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Histological Effects Of Insecticides On Liver & Kidney Of Economically Important Indian Teleosts (Cirrhinus Mrigala & Catla- Catla)

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Abstract

In modern year, the approval of new technologies in crop resistance has increased the off-take of broad spectrum synthetic insecticides used in public health practices have introduced a new category of pollutants. Sporadic numbers of large scale fish destroy and inequity in ecological cycle have caused extensive spread concern. The Organochlorine insecticides are chlorinated hydrocarbon (the compound which are made of carbon and hydrogen) to manage insects and insect-borne diseases. Therefore, common organochlorine insecticides including , dichloro-diphenyl-trichloroethane (DDT), damage to the liver, kidney, and other body organs.

Key words: Endosulphan ; DDT ; damage to body organ ; hydrocarbon.

INTRODUCTION :

The insecticides enters the aquatic environment by geological weathering industrial effluents mining effluents, domestic waste and agricultural runoff. Due to this water get polluted and produces deleterious effect on aquatic plants, animals, and micro-organisms. The life of aquatic organism has been affected by the presence of insecticides. The toxicity of insecticides on organism depends upon the nature. India has vast inland **resources** According to available estimates, the rivers and main tributaries cover a length of 27,359km, and irrigation canal, 1,12,654km. Large quantities of insecticides enter into these water bodies from the adjoining agricultural fields during rainy season. In a shorts span of two decades, the USA alone has produced over one billion pounds of insecticides. In India, the use of insecticides has increased greatly during the last two decades. The total ban on the use of some chlorinated insecticides by USA and Canada is a warning to us, although no sizeable incidents have occurred in India. Scientists of Indian Toxicological Research Centre have warned that problems caused by increased use of insecticides in this country must be thoroughly studied to avert ecological catastrophe. Some of these insecticides, widely used in India are quite persistent, accumulating in the tissues of aquatic animals, like fishes. They also leave residues in water and soil causing pollution. The edible parts of the fishes may accumulate endrin up to 214.28ppm. Apparently the consumption of resistant fish with

heavy insecticides residue in their bodies would be dangerous to all fish eaters including man. Next come industrial pollution, with the rapid increase in industrial development and urbanization the waste disposal problem also arises. The land water has to share the increasing load of waste products. The large scales fish kill in Yamuna river at Delhi (1965) has been attributed largely due to the pollution of river Yamuna by DDT, the people of Delhi showed high level of DDT concentration in their bodies. The effluents of fertilizers Corporation of India at Sindri contain chromates, alkalise, cyanides, phenols and ammonia etc. Several incidence of large scale fish mortality during dry months in Tilaiya and Panchet reservoirs have been reported due these pollutants

The toxicity of pesticides and industrial wastes to fish and fish-food organisms has received the attention of several workers (Anderson ,1960; Mathur, 1962 and soon . **In making direct approach to the solution, two basic studies are essential:**

1) The determination of the maximum concentration of various pesticides and wastes, which are not effective to aquatic organisms under continuous exposure.

2) The relationship between the amount of applied pesticides and the amount reaching into the water.

By the above methods it would be possible to specify the dosage of pesticides which can be applied in a specific area and also the amount of water to be mixed with industrial waste its discharge. The problem arising out of the increased use of pesticides in Indian and toxic waste disposal of industries must be thoroughly examined to avert the imbalance.

MATERIAL AND METHODS:

Following test animals and test solution were taken:

TEST ANIMALS :

FISHES: Fingelings of Catla- Catla and Cirrhinus mrigala , the two most economically important fresh water teleosts were taken for the study .

TEST SOLUTIONS :

INSECTICIDES : Insecticides belonging to the following three groups were taken for the study :

(a) **Chlorinated insecticides:** DDT , Endosulphan (Thiodan)

(b) **Synthetic pyrethroid :** Decamethrin

(c) **Oganophosphorus insecticides :** phosphamidon (dimecron)

COLLECTION OF TEST FISHES: Fingerlings of Catla-Catla and Cirrhinus mrigala (Av. Size 7 cm and Av .Wt. 7 gm) were collected from the nursery ponds of States fisheries Department , situated at Kalyanpur , Kanpur . Fishes were brought in oxygen filled plastic bags and were kept in outdoor plastic pools of 320L. Water capacity for acclimation . Fishes were then transferred in indoor plastic pool of same capacity for seven days prior to experiment for proper acclimation. Artificial feed consisting of rice bran and groundnut oil cake was given to the test animals . Zooplankton were also given on alternate days as feed . The fishes were starved for 24h. before the commencement of the experiment.

