J. Adv. Zool. 2018: 39(1): 06-12 **ISSN-0253-7214**

HISTOPATHOLOGICAL EXPRESSION OF DIFETHIALONE INTOXICATION IN THE LIVER OF INDIAN DESERT GERBILS, *MERIONES HURRIANAE*

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ABSTRACT: Difethialone is a potent second generation anticoagulant rodenticide. The aim of this study is to investigate Difethialone induced histopathological changes in the liver of Indian desert gerbils, *Meriones hurrianae*. Irreversible pathologic changes of the hepatic tissues were noticed in the gerbils when they were subjected to 24 hours exposure of acute median lethal dose of Difethialone, administered orally. The alterations in liver tissue of treated gerbils were recorded at intervals of 2, 4, 6 and 8 days and were photographed. Congestion of the central vein, lymphocytic infiltration and atrophy of the hepatic cells was observed and the severity of the damage inflicted on the liver was time dependent. The findings can serve to estimate the time and cost efficiency of pest control through Difethialone.

KEYWORDS: Difethialone, Hepatic, *Meriones hurrianae*, Second generation anticoagulant rodenticide.

INTROUCTION

The liver performs numerous functions and is the site of metabolism of all nutritional substances like carbohydrates, lipids, proteins, vitamins and minerals. It is the site of detoxification of various toxic substances either produced in the body or taken along with food. Different biochemical changes occur by which toxic substances are rapidly made excretable. Toxic drugs and substances are eliminated by the action of liver enzymes. Liver is instrumental in synthesizing an enzyme VKOR (Vitamin K epoxy reductase), that can recycle vitamin K, which is necessary for making blood clotting agents in blood. Extra supply of these clotting agents is stored in the body and the target animals show the toxicity effects in some days, only after this reserve is exhausted. Second generation anticoagulant rodenticides are potent, single dose poisons that are retained by the animal tissues and are more persistent, especially in liver [6] and can cause kill of rodent pests in a short time [5]. These anticoagulant rodenticides inhibit enzymes necessary in the recycling of vitamin K, producing impaired coagulation mechanism [2, 12].

Second generation anticoagulant rodenticides play an important role in the control of rodents which are resistant to first generation anticoagulants [3]. Difethialone is one such SGAR and a hydroxycoumarin derivative. It acts as a

Vitamin K antagonist and inhibits VKOR activity, thus interfering with the synthesis of blood coagulation proteins (factors I, II, VII, IX and X) in the liver and preventing recycling of Vitamin K.

Mortalities from Difethialone toxicosis was observed in 100 % gerbils. Clinical symptoms of toxicity are manifested in hemorrhages, internal as well as external bleeding from the eye, ears and claws, anaemia, general weakness. Anorexia is seen in all the treated gerbils.

MATERIALS AND METHODS

Test species

The Indian desert gerbil, *Meriones hurrianae*, was taken as the test species. It is the major pest in the arid regions of Rajasthan and occurs in kharif crop fields. Healthy adult gerbils of both the sexes, between average body weights of 80-120 grams were collected, acclimatized to the laboratory conditions for 10 days before conducting any experimental trials. The gerbils were kept in polypropylene cages of the size 435 x 290 x 160 mm and maintained on a palatable diet. 30 adult gerbils of combined sexes were used for the trial and the experiment was replicated thrice. Male and female gerbils were kept in separate cages to avoid breeding. Liver tissue was studied for the histopathological changes post treatment of gerbils with acute median lethal dose. The toxicity effects were recorded at autopsy interval 2, 4, 6 and 8 days respectively. The LD_{50} of Difethialone is 0.28 mg/kg body wt. for Indian desert gerbils, *Meriones hurrianae* [11].

Route of exposure

Oral forced feeding of the single dose of acute median lethal dose of Difethialone was done with the help of a stomach gavage needle. The vehicle control experiment was carried out with the poison free carrier, propylene glycol.

Histopathological Studies

The liver of all the control and treated group of animals was removed and stripped clean of excessive tissues. It was washed thoroughly in water and was kept for 24 hours in Bouin's fluid for fixation. The tissue was then washed in water to remove excess fixative and passed through graded series of alcohol for dehydration and then in xylene for clearing. Paraffin blocks were prepared by embedding the tissue in wax. The sections were cut at 5 microns and double staining was done with Ehrlich's haematoxylin and eosin for histological investigation as described by Wilson and Gamble [13].

