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HISTOPATHOLOGICAL CHANGES IN VITAL ORGANS OF HOUSE RATS GIVEN LETHAL DOSE OF CHOLECALCIFEROL (VITAMIN D_3)

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ABSTRACT

House rats trapped from poultry farms of PAU, Ludhiana (Punjab) were orally administered with lethal dose (36mg/kg) of cholecalciferol (vitamin D_3). Histopathological examination after single oral intake of cholecalciferol exhibited toxicity in heart in the form of calcification in the myocardium and in tunica media layer of the coronary blood vessels along with the muscular necrosis. Marked congestion of alveolar capillaries, hemorrhages in the air spaces, edema, emphysema were the observations made in the lungs of cholecalciferol fed rats. Mineralisation was evident both on mucosal and serosal sides of stomach. In kidneys, there was mineralisation in cortex and medulla. In liver, hepatic cells showed degeneration, dilation of hepatic sinusoids and marked centrilobular necrosis due to cholecalciferol toxicity.

Key words: Cholecalciferol, Histopathology, House rats, Hypercalcaemia, Toxicity, Vitamin D_3

INTRODUCTION

The house rat, *Rattus rattus* (Linneaeus) commonly called as the roof rat is one of the most abundant, widely distributed and cosmopolitan commensal rodent species known throughout the world. It causes massive destruction of food stuffs and to the infrastructure (Krishnakumari *et al.* 1992). Besides causing huge loses, house rat has a potential for the transmission of a number of fatal diseases (Weber 1982). Rodenticides play a significant role in combating rodent pests both under agricultural and commensal situations (Buckle and Muller 2000). However, their use entails hazards to other mammalian life including man, pets and domestic animals (Watt *et al.* 2005). So there is a continuous need to identify new and more potent rodenticides with low risk of secondary poisoning and non-hazardous to non-target species, especially under commensal situations. Among the various rodenticides, cholecalciferol (vitamin D_3) has been considered as a safe rodenticide (Craigmill 1988 and Eason *et al.* 2000). It is a sub-acute rodenticide with a lethal dose of 36mg/kg body weight (Kaur *et al.* 2008) and has been found to be effective for the killing of rats (Saini and Parshad 1992 and Morrow 2001). After its intake rodents suffered from hypercalcemia i.e. raising of calcium level in blood plasma (Kocher *et al.* 2008), resulting in irreversible soft tissue minerlisation, thus causing death (Jolly *et al.* 1993 and Morrow 2001). Use of cholecalciferol as a rodenticide in bait lowered the risk of secondary poisoning and minimized the toxicity of non-target species (Eason *et al.* 2000). Therefore, keeping in mind the remarkable properties of cholecalciferol as a potent and safe rodenticide, the present study was undertaken to examine the histopathological changes in vital organs of house rats (the predominant commensal rodent pest species) after ingestion of lethal dose of cholecalciferol.

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MATERIAL AND METHODS

House rats used in this study were trapped live from poultry farms of Punjab Agricultural University, Ludhiana, Punjab. The rats were acclimatized individually in laboratory cages for at least 15 days by providing them food and water *ad libitum.* Adult and healthy rats (both male and female rats having body weight more than 100 gm) were selected and weighed. Cholecalciferol (vitamin D_3) was purchased from standard sources (Ranbaxy Fine Chemicals Ltd., Across Organics). Lethal dose of vitamin D_3 i.e. 36 mg/kg body weight (Kaur *et al.* 2008) was prepared by dissolving it in vegetable oil and its single dose was orally administered to ten overnight food deprived rats. Ten rats were kept as control (untreated) also. Mortality of rats (in terms of days) after oral intake of lethal dose was recorded.

Rats died (within 6-12 days) after ingestion of lethal dose of cholecalciferol and untreated rats were necropsied and the vital organs like heart, lung, stomach, kidney and liver were collected. These organs after complete fixation in 10% formalin solution were washed in distilled water, dehydrated in graded series of ethanol, cleared in xylene and embedded in paraffin wax with melting point between 58-60^{0}C. The sections were cut at 5um thickness on a microtome and after usual dewaxing and rehydration in descending ethanol series to water, the sections were stained in haematoxylin, counter stained with eosin, dehydrated in ascending ethanol series, cleared in xylene and mounted in DPX (Luna 1968). Haematoxylin-eosin stained slides were studied under an optical microscope (Olympus microscope) for evaluating the histopathological changes caused due to lethal dose of cholecalciferol in different tissues of treated house rats.

RESULTS AND DISCUSSION

Clinical signs appeared in the form of sluggishness of house rats and softness of bones just after one day of oral administration of lethal dose (36 mg/kg body weight) of cholecalciferol. All the rats died within 6-12 days after oral intake of lethal dose of vitamin D_3, supporting the fact of its delayed toxicity like other sub-acute rodenticides (Buckle and Muller 2000).