EXPERIMENTAL PROCEDURE : 24 h , short term bioassay experiment were conducted in glass aquaria containing 20L of dechlorinated tap water . The fishes were then exposed to different concentration of insecticides to evaluate the toxicity (median tolerance limits or LC50 value). There were three replicates foe each concentration and ten fishes per replicate together with one control set. Observation was recorded during the course of experiment upto24h.

BIOASSAY OF INSECTICIDES WITH FISH-FOOD ORGANISMS

Collection of test Organisms: The fish-food organisms, Daphnis magns, Cyciops viridis and Chironomous Larvae were collected from the nearby ponds and brought to the laboratory. They were kept in glass jars and fed with dried yeast powder . The experimental animals were kept for acclimation without food for 24h. prior to the experiment.

Experimental procedure: 24h. short term bioassay experimental were conducted in large sized patridishes containing 200ml of dechlorinated tap water . The animals were exposed to the different concentrations of insecticides to evaluate the LC50 values. There were three replicates for each concentration and twenty test animals per replicates together with one control. Salient observations were recorded.

HISTOLOGICAL STUDIES

Histological studies of fishes with insecticides :

Experimental procedures: Test animal were collected in the manner described earlier. Emusifiable concentrates of insecticides were taken. Sub – lethal dosage 0.01ppm (the dosage at which fish can survive at least for 15 days) of all insecticides were prepared by dilution method . Experiment was conducted in glass aquaria of 25 L. capacity containing declorinated tap water. Five fingerlings (Av. Length 7cm Av Wt 7.0 gm) were released in each aquarium having sub-lethal dosage of insecticide. Experiments were conducted for a period of 15 days . There was one control set. After the completion of experiment, the fishes were taken out. Experimental fishes were dissected for liver, Kidney. The organ was fixed in aqueous Bouin's fixative for 16-25h. After fixation, the materials were washed in running water and then transferred to

distilled water. Materials were dehydrated in ascending series of alcohol , cleared in xylene and transferred to paraffin embedding bath. Blocks were prepared and sectioned at the thickness of 6-8u. Slides were dewaxed, dehydrated and stained in Haematoxylin –Eosin stain. After staining, the slides were mounted in Canada balsam. Observations were recorded. Photomicrographs were taken for evaluation of results.

Histological studies of fish with industrial wastes

Experimental procedure: Animals were collected in the manner described earlier. Test concentration was prepared on percent by volume basis. 100%, 10% and 1% concentration of deferent industrial waste were taken. The different tissues were taken out and processed in the manner described earlier. The tests were evaluated.

OBSERVATIONS

Effect of insecticides: During the experiments the fishes exhibited peculiar rectionsafter 12hrs. of exposure . At first , the fishes surfaced for 10-15 min , then slowly became lethargic and settled at the bottom . Endosulphan , a chlorinated insecticides was found to be most toxic to the above fishes, followed by Decamethrin> DDT> Phosphamidon .

Histological studies on Liver of C.mrigala: It is a trilobed structure and has a dirty brown colour . The two marginal lobes are long and lie on the ventral side of the alimentary canal. They are thick proximally but thin distally. The two lobes are united proximally and fused portion lies across the oesophagus ventrally. The median lobe lies dorsal to the intestinal bulb and is joined to the marginal lobes laterally and also at the junction of the two marginal lobes. The gall bladder is a prominent structure in this fishes and is moss green in colour when in is filled with bile. It is thin walled and bag like and lies over the marginal lobe of the hepatopancreas. A single duct, from the middle lobe joins the gall bladder prior to its termination into the intestinal bulb. A pair of ducts arising from the right marginal lobe, run across the gall bladder, unites and immediately opens into it. A transverse of the liver shows that's the cells are large, many sided and posses prominent nuclei. The hepatic ducts are numerous and distinct and appear to have zigzag course. There are sinusoid spaces in the liver tissue. Apparently this fish has no pancreas but lies embedded in the liver, thus in this fish the liver is not a single organ but is composed of liver and pancreas, and is therefore called the heptopancreas. The pancreatic tissue is scattered in the midst of the liver cells as lobules. The lobules may be isolated or found in clusters. The cells forming the lobules are lobules are large and distinct. In addition to their being chromophobic , the cells contain in their cytoplasm , masses or highly stained "Colloid".