RESULTS AND DISCUSSION

The liver is an essential organ of the body. It is a glandular structure, both secretory and excretory in nature. The hepatic parenchyma is composed of innumerable small lobules somewhat

pyramidal hexahedron in shape. The lobules are composed of a large number of hepatocytes arranged in long hepatic cords (Fig. 1). The hepatic cells are polyhedral with large, round, centrally located nuclei. The cytoplasm is granulated, vacuolated. Hepatocytes are mononucleate but binucleate hepatocytes are also visible. The sinusoids form a radial network that allows blood to come into contact with every parenchymal hepatic cell. The lining of the sinusoids is formed by the endothelial cells and phagocytic cells of the reticulo-endothelial system called Kupffer cells. The bile capillaries are located between the adjoining faces of the hepatic cells. Bile is formed in minute vacuoles in the hepatic cells which is discharged through five intercellular canaliculi into the bile capillaries. The bile flows from the center towards the periphery.

Oral forced feeding of Difethialone (LD_{50} - 0.28 mg/kg body wt.) resulted in several pathological changes in the liver of gerbils.

2nd day post Difethialone treatment

Histopathological alterations revealed early toxicity with benign degenerative changes. Dilated sinusoids with centrilobular congestion and mild degeneration of the endothelial lining of portal vein were observed (Fig. 2).

Hepatic cells and their nuclei were enlarged showing vacuolization (Fig. 6).

4th day post Difethialone treatment

More pronounced changes were observed on the 4th day of intoxication. The hepatocytes become much swollen. Degeneration of the cytoplasm along with its movement to the periphery was seen. This was due to the appearance of several vacuoles formed by fatty degeneration. Lymphocytic infiltration was seen. Darkly stained pycnotic nuclei were observed (Fig. 3). The endothelium lining of the portal vein ruptured and was found invading the lumen of the portal vein. Dilation of sinusoids was seen with zonal necrosis of hepatic cells (Fig. 7).

6th day post Difethialone treatment

Necrosis of the liver cells was most frequently visible on the 6th day. Hepatocytes were cloudy in appearance due to vacuolization and pycnosis of the hepatic nuclei was seen in some cells (Fig. 4). Marked lymphocytic infiltration was observed near the portal vein. Kupffer cells were also seen (Fig. 8). Binucleate cells were observed at some places.

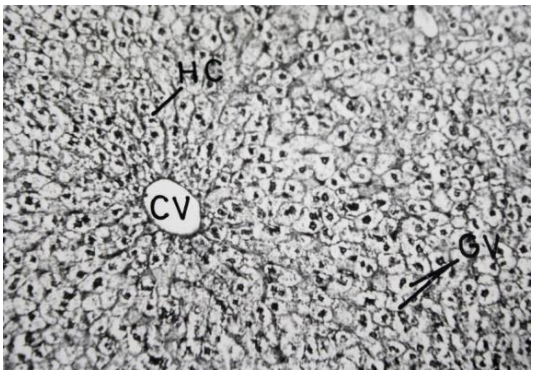

Fig.1. Photograph showing normal structure of liver composed of hepatocytes arranged in hepatic cords (HC), granulated and vacuolated cytoplasm (GV), of hepatocytes, central vein (CV) and sinusoids. H & E x 200.

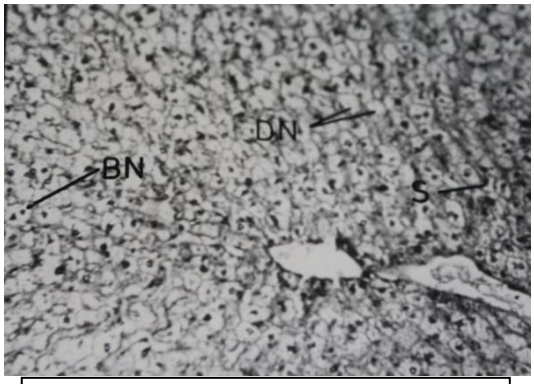

Fig.2. Liver of gerbil, *M. hurrianae*, 2nd day after single exposure of Difethialone (0.28 mg/kg b.wt.): Photograph showing binucleate cell (BN), dilated sinusoids (S). Mild degeneration of the endothelial lining of the portal vein is also visible. H & E x 200.

