ISSN 110-2047. ALEX. J. VET.SCIENCE. 2002 , VOL 18 N. 161 :174

COMPARATIVE STUDIES ON TOXICOSIS OF COMMON CARP WITH SOME PHENOLIC COMPOUNDS

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ABSTRACT :Two hundred and sixty five Common carp fish were exposed for short and/or long term intoxicated with either phenol or m-cresol. Determination of LC_{50} revealed that carp fish was more sensitive to phenol than m-cresol. Toxicosis with these phenolic compounds resulted in characteristic nervous and respiratory signs. Grossly, a thick mucous layer covering the gills and skin was a permanent alteration. Histopathologically, both the gills and brain were the most affected organs.

INTRODUCTION

Diseases of aquatic animals, particularly those of toxic origin represent the biggest hazards to these organisms and considered a major branch of the environmental pollution. Phenol and its derivatives as m-cresol are among the most toxic and ubiquitous contaminants. These compounds arised from the distillation of cool and wood, oil refineries, chemical plants, livestock dips and human and animal wastes. So, they may be released into surface water through industrial wastes and sewage pollution (Hook, 1965 b and Alabaster and Lioyd, 1982). These toxic compounds are not only harmful to fish but also to the consumer of fish flesh containing residues of these compounds. Ohshima et al. (1989) reported stomach cancer due to consumption of fish flesh containing phenolic compound residue for long time.

This study was carried out to estimate the lethal concentration 50 (LC_{50}) for phenol and m-cresol then determination of the clinical signs, gross lesions and histopathologic changes in common carp fish associated with acute and chronic toxicosis with phenol and m-cresol.

MATERIAL AND METHODS

Fish:

Two hundred and sixty five Common carp ***(Cyprinus carpio)***

fish (100 ± 20 g B. W.) were used in this study. These fish were purchased from Barsek Fish Farm, Behira Province, Egypt.

Aquaria:

Clean disinfected glass aquaria were used (90 X 50 X 35cm). This aquaria were used for holding the experimental fish throughout the period of the present study , supplied with chlorine free tap water according to ***Innes (1966).*** The water temperature was maintained throughout the experiment within 18 – 20 °C. Fish were acclimatized for 2 weeks in these aquaria before the beginning of the experiment and fed commercial diet (25% protein) at a ratio of 3% of B.W.

Phenol and m-cresol:

Phenol (C_6H_5OH) and m-cresol ($CH_3C_6H_4OH$) were purchased from El-Gomhoria Company.+

Determination of LC50 of phenol and m-Cresol

One hundred fish were used for determination of LC_{50} of phenol and m-Cresol according to the method described by Behrens and Karber (1953).

Experimental design:

One hundred and sixty five fish were classified into 6 groups namely GPs. 1, 2, 3, 4, 5 and 6. GPs 1, 2 and 3 were 35 fish of each and used for acute toxicity with both phenol and cresol. The others (GPs. 4, 5 and 6) were 20 fish of each and used for chronic toxicity of these phenolic compounds as follows:

Acute toxicity:

GP. 1: 30 ppm phenol/liter (slightly lower than LC_{50}) were added and was kept constant throughout the experiment even after renewal of the water.

GP. 2 : 60 ppm cresol/liter (slightly lower than LC50) were added.

GP.3 : was used as a control group.

The duration of each experiment was one week. Five fish were sacrificed daily (Table, 1) and daily mortalities were recorded.

Chronic toxicity:

GP. 4: 9 ppm phenol/liter (1/5 LC50) were added to aquaria.

GP. 5: 15 ppm cresol/liter (1/5 LC50) were added to aquaria.

GP. 6: was used as a control group.

The duration of the experiment for both compounds was 4 weeks. Five fish from each group were sacrificed weekly up to 4 weeks (Table, 2). Daily mortalities besides clinical signs were recorded.

Pathologic studies

Fish were sacrificed by decapitation then necropsied. Thereafter, tissue specimens from gills, skin (with underlying musculature), intestine, hepatopancrease, brain, kidneys and spleen were fixed in 10% neutral buffered formalin then processed through the conventional paraffin embedding technique. Paraffin sections were stained with hematoxyline and eosin (H, E) according to Culling (1983).

RESULTS

1- LC_{50} of phenol and m-cresol:

The results obtained from the experiment of LC50 determination revealed that the 7 days LC50 of phenol and m-cresol in common carp was 45 and 75 mg/liter, respectively.

2-Clinical signs:

The clinical signs of the acutely and chronically intoxicated fish either with phenol or m-cresol were nearly similar. These signs were nervous and respiratory manifestations in the form of rapid aimless swimming, rapid opercular movement, gasping and aggregation of fish around the source of air and jumping over the surface of the water. In addition, the fish in GP. 1 appeared depressed and very weak besides a loss of equilibrium after 3 days post exposure . However, in case of GP. 2, the same signs appeared after 30 minutes, disappeared for 10 – 15 minutes, then appeared gain for 2 – 3 minutes and so on until the end of the 1st day of the experiment. Mortalities were shown in Tables (1, 2).