KIDNEY: It is the renal excretory organ and extended dorsally behind the branchial region in the pericardial cavity upto the caudal end of the coelom. Each kidney is deep red in colour in the living condition and is covered over by thick whitish membrane. The

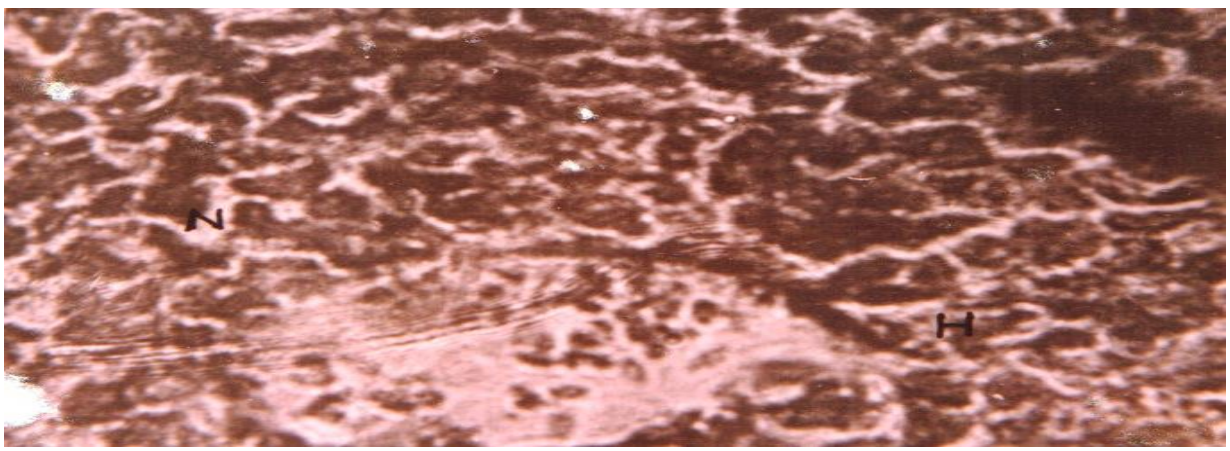
portion lying in the pericardial cavity is derived from pronephros and is called head kidney. It consists mostly of lymphoid tissues with few stray tubules scattered in the midst. The tissues lie closely adhered to the posterior cardial veins. It is a small ovoid mass connected to the main kidney in the coelomic cavity by a narrow strip of tissue running through the transverse membrane . The coelomic portion of the kidney is mesonephrons. Anterioly, the two halves of the kidney are free and lie lateral to the vertebral column. A little behind , the two halves unite, become thickened and transversely expanded . Behind the expanded region, the two halves again separate, becoming thin at the caudal end. The functional unit of the kidney is the nephron.It has at its origin, a double layered cup-like structure called the glomerulus,derived from a branch of the renal artery. Emerging from the nephron is a venule. The capsule narrows posterioly into a neck segment, a proximal and a distal convoluted tubules which terminate in the initial collecting tubules. Ultimately these discharge their content into Wolffian ducts. The entire nephron in lined by a single layered epithelium, the cells of which are large and contain prominent nuclei. In C.mrigala , only the middle portion of the kidney is functional , having distinct glomeruli. Thus the kidney tends to be divided into an anterior, middle and posterior kidney. The nephron are loosely arranged.

Treated fishes: The histological changes observed in C.mrigala in the liver, kidney by different insecticides are as below:

