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The Acute Toxicity of Deisopropylngaione in Mice

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ABSTRACT

It has become apparent that inflammation provoked by injury to renal epithelial cells serves to amplify kidney injury and dysfunction *in vivo*. Compounds including the sesquiterpene ketones generally believed to cause kidney injury by direct tubular toxicity or crystal nephropathy have been considered in different renal studies. The aim of this study was to highlight the histotoxicity of Deisopropylngaione (DIN) in mice. Ninety male quackenbush mice weighing between 30 and 35 g b.wt. were divided into five groups of fifteen mice per group and received 70, 98, 137, 192 and 268 mg kg⁻¹ DIN intraperitoneally, respectively. Haematoxylin eosin (H and E) and Von Kossa stained histology slides showed proximal tubular necrosis and hyaline cast formation. Deposition of calcium salt in residual necrotic tubular epithelium was on the increase with increase in dose rate. Besides the pulmonary lesions seen in mice following intoxication by DIN, the kidney lesions were consistent with previous studies of these compounds in mice and similar to mercuric chloride poisoning in sheep.

Key words: Acute toxicity, deisopropylngaione, nephrotoxicity, tubules, necrosis, mice

INTRODUCTION

Deisopropylngaione (DIN) is a sesquiterpene ketone and one of the essential oils found in the Theodore variety of *Myoporum deserti*. Commonly known as Ellangowon Poison Bush or Turkey Bush, has long been recognized as toxic to grazing stock (Seawright *et al.*, 1982). It is an erect shrub, usually glabrous and belongs to the family Myoporaceae. The plant has been recorded in various districts of Queensland in Australia (Allen *et al.*, 1978). Chemical investigations have revealed the existence of eleven furanoid essential oils (Hamilton *et al.*, 1973). Preliminary investigation of experimental intoxication in mice indicated that DIN was nephrotoxic. Furthermore, Ogunsan *et al.* (2003) reported that following intoxication of sheep with 150 mg kg⁻¹ b.wt. of DIN intra-peritoneally, there was impairment of renal function as depicted by decreases in the Glomerular Filtration Rate (GFR), Renal Plasma Flow (RPF) and tubular transport maximum for glucose (TMG) which were essentially pre-renal in origin. Miller *et al.* (2010) also observed decreases in Glomerular Filtration Rate (GFR) and plasma magnesium concentrations after dosing cisplatin to rats at 50 mg m⁻² body surface area and observed histological changes of acute tubular necrosis. Several agents were reported in histological studies to induce nephrotoxicity in humans and animals (Wu *et al.*, 2002; Atessahin *et al.*, 2003; Machoy-Mokrzynska, 2004; Malekinejad *et al.*, 2010) causing pathologic changes that are often reversible, however, long time

exposure to these agents may induce irreversible renal degeneration (Dehghani *et al.*, 2011). Studies of Zabulyte *et al.* (2007) have shown that exposure to high doses of fluoride causes damage to renal tissue resulting into renal dysfunction. Al-Omireeni *et al.* (2011) demonstrated in their study that sodium fluoride causes significant decrease in protein levels and total collagen content in rat kidney resulting into a disturbance of connective tissue matrix of the kidneys. Contamination of various foods consumed by animals with ochratoxin A, a mycotoxin (Okutan *et al.*, 2004) have been reported to cause the development of renal disease due to oxidative stress (Malekinejad *et al.*, 2010) accompanied by proximal tubular atrophy and cortical interstitial fibrosis (Aukema *et al.*, 1999). This mycotoxin (ochratoxin A) was also shown to cause carcinoma in male rat renal parenchyma after exposition (Adler *et al.*, 2009). The objectives of this study were to establish the dose rate(s) capable of intoxicating mice and to highlight renal histopathological features associating with DIN intoxication in male mice.

