

HISTOPATHOLOGICAL, HISTOCHEMICAL AND PHYSIOLOGICAL STUDIES ON THE EFFECT OF THE INSECTICIDE "HOSTATHION" ON THE LIVER OF THE CATFISH *CLARIAS gariepinus*

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ABSTRACT

In the present work experiments were carried out to investigate the hazardous effects of the organophosphorous insecticide (Hostathion) on the histology, histochemistry and physiology of the liver of the catfish (*Clarias gariepinus*) after exposure to LC_{50} , $\frac{1}{2}LC_{50}$ and $\frac{1}{4}LC_{50}$ of the insecticide for different periods. The results showed that the histopathological changes induced in the liver were mainly represented by liver cord disarray, cytoplasmic vacuolization of the hepatocytes, damage of blood sinusoids, blood vessel congestion and inflammatory leucocytic infiltration. The histochemical observation revealed marked reduction in glycogen contents and total protein contents of the liver cells as compared with the control material. Physiologically, different treatments at different time intervals resulted in variable degrees of inhibition in 5'-nucleotidase and alkaline phosphatase activities. These changes were both concentration- and time- dependent.

INTRODUCTION

Pesticides have been widely used all over the world for several decades to eliminate pests. Random and indiscriminate use of pesticides have been continuously creating serious problems to aquaenvironment since these pesticides usually find their way to the aquatic ecosystem through various routes (Caldas *et al.*, 1999 and Hassanein, 1999). Therefore, it is necessary to study in detail the possible impacts of these hazardous chemicals on the aquatic

organisms with special emphasis on the most economic ones, viz, the fishes. Such pesticides are also known to have affinity for residing in animal tissues, especially in the fatty ones (Deichmann *et al.* 1975).

There is considerable information indicating that pesticides are responsible for many adverse impacts in fishes and other animals from the histopathological, histochemical and physiological points of view (Bansal *et al.*, 1979; Verma *et al.*, 1983; Sastry and Siddique, 1984; Abdel-Aziz, 1985; Hassanein, 1991; Soliman *et al.*, 1993; Elezabi *et al.*, 2001).

In Aswan governorate, which represents the local area of the present investigation, thousands of tons of insecticides, especially organophosphorous compounds, are dumped and used for eradication of insects around the shore of High Dam lake as such area can be a passway to the invasion of dangerous insects to Egypt from the Sudan. Of these insects, is the mosquitos, *Anopheles gambiae*, which carries the causative agent *Falci-parum malaria*. For eradication of these dangerous insects the Malaria station in such areas uses all types of insecticidal protection at all possible modes of application in terms of using dusting powder, solutions, emulsions and water suspensions. These insecticides find their way directly to High Dam lake causing water pollution which became a great hazard for aquatic life. This hazardous problem attracted the attention of a number of investigator to initiate a series of investigations. In the present investigation, the organophosphorous insecticide hostathion was chosen to study its effects on histological, histochemical and physiological aspects of the liver of one of the economic fresh water fish namely the catfish "*Clarias gariepinus*".

MATERIALS AND METHODS

Experimental animals:

Living samples of *Clarias gariepinus* were collected from the High Dam Lake, each weighing 500-750 g. The fish samples were transported in well-aerated containers to large tanks in the laboratory. These tanks contain Nile water and were continuously aerated using air pumps. The fish samples were kept for at least 24 hours in these tanks before experimentation. The fishes were provided with suitable food of algae and grass.

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Tested pollutant:

An organophosphorous insecticide was used in the present study, namely Hostathion. This insecticide is widely used for eradication of gambian mosquitoes at the region of High Dam Lake. Its active ingredient in the commercial compound was 40%. Dilutions of different concentration were prepared by emulsification with water.

Determination of LC₅₀:

The concentration of hostathion which causes 50% live-death response (LC₅₀) at 96 hours was determined. Groups of 10 fishes were exposed to gradual concentrations (Table 1) of the insecticide for 96 hours. LC₅₀ value was obtained by plotting the percentage of mortality versus concentration on semi-log paper (Fig. 1).

Experimental design:

The experimental fishes were divided into four groups.

The 1st group: 25 fishes were exposed to LC₅₀ (3 mg / l) of hostathion for 24 hours exposure period in specially equipped aquaria (80 x 50 x 50 cm). During this period, the fishes that die were immediately removed. On the other hand, the surviving individuals were decapitated after the intervals of 6, 12 and 24 hours post exposure.