3-Gross lesions:-

The gross lesions of the acutely and chronically intoxicated fish either with phenol or cresol were nearly similar. These lesions consisted of excess mucus covering the skin and gills, focal areas of skin erosions (Fig. 1) and severe congestion of the spleen, kidneys, heart and intestine. In addition, the fish in GP.1 showed palness of the liver , but other few cases showed severely congested liver. Moreover, the brain was severely congested and the meningeal blood vessels were tortuous. However, in case of GP. 5 ascitis and mild congestion of the brain and internal organs were evident (Fig. 2).

4.Histopathological findings:-

***Phenol (30 PPm/liter for 7 days as short term exposure).**

Gills: the gills suffered from hypertrophy and both grade I and II gill hyperplesia. In grade I hyperplesia, there was only slight thickening of secondary lamellae (Fig. 3); otherwise, few cases showed grade II hyperplesia where in some secondary lamellae were fused with each others (Fig. 4). From the 5th day of the experiment and onward, grade III gill hyperplesia (i.e. adhesion of most secondary lamellae) besides lamellaer edema were the most characteristic findings (Fig. 5).

Brain:

Throughout the experiment, the meningeal blood vessels were severely congested with previscular edema and/or lymphocytic cuffing (Fig. 6).

Skin and underlying musculature:

The noticeable skin lesions were epidermal thickening which attributed mainly to intercellular epidermal edema or spongiosis separating the Malpighian cells from each others forming cystic-

like structure. Furthermore, hyperactivation of melanomacrophage centers was rare except for cases that showed skin erosions (Fig. 7).

Hepatopancreas:

The constant lesions of the hepatocytes allover the experiment were focal vacuolar and hydropic degeneration (Fig. 8).

Intestine:

The superficial epithelial desquamation besides coagulative necrosis of the tips of intestinal villi were noticed.

Heart, kidneys and spleen:

Except for few circulatory disturbances as congestion and mild haemorrhages, the microscopical examination of these organs did not show any changes.

***m-cresol (60 ppm/liter for 7 days).**

Gills: Grade I and II gill hyperplesia were observed in the majority of the examined cases; however, grade III and IV hyperplesia were only seen in one case on the 6th day which showed adhesion of the primary lamellae (grade IV gill hyperplesia) with eosinophilic granular cells infiltration rather than lymphocytes.

Brain, skin and underlying musculature, hepatopancreas, intestine, heart, kidneys and spleen:

The lesions in these organs were nearly similar to those described in acute intoxication with phenol.

B-Chronic Toxicosis:

***Phenol (9 ppm/liter for a month):**

Gills: Grade III gill hyperplesia was the predominant form of gill hyperplesia at this stage of the experiment. Sublamellar edema with eosinophilic granular cells infiltration were also seen (Fig. 9).

Brain:

Severe congestion of cerebral and meningeal blood vessels with perivascular edema and/or lymphocytic cuffing were the main lesions during the first 2 weeks. Moreover, hemorrhages were also seen (Fig. 10). In addition, neuronal degeneration (Fig. 11), satellitosis, gliosis and neuronophagia were evident.

Skin and underlying musculature:

The examination of the skin revealed moderate epidermal thickening due to spongiosis, dermal edema and hyperactivation of the mucous and alarm cells.

Hepatopancreas:

Severe focal and/or diffuse hydropic degeneration of the hepatocytes (Fig. 12) besides congestion of both hepatic and pancreatic blood vessels were the main lesions.

Intestine:

Hyperactivation of goblet cells with submucosal congestion and infiltration of the lamina propria

with numerous lymphocytes were the characteristic lesions.

Heart:

Focal areas of sarcoplasmolysis and hyperactivation of the atrial reticuloendothelial cells (endotheliosis) were observed in few cases.

Kidneys:

There were mild cellular swelling of the tubular epithelium with congestion only during the last week of the experiment.

Spleen:

Areas of lymphoid cell depletion were seen on the last week of the experiment.

***m-cresol (15 PPm/liter for 1-month):**

The histopathological changes were nearly similar as in chronic intoxication of phenol.

Table (1): Number of both sacrificed and dead fish acutely intoxicated with phenol and m-cresol throughout the experiment (7 days).

Days	GP.1 30 PPm Phenol/L		GP.2 60 PPm Cresol/L		GP3 Control		Total
	Dead	Sacrificed	Dead	Sacrificed	Dead	Sacrificed	
1st	2	3	1	4	-	5	15
2nd	2	3	1	4	-	5	15
3rd	1	4	-	5	-	5	15
4th	1	4	-	5	-	5	15
5th	-	5	-	5	-	5	15
6th	1	4	-	5	-	5	15
7th	-	5	-	5	-	5	15
Total	7	28	2	33	-	35	105
%	20	80	5.7	94.3	0	100	

Table (2): Number of both sacrificed and dead fish chronically intoxicated with phenol and m-cresol throughout the experiment (4 weeks).

