# PROTECTIVE EFFECTS OF VERAPAMIL AGAINST HEXACHLOROBUTADIENE NEPHROTOXICITY IN RATS

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## ABSTRACT

In order to evaluate the protective effect of verapamil against hexachlorobutadiene (HCBD) nephrotoxicity, 30 adult Wistar albino rats were divided into five equal groups, A, B, C, D and E and were given intra-peritonially corn oil (I ml/kg; control), HCBD (50 mg/kg), verapamil (50 µg/kg) with HCBD (50 mg/kg), verapamil (100 µg/kg) with HCBD (50 mg/kg) and verapamil (100 µg/kg) with HCBD (100 mg/kg), respectively. Verapamil was given one hour before HCBD treatment. All animals were killed after 24 hours. Serum concentrations of urea and creatinine were higher ($P<0.05$) in HCBD-treated rats compared with the controls, as well as verapamil treated groups. Histopathological examination of kidneys showed substantial necrosis in straight portion of the proximal tubules in HCBD treated rats. In verapamil-treated groups, kidneys appeared normal. It was concluded that verapamil can protect kidneys against toxic effects of HCBD in rats.

**Key words**: Rats, nephrotoxicity, verapamil, hexachlorobutadiene.

## INTRODUCTION

Calcium is an important mediator of cell death in xenobiotic-induced cell injury. Alterations in intra-cellular calcium levels play an important role in the cysteine conjugate induced cell death. Some studies have shown that calcium channel blockers, such as verapamil, protect kidneys against nephrotoxins such as mercuric chloride (Girardi and Elias, 1998), gentamycin (Ali *et al*., 2002), cisplatin (Sleijfer *et al*., 1987), cephalosporins (Browning, 1990), and cyclosporine (Koo *et al*., 2003). This paper presents the results about the protective effect of verapamil against HCBD nephrotoxicity in rats.

## MATERIALS AND METHODS

In this study, 30 adult Wistar albino rats of either sex, weighing 150-200g, were used. They were kept under 12 hour light/dark cycle at 20ºC and 50% humidity. After acclimatization, they were randomly divided into five equal groups, i.e. A, B, C, D and E. These rats received single dose of the respective treatment via intraperitonial route, as shown in Table 1.

All animals were killed under light ether anesthesia 24 hours after treatment. Blood samples collected from each rat were used for the determination of urea and creatinine concentrations, using Technicon RA-1000 Autoanalyser. The right kidney of each rat was removed and fixed in 10% neutral buffered formalin, sectioned at 5 µm and processed for Haematoxylin and Eosin staining for histopathological studies. The data on serum urea and creatinine concentrations were analyzed using one-way analysis of variance and Tukey's test.

## RESULTS AND DISCUSSION

Rats of group B treated with HCBD alone showed significantly higher serum urea and creatinine concentrations compared to control (corn oil) and verapamil treated groups (Table 2). However, non significant difference was observed between corn oil and verapamil treated groups.

In control and verapamil treated groups, glomerulus, Bowman's capsule, proximal, distal and collecting tubules of kidneys appeared normal histo-logically. However, in HCBD treated group, an extensive damage in straight portion of proximal tubule was observed. Other parts of kidney, including cortex and medulla, appeared normal.

**Table 1: Protocol of treatments for rats of five groups**

| Group | Treatment detail |
|---|---|
| A | Control: corn oil (1 ml/kg) |
| B | HCBD (50 mg/kg) |
| C | Verapamil (50 µg/kg) one hour before HCBD (50 mg/kg) |
| D | Verapamil (100 µg/kg) one hour before HCBD (50 mg/kg) |
| E | Verapamil (100 µg/kg) one hour before HCBD (100 mg/kg) |

**Table 2: Serum urea and creatinine concentrations in rats treated with various doses of HCBD with and without verapamil**

| Groups | Urea (mg/dL) | Creatinine (mg/dL) |
|---|---|---|
| A: Corn oil (1ml/kg; control) | 33.8 ± 1.3a | 0.53 ± 0.05a |
| B: HCBD (50 mg/kg) | 81.7 ± 4.7b | 1.08 ± 0.30b |
| C: Verapamil (50 µg/kg) with HCBD (50 mg/kg) | 40.3 ± 1.1a | 0.53 ± 0.05a |
| D: Verapamil (100 µg/kg) with HCBD (50 mg/kg) | 38.0 ± 5.4a | 0.50 ± 0.01a |
| E: Verapamil (100 µg/kg) with HCBD (100 mg/kg) | 44.8 ± 6.2a | 0.60 ± 0.01a |

Values bearing different letters in a column differ significantly (P<0.05).

These results showed that verapamil has a protective effect against HCBD-induced nephrotoxicity in rats. HCBD is a potent nephrotoxin which can cause degeneration, necrosis, and regeneration in renal tubular epithelial cells (Berndt and Mehendale, 1979; Kirby and Bach, 1995). Its toxicity is due to its conjugation with glutathione to form glutathione S-conjugate, and finally to the related cysteine-conjugate. This metabolite is then actively taken up by kidneys and cleared through the renal tubular epithelial cells as a reactive thiol derivative by the enzyme-lyase, which covalently binds to macromolecules (Schrenk and Dekant, 1989). The S3 region (pars recta) of the proximal tubule of rat's kidney is the most susceptible organ to the nephrotoxicity induced by cysteine-conjugates.

Calcium has been shown to have an important role in cellular toxicity (Ali *et al*., 2002). As calcium homeostasis is precisely controlled, any alteration in intracellular calcium level could play an important role in the cysteine conjugate-induced cell death. Thus, calcium channel blockers like verapamil may affect the role of calcium in cellular toxicity. Toxicological consequences of increased intracellular calcium concentration could be: i) activation of calpains, leading to disruption of the protein components of cytoskeleton, ii) activation of Ca-dependent endonuclease, resulting in DNA single and double strand breaks and apoptotic or necrotic cell death, iii) activation of Ca-dependent phospholipases, causing liberation of arachidonic acid and disruption of membrane integrity with ensuing cell death, and iv) activation of gene expression (Watson *et al*., 1987). The mechanism(s) for protective effect of verapamil is not clear, but may be mediated by: a) enhancing elimination of HCBD and/or its toxic metabolite, b) inhibiting the activation of Ca-dependent proteases (calpains), endonucleases and phospholipases, c) improving renal blood flow due to vasodilatory effect, which will reduce toxicity and d) preventing gene expression (Ali *et al*., 2002).

Several investigations have been carried out on the role of calcium channel blockers against nephrotoxicity of mercuric chloride (Ali *et al*., 2002), gentamycin (Watson *et al*., 1987), cephalosporines (Sleijfer *et al*., 1987), cyclosporine (Koo *et al*., 2003) cadmium (Browning, 1990) and cisplatin (Sleijfer *et al*., 1987). Verapamil can protect kidneys against mercuric chloride toxicity. Animals treated with verapamil (75 µg/kg, i.p) prior to mercuric chloride showed normal kidney appearance (Girardi and Elias, 1998).

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