
Group-A	90.21 ± 6.82 *	1.76 ± 0.16 *	2.07 ± 0.11 *	41.21 ± 7.13 *	58.28 ± 4.16 *
Group-B	70.26 ± 7.32 #	3.96 ± 0.27 #	0.81 ± 0.07 #	81.86 ± 4.18 #	87.71 ± 6.13 #

Values are expressed as Mean ± SEM, n = 12.
* $p < 0.05$, when compared with control.
# non-significant when compared with control.