Weeks	GP.4 30 PPm Phenol/L		GP.5 60 PPm Cresol/L		GP6 Control		Total
	Dead	Sacrificed	Dead	Sacrificed	Dead	Sacrificed	
1st	1	4	-	5	-	5	15
2nd	-	5	1	4	-	5	15
3rd	-	5	-	5	-	5	15
4th	-	5	-	5	-	5	15
Total	1	19	1	19	-	20	60
%	5	95	5	95	0	100	

DISCUSSION

The encountered signs in this study were severe in phenol Toxicosis (GPs. 1 and 4) or mild to moderate in m-cresol Toxicosis (GPs. 2 and 5). These signs were nervous and respiratory in nature. Alabaster and Lloyed (1982) stated that cresol is less toxic than phenol and subsequently the associated signs to phenol Toxicosis will be more severe than that of cresol Toxicosis. These signs could be attributed to the severest gill and brain lesions or due to the direct contact between the tested chemicals and gill tissue.

The above-mentioned signs were nearly similar to those recorded by Waluga (1966 a), Alabaster and Lloyd (1982) El-Manakhly and Soliman (1993) and Safinaz (2000)

The determined LC_{50} and mortality rates in this study were greatly different from those recorded in Common carp (Alabaster and Lloyd, 1982) and in Grass carp (El-Manakhly and Soliman, 1993). Post (1987) reported that the toxic dose of any phenolic compound greatly varied among different fish species and under different environmental factors (i.e. temperature, oxygen content and water pH).

With regard to the pathological findings, the main macroscopic and microscopic changes were present in the gills and brain followed by skin then the intestine. A possible explanation of this is the direct contact between gills, skin and intestine to the dissolved phenolic compounds in water in addition to that these compounds may have an affinity to the nervous tissue (neurotoxicity).

The encountered lesions in gills, brain, intestine and skin were nearly similar to those mentioned by Waluga (1966 a), Roberts (1978), Alabaster and Lloyed (1982), Post (1987) and El-Manakhly and Soliman (1993) and Safinaz (2000)

Concerning the microscopical examination of the hepatopancreas, kidneys, spleen and heart, it was found that the hepatic tissue was the most affected tissue. This may be due to that the liver is the site of detoxification of any toxic materals Moreover, phenol or cresol metabolites are detected mainly in bile not in urine. This may explain the absence of renal lesions.

These results were in partial agreement with those of Schulze (1961), Walgua (1966 a) and Meldahal et al. (1996). However, these results were completely different from those of Waluga (1966 b), Alabaster and Lloyd (1982), post (1987) and E-Manakhly and Soliman (1993) who recorded severe renal and splenic lesions due to phenolic compounds toxicosis. These

differences may be due to variation in the toxic dose of the phenolic compounds, environmental factors and species of the fish or a combination of these factors.

Finally, it could be concluded that phenol was more toxic to Common carp than m-cresol Further work is needed to evaluate the risk of tissue residue of these compounds in fish flesh to the consumers besides their effects, on the reproductive organs of mature fish.

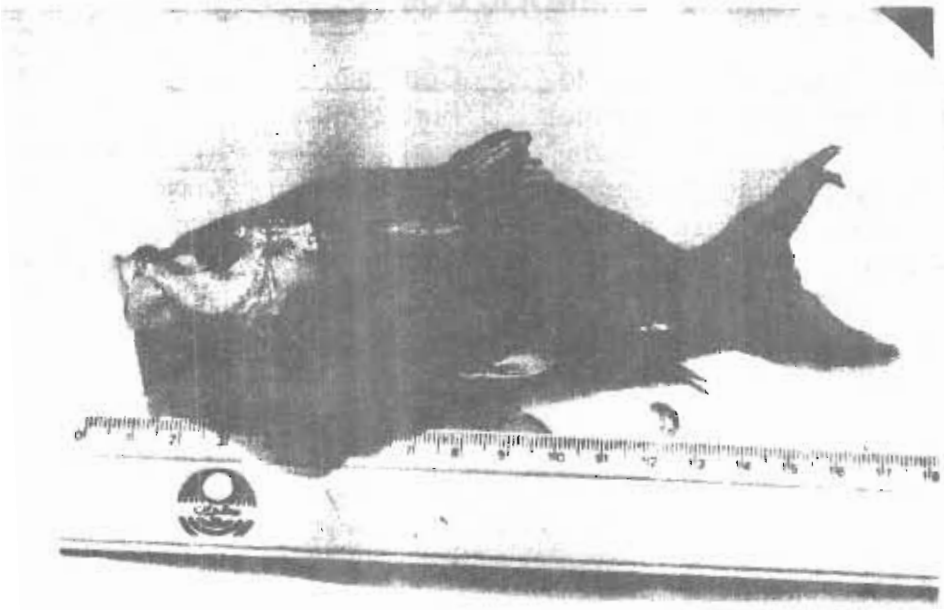

Fig. (1): Common carp fish after 3 days from acute intoxication with phenol: Skin erosions (arrow).