MATERIALS AND METHODS

Ninety male quackenbush mice weighing between 30 and 35 g b.wt. obtained from the laboratory animal breeding station of the Department of Veterinary Pathology and Public Health, University of Queensland, Brisbane, Australia were used in this study. The study which lasted for 30 days was carried out in the preparation room of the post mortem Toxicology Laboratory, Veterinary Pathology, University of Queensland, Australia. The mice were divided into 5 groups of fifteen mice to a group. A 1:19 dilution of DIN in arachis oil was dosed to the experimental mice Intraperitoneally (i.p.) One group of 15 mice received 0.3 mL total dose of arachis oil IP and served as the control group. The five groups of mice were dosed with DIN by the intraperitoneal route at 70, 98, 137, 192 and 268 mg kg⁻¹ b.wt., respectively. The mice tails were marked with different colours for proper identification while their cages were tagged according to the dose rates. The experimental mice were fed proprietary mice pellets and water was offered *ad libitum*. Mice on high dose rate of 268 mg kg⁻¹ b.wt. started dying 12 h post exposure and surviving mice were euthanized and necropsy performed immediately. Surviving mice in each group of different dose rate were also euthanized and post mortem done on them progressively up to 10 days post exposure to DIN. At postmortem, samples of kidney were obtained, diced and fixed in 10% buffered formalin which was changed 24 h later. The fixed tissues were processed on automatic tissue processor (Duplex Processor) supplied by Shandon Elliott through graded alcohol of 70, 80, 90 and 95%; absolute 1, 2 and 3 and xylene 1 and 2, respectively. The prepared tissues were sectioned on Rotary Microtome HM 340 (Thermo Scientific, Japan). Haematoxylin eosin staining technique was employed to produce histopathology slides and a further set of tissues were stained Von Kossa to demonstrate calcium salt deposition. The slides were viewed at low and high powers microscope (Nikon 225621).

RESULTS

Clinicopathology: Following administration of deisopropylnigaone, all the mice appeared ill evidenced by depression most especially at higher dose rate of 268 mg kg⁻¹ b.wt. There was loss of appetite as animals huddled to corner of their cages away from feed cans. Mice dying 8-12 h post DIN intoxication suffered from respiratory distress. There was jerky, strong thoracoabdominal breathing in animals on sternal recumbency. The condition of surviving animals at lower doses started to improve 36 h post intoxication, evidenced by increased activities within their cages and return of appetite. They were euthanized 10 days post-exposure and autopsied. Figure 1a shows mouse on 150 mg kg⁻¹ b.wt. of DIN dying 48 h post exposure. The right kidney was markedly pale.