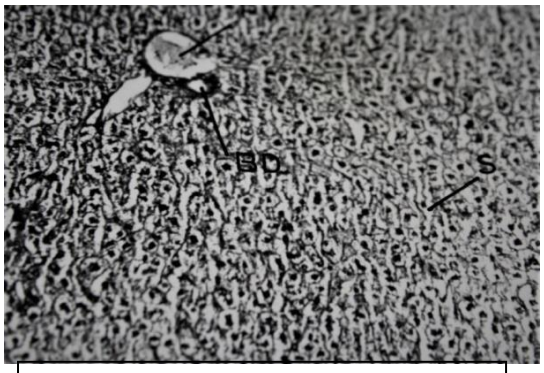

Fig.3. Liver of gerbil, *M. hurrianae*, 4th day after single exposure of Difethialone (0.28 mg/kg b.wt.): Slide showing ruptured endothelium of the portal vein invading its lumen (PV), bile duct (BD) and widening of the sinusoidal space (S). H & E x 200.

Fig.4. Liver of gerbil, *M. hurrianae*, 6th day after single exposure of Difethialone (0.28 mg/kg b.wt.): Slide showing various cytoplasmic vacuoles (V), pycnosis of the hepatic nuclei and necrosis (N) of some hepatic cells. H & E x 200.

8th day post treatment

Severe degenerative changes were observed after 8th day of treatment. Architecture of the liver showed degenerative changes. Karyolysis and karyorrhexis of the nuclei were observed. The nuclei become irregular in shape and have degenerated completely in some of the cells (Fig. 5). There was considerable amount of lymphocytic infiltrate and hyperplasia of cells was common. Cytoplasm becomes granular and atrophy of the cell membrane was seen (Fig. 9).

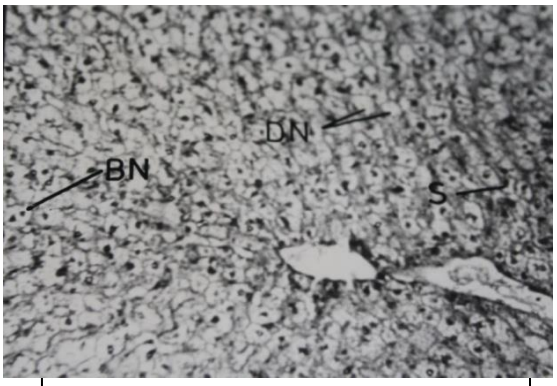

Fig.5. Liver of gerbil, *M. hurrianae*, 8th day after single exposure of Difethialone (0.28 mg/kg b.wt.): Photograph showing karyolysis, karyorrhexis, binucleate cells (BN), dilated sinusoids (S) and degenerated nuclei in many cells. H & E x 200.

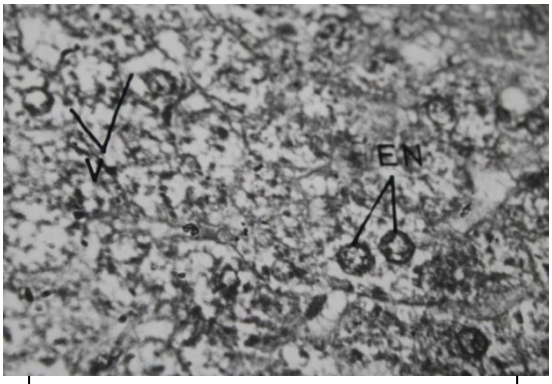

Fig.6.Enlarged hepatocytes showing vacuolization (V) and enlarged nuclei (EN), 2nd day after Difethialone treatment. H & E x 400.

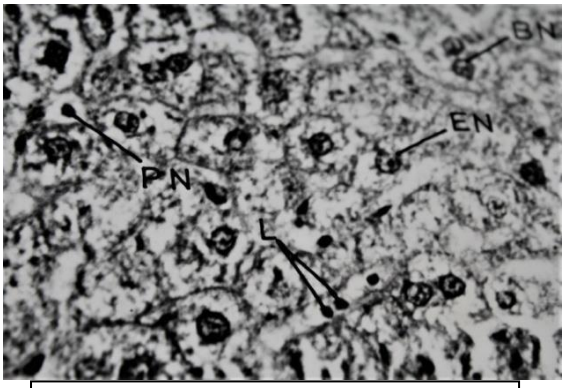

Fig.7. Photomicrograph showing binucleate cell (BN), enlarged nuclei (EN) of the hepatocytes, darkly stained pycnotic nuclei and lymphocytic infiltration, 4th day post Difethialone exposure. H & E x 400.

