PATHOLOGIC AND TOXICOLOGIC STUDIES ON HERBICIDE (ARGOLD) IN COMMON CARP

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ABSTRACT

One hundred and eighty common carp fish (70-85 gm B.wt.) were equally divided into 3 groups (gps.) to study the clinical signs , lesions and residues of herbicide Cinmethylin (argold) . Gp.(1) was kept as untreated control. Gps. (2&3) were exposed to 0.1 and 0.2 ppm Cinmethylin/liter respectively for 28 days. The clinical signs were recorded and 15 fish from each group were necropsied after 1,2,3 and 4 weeks from the beginning of experiment. Samples were taken from fish muscles to detect Cinmethylin residues . Specimens were collected from the liver , kidneys, spleen , gills, stomach , intestine and brain then fixed in 10 % neutral buffered formalin . Paraffin sections, 5u thick, were prepared and stained with hematoxylin and eosin and examined microscopically .

The clinical signs were severe in gp.(3) particularly 4 weeks after exposure and were represented by hyperexcitation and respiratory manifestations (swimming near water surface with opened mouth). The fish showed muscular spasm , loss of balance and tried to jump out from the aquarium. The lesions were similar among fish of gps. (2&3) . The severity of the lesions increased after 4 weeks of exposure (gp.3) . Macroscopically , the viscera and gills were congested . Microscopically , the hepatic cells showed vacuolar and hydropic degenerations. The portal areas showed fibroblastic proliferation infiltrated with numerous mononuclear cells. The hepatocytes, around the central veins, were necrotic and replaced with extravasated blood. The hepatic capsule was thickened with hyalinized fibrous connective tissue, infiltrated with round cells. Some pancreatic acinar cells were vacuolated, necrotic and the acini contained few inflammatory cells. The kidneys showed congested glomeruli beside degenerative changes in some renal tubules. The epithelial lining of some renal tubules showed hyaline droplet degeneration and coagulative necrosis . The renal capsule was thickened by fibrous tissue. Numerous proliferating melanomacrophage centers were no-

ticed among the renal tubules. The spleen showed necrosis of the hemopoietic tissue. The gill filaments showed congestion beside telangiectasis of the secondary lamellae. Desquamation of the epithelial covering of the secondary lamellae was noticed. The stomach and intestine showed metaplasia of the epithelial lining to goblet cells. Focal necrosis of the gastric glands was noticed. Congestion and edema with the presence of eosinophilic granular cells (ECG) and lymphocytes were noticed in the gastric and intestinal lamina propria. The brain showed degenerated neurons , satellitosis, neurophagia and focal gliosis. Degenerated Purkinje cells were noticed in the cerebellum . Demyelination of some nerve fibers was encountered in fish of gp.(3) which survived till the end of the experiment .

Significant levels of Cinmethylin residues were encountered throughout the period of exposure. The greater concentration was induced with prolonging the period of exposure.

It could be concluded that exposure of the common carp to both concentrations of Cinmethylin induced a great toxicosis to fish, manifested by clinical signs and lesions with presence of its residues in the muscles which could induce serious hazards upon their consumers .

INTRODUCTION

Environmental pollution is one of the serious problems in the world . Herbicides are used to control aquatic vegetation in operation of irrigation drainage system. Cinmethylin (Argold) is a relatively new herbicide for the control of annual grass weeds and the suppression of a wide spectrum of broad leaf weeds and sedges in transplanted rice. It is considered as a selective herbicide for use in padding rice . Moreover it is widely used in rice fields as a herbicide to kill undesired weeds and grasses **(Peterson et al. 1983)**. **Schulz (1972)** described interstitial hemorrhage and fatty change of tubular epithelium in kidneys of Carp exposed to herbicide Kamex at concentrations of 6 and 12 ppm . The liver showed focal necrosis , hemorrhages and cholangitis. Moreover endocarditis and myocarditis were noticed . Similar lesions were described in Common carp exposed to Rifit herbicide **(El-Bouhy et al . 1992)**. Tilapia exposed to 22 ppm Dichlobenil showed mild lesions in the gills, liver and brain **(Diab 1992)**. He added that the induced lesions were severe by extension of the exposure time. **Lam et al. (1983)** found that the liver of rats, orally given Cinmethylin, showed centrolobular degenerative changes. On the other hand **Larson (1988)** didn't find any lesions in dogs, treated orally with Cinmethylin .

