Histological changes induced by cadmium ion in the Gills, Liver and Intestine of juvenile *Carassius carassius* (L.)

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Abstract

Evalutions of histopathological lesions in gill , liver and intestine tissue were carred out in juveniles of fish *C.carassius* following 8 dayes of exposure to (o.2&o.5)ppm of cadmium. This study was showed on gills (1) fusion of adjacent lamellar epithelium (2) sepration of epithelial layer (3) epithelial necrosis (4) loos in regular shape (5) hyperplasia of secondary lamellar epithelium . But in liver recorded (1) haemorrahgein liver sinuses (2) hypertrophy with hepatic cells (3)diploid nucleus (4) necrosis in hepatic cells (5) deposite material . While in intestine observed (1) shortining villi (2) inflammation response with degeneration in mucusa and sub mucusa .

التغيرات النسيجية الناتجة عن تاثير ايون الكادميوم في غلاصم وكبد وامعاء يافعات اسماك كارب الكراسين Carassius carassius (L.)

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الخلاصة

قدر اضرار الانسجة المرضية في نسيج الغلاصم والكبد والامعاء التي نقلت من يافعات اسماك كارب الكراسين المعرضة لمدة 8 ايام الى تركيزي (0.2 و 0.5) جزء بالمليون من عنصر الكادميوم . وقد لوحظت التاثيرات التالية على الغلاصم (1)اندماج الصفائح الغلصمية الثانوية (2)انفصال الطبقة الطلائية(3)نخر للخلايا الطلائية (4)فقدان في الشكل النظامي (5)فرط التنسج في الصفائح الغلصمية الثانوية. اما في الكبد فكانت التغيرات: (1) نزف في الجيبانيات الكبدية (2) فرط التنسج في الخلايا الكبدية (3) زيادة في اعداد الانوية (زيادة في الانقسام) (4) نخر في الخلايا الكبدية (5)ترسب في بعض المواد .بينما في الامعاء لوحظ(1)قصر في طبقة الاهداب (2)استجابة التهابية مع تحطم في الطبقة المخاطية والطبقة تحت المخاطية.

Introduction

Cadmium is considered to be one of the most toxic heavy metals. It is anon essential trace element with no biological function (Viarengo,1985). Cadmium occurs primarily in fresh waters as divalent forms including free cadmium (II) ion, cadmium chloride and cadmium carbonate (Dwaf,1996). Cadmium does not break down in the environment , but can change form . Cadmium can remains in the body for long time and can bioaccumulation for many years after exposure to low levels of this metals (Groundwork ,2002) . The natural level of cadmium varies from 0.1 to 10µg/L in fresh water (Singhal & Jain , 1997). Fish are relatively sensitive to changes in the surrounding environment including an increas in pollution. Early toxic effects of pollution may , however , only be evident on cellular or tissue level before significant changes can be identified in fish behaviour or external appearance , and these have shown by previous studies.

According to Morsey & Protasowicki (1990) cadmium caused pathological alterations in the gill filaments , respiratory lamellae and necrosis of hepatic cell of cyprinus carpio . Metals may be taken up by aquatic organisms via the general body surface , across the gills, and through the gut lining following the ingestion of food and metal containing particulate material (Memmert,1987;Sanders,1997) . Al-Mansoori (2004) studied the fish juveniles *C. carassius* was exposid to concentrations (0.4 and 15) ppm of Cu and Pb respectively and seen effect on gills caused hyperplasia and fusion between lamellae . Abdullah (2001) observed epithelial necrosis and rupture of the gill epithelium are directed responses induced by the action of zinc ions . Thophon *et. al.* , (2003) demonstrated that gill lamellae and kidney tubules were the primary target organs for the acute toxic effect of Cd after exposure to 10 and 0.8 mg/l for 96h, While showed marked changes in the intestine after exposure of two lethal concentrations 25 and 40 mg of Cd on *Lepidocephalichthy guntea* after 96h (Shivaraj & patil,1994). Crespo *et.al.* , (1986) reported morphological changes in intestine trout and showed

