4-Vinylcyclohexene diepoxide elicits hepatotoxicity through induction of oxido-inflammatory signaling in rats

[1]Ikenna C. Maduako, [2]Chidinma N. Igwe

[1]Chemoprevention & Toxicology Laboratory, Department of Medical Biochemistry, College of Medical Sciences, Benson Idahosa University, Benin City, Nigeria

[2]Department of Public Health, Imo State University, Owerri, Nigeria.

QR CODE

Doi: https://doi.org/10.21816/ijf mi.v5i1

*Corresponding author email:
imaduako@biu.edu.ng

Abstract

The incidence of liver toxicity is gradually receiving public health attention, as it is associated with environmental contamination with toxins and xenobiotics. The present study examined the effects of 4-vinylcyclohexene diepoxide (VCD) on indices of oxido-inflammatory pathways in the liver of Wistar rats following treatment with 25, 50, and 100 mg/kg for 7 days by oral gavage. Administration of VCD markedly decreased liver antioxidant enzyme activities and glutathione levels, with corresponding increases in myeloperoxidase activity, nitric oxide levels, and malondialdehyde levels. ELISA analysis showed that VCD exposure increased the protein levels of pro-inflammatory cytokines, including inducible nitric oxide synthase and cyclooxygenase-2, in the livers of rats. Our data indicate that exposure to VCD induces liver injury via oxido-inflammatory signaling in rats, thereby confirming its hepatotoxic effect.

Introduction

Both industrial and environmental toxins can significantly impair liver function. As the body's largest internal organ, the liver is essential for regulating metabolism and eliminating waste products (Parkinson, 1996). Homeostasis refers to the regulation of the body's internal environment, where excretory processes are adjusted to balance intake, metabolic production, and losses. Conversely, clearance describes the specific rate at which the body eliminates toxic substances (Randers et al., 1998). The inability of these organs to carry out these functions triggers systemic breakdown and disruption of vital biochemical processes (Kumar et al., 2020).

4-vinylcyclohexene diepoxide (VCD) is industrially produced through the metabolism of 4-vinylcyclohexene (VCH). VCH itself is a byproduct generated during the homodimerization of 1,3-butadiene, a process common in the manufacturing of rubber, plastics, flame retardants and pesticides (Felter et al., 2021). Human exposure to occupational and environmental VCD can disrupt normal liver which serves as the primary site for xenobiotic metabolism and detoxification of environmental toxicants. In liver, VCH is bioactivated to the more toxic diepoxide metabolite (VCD) primarily by cytochrome P450 (CYP) isoform CYP2A and CYP2B, though CYP2E1 contributes in ovarian tissue (Fontaine et al., 2001). VCD induces oxidative stress, promotes lipid peroxidation, glutathione depletion, iron release and protein adduct formation, ultimately overwhelming cellular defenses against reactive oxygen species (ROS), leading to cellular damage (Fontaine et al., 2001). While the reproductive health consequences of VCD are well-documented, initial biochemical and molecular mechanisms driving its toxicity in the liver remain undocumented. This study, therefore, investigated, for the first time, specific sub-cellular and molecular alterations in the rat hepatic system following oral administration of VCD.

Materials and methods

Reagents

4-vinylcyclohexene diepoxide (VCD, purity > 96%) was obtained from Sigma-Aldrich (St Louis, MO, USA). Alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase were sourced from Randox. All remaining chemicals were of analytical grade from British Drug Houses Dorset. Levels of COX-2, and iNOS proteins in the supernatant were measured using commercially available ELISA kits in accordance with the manufacturer's instructions (CUSABIO Life Science Inc., WUHAN, China).

Experimental animals and regimen

Adult male albino Wistar rats (weighing 185 – 190 g) purchased from the Animal facility of the Anatomy Department, University of Benin, Benin City, Nigeria. The acclimatization period of one week was conducted in hygienic conditions, housed in plastic cages under standard laboratory settings with a 12-hour light/dark cycle, and provided ad libitum access to standard chow and potable water. All experimental procedures adhered to the principles set in the NIH Guide for the Care and Use of Laboratory Animals (1985). All experimental procedures were conducted following approval from and observing the guidelines of Benson Idahosa University Animal Care and Use Research Ethics Committee (BIU-ACUREC file No: 0012/med/064). The rats were distributed into four groups (n=7 each). The group 1 serve as control, and administered with corn oil while others were administered with VCD orally via gavage at doses of 25, 50, or 100 mg/kg (in corn oil) daily for seven consecutive days. These doses were selected based on reproducible biochemical and histological changes observed in preliminary study. The study duration is seven days.

Preparation of homogenates

Twenty-four hours after the final treatment, all rats were weighed and euthanized under anesthesia with a ketamine (87.5 mg/kg) and xylazine (12.5 mg/kg) combination. Succinctly excised livers were rinsed in

ice-cold phosphate-buffered, blotted and weighed. Followed by homogenization and centrifugation the resultant supernatants kept in freezers for biochemical endpoint assays.