The aim of the present work was to study the clinical signs, lesions and muscle residues of Cinmethylin herbicide in the Common carp (Cyprinus caprino).

MATERIAL AND METHODS

One hundred and eighty Common carp (Caprinus carpioo) were collected from Abbassa fish farm. The average weight and length were 70-85 gm and 15-17 cm respectively. Fish were brought alive to the laboratory and kept in a well areated glass aquaria measuring 100 x 50 x50 cm. Fish were acclimatized on dechlorinated tap water for 15 days. Each aquarium was supplied with two air pumps. The water temperature was fixed at 27°C ± 2 . The total hardness and dissolved oxygen content were 50.8 mg/L as (Ca CO3) and 5.6 mg/dl O2 respectively. The pH was 7-8.5. The fish were fed on commercial pelleted ration at a rate of 20% body weight, once daily. The fish were divided into 3 equal groups. Gp (1) was kept as untreated control. Gps. (2&3) were respectively exposed to 0.1 and 0.2 ppm Cinmethylin /liter for 28 days.

The clinical signs were recorded, and 15 fish from each group were necropsied after 1,2,3 and 4 weeks from the beginning of the experiment (**Plump and Bowser 1983**). Samples were taked from the fish muscles to detect Cinmethylin residues according to **Lee et al. (1990)** after modification of **Ramsteiner et al. (1974)**.

Specimens were collected from the liver , kidneys , spleen , gills, stomach, intestine and brain and fixed in 10% neutral buffered formalin. Paraffin sections of 5 u thick were prepared and stained with hematoxylin and eosin and examined microscopically (**Luna 1968**).

RESULTS AND DISCUSSION

Clinical signs :

The clinical signs varied according to the concentration and period of exposure. The clinical signs were sever among fish of gp. (3) particularly after 4 weeks of exposure and were represented by hyperexcitability and respiratory manifestations (Swimming near to the water surface with opened mouth) The fish showed muscular spasm and loss of balance beside trying to jump out from the aquarium . 4 weeks after exposure .

Macroscopically, the common lesions in all the necropsied fish of gps.(2&3), were the accumulation of fluid in the abdominal cavity with congested viscera and gills. Microscopically, fish of gps.(2&3) exhibited nearly similar microscopic lesions, in the examined organs. The severity of the lesions increased in fish exposed to the high concentration (gp.3). The hepatic cells showed vacuolar and hydropic degenerations. The portal areas showed fibroblastic proliferation infiltrat-