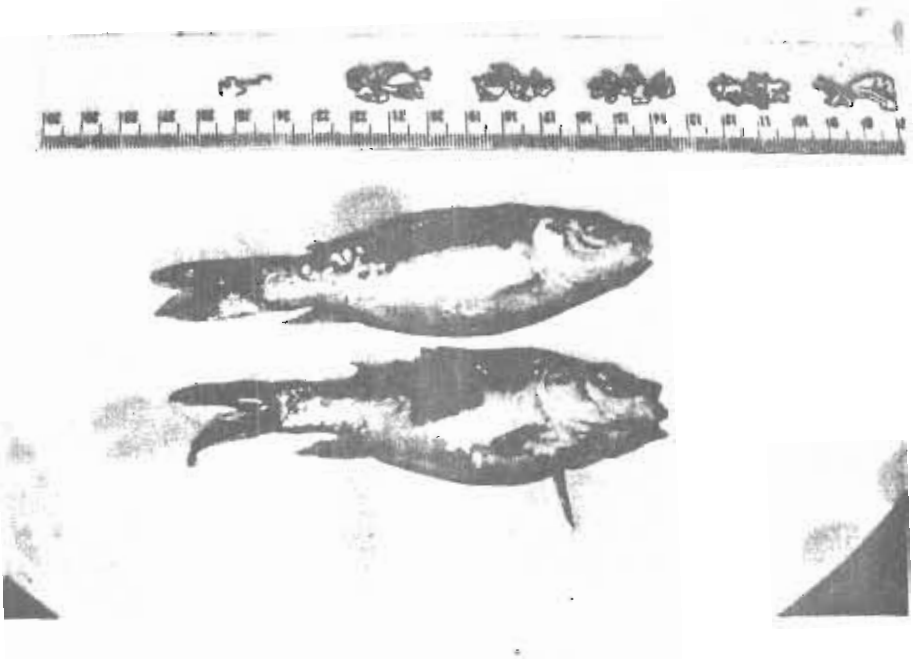

Fig. (2): Common carp fish after 15 days from chronic intoxication with m-cresol : Severe asicitis.

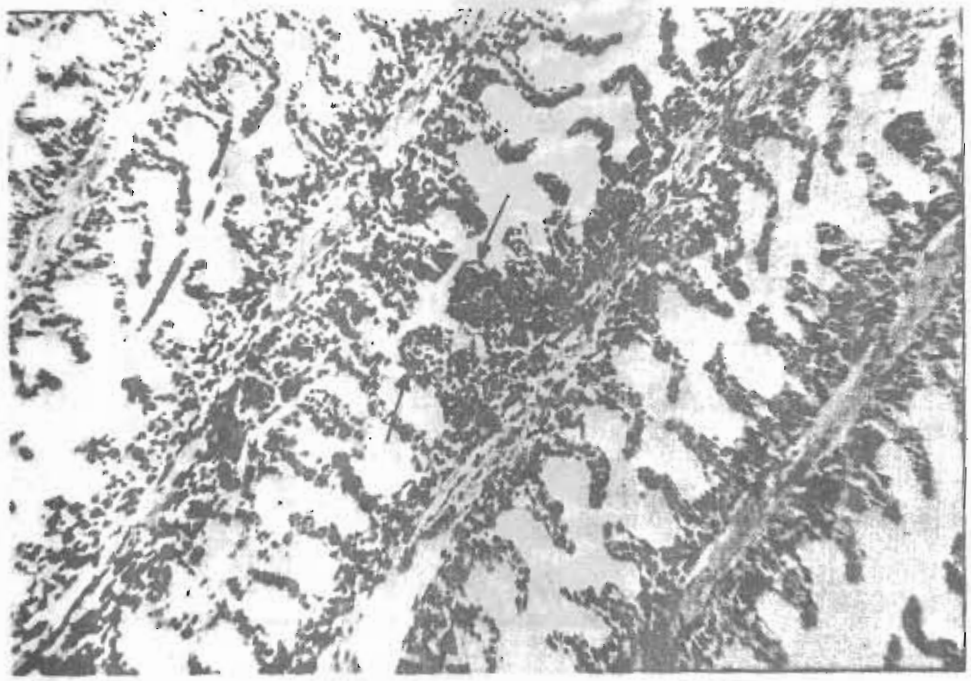

Fig. (3): Gills, of Common carp 3rd day of acute phenol Toxicosis: Grade I hyperplasia with slight thickening of the secondary lamellae (arrows). H & E (X = 160).