swollen and detection in epithelial layer extensive in intracellular and degenerated epithelial layer after exposure to Cd and Pb . Results from histopathological studies are useful in establishing water quality criteria (FAO,1987) . Histological analysis appears to be a very sensitive parameter and is crucial in determining cellular changes that occur in target organs such as the liver (Dutta , 1996) . The liver is a detoxification organ and essential for both the metabolism and excretion of toxic substances . Exposure to toxicants may cause histological changes in the liver, which in turn could be used as abiomarker to indicate prior exposure (Hinton & Lauren ,1990) .The liver has the ability to degrade toxic compounds , but its regulating mechanisms can be overwhelmed by elevated concentrations of these compounds , and could subsequently result in structural.

Similar studies on various fish species , exposed to various toxicants , showed histopathological changes in the livers of those specimens.Ghosh & Chakrabarti (1993) showed histological changes in liver of fish *Heteropeustes fossils* after exposure to 57 mg of cd for 30 days , rupture of cell membrane of hepatocytes , damage of connective tissue . Cellular swolling or hydropic degeneration was observed in a few livers of fresh water fish species *Oreochromis mossambicus* specimens over 96h in 5 ppm of cd these cells were swollen and the cytoplasm appeared cloudy and granular (VanDyk , 2003) .This study attempts to evaluate the nature and extent of histopathological lesions caused by cadmium in the gills, liver and intestine of juveniles fish *C. carassius* potentially subjected to environmental cadmium pollution.

Materials & Method

Specimens of the fish juveniles *C. carassius* were collected from Garmet Ali river with weighted 15g during September to November ,2000. The animals were starved for (24)h before use . The active individuals from

fish were taken with three replicates for two concentration of cadmium in addition to control.

An aqueous stock solution of 1,000 part per thousand (ppt) was prepared by dissolving 1.9446 of metal $CdCl_2$. $2H_2O$ in litter of distilled water .

All fish exposed to period LT50 high concentration 8 days to cadmium metal.

Gills , liver and intestine specimens from the control and experimental animals were fixed in bouins solutions for 24h and then washed for 2h in running tap water and dehydrated in a graded series of alcohol, cleared in xylene and embedded in paraffin wax . Section of (7µ) were cut using a rotary microtome and stained with harris hematoxyline and eosin.

Result

Histology of gills:

Fish gills composed of two morphologically distinct epithelia the multilayed filament epithelium which is largely involved in ion exchange and the bilayered lamellar epithelium involved in gas exchange .The chloride cells are mainly situated in the filament epithelium (fig-1).

Histological changes of gills :

The exposure to 0.2 ppm cd caused start fusion of adjacent secondary lamellae ,separation of epithelial layer, destroyed cells of epithelium layer , adjacent lamellae curved and hyperplasia (fig-2)but after exposure to 0.5 ppm cd caused loos in regular shape, fusion of adjacent secondary lamellae , hyperplasia of secondary lamellar epithelium, separation of epithelium layer and congestion of blood vessels (fig.3,4).

Histology of liver:

Tissue in fish consist of hepatocytes which organized as longitudinal cords included Sinuses and hepatic capillaries (fig-5).

Histological changes of liver:

The exposure to 0.2 ppm caused haemorrahge in liver sinuses , hyperplasia with hepatic cells and increas mitosis (diploid nucleus), necrosis

in liver cells, irregular hepatic plates and degeneration in hepatic cells membrane and extended in sinuses (fig.6,7) but after exposure to 0.5 ppm caused irregular hepatic plates sever necrosis in hepatic cells, increased in cell number (deposite material)and hyperstaining in nucleus (fig-8,9).

Histology of intestine :

Consist of four layers and its from the intestine to the outside , respectively : **(1)** Mucosa: the layer which lining the digestive canal , its asingle layer from columnar epithelial cells which contain few number of secretory cells called Goblet cells which increase in number at the forest parts of the intestine .The mucosa contain a thin layer of a loose connective tissue and blood vesseles called lamina propria.

(2) Sub mucosa : The thickest layer of the intestine and its aloos connective tissue.