ed with numerous mononuclear cells particularly around the bile ducts. The latter showed retained bile inside its lumina (Fig. 1). The hepatocytes showed necrotic changes and were replaced with extravasated blood, particularly around the central veins, 2-3 weeks exposure. The hepatic capsule was thickened with hyalinized fibrous tissue and round cell infiltration. Some pancreatic acinar cells appeared vacuolated and necrotic. The acinar lumina contained few inflammatory cells (Fig. 2). The hepatic sinusoids and central veins were highly congested .Numerous bile ducts with periductal fibrosis (Fig. 3) were encountered mainly in fish which survived for long periods (3-4 weeks). The kidneys revealed overdistension of the peritubular blood vessels with immature erythrocytes and focal hemorrhages in all necropsied fish (Fig. 4). The hemopoietic elements showed variable degrees of activation. Congestion of the glomeruli beside degenerative changes of some renal tubules could be noticed in fish exposed for 2 weeks (Fig. 5). The epithelial lining of some renal tubules showed hyaline droplet degeneration, particularly in fish necropsied after 1 & 2 weeks exposure (Fig. 6). Moreover, coagulative necrosis of some renal tubules, represented by dissociated renal epithelium with eosinophilic homogenous cytoplasm and pyknotic nuclei, was noticed in fish necropsied after 3 &4 weeks exposure. Hyaline casts were noticed inside the lumina of some renal tubules. The renal capsule appeared thick by fibrous tissue with numerous proliferating melanomacrophage centers (Fig. 7) which were distributed among the renal tubules. The spleen showed necrotic lymphoid tissue which was replaced by edema and eosinophilic debris, particularly in fish exposed for 4 weeks (Figs. 8 & 9). Hyperplasia of the melanomacrophage centers was encountered. Depletion of the hemopoietic elements was noticed in fish which survived to the end of the experiment. The gills showed severe congestion of the gill filaments in all the necropsied fish of gps. (2 & 3) (Figs. 10 & 11). Telangiectasis of the secondary lamellae was seen after 2 weeks exposure. Desquamation of the epithelial covering of the secondary lamellae or complete sloughing of the secondary lamellae could be seen in some cases, 3 & 4 weeks exposure. Mild focal proliferation of the epithelial covering of the free portion of the secondary lamellae with fusion could be seen in some cases (Fig. 12). Vacuolation of the epithelial cell covering of the gill rackers with aggregations of numerous eosinophilic granular cells (EGC) were common in fish of gps. (2 & 3) (Fig. 13) . The gill arch revealed congestion beside hemorrhage and edema . The stomach showed metaplasia of the epithelial lining to goblet cells in the majority of fish in gps. (2 &3) at different periods. Focal necrosis of the glandular epithelium of the gastric glands, represented by pyknosis, was noticed 2 & 3 weeks after exposure. Edema and congestion with the presence of EGC and some lymphocytes could be seen in the lamina propria after 2, 3 and 4 weeks exposure (Fig. 14). The smooth muscles showed focal hyaline degeneration and edema 4 week after exposure. The intestine showed goblet-cell metaplasia of the epithelial covering of the villi with the presence of basophilic mucus adherent to the intestinal epithelium in fish exposed for 3 weeks (Fig. 15). The lamina propria

and submucosa showed congestion , edema and infiltration with lymphocytes and EGC after 4 weeks exposure. Moreover, the smooth muscles showed hyaline degeneration. The brain showed degenerated neurons, satellitosis, neuronophagia and focal gliosis in diencephalon of fish of gp. (3) necropsied after 3 & 4 weeks exposure. Congestion, edema and few round cells were focally observed in the meninx primitiva (covering of the brain). Degenerated Purkinje cells were noticed in the cerebellum. Demyelination of some nerve fibers was encountered mainly in fish of gp.(3) which survived till the end of the experiment (Fig.16).

Rsidues of Cinmethylin :

Table (1) shows the residues of Cinmethylin in the muscles of Common carp (gps. 2 &3) exposed to 0.1 and 0.2 ppm respectively compared with (gp.1), untreated control. The residues were dose and time dependant .

The present study declared serious effects of Cinmethylin in the Common carp. The used doses did not induce mortalities but induced clinical signs represented by hyperexcitation of fish, swimming near the water surface with respiratory and nervous troubles, particularly in fish of gp. (3). Such clinical signs could be attributed to the gills and brain lesions. The absence of deaths in our work may be due to the low concentration of the used herbicide. These findings are in agreement with **El-Bouhy et al. (1992)** who mentioned that the concentration of Rifit herbicide showed no observable acute toxicity on fingerlings of common carp.

The postmortem findings indicated toxicosis, represented by accumulation of fluids in the body cavities and congestion of the viscera and gills. These lesions explained the action of herbicide on the vascular endothelium and liver tissue (**Johns et al. 1997**). **Boller et al. (1973)** showed ascites in fish intoxicated with dichlobenil. The histopathologic changes in the liver were mainly the picture of toxic hepatitis beside destruction of hepatopancreatic tissue and periductular fibrosis. Similar results were reported by **Diab (1992)** who exposed tilapia fish to 0.22 ppm dichlobenil for one month. He described degenerative changes in the hepatocytes. The difference in the intensity of lesions could be due to the difference of the used herbicide and type of fish. The renal lesions declared nephropathy represented by hyaline and hydropic degenerations and necrotic changes of the tubular epithelium accompanied with hemorrhages and activation of hemopoietic elements . The forementioned results are partially in concurrence with **El.Bouhy et al (1992)** who described activation of the hemopoietic elements with severe renal congestion in the Common carp, exposed to Rifit herbicides. The gill lesions were severe and indicated the serious effect of the used herbicide on the respiratory organs. These lesions were