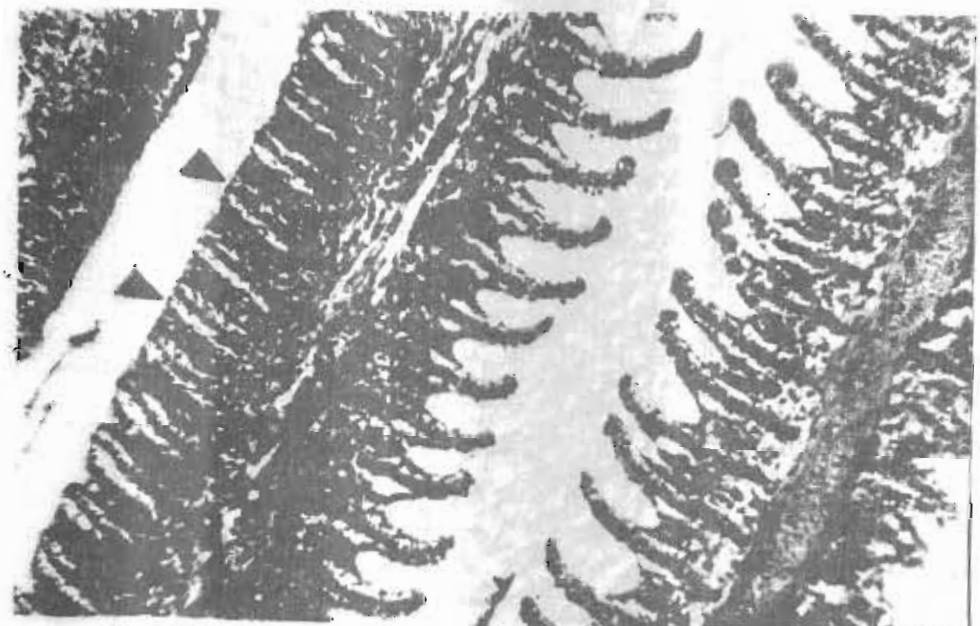

Fig. (4): Gills, of Common carp 3rd day of acute phenol Toxicosis: Grade II hyperplasia with fusion of some secondary lamellae (arrows heads). H & E (X = 160).

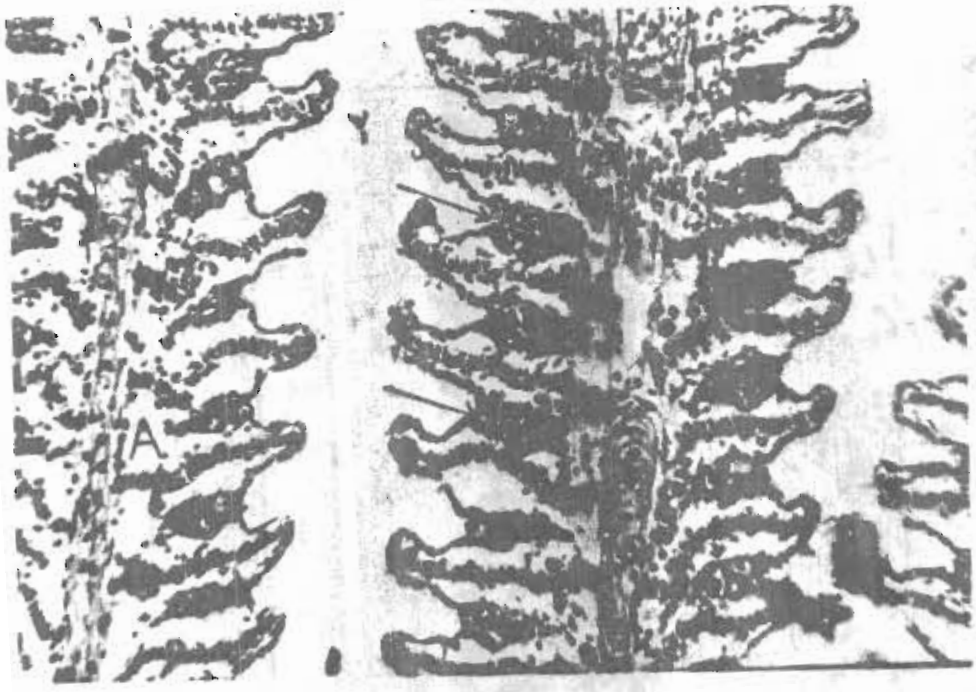

Fig. (5): Gills,of Common carp 4th day of acute phenol toxicosis. Grade III hyperplasia with fusion of the most of secondary lamellae (arrows) besides lamellar edema (A). H & E (X = 250).