(3) Muscularis : It is two types:

(a) External longitudinal muscles

(b) Internal circular muscles .

(4) Serosa : It is a thin layer of aloos connective tissue (fig-10).

Histological changes of intestine :

The exposure to 0.2 ppm of Cd caused shortining villi (enlarged Goblet cell) inflamation response in mucusa and submucusa layer and degeneration submucusa and serosa (fig-11,12) but after exposure to 0.5ppm of cd caused degeneration in mucosa, submucosa and muscularis, damage in mucosa, inflamation response with mucosa and submucosa, shortining villi with degeneration in up villi (fig-13,14,15).

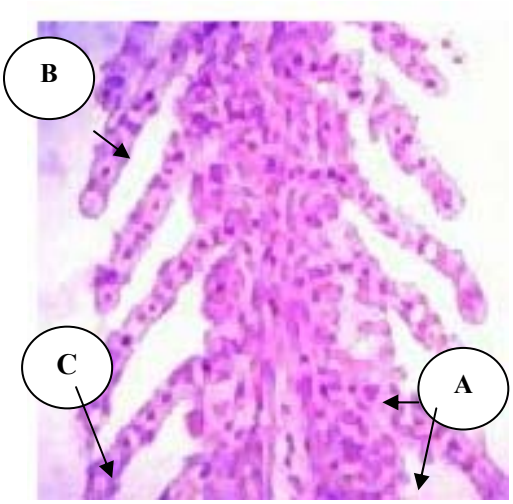

Fig (1) .longitudinal section in gills of control juvenile fish *C. Carassius* (A) Secondary lamellae (B) pillar Cells (C) red blood cells (H & E 400x)

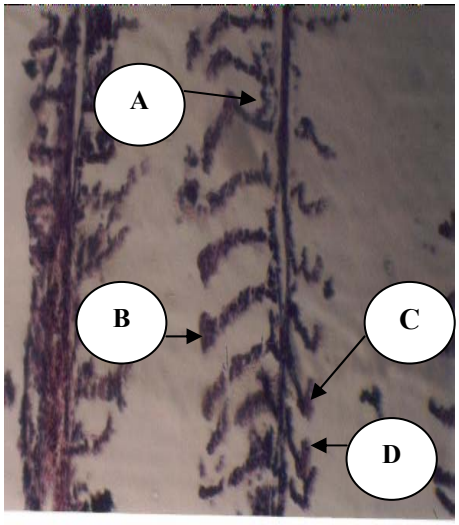

Fig (2) . longitudinal section in gills of juvenile *C. Carassius* after exposure to 0.2 ppm of Cd showed (A) sepration of epitheliallayer (B) Hyperplasia (C) Adjacent lamellae curved (D) Fusion of adjacent lamellae (H & E 400X)

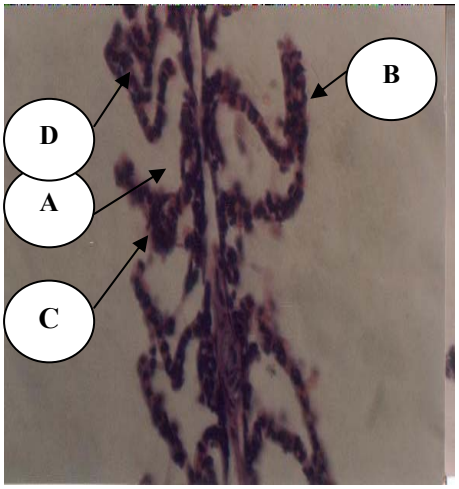

Fig (3).longitudinal section in gjills of juvenile *C. Carassius* after exposure to 0.5 ppm of cd showed congestion (A) blood vessels (B) fusion of adjacent secondary lamellae (C) separation of epithelial layer (D) adjacent lamellae curved (& E 400x)