congestion, telangiectases, hemorrhages and edema accompanied by sloughing of the secondary lamellae (gp. 3) and desquamation with hyperplasia of lamellar epithelium on the tips of gill filaments (gp. 2). These results could be due to the direct contact between tissue of gills and the used herbicide. Similar gill lesions were reported by **Diab (1992)**. **Wiersma Roem et al. (1978)** noticed hyperpalsia and hypertrophy of gill epithelium in fish intoxicated with dichlobenil. The splenic lesions, in our work, were necrosis of the lymphoid tissue, depleted hemopoietic centers and hyperplastic melanomacrophages. These lesions could be due to toxemia induced by the prolonged exposure to the used herbicide. Similar results were reported by **El-Bouhy et al.(1992)**. Both the gastric and intestinal lesions, in our investigation, were related to the local action of the herbicide on the mucosae. The brain lesions, in our results, included degenerated neurons, focal gliosis, edema, congestion and demyelination of some nerve axons and indicated that the used herbicides induced neural injury. These results are partially in accordance with **Diab (1992)** who found brain edema induced by the used 2 levels of exposure to dichlobenil (0.22 and 2.2 ppm) in Oreochromis niloticus. Significant levels of Cinmethylin residues were encountered throughout the period of exposure. The greater concentration was induced with prolonging the period of exposure. Our results are consistent with **Lee et al. (1990)** who found significant amount of Cinmethylin residues in muscles of blue gill sun fish .

It could be concluded that the exposure of common carp to both concentrations of Cinmethylin induced toxicosis in the fish manifested by clinical signs and lesions with the presence of its residues in the muscles which could induce serious hazards upon their consumers .

Table 1 : Groups, Concentrations and residues of Cinmethylin (ppm) in muscles at different periods of exposure .

Groups	Concentration of Cinmethylin	Residues of Cinmethylin (ppm) in muscles			
		7 day	14 days	21 days	28 days
1	Control	0.0	0.0	0 0	0 0
2	0.1 ppm	49 12	51 72	60.11	60.44
3	0.2 ppm	99 84	118.16	136.61	261 97

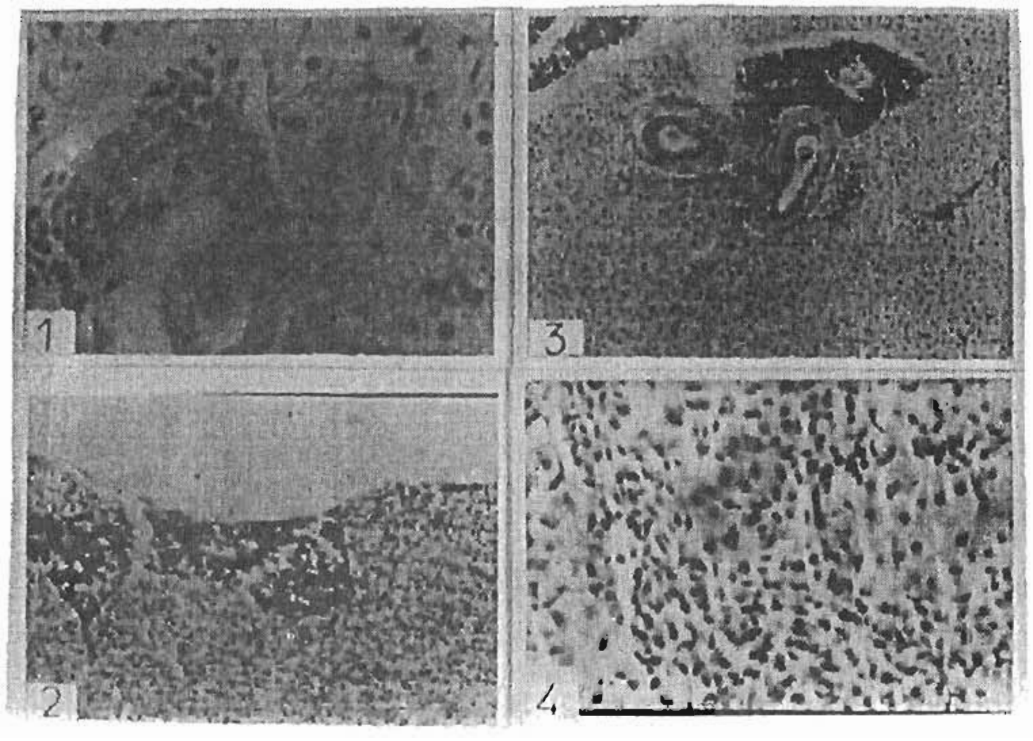

Fig. (1). Liver, (gp.2) one week exposure, showing mononuclear cell infiltration, fibroblastic proliferation and retained bile in the lumens of bile ducts . H&E , X 600.