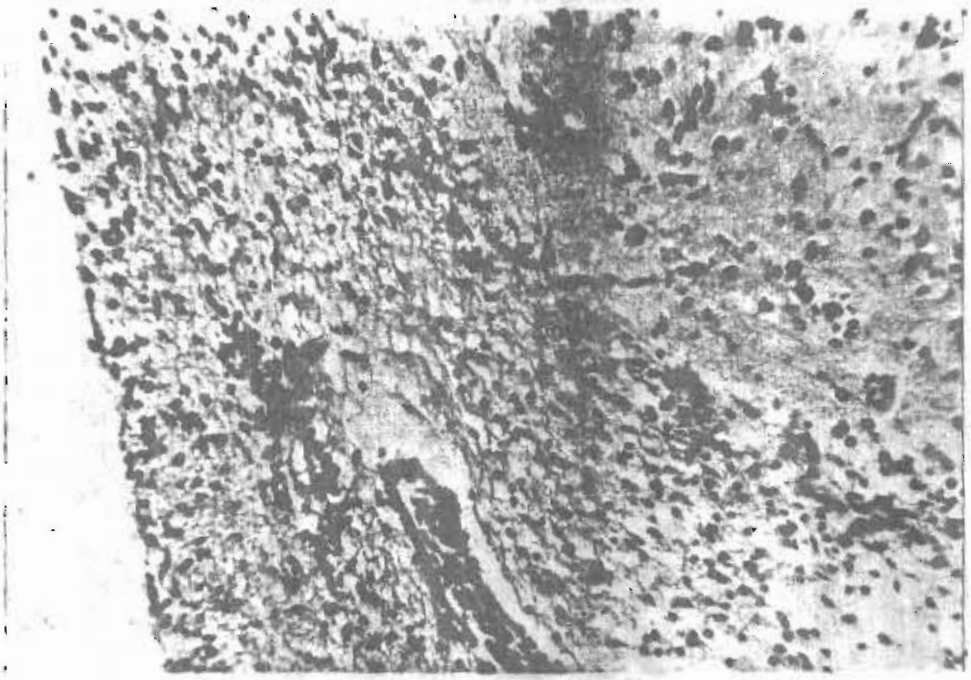

Fig. (6): Brain of Common carp, 2nd day of acute phenol Toxicosis: Congested blood vessel (arrow) with slight perivascular edema and lymphocytic cuffing. H & E (X = 250).

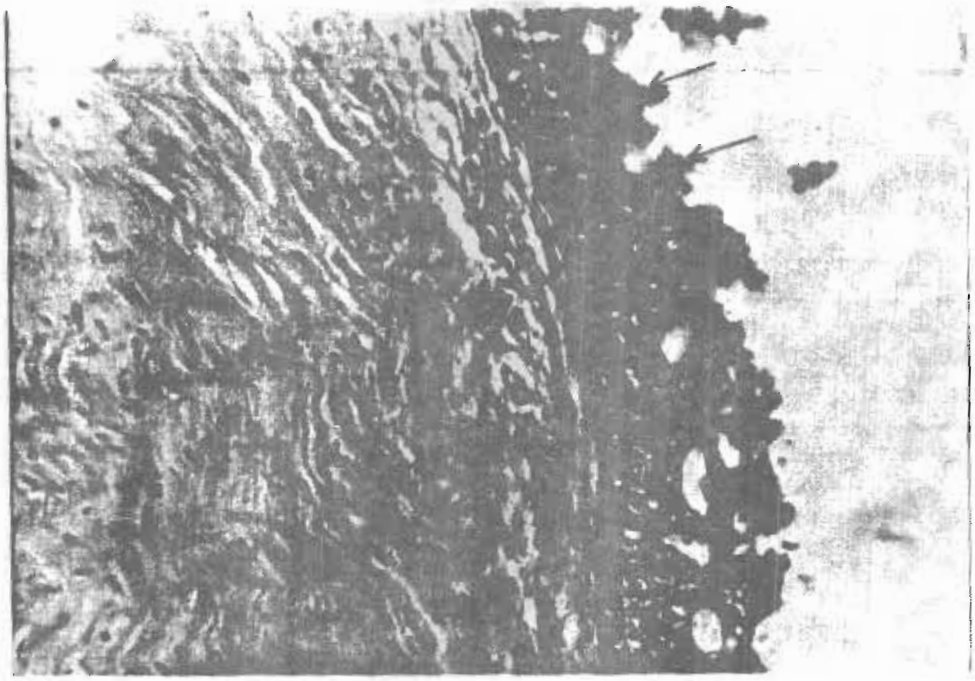

Fig. (7): Skin, 6th day of acute phenol toxicosis: showing detchament of some epithelial cells (arrows) with cystic cavitations (arrowheads) and dermal melanosis (A). H & E. (X = 250).

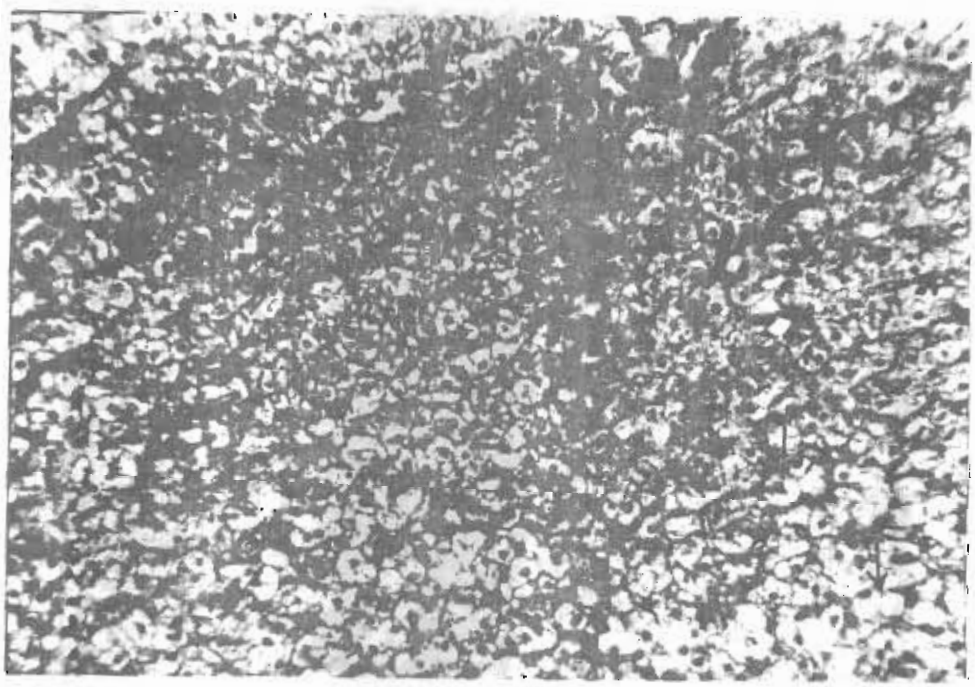

Fig. (8): Hepatopancreas, 2nd day of acute phenol toxicosis: showing hydropic degeneration of the hepatocytes (arrows). H & E. (X = 250).

