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Effects of subacute 3-monochloropropane-1,2-diol treatment on the kidney of male albino rats

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ABSTRACT

3-Monochloropropane-1,2-diol (3-MCPD) is a well-known food contaminant. Although the kidney is thought to be a target organ for 3-MCPD toxicity, nephrotoxic structural changes are relatively unstudied. We investigated the renal alterations caused by 3-MCPD in male albino rats. 3-MCPD was administered orally, at a dose of 60 mg/kg for 7 days. 3-MCPD caused significant elevation of serum creatinine and urea levels together with hydropic degeneration, necrosis and shedding of the cells of the proximal convoluted tubules, urinary casts in the distal convoluted tubules and interstitial inflammatory cell infiltration. Administration of 3-MCPD for a period as short as 7 days causes acute renal failure in male albino rats.

KEYWORDS

Acute renal failure; kidney; 3-MCPD; 3-monochloropropane-1; 2-diol; tubular necrosis; rat

3-Monochloropropane-1,2-diol (3-MCPD), formerly known as alpha-chlorohydrin, is a well-known food contaminant (Mahmoud et al. 2018). It is formed in food by heat processing food containing salt and fat, acid hydrolysis of vegetable proteins or 3-MCPD ester hydrolysis (Fao 2007; Baer et al. 2010). 3-MCPD is present in soy sauce, oyster sauce, margarine, vegetable oils (excluding walnut oil), bread, fine bakery products, infant formulas, preserved meats, soup, gravy mixes and stock cubes. It is found also in drinking water treated with epichlorohydrin resins (El Ramy et al. 2007; Zhang et al. 2012).

The kidney has been reported to be sensitive to 3-MCPD toxicity (Lee et al. 2015). Cho et al. (2008) reported a significant increase in the relative weight of the kidney of B6C3F1 mice that received 37 mg/kg doses of 3-MCPD/day for 13 weeks. Acute renal failure was reported in 20–50% of rats exposed to 29.5 mg/kg 3-MCPD orally for 90 days (Barocelli et al. 2011). Eosinophilic bodies were reported in the proximal tubules of F344 rats treated with 40 mg/kg 3-MCPD for 4 weeks (Onami et al. 2014). Tubule basophilia was observed in the kidneys of CB6F1-non-Tg rasH2 mice treated with 3-MCPD at doses up to 100 mg/kg for 28 days (Lee et al. 2015). Small vesicles and hydropic degeneration were reported in the kidneys of Wistar rats treated with 3-MCPD (60 mg/kg) for 35 days (Ji et al. 2016). We investigated the renal structural alterations caused by short-term administration of 3-MCPD.

Material and methods

Chemicals

3-Monochloropropane-1,2-diol (Epibloc®) was purchased from Sigma Chemical Co. (St. Louis, MO). All other chemicals were of analytical grade and were obtained from standard commercial suppliers.

Animals

We used 12 120–140 g adult male Wistar albino rats (*Rattus norvegicus*) obtained from the Veterinary Serum and Vaccine Research Institute (Cairo, Egypt). The animals were housed and acclimatized to laboratory conditions for 1 week before beginning the experiments. The animals were reared in polypropylene cages with clean wood shaving bedding at 25 ± 1° C, 50% relative humidity and 12 h light/12 h dark cycle. The animals were allowed free access to water and standard rodent food pellets (Agricultural-Industrial Integration Co., Cairo, Egypt). Animal handling procedures were in accordance with the Helsinki Declaration of 1975 as revised in 1983.

Experimental design

The animals were divided into two groups of six: group 1, rats were given distilled water and served as the control group; group 2 rats were given 60 mg/kg 3-MCPD (Ji et al. 2016) for 7 days.

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Sample collection

Twenty-four hours after the last dose, rats were anesthetized with diethyl ether and weighed. After necropsy, blood samples were collected by cardiac puncture, left to clot, centrifuged at 1,800 x g for 10 min. The supernatent serum was frozen at −20° C until assay for biochemical parameters. The kidneys were excised, blotted, weighed then processed for histological and histochemical assessments.

Biochemical assays

Stored serum samples were analyzed for urea and creatinine according to the colorimetric methods of Patton and Crouch (1977) and Henry et al. (1974), respectively, using commercial kits from Biodiagnostics Co. (Dokki, Egypt).

Histology and histochemistry

Small pieces, approximately 15 × 15 × 5 mm, of the kidney were fixed in alcoholic Bouin's solution at room temperature for 24 h. Samples were dehydrated through increasing concentrations of ethyl alcohol, cleared in terpineol and embedded in paraffin. Sections were cut at 5 µm and mounted on slides. Slides were de-waxed in xylene, then re-hydrated through decreasing concentrations of ethyl alcohol. Sections were stained with hematoxylin and eosin (H & E) (Drury and Wallington 1980) for routine histology, periodic acid-Schiff (PAS) for glycoproteins (Hotckiss 1948), or mercuric bromophenol blue for total proteins (Mazia et al. 1953).