Histopathological study of heart of cholecalciferol toxic rats, exhibited varying stages of mild to severe degenerative necrosis of the heart muscles. Calcification was observed in the myocardial muscles which had undergone degeneration. Besides these changes there was evidence of mild focal infiltration of lymphomononuclear cells in the myocardium (Fig.1A). Post-mortem examination of possums poisoned with bait containing cholecalciferol have also shown the widespread mineralisation of cardiac muscle fibres, myocardium and of blood vessel walls in heart (Beasley *et al.* 1997).

Lung parenchyma exhibited varying degree of mineralisation ranging from mild to diffuse deposits and marked congestion of alveolar capillaries and hemorrhages in the air spaces (Fig.2A). Calcification of alveolar septa, bronchial sub-mucosa and wall of arteries has also been observed in the pigs having cholecalciferol toxicity (Chineme *et al.* 1976).A marked diffuse mineralisation was observed on the serosal side (Fig. 3A) as well as in the gastric mucosa of stomach (Fig.3A) of cholecalciferol toxic house rats. At some locations, a mild lymphomononuclear cell infiltration in the subepithelial layer of the stomach was found.

Marked glomerular congestion along with the degenerative changes of varying severity in the renal tubular epithelium and evidence of hemorrhage in intestitium was also observed. In the central regions of the renal tubules, casts were present, whereas towards the medullary region marked diffuse mineralisation was observed (Fig.4A). In case of vitamin D_3 toxicity in sheep, medulla region of collecting tubules has also been observed to be the major mineralisation site and the cortex as a minor mineralisation site (Simesen *et al.* 1978). Liver sections of vitamin D_3 toxic rats revealed congestion, hemorrhages and degeneration of hepatic cell (Fig.5A). Hepatic cells showed degeneration along with the evidence of vacuolar and albuminous

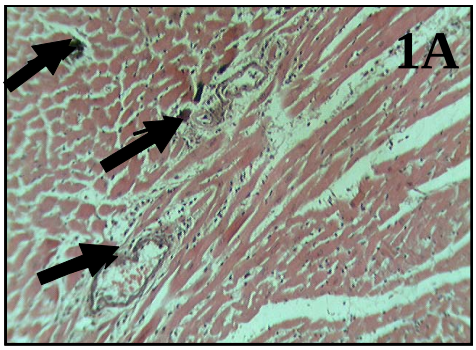

Fig 1A : Section of heart of house rat showing areas of mineralisation in myocardium (arrow) after ingestion of lethal dose of cholecalciferol. H & E X 150.

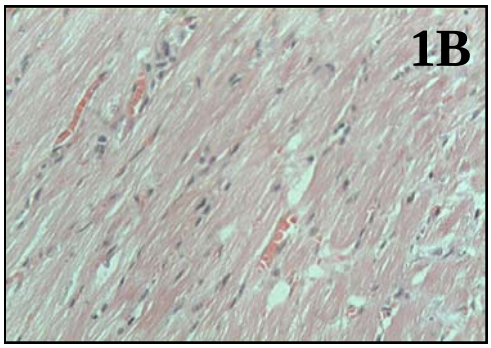

Fig 1B : Section of heart of house rat showing normal histology. H & E X 150.

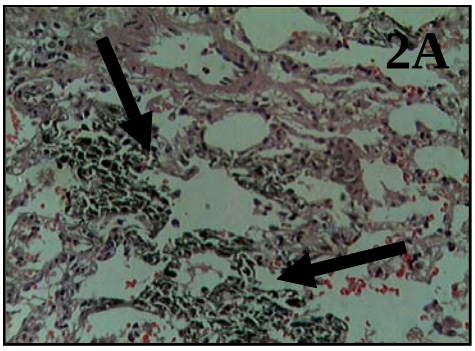

Fig 2A : Section of lung of house rat showing mineralisation (arrow) after ingestion of lethal dose of cholecalciferol. H & E X 150.

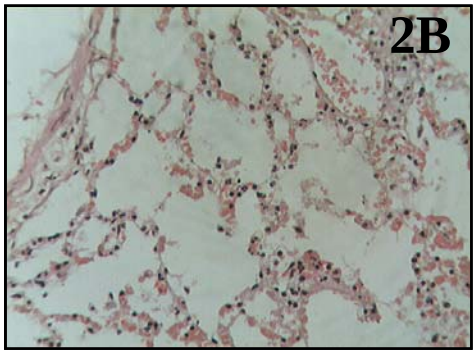

Fig 2B : Section of lung of house rat showing normal histology. H & E X 150.