Fig. (2). Liver, (gp.2) 3 week exposure, showing vacuolated and necrotic pancreatic acini. H & E., X 150

Fig. (3). Liver, (gp 3) 4 week exposure, showing fibrosis around the bile ducts. H & E., X 150.

Fig. (4). Kidney, (gp 2) one week exposure, showing local hemorrhage among the renal tubules . H & E., X 600

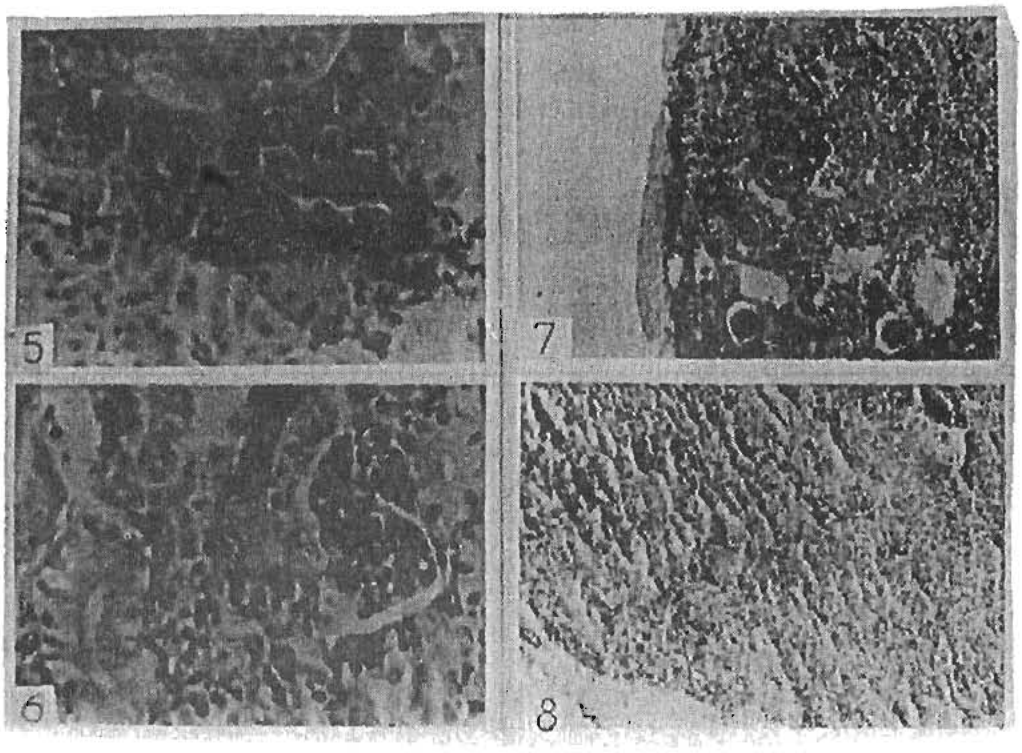

Fig.(5). Kidney, (gp 2) 2 weeks exposure, showing hypercellularity of the glomeruli beside degenerative changes in the renal tubules . H&E., X 500.

Fig. (6). Kidney, (gp.3) 2 week exposure, showing hyaline droplet and hydropic degenerations in the tubular epithelium beside glomerular congestion . H&E. X600.

Fig. (7). Kidney, (gp.2) 4 week exposure , showing thickened renal capsule with presence of numerous melanomacrophage centers . H&E., X 150.

Fig. (8). Spleen, (gp3) 3 week exposure, showing local necrosis, H&E., X 150.