Determination of liver function test

Blood samples were collected via cardiac puncture from each rat. Samples were transferred to non-heparinized tubes, allowed to clot at room temperature, and then spun at 5,000 rpm for 10 minutes to obtain serum. The separated serum was transferred to Eppendorf tubes and stored until used for liver function markers, including alanine aminotransferase (ALT), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), and aspartate aminotransferase (AST), using commercially available RANDOX kits protocol.

Assay of oxidative stress biomarkers

Total protein levels in hepatic tissue were quantified using the Bradford assay (1976). Superoxide dismutase (SOD) activity was measured based on its inhibition of epinephrine autoxidation, as reported by Misra and Fridovich (1972). Catalase (CAT) activity was determined by monitoring the decomposition of hydrogen peroxide spectrophotometrically, following the protocol described by Claiborne (1995). Reduced glutathione (GSH) levels were quantified using the spectrophotometric method involving reaction with 5,5'-dithiobis (2-nitrobenzoic acid) (DTNB), as described by Jollow et al. (1974). Lipid peroxidation (LPO) was evaluated by measuring malondialdehyde (MDA) content via the thiobarbituric acid reactive substances (TBARS) following the established protocol (Farombi et al., 2000).

Determination of proinflammatory markers

The spectrophotometric method of Green (1982) was used to determine the nitrite level in the liver tissue. Succinctly, equal volumes of tissue sample and Griess reagent were mixed, incubated for 15 minutes, and the absorbance was read at 540 nm. NO concentrations were derived from a sodium nitrite standard curve and normalized to protein content (units/ mg protein). Myeloperoxidase (MPO) activity in hepatic tissue was quantified using a spectrophotometric assay involving the oxidation of a chromogenic substrate (o-dianisidine) in the presence of hydrogen peroxide according to the protocol of Granell et al. (2003).

Statistical analysis

Data were analyzed using one-way analysis of variance (ANOVA), followed by Bonferroni's post hoc test for multiple comparisons between groups. Statistical significance was set at $p < 0.05$. Analyses were performed with SPSS software (version 17).

Results

Effects of VCD on biomarkers of hepatic function in challenged rats

Table 1 displays the results for hepatic biomarkers in rats exposed to increasing doses of VCD. Serum levels of AST, ALT, ALP, and LDH showed significant dose-dependent elevations in VCD-treated groups relative to controls ($p < 0.05$).

Table 1. Effect of VCD on the liver function biomarkers in treated rats for seven consecutive days.

Parameters	Control	VCD 25mg/kg	VCD 50mg/kg	VCD 100 mg/kg
AST (U/L)	39.72±2.16	66.20±3.29*	82.08±2.12*	108.13±3.14*
ALT (U/L)	20.22±1.90	32.52±2.64*	56.69±3.19*	78.52±1.24*
ALP (U/L)	30.25±2.25	43.51±2.20*	68.61±2.22*	80.90±2.20*
LDH (U/L)	1.22± 0.13	5.15±0.30*	10.18±0.10*	17.80±0.27*

Values are expressed as mean ± SD of 7 rats. *$p < 0.05$ vs. control

Effects of VCD on the liver redox status and inflammatory indices in exposed rats

Tables 2 and 3 illustrate the impact of VCD on hepatic oxidative stress markers in rats. VCD treatment at all doses significantly reduced hepatic activities of CAT and SOD, as well as GSH levels, compared with the control group ($p < 0.05$). Furthermore, significant increases in hepatic MDA levels were observed only at the higher acute oral doses of 50 and 100 mg/kg VCD in rats ($p < 0.05$). All tested VCD doses markedly elevated hepatic MPO activity and NO concentrations compared to controls ($p < 0.05$).

Table 2. Effect of VCD on hepatic redox status in treated rats for seven consecutive days.

Parameters	Control	VCD 25mg/kg	VCD 50mg/kg	VCD 100 mg/kg
SOD	27.22±1.20	12.80±1.11*	7.78±0.20*	4.08±0.11*
CAT	18.73±0.10	10.18±1.02*	6.12±0.11*	2.03±0.22*
GSH	35.25±1.21	21.27±0.82*	14.07±1.04*	8.18±1.11*
LPO	0.56 ± 0.05	1.28 ± 0.19*	*1.98 ± 0.07*	2.28 ± 0.19*

SOD activity (nmoles epinephrine oxidized/min/mg protein); CAT activity (µmole H_2O_2 consumed/min/mg protein); GSH level (µmole/mg protein); LPO level (µmol MDA/mg protein). Values are expressed as mean ± SD of 7 rats. *p < 0.05 vs. control

Table 3. Effect of VCD on inflammatory indices in treated rats for seven consecutive days.