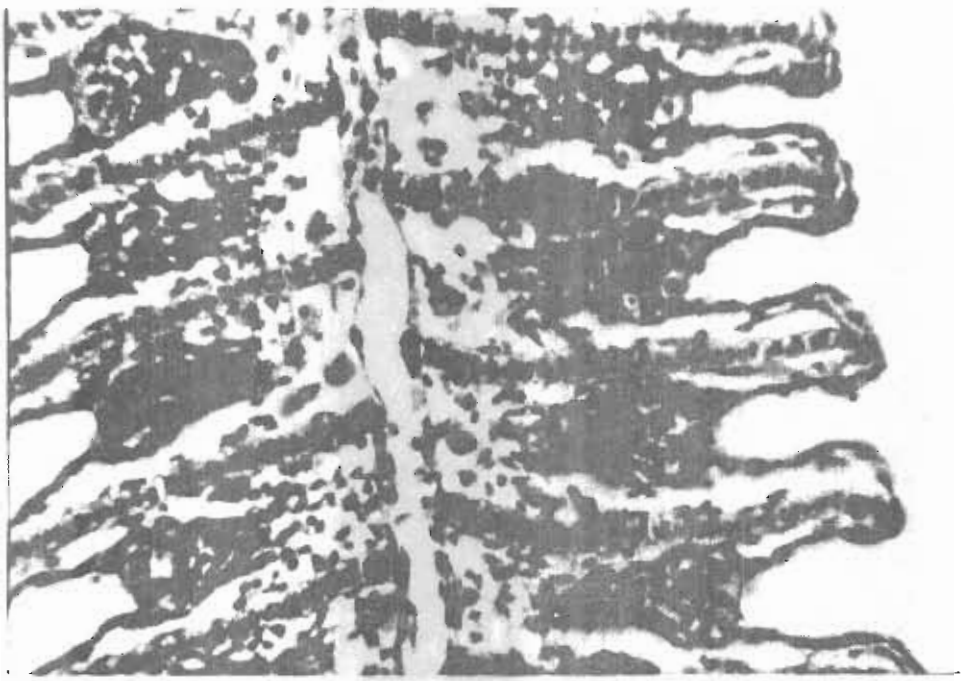

Fig. (9): Gills, 4th week of chronic phenol toxicosis: showing severe lamellar edema with eosinophilc cell aggregation (arrowheads). H. & E (X = 400).

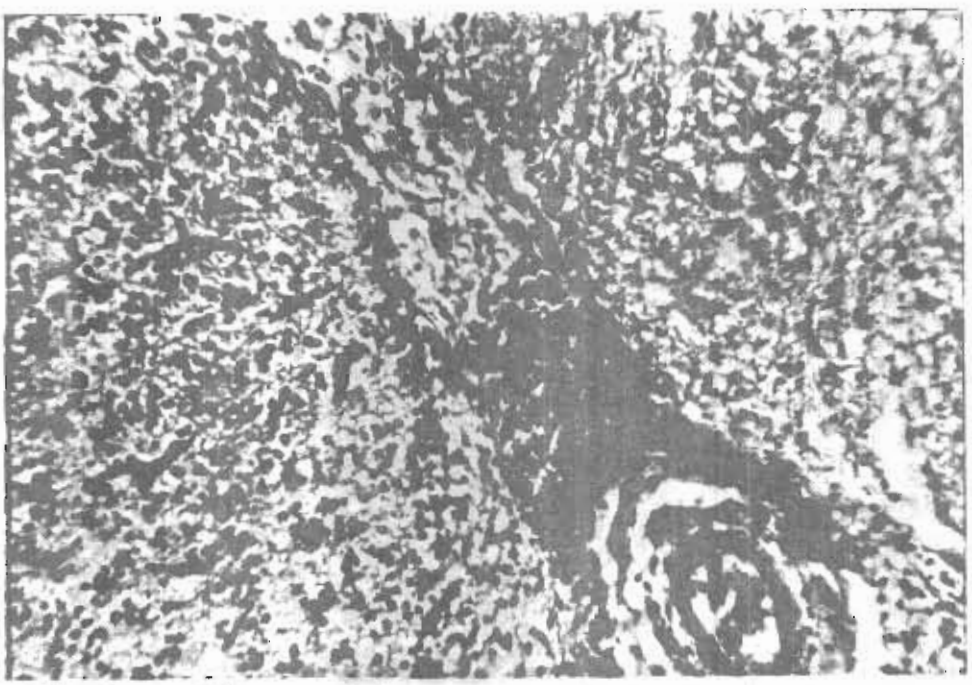

Fig. (10): Brain, 2nd week of chronic phenol toxicosis: showing congestion (arrow) and hemorrhage (arrowheads). H & E (X = 250).