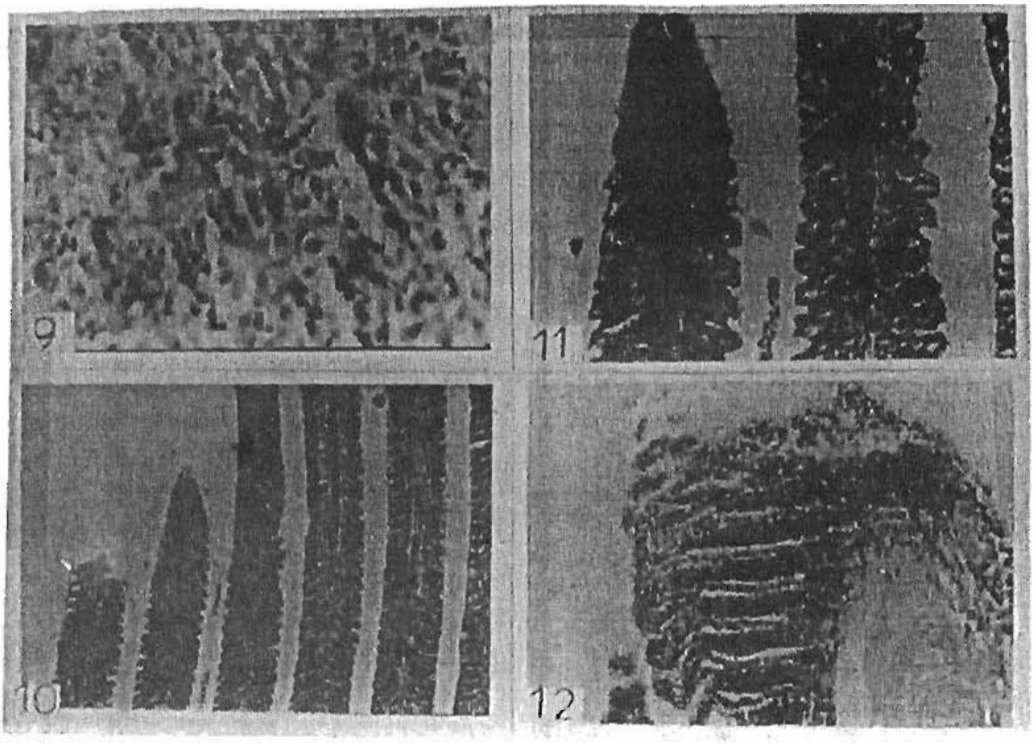

Fig. (9). A high power of fig .(8) to show the necrotic debris and edema replacing the lymphoid tissue. H & E., X 600

Fig. (10). Gills, (gp.2) 2 week exposure, showing congested gill filaments . H&E., X 150.

Fig. (11). A high power of fig (10) to show the severe congestion of the gill filaments . H&E , X 600.

Fig. (12). Gills, (gp.3) 4 week exposure , showing hyperplastic epethelial covering and fusion of the secondary lamellar epithelium . H&E., X 150.