Parameters	Control	VCD 25mg/kg	VCD 50mg/kg	VCD 100 mg/kg
NO	1.01 ± 0.04	4.24 ± 0.27*	7.19 ± 0.22*	10.20 ± 0.13*
MPO	0.89 ± 0.02	1.97 ± 0.24*	7.32 ± 1.11*	11.83 ± 0.17*

NO (units/mg protein); MPO activity (units/mg protein). Values are expressed as mean ± SD of 7 rats. *p < 0.05 vs. control

Effects of VCD on the level of pro-inflammatory proteins of challenged rats

Table 4 presents the effects of graded VCD doses on hepatic pro-inflammatory markers in rats. Exposure to VCD significantly increased protein levels of iNOS and COX-2 in the liver compared with the control group ($p < 0.05$).

Table 4: Effect of VCD on hepatic pro-inflammatory proteins in treated rats for seven consecutive days.

Parameters	Control	VCD 25mg/kg	VCD 50mg/kg	VCD 100 mg/kg
iNOs	1.51 ± 0.41	8.80 ± 1.01*	13.13 ± 2.02*	18.20 ± 1.09*
COX-2	3.01 ± 1.12	9.11 ± 0.21*	11.07 ± 0.17*	15.33 ± 1.21*

iNOS (pg/mL); COX-2 (pg/mL). Values are expressed as mean ± SD of 7 rats. *p < 0.05 vs. control

Discussion

Pesticides are utilized globally across agriculture, industrial, and domestic sectors to manage pests and protect public health. However, many of these substances, such as VCD are known to trigger systemic inflammation and oxidative stress in the body (Beier et al., 2025). In event of hepatic inflammation or oxidative damage, the structural integrity of liver cells is often compromised. This allows cytoplasmic enzymes, transaminases, and LDH to seep into the blood, where they serve as measurable biomarkers of the severity of the injury. Our study examined the hepatotoxic effects of VCD in male Wistar rats. Oral administration of increasing VCD doses caused hepatic damage, as indicated by marked elevations in serum AST, ALT, ALP, and LDH activities compared with control.

Enzymatic and non-enzymatic antioxidants within liver cells maintain the hepatic redox balance. SOD neutralizes superoxide radicals generated during mitochondrial respiration and inflammation, converting them to hydrogen peroxide, which CAT subsequently decomposes into water and oxygen (Halliwell & Gutteridge, 2015). In this study, VCD-induced hepatotoxicity is associated with a marked decline in CAT and SOD in the hepatic cells. Furthermore, the observed reductions in hepatic SOD and CAT activities suggest enzyme inactivation and consequent oxidative stress in the livers of VCD-treated rats (Maduako & Farombi, 2025; El-Bassyouni et al., 2015), which likely stems from the surge of free radicals produced during VCD metabolism by the liver CYP enzymes (Bölck et al., 2014). The initiation of hepatoxicity is often linked to oxidative stress, characterized by the production of more pro-oxidants than antioxidants. This leads to generation of reactive oxygen species (ROS) and LPO that compromise cellular integrity and exhaust natural enzymatic defenses (Zhu et al., 2020). Unabated increases in ROS and LPO are usually associated with suppressed activities of antioxidant enzymes and GSH levels (Jomova et al., 2024). Our study data showed a notable reduction in hepatic GSH concentration in VCD-treated groups, whereas LPO levels showed a significant dose-dependent increase. Our data was supported by previously reported study (Bhattacharya & Rao, 2001).

Elevated MPO activity, usually released by activated neutrophils, is associated with inflammation and oxidative stress. By producing hypochlorite from hydrogen peroxide, MPO exacerbates ROS generation, leading to significant structural damage in the liver (Maduako & Farombi, 2025; Davies & Hawkins, 2020). The induction of iNOS during systemic inflammation facilitates the overproduction of NO, which can subsequently interact with reactive species to exacerbate tissue damage (Sharma et al., 2007). The hepatotoxic impact of VCD from our study was further substantiated by a significant rise in

MPO activity, reflecting the systemic infiltration of mononuclear cells into the liver tissue of the treated rats, along with a significant concomitant rise in the production of NO, likely resulting from the activation of inducible inflammatory pathways. This earlier report (Maduako & Farombi, 2025) supports our findings.

iNOS and COX-2 were assessed to ascertain their role in VCD-induced liver toxicity. To confirm the role of nitrosative stress in VCD-induced hepatotoxicity, hepatic iNOS level was evaluated in rats using ELISA technique. From our study, the observed upregulation of iNOS and concomitant rise in nitric oxide levels in VCD-treated livers likely promote liver injury through enhanced inflammation and nitrosative stress. COX-2 is a highly inducible enzyme that facilitates arachidonic acid conversion, playing a central role in the cellular response to inflammation (Ricciotti & FitzGerald, 2011). Consistent with VCD's ability to promote inflammation and oxidative stress (Abolaji et al., 2016), our study data indicated a substantial increase in liver COX-2 level among VCD-exposed rats. COX-2 upregulation is associated with inflammatory pathology (Ricciotti & FitzGerald, 2011).

Conclusion

In conclusion, our study showed that VCD induced a dose-dependent hepatotoxicity. The hepatotoxic effect of VCD involved oxido-inflammatory stress.

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