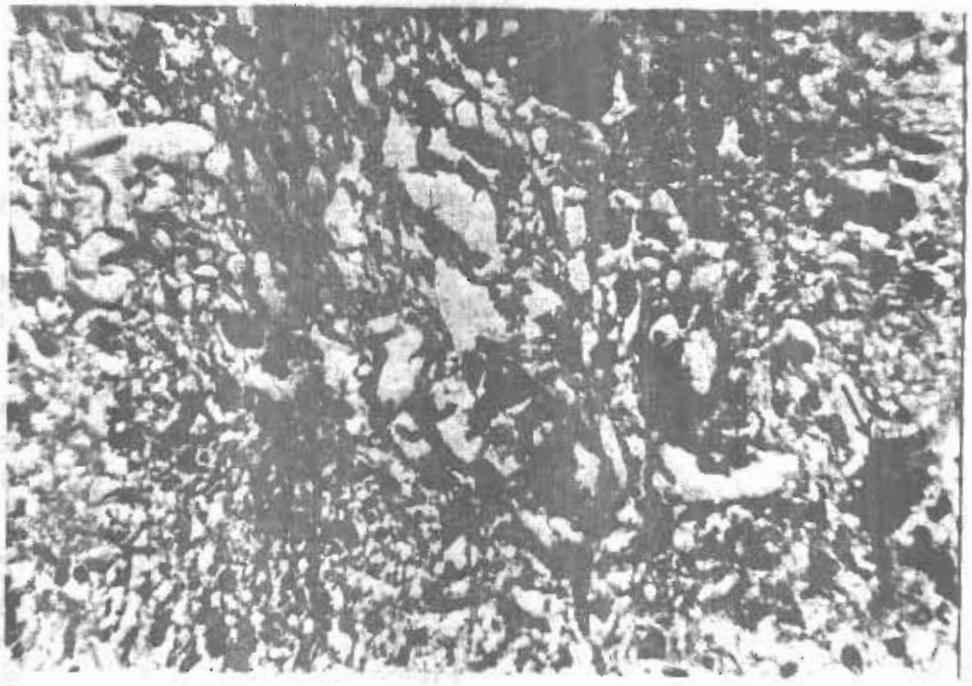

Fig. (11): Brain, 4th week of chronic phenol toxicosis: showing neuronal degeneration (arrow) H & E (X = 400).

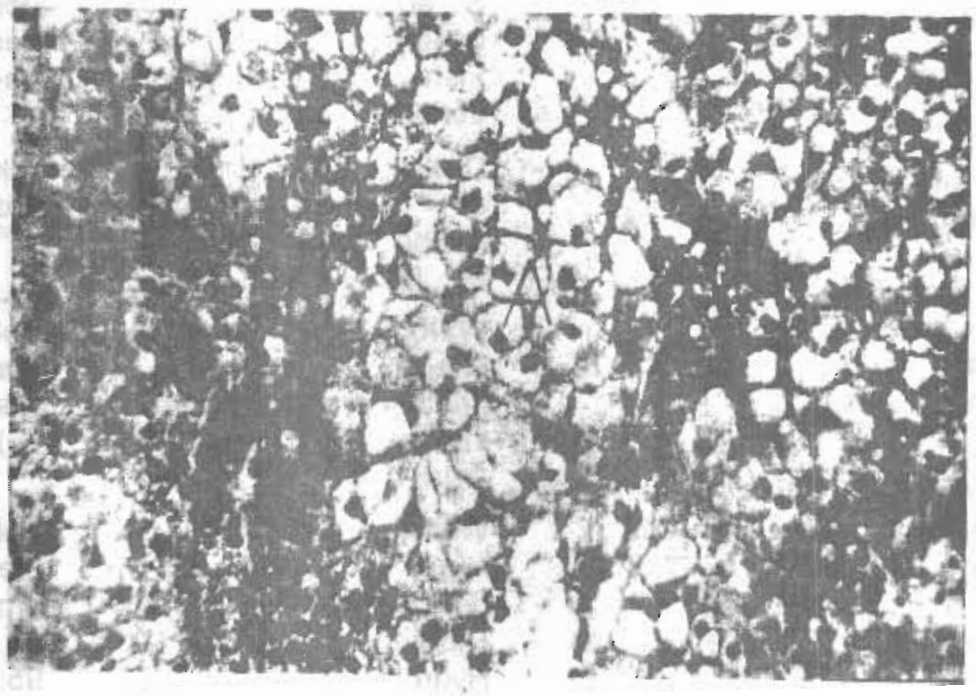

Fig. (12): Hepatopancreas, 1st week of chronic phenol toxicosis. showing severe hydropic degeneration (A) of the hepatocytes. H & E (X = 400).

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