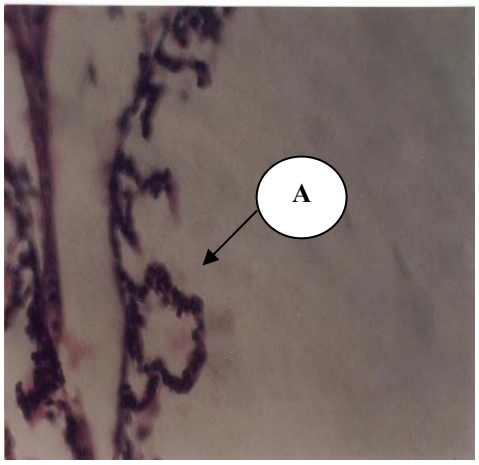

Fig (4) . longitudinal section in gjills of juvenile *C. Carassius* after exposure to 0.5 ppm of cd showed (A) losse in regular shape , fusion of adjacent secondary lamellae (H & E 400x) .

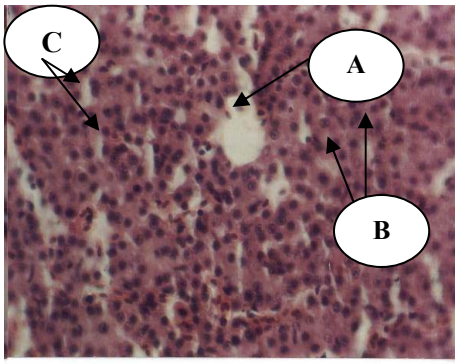

Fig (5) longitudinal section in liver of control juvenile fish *C. Carassius* showed (A) central vien (B) hepatic cord (C) sinuses (H & E 400X)

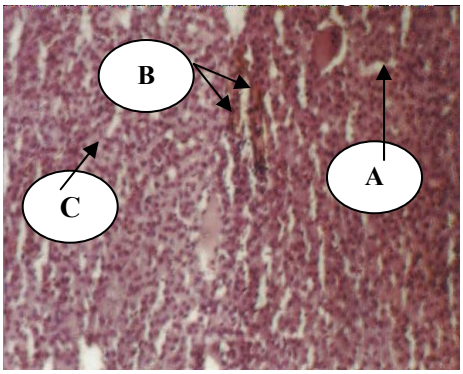

Fig (6) . longitudinal section in liver of juvenile *C.Carassius* after exposure to 0.2 ppm of Cd showed (A) necrosis (B)haemorrahge in liver sinuses (C) hyperplasia (H & E 200X)

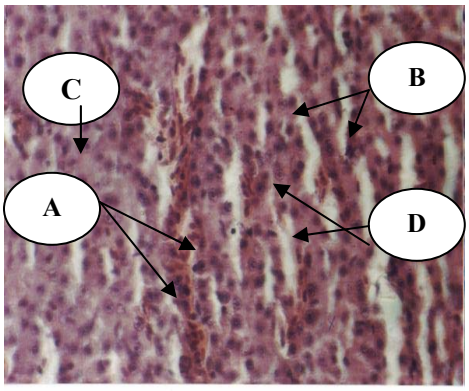

Fig (7) . longitudinal section in liver of juvenile *C.Carassius* after exposure to 0.2 ppm of cd showed (A) irregular hepatic plates, haemorrahge in liver cells (B) extended in sinuses(C) degeneration in hepatic cells membrane (D) diplod nucleus (H & E 400X)

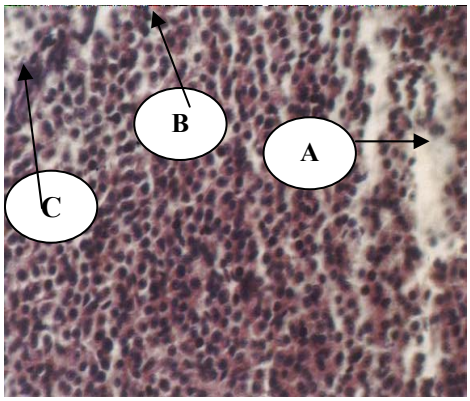

Fig (8) . longitudinal section in liver of juvenile fish *C.Carassius* after exposure to 0.5 ppm of cd showed (A) necrosis (B) hyper staining in nucleus(C) degeneration in hepatic cells (H & E 400X)