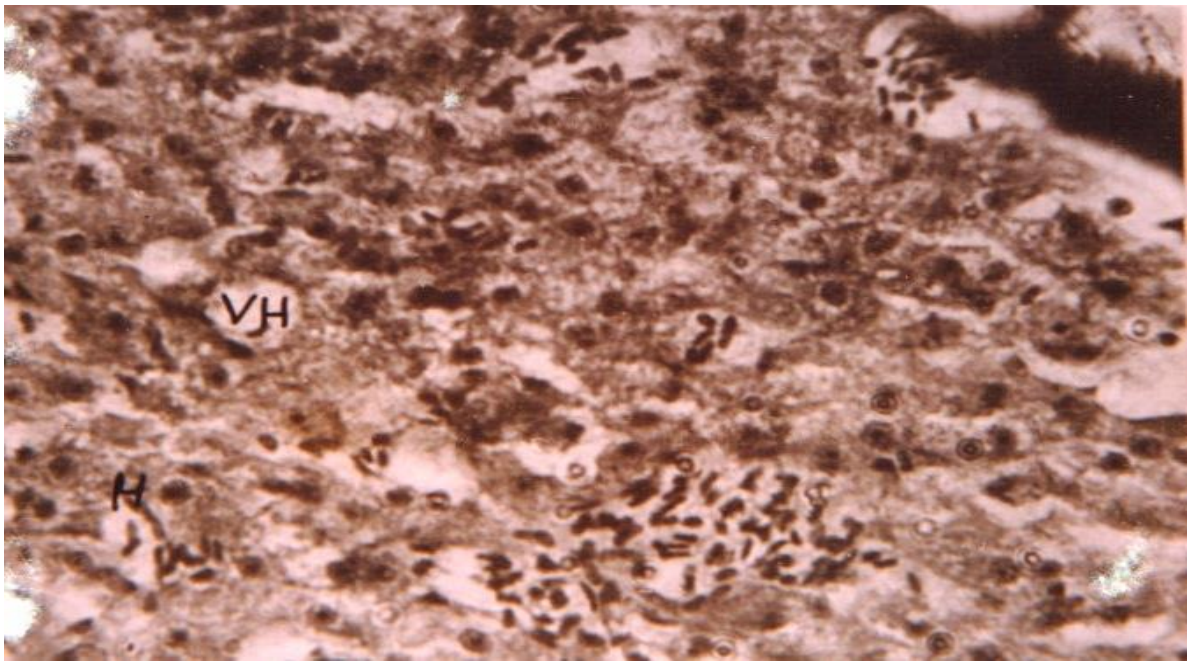
LIVER: The following changes were observed in the liver:

DDT: Severe histological changes were induced by DDT in the liver of C.mrigala. Cytoplasm of the cells disappeared and due to its absence, cells became hollow. Much accumulation of stain in peripheral cells as compared with the central ones was observed. The peripheral cells were more dense. The polygonal shape of the cells was deformed. Due to this deformity the entire liver cells looked like complicated mass at some places. The hepatic cells showed marked degeneration. At places necrosis was marked. Disruption of sinusoid was noticed and at some places it was difficult to mark cell boundaries.

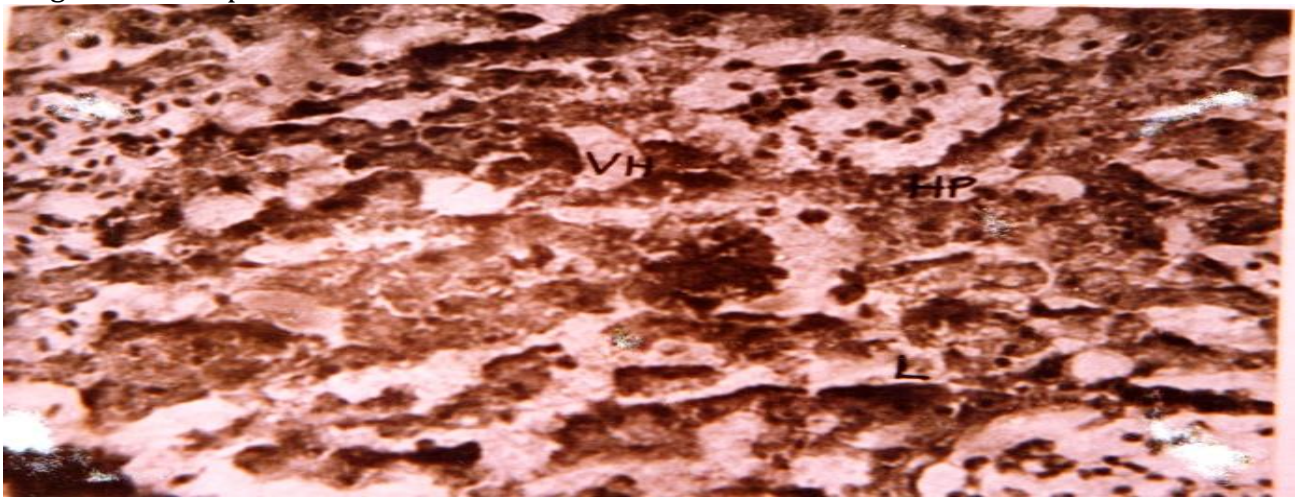
Endosulphan: Hepatic cells appeared highly vacuolated and showed cloudy swelling. Deformity and hypertrophy of the hepatic cells and nuclei along with disruption of sinusoids was observed at some places. Necrosis was more pronounced in the peripheral cells than the central cells.