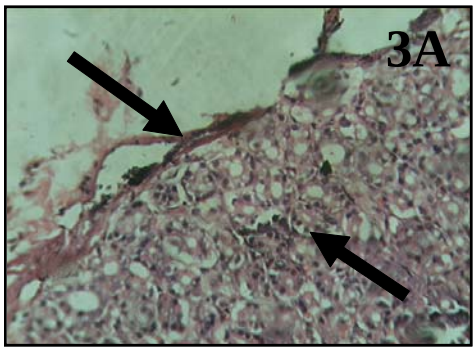

Fig 3A : Section of stomach of house rat showing mineralization (arrow) after ingestion of lethal dose of cholecalciferol. H & E X 150.

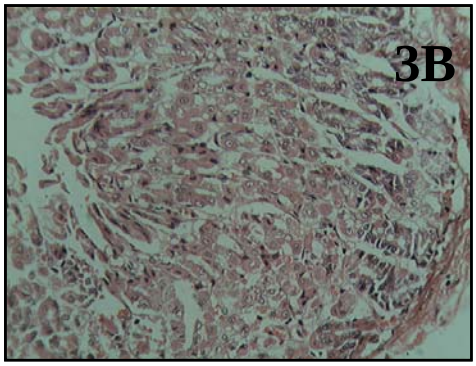

Fig 3B : Section of stomach of house rat showing normal histology. H & E X 150.

degeneration, dilation of hepatic sinusoids and marked centrilobular necrosis. Mineralisation of crystals of calcium salts has also been found in the liver sections of vitamin D_3 toxic animals (Hilbe *et al.* 2000). Control rats (not given treatment with collectciferal Fig. 5A showed normal historogy of all the above mentioned tissues (Fig. 1B, 2B, 3B, 4B, 5B)

From the results of this study it can be concluded that oral intake of lethal dose of cholecalciferol (vitamin D_3) by house rats led to their toxicity in the form of mineralisation in the vital organs and resulted in the mortality of rats. Hence vitamin D_3 can be used as a safe rodenticide for the control of house rats, especially under commensal

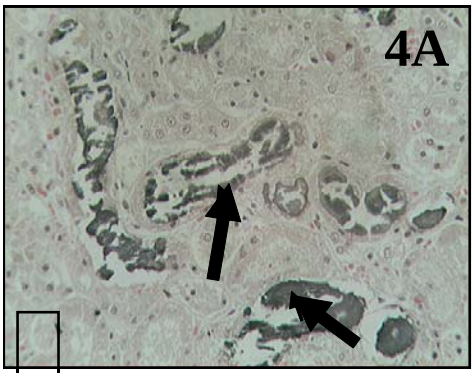

Fig 4A : Section of kidney of house rat showing marked diffuse mineralisation in tubules (arrow) after ingestion of lethal dose of cholecalciferol. H & E X 150.

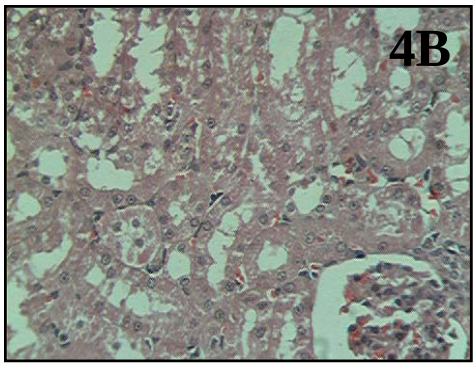

Fig 4B : Section of kidney of house rat showing normal histology. H & E X 150.

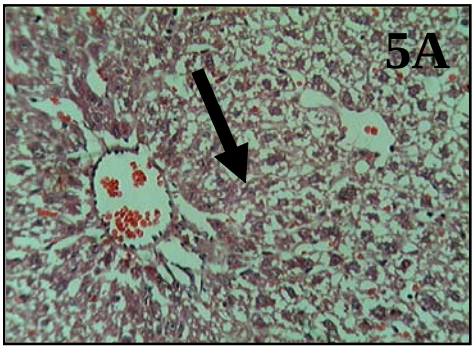

Fig 5A : Section of liver of house rat showing degeneration of hepatic cells (arrow) after ingestion of lethal dose of cholecalciferol. H & E X 150.

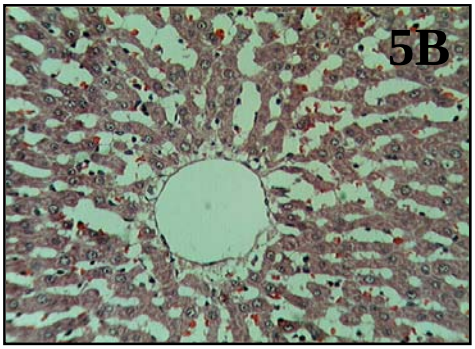

Fig 5B : Section of liver of house rat showing normal histology. H & E X 150.

situations because of its potent rodenticidal characteristics and target specific killing property.

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