The 2nd group: 25 fishes were exposed to acute treatment ($\frac{1}{2}$ LC₅₀) of hostathion. This concentration was prepared by emulsifying 1.5 mg/l in the Nile water. Fishes were kept under observation for 4 days exposure period and sampled after 2, 3, 4 days post exposure.

The 3rd group: 25 fishes were exposed to chronic treatment ($\frac{1}{4}$ LC₅₀) (0.75 mg / l). The experiment continued for 15 days and sampled after 5, 10 and 15 days from the beginning of exposure.

The 4th group: 10 fishes were used as a control and kept in Nile water without any treatment.

At the end of each exposure time, fishes were decapitated and the liver was taken for determination of different investigations.

Histological and histochemical investigations:

Immediately after decapitation, the fishes were dissected and small pieces of the liver were fixed in 10% formalin and Carnoy's fluid. The fixed samples were dehydrated in ascending series of

ethanol, cleared in methyl benzoate and embedded in paraffin wax. Sections of 5-7 μm thickness were cut, mounted and stained with different stains according to the target of investigation. For histopathological investigations, 10% formalin - fixed sections were stained with haematoxylin and eosin. For histochemical investigations, materials fixed in Carnoy's fluid were stained with periodic acid Schiff's (PAS) technique (Hotchkiss, 1948) for demonstration of polysaccharides (liver glycogen). Total proteins were demonstrated by the mercury bromophenol blue method (Mazia *et al.*, 1953).

Physiological investigation

Immediately after decapitation, the liver was removed, washed, homogenized and kept in deep freezer at -20°C until used. 5'-Nucleotidase assay was performed using the method of El-Aaser and El-Merzabani (1975). The principle of this method depends on the incubation of the specific substrate adenosin-5-monophosphate with the enzyme at pH 7.5 and determination of the liberated inorganic phosphorous. The formed colour is estimated at wave length 640-720 nm. Alkaline phosphatase activity was estimated using the method of Kind and King (1954). The method is based on the hydrolysis of disodium phenyl phosphate with the liberation of phenol and formation of disodium phosphate. In the presence of alkaline oxidizing agents 4-amino-phenazone, 4-amino-antipyrine, a red or purple colour is formed with compounds containing a phenolic group. The amount of the formed phenol is estimated colourimetrically at wave length 520 nm.

RESULTS

Histopathological results:

In liver sections of normal fish the hepatocytes form a rather cord-like pattern (Fig. 2). These cords are arranged around tributaries of the hepatic vein. The liver cells are large in size, polygonal in shape with homogenous eosinophilic cytoplasm and centrally located nuclei. A large number of blood sinusoids were also observed.

Haematoxylin and eosin stained sections of the fish liver have displayed pathological changes after treatment with different concentrations of the applied insecticide.

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Effect of LC₅₀:

Exposure of *Clarias gariepinus* to LC₅₀ (3 mg / l) of hostathion for 6 hours had induced obvious histopathological changes in the liver. These changes included marked pathological changes of hepatic cells, inflammatory leucocytic infiltrations and congestion of the intrahepatic blood vessels (Fig. 3). After 12 hours, the hepatocytes have lost their normal architecture and a large number of these cells appeared with pyknotic nuclei. The sinusoidal lumina were constricted and congested with blood cells (Fig. 4). The histopathological changes of the liver were more pronounced after 24 hours; the liver cells were degenerated and suffered from cytoplasmic vacuolization (Fig. 5). The intrahepatic blood vessels were dilated and congested .

Effect of ½ LC₅₀

Treatment of fishes with this concentration for 2 days induced remarkable pathological changes. The hepatocytes lost their cord-like arrangement and the tissue displayed some necrotic areas (Fig. 6). Marked congestion of blood vessels was observed after 3 days of treatment (Fig. 7) and the lumens of hepatic sinusoids were narrowed. Later on, after 4 days, clear degeneration of hepatocytes was detected; the cells exhibited large number of cytoplasmic vacuoles and pyknosis of nuclei. The blood sinusoids could not be seen as they suffered great narrowing (Fig. 8).