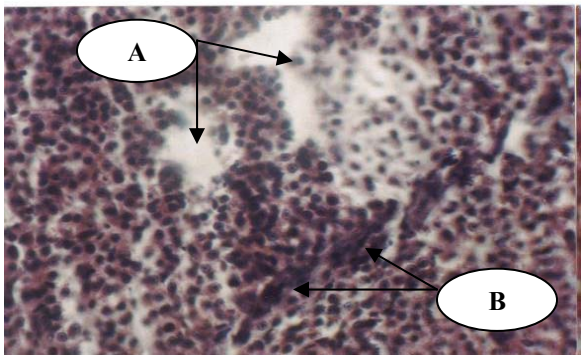

Fig (9) . longitudinal section in liver of juvenile fish *C. Carassius* After exposure to 0.5 ppm of cd showed (A) losse in regular shape , sever necrosis (B) hyper staining in nucleus (H & E 400 X) .

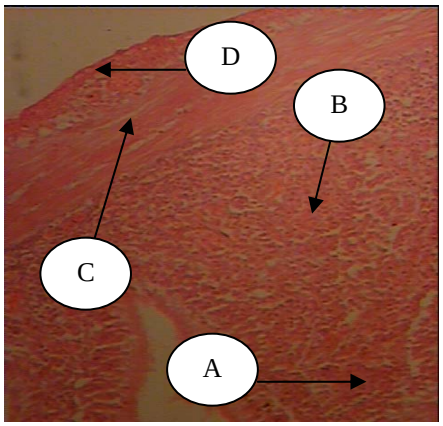

Fig (10) . longitudinal section in intestine of control juvenile fish *C . Carassius* showed (A) mucosa (B) Submucosa (C) muscular (D)Serosa (H & E 400x) .

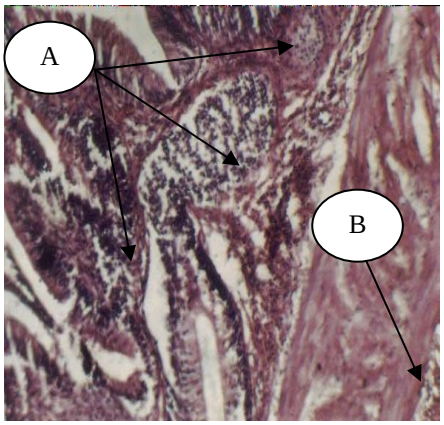

Fig (11) . longitudinal section in intestine of control juvenile fish *C . Carassius* after exposure to 0.2 ppm of cd showed (A) inflammation responses in mucosa and submucosa (B) degeneration in serosa (H & E 200x) .

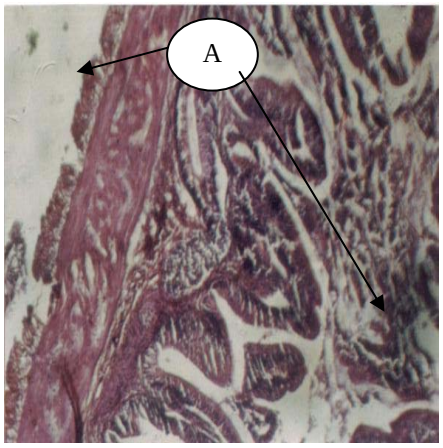

Fig (12). longitudinal section in intestine of juvenile fish *C.Carassius* after exposure to 0.2 ppm of cd showed (A) degeneration in submucosa and serosa (H &E 200x) .