Statistical analysis

Data were expressed as means ± SEM. The differences among the normally distributed means were tested using the t-test for independent samples using GraphPad Prism™ (version 5.0, GraphPad, San Diego, CA). Values for $p \leq 0.05$ were considered statistically significant.

Results

Histological and histochemical results

The kidneys of the control rat kidneys exhibited normal appearance, size and color, and no signs of toxicity. H & E stained sections of the kidneys of the control rats exhibited normal renal architecture (Figure 1a). The glomeruli and proximal and distal covoluted tubules appeared normal. The cells of the proximal tubules are cuboidal with abundant microvilli that formed a normal brush border. Each transverse section of a proximal tubule typically contained three–five cells with round nuclei. The cuboidal cells of the distal convoluted tubules are flatter and smaller than those of the proximal tubule and have no brush border (Figure 1b). PAS stained sections exhibited strong staining of the basement membrane and moderate staining of the cytoplasm of the tubule cells, while the brush border of the proximal tubules and the glomeruli were stained intensely owing to muccopolysaccharides (Figure 2a). Sections stained with mercuric bromophenol blue showed that the cytoplasm, nucleus and cell membrane of renal cells and the glomeruli were stained strongly for proteins (Figure 3a).

On the other hand, 3-MCPD treated rats were thin, hypoactive and exhibited oligouria. At necropsy, the kidneys of these rats appeared swollen and pale with increased weight compared to controls (Table 1). Histological examination of the kidneys of 3-MCPD treated rats showed architectural disruption (Figure 1c). Most of the glomeruli were congested. The cells of the proximal tubules showed various degrees of degeneration as evidenced by hydropic changes, cloudy swelling, necrosis and exfoliation. Interstitial inflammatory cell infiltration was observed adjacent to necrotic tubules. Most of the distal convoluted tubules contained eosinophilic hyaline casts (Figure 1b). In PAS stained sections of this group, the cytoplasm of the tubule cells with hydropic changes was stained weakly, while the remainder of the renal tissue was stained moderately. Urinary casts were stained intensely by PAS for muccopolysaccharides (Figure 2b). In sections stained with mercuric bromophenol blue, the cytoplasm of the tubular cells with hydropic changes was stained weakly; tubule basement membranes, necrotic cells and glomeruli were stained moderately. Urinary casts were stained intensely for protiens (Figure 3b). The structural observations were accompanied by significant elevation serum creatinine and urea levels compared to controls (Table 1).

Biochemical results

Biochemical analysis of kidney biomarkers of 3-MCPD treated rats showed signs of nephrotoxicity. Creatinine and urea levels were significantly elevated ($p < 0.001$) compared to controls (Table 1).

Discussion

We investigated the effect of oral administration of 60 mg/kg 3-MCPD on the kidney of male albino rats. Macroscopic examination of 3-MCPD treated rats revaeled pale and edematous kidneys. Kidney pallor