Effect of ¼ LC₅₀

Treatment of fishes with this concentration of hostathion for five days had caused disarray of hepatic cords, the blood sinusoids appeared containing degenerated blood cells. The toxic change was more severe after 10 days of exposure to this concentration, the tissue showed marked inflammatory leucocytic infiltrations and congestion of blood vessels (Fig. 9). The histopathological pathological lesions observed at the end of treatment (15 days) can be summarized as follows: the hepatocytes appeared enlarged and their nuclei suffered from karyolysis (Fig. 10). Some specimens showed hepatocytes with severe cytoplasmic vacuolization and a large number of binucleated cells were observed (Fig. 11). The blood sinusoids became so narrow that they almost became obscure (Fig. 11).

Histochemical results:**Glycogen content**

In normal liver of *Clarias gariepinus*, large amounts of glycogen were observed in the cytoplasm of the hepatocytes as indicated by large number of reddish fine granules of different sizes (Fig. 12a).

After exposure of fishes to LC₅₀ (3mg / l) of hostathion for 6 hours, glycogen content of the liver cells decreased (Fig. 12b). This diminution of glycogen inclusions became more pronounced after 12 hours of treatment. Later on and after 24 hour of treatment, glycogen loss was more prominent in the hepatic tissue. This diminution was quite evidenced in the amount and stainability as represented in Fig. 12c. After treatment with ½LC₅₀ for two days, the liver tissues showed a highly decrease in its glycogen content in the cytoplasm of most of the hepatocytes (Fig. 12d). The above feature became more marked after 3 and 4 days in both quantity and stainability (Fig. 12e). A marked reduction of glycogen contents was noticed in different hepatocytes as noticed after 5 days of exposure to ¼LC₅₀. Most of these hepatocytes embodied large vacuoles in their cytoplasm and glycogen inclusions were confined to the intervacuolar areas. The reduction of glycogen inclusions became more marked after 10 days of treatments. This diminution was more evidenced in both amount and stainability of these substance located in between the extensive cytoplasmic vacuoles (Fig. 12f). At the end of exposure period (after 15 days), glycogen content displayed faint stainability and became hardly detectable.

Total proteins

In the hepatocytes of normal *Clarias gariepinus*, total proteins appeared as intensely dark blue coloured inclusions in the cytoplasm. Chromatin bodies and nucleoli exhibited a deep colouration with bromophenol blue (Fig. 13a).

Total proteins were found to exhibit a slight decrease in cytoplasm and nucleus of the liver cells of *Clarias gariepinus* treated with LC₅₀ (3mg/l) of hostathion after lapse of 6 hours as designated in (Fig. 13b). After 24 hours of treatment, such decrease was more pronounced in the hepatocytes which appeared vacuolated (Fig. 13c). A higher decrease in protein content was detected in both the amount and stainability after exposing the experimental fishes to ½LC₅₀ followed by examination after lapse of 2 days (Fig. 13d). This decrease of proteinic inclusion was found to be more striking in both

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the relative amount and intensity in the hepatocytes examined After treatment ($\frac{1}{4}LC_{50}$) with hostathion, considerable decrease of proteinic materials was observed in the liver. After 15 days, there was an obvious reduction in the protein contents and their remnants were mainly located at the peripheries of the hepatic cells which showed sever cytoplasmic vacuolation (Fig. 13f).

Physiological results:

5'-nucleotidase

Treatment of *Clarias gariepinus* with the LC_{50} of hostathion for 24 hour had induced a highly significant ($P < 0.01$) reduction in the activity of 5'-nucleotidase. The enzyme activity was reduced by 68.76%, 72.88% and 77.47% after 6, 12 and 24 hours of treatment, respectively. Exposing fish to $\frac{1}{2}LC_{50}$ of hostathion induced a great inhibition in the 5'-nucleotidase enzyme activity in the liver. The percentages of the inhibition were 72.53%, 74.04% and 78.36% after 2, 3, 4 days post treatment, respectively. These inhibitions were found to be highly significant ($P < 0.01$). Chronic treatment of fishes with ($\frac{1}{4}LC_{50}$) of the insecticide had induced marked and highly significant ($P < 0.01$) inhibition in the activity of 5'-nucleotidase in the liver. The percentage of this inhibition recorded its highest value (81.87%) after 5 days post-exposure, then its decline was 78.04% and 74.76% after 10 and 15 days respectively (Table 2).