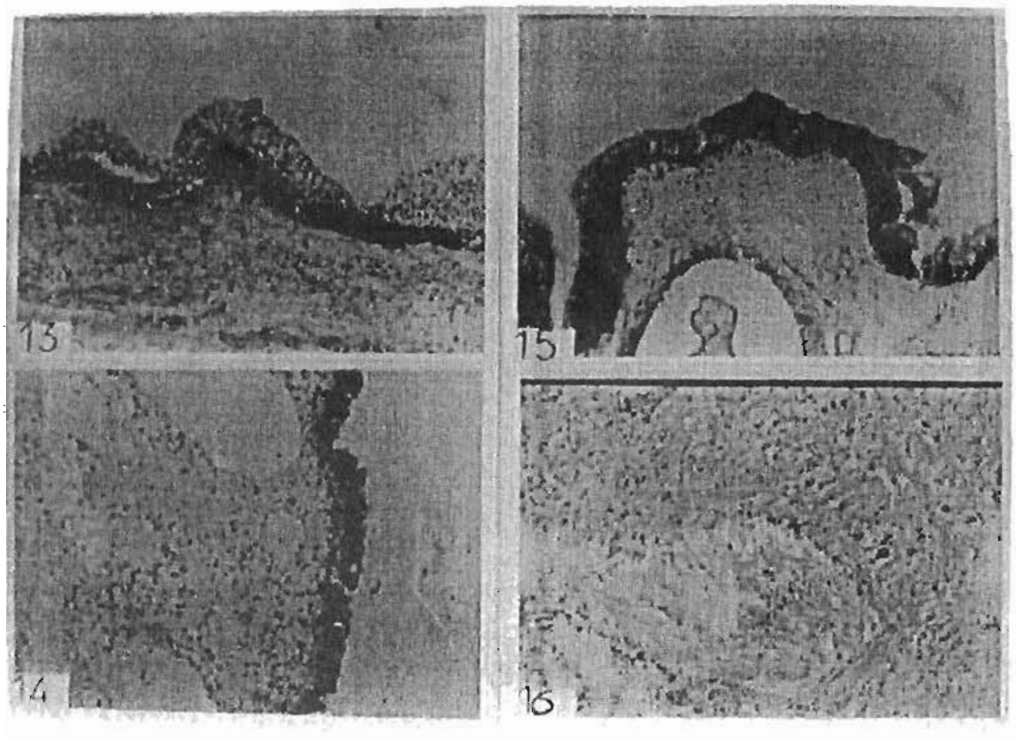

Fig. (13). Gills, (gp. 3) 4 week exposure, showing vacuolated epithelial covering of the gill rackers. H & E., X 150

Fig. (14). Stomach, (gp.3) 2 week exposure, showing edema, EGC and lymphocyles in the lamina propria , H&F ., X 150.

Fig. (15). Intestine, (gp 2) 3 week exposure, showing mucus covering the intestinal epithellum, H&E., X 150.

Fig.(16). Brain, (gp.3) 4 week exposure, showing demyelination of some nerve fibers . H&E , X 150.

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الملخص العربى

دراسات باثولوچية وسمية على مبيد الحشائش (الأرجولد) فى أسماك المبروك

المشتركون فى البحث

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تمت هذه الدراسة على ١٨٠ من أسماك المبروك تراوح وزنها حوالى ٧٠-٨٥ جم قسمت إلى ثلاث مجموعات متساوية لدراسة الأعراض الإكلينيكية والتغيرات المرضية ونسب بقايا المبيد فى الأنسجة الناتجة من التعرض لمبيد الحشائش الأرجولد. المجموعة الأولى وتركت كضابط للتجربة. المجموعة الثانية والثالثة تعرضت إلى ١ر٠-٢ر٠ جزء فى المليون من المبيد لكل لتر ماء على الترتيب لمدة ٢٨ يوم. كانت الأعراض ممثلة فى أعراض تنفسية والطفو بالقرب من السطح مع فتح الفم وانقباض العضلات وفقد التوازن وكانت التغيرات المرضية ممثلة فى إحتقان الأحشاء الداخلية والخياشيم أما التغيرات تحت الميكروسكوب فكانت متماثلة فى كل من المجموعة ٢ر٣ ولكنها كانت شديدة فى المجموعة الثالثة خصوصاً بعد ٤ أسابيع من التعرض للمبيد وكان أهمها فى الكبد هو إمتلاء مناطق الحيز البابى بالنسيج الليفى والعديد من الخلايا الليمفاوية وتنكرز فى خلايا الكبد حول الأوعية الدموية والتى حل محلها نزيف دموى بالإضافة إلى تنكرز واضح فى البنكرياس وأوضحت الكلية إحتقان شديد وتنكرز فى النبيبات الكلوية مع زيادة فى مراكز الميلانوماكروفاج وكان هناك موت لبعض الخلايا الليمفاوية فى الطحال أما الخياشيم فكان بها إحتقان واتساع فى الأوعية الدموية بالإضافة إلى موت ووقوع الخلايا المبطنة للصفائح الثانوية، وكان هناك تحول فى خلايا النسيج المبطن لكل من المعدة والأمعاء إلى خلايا كأسية مع وجود ارتشاحات واحتقان فى الأوعية الدموية. وأظهر المخ اضمحلال فى الخلايا العصبية وموتها والتهامها بواسطة خلايا الميكروجلايال واختفاء غشاء الميالين من حول الأطراف العصبية فى المراحل النهائية.