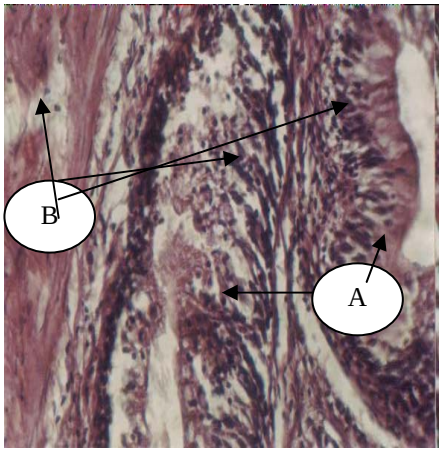

Fig (13) . longitudinal section in intestine of juvenile fish *C. Carassius* after exposure to 0.5 ppm of cd showed (A) inflammation response in mucosa , submucosa and Muscularis (B) degeneration in mucosa , submucosa and. musecules (H & E 400 x)

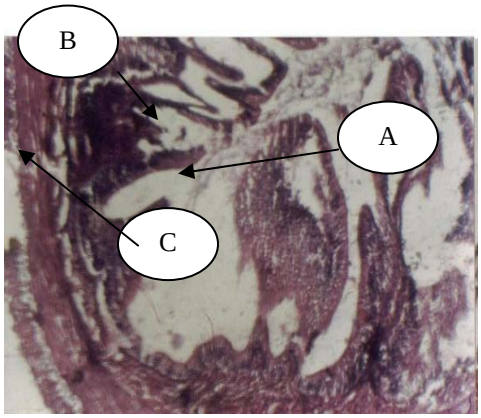

Fig (14) .longitudinal section in intestine of juvenile fish C. Carassius after exposure to 0.5 ppm of cd showed (A) end normal villi (B) degeneration in up villi (C) degeneration in serosa layer (H & E 400 x)

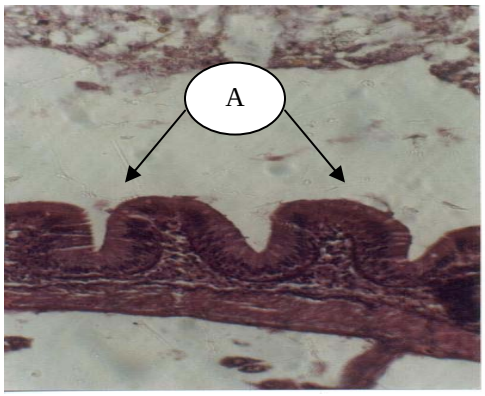

Fig (15). longitudinal section in intestine of juvenile fish *C. Carassius* after exposure to 0.5 ppm of cd showed (A)shortening in villi (H & E 400x) .

Discussion

Both specific and non specific pathologies were detected in histological analysis of many tissues. But most pathologies were specific for cadmium in addition the prevalence of Pathologies increased with increase the concentration The cadmium induced histopathological changes observed in gills, liver and intestine of *C. carassius* were similar to pathological changes observed in other fishes due to heavy metals toxicity in regional studies or strange studies (Al- Mansoori, 2004 ; Shivaraj & Patil, 1994 ; randi *et al.*, 1996). In order to conduct aproper histological investigation on a specific organ of an exposed specimen it is important of first examine and investigate the histology of the same organ of control specimens, as summed to reflect the normal histological structure of that organ (Van Dyk , 2003) . Gills are suspected of being the critical organ for fish exposed to heavy metals. In present study showed fusion of adjacent in primary and secondary lamellae, necrosis of epithelium and hyperplasia this is a greement with Randi *et. al*. (1996) were demonstrated the gills after exposure to1.5 mg/l of Cd following 30 days in fresh water fish *Nacropsobrycon uruguayanae* observed hyperplasia , sepration of

respiration epithelium and necrosis while Chacrabarti *et .al*. (1994) observed in *Mystus vittatus* after exposure to 20mg of cd for 70 days were fusion and disruption of linguiform process,necrosis of mucosal border of the olfactory lamella,disruption of sensory receptor cells .