Alkaline phosphatase

Fishes exposed to LC_{50} of hostathion showed that the activity of alkaline phosphatase in the liver was subjected to significant ($p < 0.05$) inhibition after 12 and 24 hours of treatment. A marked inhibition of this enzyme was recorded after treatment with $\frac{1}{2}LC_{50}$. The inhibition percentages were 66.92%, 69.45% and 71.91% after 2, 3 and 4 days respectively. All these inhibitions were highly significant ($P < 0.01$). A highly significant ($P < 0.01$) reduction in the alkaline phosphatase activity was recorded after treatment with $\frac{1}{4}LC_{50}$ of hostathion. The percentage of this reduction were 65.80% after the first 5 days and increased through 10 days of the experiment (71.54%) where it reached its highest value (74.71%) after 15 days of treatment (Table 2).

DISCUSSION

Numerous organophosphorous insecticides are widely used in many parts of Egypt to control insects, pests and disease vectors. Many of the treated areas contain freshwater resources like streams, lakes and ponds which harbour diverse aquatic fauna and flora. In the present investigation a study was carried out on the effect of the organophosphorous insecticide "hostathion" on the Nile fish *Clarias gariepinus*. This organophosphorous compound is dumped near and around the shores of High Dam Lake to eradicate the Gambia mosquito.

It was found that hostathion induced marked histopathological changes in the liver of catfish at different intervals and treatment with three concentrations i.e., LC_{50} , $\frac{1}{2}LC_{50}$ and $\frac{1}{4}LC_{50}$.

It is well known that the liver is the first target organ in toxicological prospects regarding its role in detoxification, biotransformation and excretion of xenobiotics. After enteric uptake of injurious materials, it is the first organ to be exposed to such hazards via the portal circulation (Wester, 1988).

In the present study, examination of liver sections obtained from fishes exposed to different concentrations of hostathion has shown marked histopathological lesions. These lesions included disarray of hepatic cords, cytoplasmic vacuolization of the hepatic cells, nuclear pyknosis, inflammatory leucocytic infiltrations and congestion of blood vessels.

Such lesions were previously observed by many investigators in the liver of different fish species under the effects of different types of insecticides. Eller (1971) found that there were cellular changes in the liver of *Salmo clarki* following food or water exposure to 0.01 mg / kg b.w. or 0.01 ppm of the insecticide endrin. Malathion was found to induce histopathological lesions in the liver of *Channa punctatus* (Dubale and Shah, 1979). Mandal and Kulshrestha (1980) studied the effects of sublethal concentration of sumithion on liver, kidneys and intestine of *Clarias batachys*. They observed liver necrosis, vacuolization and breakdown of the cell boundaries. They also observed vacuolization of epithelial cell of uriniferous tubules and degeneration of the glomeruli in the kidney, while in the intestine, they noticed lesion formation in the villi and enlargement of mucous cells. Histological changes in the liver of *Tilapia mossambica* after exposure to the organophosphate monocrotophos were reported by Desai *et al.*, (1984). At the initial stage of intoxication, necrosis and

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vacuolization of hepatocytes were recorded, while fatty degeneration was observed later on. Treatment with endrin produced acute pathological changes in the liver of *Channa punctatus* (Sastry and Sharma, 1979).

Hassanein (1991, 1999) reported that exposure of *Gambusia affinis* or Nile Bolti *Oreochromis niloticus* to different concentrations of malathion or goal has resulted in liver cord disarray, necrosis, cytoplasmic vacuolization of hepatic cells and disintegration of blood materials. These lesions were both concentration and time dependent. Similar findings were also presented by Soliman *et al.*, (1993) in *Gambusia affinis* exposed to acute and chronic concentrations of malathion. Elezabi *et al.* (2001) studied the effect of malthion on the fish *Oreochromis niloticus* and their results showed that this insecticide induced many histopathological changes in the liver and gills of the fishes. These changes were hemorrhage, necrosis and destruction of lamellae of the lungs, and necrosis and lipidosis in the liver.

It was found that different concentrations of hostathion induced gradual and marked reduction in glycogen content in the hepatocytes. These results are in full agreement with those achieved by some authors who have investigated the effects of different pesticides on fishes.