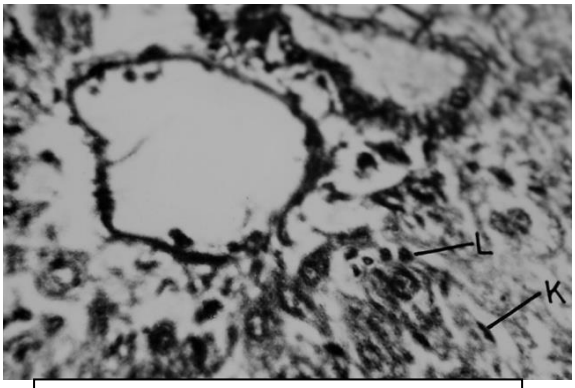

Fig.8. Photomicrograph showing lymphocytic infiltration (L) near the portal vein. Kupffer cells (K) are distinctly visible, 6th day post Difethialone exposure. H & E x 400.

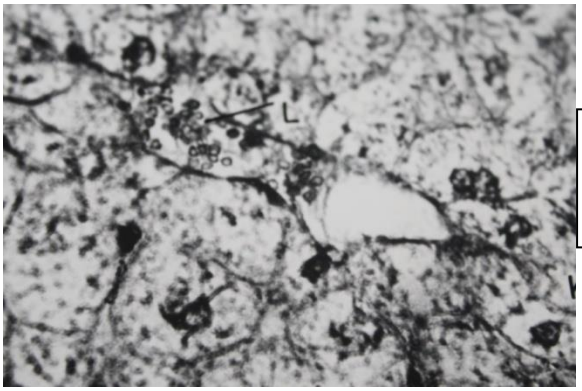

Fig.9. Liver architecture shows severe degenerative changes 8th day after Difethialone exposure. Considerable infiltration of lymphocytes (L) and hyperplasia of cells with granular cytoplasm can be observed. H & E x 400.

The liver is a large, functionally heterogeneous and complex organ concerned with biotransformation of alien bodies. The biochemical and pathological changes caused by various toxins affect the liver as the hepatic cells are the major sites where the breakdown of toxic substances into their metabolites takes place. Several drugs and toxicants induce damage to the liver on administration of calculated single dose to the gerbils. The extent of damage caused depends on the toxicant used and its exposure period. The location of detoxifying enzymes in the liver makes hepatocytes a target for chemical and drug related injury. Mild effects may be diagnosed by a rise in the serum enzymes. Severe injury leads to massive necrosis and death.

The administration of acute median lethal dose (LD_{50}) of Difethialone to *Meriones hurrianae* for an exposure period of 24 hours leads to hepatic injury and a direct toxic reaction on the liver of experimented animals that is dose related and fairly prompt. Initially after 2 days of intoxication, a variety of lesions were observed in the hepatic cells including cell ballooning and enlarged nuclei, vacuolization, disruption of the endothelial lining of sinusoids and central vein. Similar results have been reported with Bromadiolone and Chlorophacinone induced hepatotoxicity in albino rats [8, 9]. Dilated sinusoids with kupffer cells and centrilobular congestion were noticed followed by degeneration of the cytoplasm along with its peripheral movement, fatty degeneration and lymphocytic infiltration of the hepatic tissue. This may be due to the inflammatory lymphoid and reticuloendothelial response to the rodenticide. The abundance of leucocytes, particularly lymphocytes in body tissues facing any injurious impacts has been reported by El-Banhawy et al. [4], on the liver of rats treated with Brodifacoum. Bromadiolone, a second generation anticoagulant, when tested on *Mus musculus* resulted in renal and hepatic necrosis [10].

In the later stages, dilatation of central veins and sinusoids was noticed with the atrophy and disappearance of liver cells. The toxin reaches the hepatic tissue by way of lymph and chylomicrons, and increase in the level of its active metabolite results in the dilatation of the sinusoids. On the 8th day, more severe hepatotoxic changes were recorded. Hyperplasia and pycnosis of the nuclei was observed. There was inflammation of the portal tracts and cell necrosis was seen. Severe necrosis leaves only a spongystromal network. The adult male Wistar albino rats treated with LD_{50} dose of SGAR Difenacoum and Bromadiolone show marked alteration of liver morphology [7]. Loss of hepatic lobules architecture, infiltration of inflammatory cells and other debris in different zones of hepatic lobules has been reported in the histology of liver of 0.25 and 0.5 mg/kg

Difenacoum treated rats, *Rattus norvegicus* [1]. The oral administration of rodenticides results in the accumulation of the toxins in the hepatocytes. These are metabolized to intermediates that form a covalent linkage to macromolecules that are necessary for vital function of the cell, ultimately leading to cell necrosis. Intoxication of Difethialone (LD_{50}) results in excess mobilization of fats and their accumulation in liver, usually in the center of the lobules. This interferes with the intracellular metabolism of lipids causing recognizable fatty changes and disfunctioning of the cell organelles.

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