The results of this study agreement with Abdullah (2001) which showed epithelial necrosis and rupture of the gill epithelium are directed res ponses induced by the action of zinc ions , the epithelium lifting appears to be one of the initial reactions of the gill to variety of pollutants. But Albaster & Lloyd (1982) refer to exposure to heavy metals causes unreservial protein complex forming toxicity effects on gills results form necrosis and disruption in epithelial layer while fusion of the adjacent secondary lamellae,one of gill pathologies specifically caused by cadmium, clearly implies a reduction in the respiratory surface (Randi *et.al*.,1996). Das& Kaviraj (1994) showed similar effects in gill epithelium of common carp exposed to cadmium 2.5 mg /L cadmium induced edema of primary and secondary gill lamellae , nuclear swelling , and necrosis and hypertrophy of epithelial cells of the secondary gill lamellae All this demonstrated by many reginal studies on fresh water fishes after exposure to sublethal concentration of heavy metals result permeability gills membranes to water and ions from environment results to swelling on epithelial layer and in the end disruption (Al-Mansoori &Suad,2002; Al-Mansorri,2004 ; Al-Ali , 2004).Shivaraj & Patil (1994) reported cellular exudated was seen the lumen of the intestine , mucus cells showed hyper activity and are filled with secretary material,erosion and degeneration of mucusal folds. But Abdullah & Al-Mansoori (2002) found that the effect of Cu and Zn concentration on mid gut causes a great shrink in tissue , reducing in central cavity and damage in the cells as well as pyknosis case .This is agreement with this study which found enlarged in goblet cell and damage in mucusa.

But studies of fishs liver structure can indeed be considerd as a starting point for comparative studies among vertebrates .The liver is a detoxification organ and essential for both the metabolism and excretion of

toxic substances.The liver is therefore situated directly in the way of blood vessels that convey substances absorbed from the digestive system and the liver can be regarded as the bodies detoxification organ and hence a target organ of various xenbiotic substances (Van Dyk,2003). The liver is one of the most biochemically complex organs in the body (Marieb,1995). The liver accomplish these detoxification actions by means of three mechanisms as summarised by (Cabts,2000): (1) A filtering system of large macrophages called kupffer cells lining the blood sinusoids ; (2) phase one detoxification pathway ; and (3) phase two detoxification pathway .In this study showed haemorrahge in liver sinuses , increas mitosis and irregular hepatic plates, this is agreement with Arellano(1999) who found some ultrastructural alterations such as partial disruption of the microvilli and endothelial lining of the sinusoids in the hepatocytes. According to Hinton & Lauren(1990) a differentiation must be made between cytolytic and coagulative necrosis. Cytolytic necrosis involves rapid disintegration of hepatocytes and focol cytolitic necrosis is recognized by replacement of hepatocytes by reactive inflammatory cells (Popper , 1988) . After necrosis , surviving hepatocytes may undergo hyperplasia,ther by regenerating sufficient hepatocytes to replace those that were lost .According to Hinton & Lauren(1990) regenerating hepatocytes are basophilic and occur as small as lands of irregular shape. Sultan & Mahmmode (1999) showed necrosis in liver after exposure to hermful factores , haemorrahge and degeneration this is responsbal to factores one of the methods either regeneration or replacement fibrosis. But reported increase in the number of lysosomes and the presence of electron dense deposits in hepatocytes from trout exposed to both copper and zinc (Hinton & Lauren ,1990). And these demonstrated by Van Dyk (2003) the liver exposure to different contaminated materiales resultes to appear pathological changes in liver cells result changes in normal liver function while Anderson and Spear (1980) showed cells atrophy cause inflamations forming by blood vesseles congestion this effected on prepearing cells in gobbletion region by oxygen and food .While Hinton & Lauren (1990)

reported hepatocyte hypertrophy should be distinguished from simple swelling due to ion and water influx hypertrophy of cells is due to organelle hyperplasia and hypertrophy of hepatocytes occurs with the exposure to compounds which induced proliferation of endoplasmic reticulum membranes. The sinusoidal endothelial cells surround the vascular space and seprate it from the interstitial space and also from the adjacent hepatic parenchymal cells extens necrosis has been after exposure to hepatotoxins (Droy&Hinton,1988) initial morphological alterations were the destruction of sinusoidal endothelial cells with loss of sinusoidal endothelium, hemorrhage into the space of diss and interhepatic space was associated with hepatocytes shrinkage and hemorrahage necrosis.

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