In this concern, Singh and Srivastava (1981) mentioned that carbohydrates decreased as a result of exposure to a sublethal concentration of a mixture of aldrin and formothion. These insecticides had induced marked diminution in the glycogen content of the liver and muscles of this fish. Exposure of freshwater fish *Mystus vittatus* to sublethal concentrations of the two pesticides thiothox and dichloruos for one month was found to induce marked depletion in both liver and muscle glycogen (Verma *et al.*, 1983). Sublethal concentrations of quinalphos resulted in reduction of glycogen in the liver of *Channa punctatus* (Sastry and Siddique, 1984). Shalaby (1985) remarked that carbohydrate contents in the intestinal mucosal cells of the catfish *Clarias gariepinus* were subjected to marked changes after exposure to a sublethal concentration of the insecticide cyolane. The author suggested that carbohydrate depletion might be due to the disturbed role of the Golgi apparatus in polysaccharide synthesis under the insecticide stress, which in turn, pushes the fish to utilize their reserve glycogen for energy needs.

Total protein contents of the liver of *Clarias gariepinus* showed a noticeable decrease after treatment with hostathion. These results agree with those of Hassanein (1991, 1999) and Soliman *et al.* (1994) who reported that treatment of fishes with malathion had induced a highly significant decrease of protein contents of the liver, testis and ovary. Hepatic total protein content significantly decreased after treating freshwater fish *Labeo rohita* with chlordane (Bansal *et al.* 1979). Sancho *et al.* (1998) reported that total proteins had decreased in liver of the European eel, *Anguilla anguilla* after fenitrothin exposure for different time intervals up to 96hrs. Hagazi *et al.* (1988) investigated the effect of dursban on RNA and proteins in white muscles of *Clarias gariepinus*. Their results proved that RNA and proteins significantly decreased and the authors attributed such decrease to an increase in the rate of protein catabolism in the muscle for energy maintenance.

The data of the present investigation reflect marked reduction in the activity of 5'-Nucleotidase in the liver of *Clarias gariepinus* after exposure to different concentrations of hostathione. 5'-Nucleotidase is a specific phosphatase enzyme acting on both pyrimidine and purine nucleotides with a phosphate group attached to the fifth carbon atom of the pentose ring (Sherlok, 1981).

The present results are in agreement with the results of Abdel-Aziz (1985) who studied the effect of dimethoate, malathion and hostathion on the freshwater fish *Oreochromis niloticus* and found that the three insecticides had caused a significant reduction in the enzyme activity. The author marked that malathion was the most potent toxicant and reported no complete recovery in the enzyme activity after discontinuation of exposure of fishes to sublethal concentration for 24 hours. Also, Hassanein (1991, 1999) recorded a highly significant inhibition of 5'-nucleotidase activity in the liver, testis and ovary of fresh waterfish *Gambusia affinis* and the liver and kidneys in the *Oreochromis niloticus* after both acute and chronic exposure to the malathion and goal. There was no complete recovery in the enzyme activity after exposure of the fishes to fresh nontoxicated water for 15 days. It seems that the reduction in the 5'-nucleotidase activity in some tissue might affect the synthesis of nucleic acid, and is ascribed to the unavailability of specific nitrogen bases necessary for the biosynthesis processes (Abdel-Aziz, 1985).

Data obtained in the present study revealed marked inhibition of the activity of alkaline phosphatase in the liver of *Clarias gariepinus* after different treatments with hostathion. This is

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presumably attributed to the fact that the liver is the first target organ of detoxification of such hazardous compounds. This result is in agreement with that of many authors who have investigated the impacts of different pesticides on fishes. In this regard, Verma *et al* (1983) observed marked reduction in the activity of alkaline phosphatase in the liver, kidneys and gills of *Mystus vittatus* after exposure to sublethal concentrations of the three pesticides, thiothox, dichloroous and carbofuran. Also, Hassanein (1999) reported inhibition of alkaline phosphatase in the liver of *O. niloticus* after treatment with different concentration of goal and attributed this reduction to a decrease in the transphosphorylation activity in the liver, which in turn indicates a disturbance in liver functions.

In conclusion, the present study showed that hostathion affected the structure and functions of the liver of *Clarias gariepinus* and this effect was concentration- and time-dependent.