Decamethrin: The liver of the exposed fish showed severe histological changes. Hypertrophy of the hepatic cells, necrosis and margination of the cells were observed. The liver damage was indicated by a scattered distribution of parenchymatous cells. The principal lesion was the vacuolar degeneration of the cytoplasm of the cells. In severely affected liver, the centre of the calls appeared empty. The peripheral cells were more dense and deeply stained and showed displacement of the nucleus from its original central position

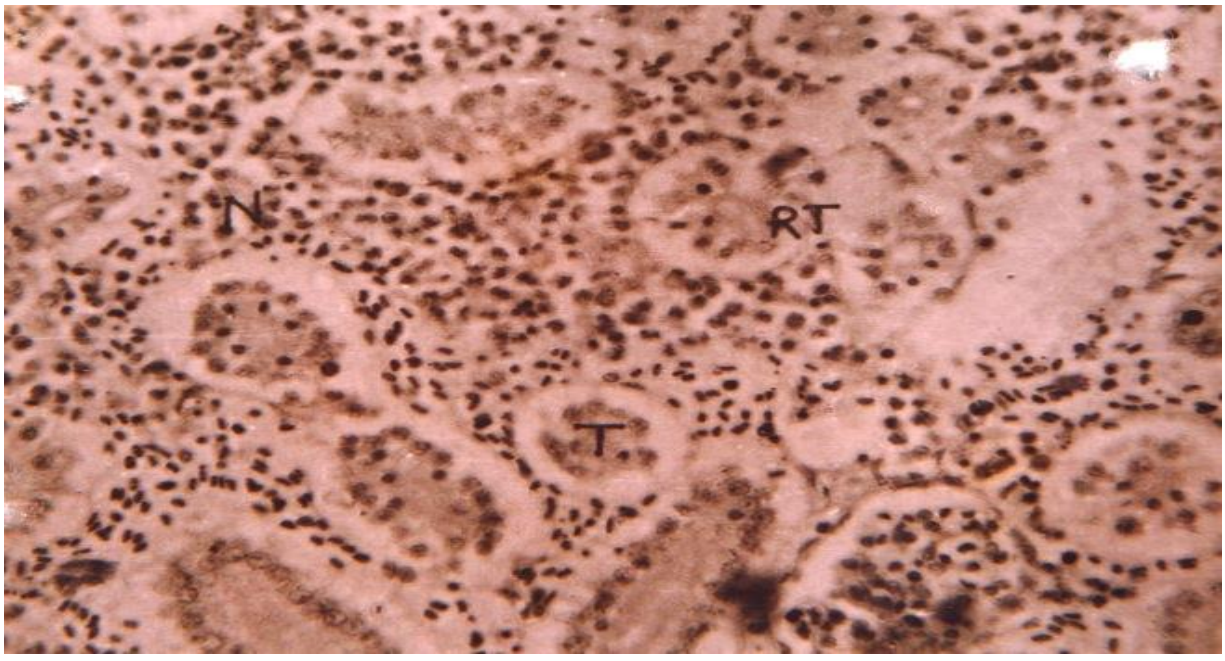


Phosphamidon : The cells were scattered and few central cells were slightly atrophied . Changes in parenchymatous cells consisted of margination and hypertrophy. Localized necrosis of cells was also noticed. The lesions were more in the central than in the peripheral cells. The central cells stained less deeply than the peripheral ones. Clumping of cells was noticed at few places. There was a slight displacement of nucleus from its original central place.