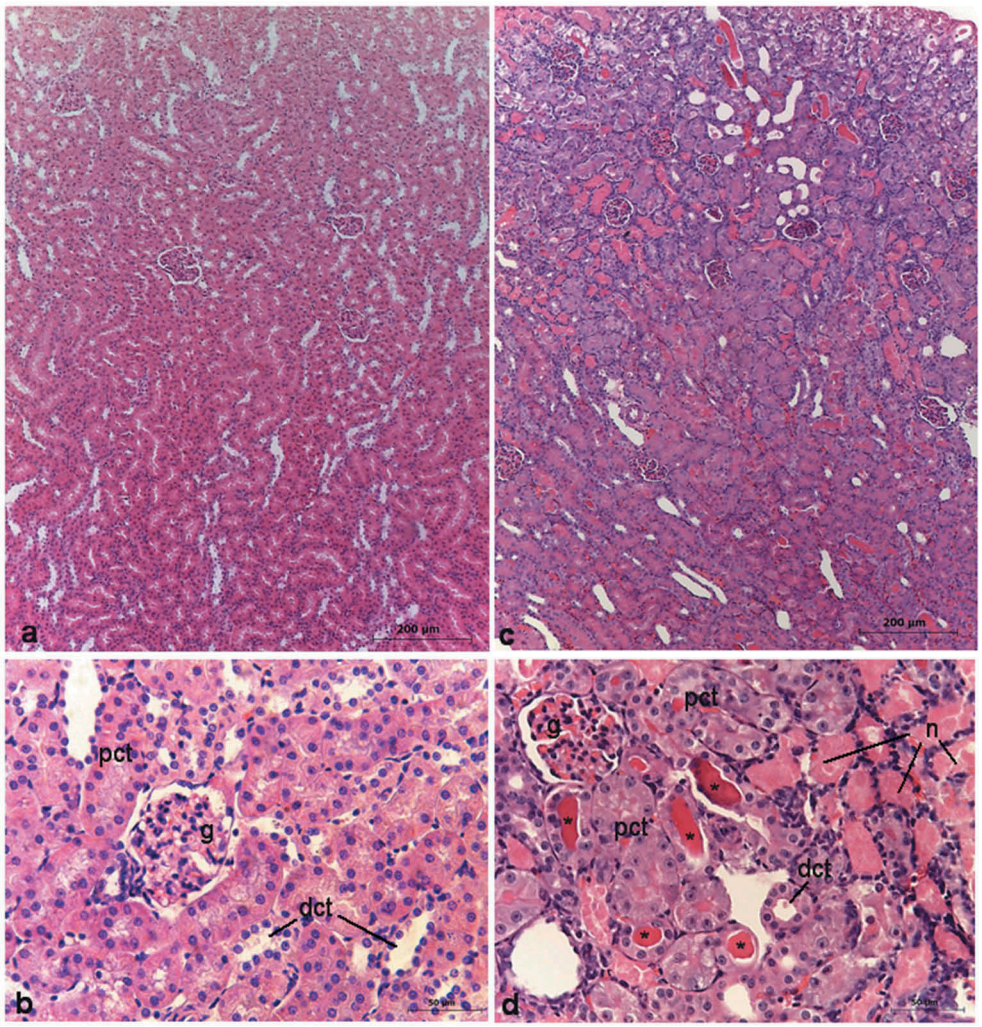

Figure 1. Kidney sections stained with H & E. a) Control rat showing normal renal architecture. b) Normal glomerulus (g), proximal convoluted tubules (pct), and distal convoluted tubules (dct). c) 3-MCPD-treated group showing renal architectural disruption. d) Higher magnification of (c) shows congested glomeruli (g), proximal tubules with hydropic changes (pct), proximal tubular necrosis (n) with adjacent inflammatory cell infiltration, and distal convoluted tubules containing urinary casts (asterisks).

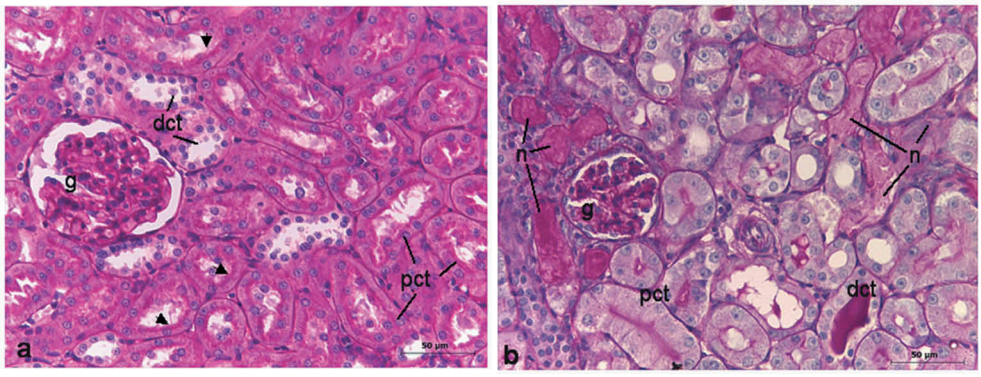

Figure 2. Kidney sections stained with PAS. a) Kidney of control rat showing intensely stained cytoplasm in the tubule cells. The brush border of the proximal tubules was stained intensely (arrow). b) 3-MCPD-treated group showing weak staining in cells of proximal tubules with hydropic degeneration (pct) and moderate staining in the remainder of the renal tissue. Urinary casts in distal convoluted tubules (dct) exhibit intense PAS staining.

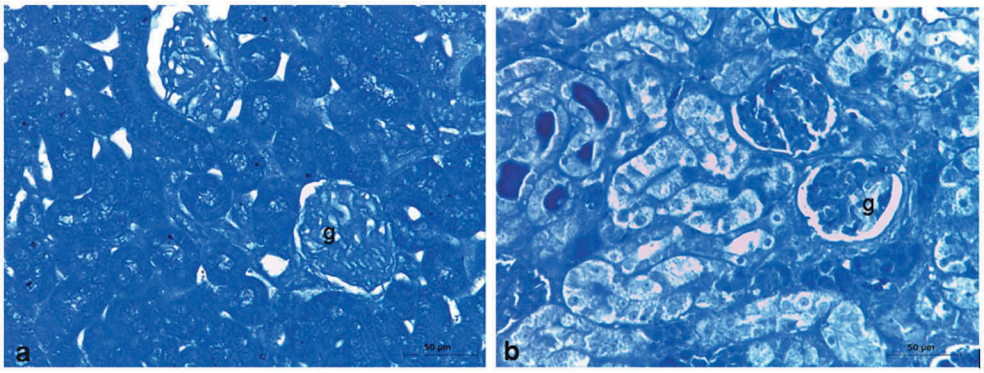

Figure 3. Kidney sections stained with mercuric bromophenol blue for total protein. a) Kidney of control rat showing intensely stained cytoplasm, nucleus and cell membrane of tubule cells and the glomeruli (g). b) 3-MCPD-treated group showing weak staining in the tubule cells with hydropic degeneration and moderate staining in the remainder of the renal tissue. Urinary casts in distal convoluted tubules (dct) were stained intensely for proteins.