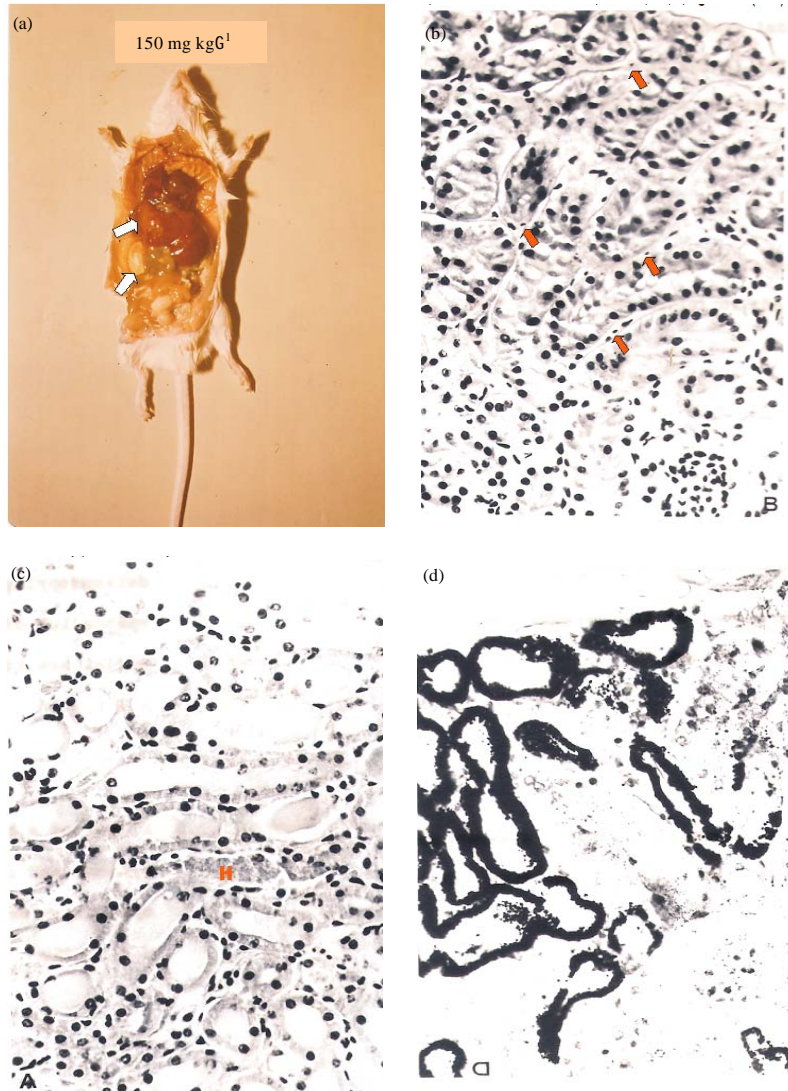


Fig. 1(a-d): Histopathological and gross lesions in the kidney of mice intoxicated by deisopropylglyoxane (DIN) showing, (a) Zonal mottling of liver and markedly pale kidney (arrow), (b) Nuclei of the tubular epithelium were pyknotic, (arrow) H and E x250, (c) Shrunken tubular epithelium and karyorrhexic nuclei, H: Hyaline cast deposition on the increase and (d) Von Kossa staining technique showing extensive calcification of necrotizing

Microscopic slides of haematoxylin eosin stained kidney revealed pyknosis of proximal tubular epithelium of the kidney (Fig. 1b). At higher doses such as $268 \text{ mg kg}^{-1} \text{ b.wt.}$, the tubular epithelial cells were shrunken and thinner and karyorrhexis of tubular cells ensued. Also at this dose rate, hyaline cast deposition within the tubular lumen was on the increase (Fig. 1c). Calcification of necrotizing tubular epithelial cells was demonstrated using Von Kossa staining technique (Fig. 1d).

DISCUSSION

The study confirmed the nephrotoxicity of mice intoxicated with deisopropylngaione. Mantle *et al.* (2010) administered in feed culture of *Penicillium polonicum* to rats and observed histopathological response in kidney involving apoptosis, abnormal mitosis and karyomegaly in proximal tubules. This suggests that *Penicillium polonicum* is carcinogenic. Whereas the kidney involvement is consistent with this study, in contrast, histopathologic slides of H and E stained kidney tissues revealed pyknosis of proximal tubular epithelium (Fig. 1b). In addition to that and at higher dose rate such as 260 mg kg⁻¹ b.wt., the tubular epithelial cells were shrunken and thinner and Karyorrhesis of tubular cells became evident. It is assumed that DIN may not be carcinogenic when compared to the observations made by Mantle *et al.* (2010), where renal proximal tubular distortion (enlarged nuclei in cells) became striking on account of Karyocytomegaly in rats fed culture of *P. polonicum*. A variety of pathogenic mechanisms play key role in nephrotoxicity caused by plant extracts, including glomerular disease, interstitial nephritis and direct cytotoxicity which may result in tubular epithelium cell death (Yohannes and Wolf, 2010). Studies in mice and rats on the toxicity and lesions caused by 2-substituted furans and thiophene, which produce renal injury similar to those caused by the 3-substituted furans, suggest that the lesions are due to metabolic activation to toxic intermediates by microsomal mixed-function oxidases present in the respective organs concerned (McMurtry and Mitchell, 1977; Seawright *et al.*, 1982). Microsomal cytochrome p-450 dependent mixed-function oxidases have been identified in the proximal renal tubules of sheep (Wattenberg *et al.*, 1968; Blackburne and Sutherland, 1972; Fowler *et al.*, 1977). Thus, the nephrotoxicity of DIN in mice could be associated with activation in the kidney by such an enzyme system. Comparatively, the kidney lesions seen in mercuric chloride intoxication in sheep were similar to those seen in mice intoxicated by DIN (Ogunsan *et al.*, 2007). Besides the pulmonary lesions seen in the lungs which is consistent with clinical observations and histopathology in DIN intoxication in sheep (Ogunsan *et al.*, 2003), the kidney lesions, typified by proximal tubular necrosis was remarkable in mice dying 24 h post exposure to DIN. Although toxic activation by the microsomal mixed-function oxygenase and 3-substituted furan compounds can be potential hazards in the diets of man and animals, they can be responsible for injuries in the lungs and kidneys. This is a factor which should be taken into account in drug design where furan moieties are part of the molecules.

CONCLUSION

An important finding of particular interest is that, besides the pulmonary lesions encountered in mice following intoxication with DIN, the kidney lesions were still consistent with previous studies of this compound in mice and similar to mercuric chloride poisoning in sheep. They include early stage tubular cell degenerations, proximal tubular necrosis, hyaline cast deposition and calcium salt infiltration into necrotizing tubular epithelium in Von Kossa stained histopathology slides.

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