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Table 1: Determination of 96h. LC₅₀ of the insecticide hostathion to the catfish (*Clarias gariepinus*)

Concentration (mg/l)	% Mortality
1.0	10
1.5	20
2.0	30
2.5	40
3.0	50
3.5	60
4.0	70
4.5	80
5.0	90
5.5	100

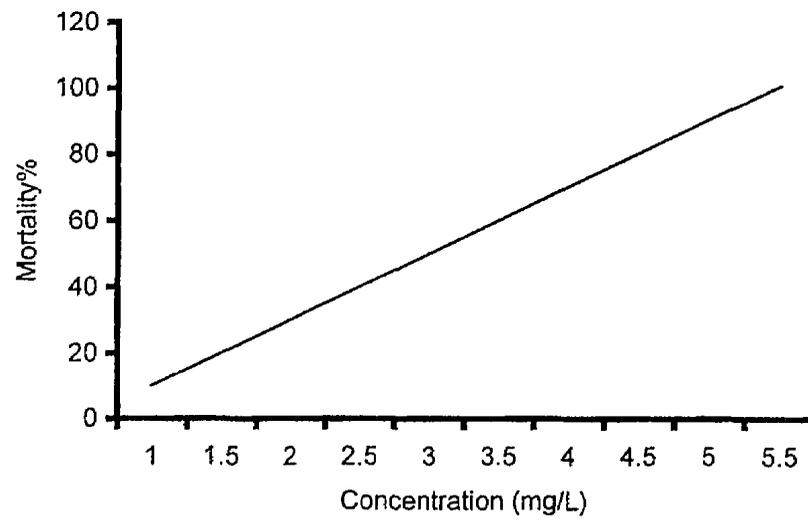
Table (2). Change in 5'-nucleotidase and alkaline phosphatase activity in the liver of catfish after treatment with different concentrations of hostathion.

Conc.	Exposure time	5'-nucleotidase		Alkaline phosphatase	
		Mean ± SD	% inhibition	Mean ± SD	% inhibition
LC ₅₀	0 (control)	2.250±0.04	0	38.85±2.25	0
	6 hrs.	0.705±0.01**	-68.67	28.05±1.18	-27.79
	12	0.610±0.05**	-72.88	21.08±2.01*	-45.74
	24	0.507±0.06**	-77.47	18.75±0.50*	-51.37
½ LC ₅₀	2 days	0.618±0.04**	-72.53	12.85±0.78**	-66.92
	3	0.584±0.03**	-74.04	11.84±0.81**	-69.45
	4	0.487±0.02**	-78.36	10.91±0.080**	-71.91
¼ LC ₅₀	5 days	0.408±0.05**	-81.87	13.28±1.09**	-65.80
	10	0.494±0.07**	-78.04	11.05±1.07**	-71.54
	15	0.568±0.08**	-74.76	10.21±1.03**	-74.71

- Activity expressed as μ mol/Pi/min/g. fresh tissue in 5'-nucleotidase and as kind and king unit/g. fresh tissue in alkaline phosphatase.

(*). Significant at $p < 0.05$, (**) significant at $p > 0.01$

Fig. (1): Lethal-mortality curve of hostathion on the catfish



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- Fig. 10. Section in the liver of a fish treated with $\frac{1}{4}LC_{50}$ for 15 days showing hepatocytes with nuclei suffering from karyolysis (arrows), X320.
- Fig. 11. Section in the liver of a treated fish showing hepatic cells with cytoplasmic vacuolization and binucleated liver cells (arrows), X320.
- Fig. 12. Sections in the liver stained with PAS technique for demonstration of glycogen. a: control fish; b: 6 hours after exposure to LC_{50} of hostathion; c: 24 hours after exposure to LC_{50} of hostathion; d: 2 days after exposure to $\frac{1}{2}LC_{50}$ of hostathion; e: 4 days after exposure to $\frac{1}{2}LC_{50}$ of hostathion; f: 10 days after exposure to $\frac{1}{4}LC_{50}$ of hostathion.
- Fig. 13. Sections in the livers stained with mercury bromophenol blue for total proteins. a: control fish; b: 6 hours after exposure to LC_{50} of hostathion; c: 24 hours after exposure to LC_{50} of hostathion; d: 2 days after exposure to $\frac{1}{2}LC_{50}$ of hostathion; e: 4 days after exposure to $\frac{1}{2}LC_{50}$ of hostathion, f: 15 days after exposure to $\frac{1}{4}LC_{50}$ of hostathion.