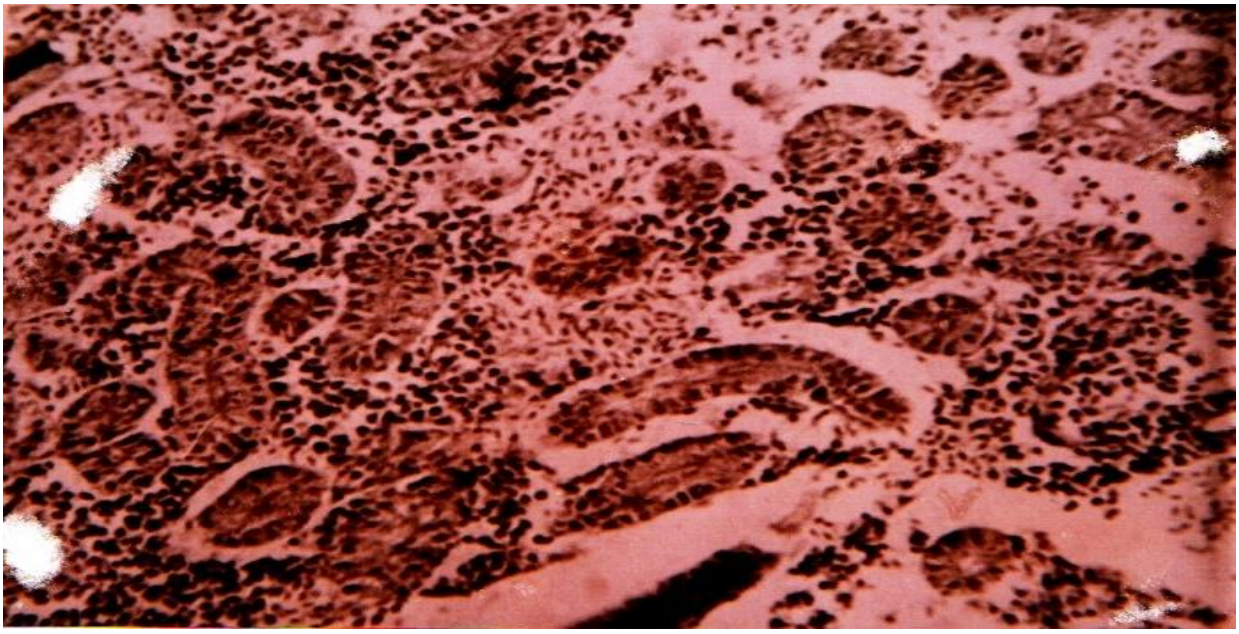
KIDNEY : The following histological changes were observed :**DDT:** The DDT induced pathology was severe in case of test fish C.mrigala. The cellular lining of the tubules cells and connective tissue were disorganized. Glomeruli were clumped at some places with severe rupture. The Bowmen's capsule were swollen.

Endosulphan: The changes in tubules were mild. The tubular epithelium showed vacuolation and some of the tubules appeared shrunken with irregular nuclei. At some places renal tubules appeared widely separated from one another due to marked increase of interstitial haemopoietic tissue (Hyperplasia). Glomeruli were swollen, deformed and at some places showed bursting.



Decamethrin : The histological changes observed in the Decamethrin treated kidney showed epithelial cells of the tubules disquamated and vacuolated . At some places renal tubules cells nuclei appeared like hyaline dots. Glomeruli undergo shrinkage and degeneration and the cells of Bowman's capsule were swollen. At some places the expanded glomeruli and their epithelial cells showed marked vacuolation

Phosphamidon : The cells of the collecting tubules were extremely vacuolated and swollen and their cytoplasm and nuclei together formed a dark coloured round mass. The cells of the proximal tubules were shrunken causing their lumen to appear enlarged. At some places bursting of the tubules was observed.



Histopathological changes in liver: Insecticides and industrial wastes produced significant histological changes in liver. The changes have been extensively studies on this organ by many workers. The liver is most suspectible to insecticides and chemicals and also most consistently damaged organ. The literature indicates that the earlier workers have been used different insecticides in lethal dosages on different fish and fish-food organism and their findings differ to a great extent in different cases. However, out of histological changes reported , some of them occur more consistently than others. The changes are in the following order: necrosis, vacuolation , hepatic cord disarray, swelling of hepatocytes, binucleated or multinucleated cells , increased cytoplasmic granularity , pyknosis of nuclei, clumping of parenchyma and cellular shrinkage.

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