Table 1. Effect of 3-MCPD on relative weight of the kidney and levels of serum creatinine and urea of albino rats.

	Control	3-MCPD
Relative weight	0.39 ± 0.01	0.67 ± 0.0
Creatinine (mg/dl)	0.66 ± 0.13	1.52 ± 0.3*
Urea (mg/dl)	33 ± 1.95	187.4 ± 34.85*

Data are means ±SEM, n = 6. *Mean value was significantly different from control group, $p < 0.001$.

could be due to hydropic changes and tubular necrosis (Mahmoud 2017). We also found that the kidneys of 3-MCPD treated rats weighed more than control kidneys. Increased relative weight of the kidneys has been reported for B6C3F1 mice treated with 37 and 76.79 mg/kg 3-MCPD for 13 weeks (Cho et al. 2008). Similarly results have been reported for rats treated with 29.5 mg/kg 3-MCPD for 90 days (Barocelli et al. 2011), and F344 rats treated with 40 mg/kg 3-MCPD for 4 weeks (Onami et al. 2014). Neither previous report included an explanation for the increase in relative kidney weight, but we believe it could be attributed to the parenchymal cell swelling that we observed.

3-MCPD-treated rats exhibited many structural alterations including hydropic changes, swelling and necrosis of proximal tubule cells, urinary casts in distal convoluted tubules and interstitial inflammatory cell infiltration. Similar observations have been reported for rats treated with 29.5 mg/kg 3-MCPD for 90 days (Borcelli et al 2011). Onami et al. (2014) reported only tubule necrosis in F344 rats treated with 40 mg/kg 3-MCPD for 4 weeks. Tubule basophilia and occasional inflammatory reactions were observed in the kidneys of CB6F1-non-Tg rasH2 mice treated with 3-MCPD up to 100 mg/kg for 28 days (Lee et al. 2015). Small vesicles and hydropic degeneration were reported in the renal cortex of Wistar rats treated with 60 mg/kg 3-MCPD for 35 days (Ji et al. 2016).

By contrast to previous reports, we report nephrotoxicity and acute renal failure after sub-acute administration of 3-MCPD.

The nephrotoxicity of 3-MCPD is due to its inhibition of glycolysis (Jeong et al. 2010). In mammals, 3-MCPD is oxidized by alcohol dehydrogenase into β-chlorolactate with further conversion to β-chlorolactaldehyde and oxalic acid (Bakhiya et al. 2011). β-chlorolactaldehyde inhibits glyceraldehyde-3-phosphate dehydrogenase and triose phosphate isomerase, which participate in glycolysis (Jones and Porter 1995; Peng et al. 2016). Impairment of the glycolytic pathway and impaired production of energy in the form of adenosine triphosphate (ATP) could contribute to kidney damage (Peng et al. 2016). Also, accumulation of oxalic acid, the degradation product of β-chlorolactic acid in the kidney, is thought to contribute to the kidney toxicity of 3-MCPD (Jones et al. 1981; Jeong et al. 2010). Consequently, depletion of ATP causes failure of ATP-dependent ion channels, such as the Na^+/K^+ pump, which results in influx of Na^+ and water, cell swelling and subsequent cell collapse and necrosis (Padanilam 2003). Nonviable cells are shed into the tubule lumen, which results in luminal obstruction and the formation of obstructing casts in the distal tubules and collecting ducts. The hyaline casts consist of urinary glycoprotein (Tamm-Horsfall protein) normally secreted by tubule cells. Luminal obstruction contributes to reduction of the glomerular filtration rate, which leads to nitrogenous waste retention and increased urea and serum creatinine (Schrier et al. 2004).

3-MCPD (60 mg/kg) caused nephrotoxicity as evidenced by significant elevation of serum creatinine and urea levels together with hydropic degeneration, cloudy swelling and necrosis of the cells of the proximal convoluted tubules, urinary casts in the distal convoluted tubules, and interstitial inflammatory cell infiltration. Ours is the first report of acute renal failure in male

albino rats treated with 3-MCPD for a period as short as 7 days, which raises concerns about accumulative exposure to this food contaminant.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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