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THE EFFECT OF MALATHION ON THE HISTOPATHOLOGICAL CHANGES IN THE LIVER OF *Gambusia affinis*

BY

Faiza M. Soliman*; Ibrahim A. El Elaimy and Hamdy M. Hamada***

* Dept. of Zoology, Faculty of Science (Sohag), Assiut University, Egypt.
** Dept. of Zoology, Faculty of Science, Al Monofeya University, Egypt.

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ABSTRACT

The histopathological changes in the liver of fresh water fish ***Gambusia affinis*** were studied after acute and chronic exposure to malathion. Acute treatment with high dose (2.2 mg / L) of malathion induce pathological lesions in the liver. These lesions included liver cord disarray,necrosis nuclear disarray,enlargement of the hepatocytes and loss of their polygonal shape. Disintegration of blood materials and blood sinusoids were also observed. A gradual increase in the damage was noticed by longer period of exposure. During chronic exposure to low dose (0.044 mg / L), the histopathological changes in the liver were similar to those observed during acute treatment but in lower intensity. The liver returned, partially, to its normal picture after 15 days in fresh untreated water (recovery period).

INTRODUCTION

Treatment of several species with different pesticides induced , not only marked changes in weight and functions of different organs , but

also obvious histopathological changes in these organs. Several organophosphate pesticides (OP) were found to be potential inducers for certain pathological symptoms in liver of fishes. Dubale and Shah (1979) investigated the histopathological lesions induced by malathion in the liver of ***Channa punctatus***. They observed destruction of cytoplasmic and nuclear material , nuclear disarray and shrinkage lading to damage of the protoplasmic material , consequent vacuolization of the hepatocytes and necrosis. They reported also that the liver damage was dose and period-dependent. Mandal and Kulshrestha (1980) studied the pathological symptoms which induced by sumithion in the liver of ***Clarias batrachus***. They observed liver necrosis, vacuolation and breakdown of cell boundaries after exposure to sublethal dose of the pesticide. Desai et al.(1984) investigated the histopathological changes in the liver of ***Tilapia mossambica*** after treatment with monocrotophos. They observed necrosis and vacuolation of hepatocytes at the initial stage of intoxication , while fatty degeneration observed later. They also noticed normalization of the histological picture of liver with only scattered lesions after stopping the treatment. The authors added that the necrotic changes were observed again with further treatment with the drug. Gabr (1986) reported that exposure of ***Tilapia nilotica*** to increased sublethal doses of diazinon caused pathomorphological changes in liver including vacuolation of the cytoplasm and protrusion in the nuclear membrane at several portions and the magnitude of changes was dose-dependent.

In order to obtain better insight into the present environmental health problem,the present study was undertaken to investigate the morphological changes in the liver of ***Gambusia affinis*** following malathion intoxication and thus to evaluate the pathogenesis of liver lesions in malathion intoxication.

MATERIALS AND METHODS

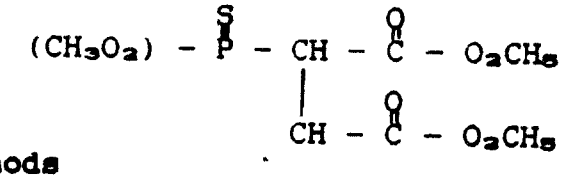
I- Materials

Experimental animals:

Gambusia affinis (F. Pocellidae) was used in the present study for its availability and high living tolerance. Living samples of *G. affinis* were collected by hand nets from the River Nile at Sohag area and were transported in well areated containers to large tanks in the laboratory. These tanks were filled with Nile water and continuously areated using air pumps. The fish samples were maintained for 10 days in these tanks for acclimatization purposes. Fishes were provided with suitable food of mosquito larvae and fresh liver tissue of frogs.

Tested compound:

An organophosphorus pesticide, malathion was used as commercial material with concentration of 59 gm/100 ml , from which different dilutions were prepared by emulsification in water.
The chemical structure of malathion is:



II- Methods

From the dose-response curve, the 96 h LC_{50} was found to be 2.2 mg/L. This concentration was selected to be acute while the chronic one was considered as 1/50 from the acute concentration (Hamada, 1991).

Acute treatment:

Effect of the acute treatment with high dose (2.2mg/L) of malathion on the histology of liver was studied at this concentration. This dose was prepared by emulsifying 2.2 mg/L in the Nile water specially equipped aquaria (70x40x50 cm).

The experiment continued for 8 days,of which the first 4 days were control period and the other followed 4 days were treatment period. During every day of the control period , 10 fishes of each sex were sacrificed. Then 80 fishes were transferred to two toxin treated aquaria , 40 fishes in each. These fishes were kept under observation for 4 days exposure period.During this period , the fishes that died were immediately removed. 10 surviving fishes were removed and sacrificed after intervals of 24 hours.

Chronic treatment:

Effect of the chronic treatment with low dose of malathion (0.044 mg/L) on the histological changes in the liver was also determined. The experiment continued for 45 days, a control period (first 15 days) followed by the treatment period (second 15 days) where fishes were given the drug in the water (0.044 mg/L) daily, then the last 15 days were considered as recovery period. Every 5 days, a sample of 10 fishes were sacrifices. The liver was taken for determination of the histological changes.

Histopathological changes:

Immediately after decapitation , the fishes were dissected and liver was fixed in Bouin's fluid for 24 hours. The fixed organs were dehydrated in a gradual series of ethanol, cleared using methyl benzoate followed by toluene, then embedded in paraffin wax. Sections (7 um thick) were stained with haematoxlin and eosin and examined histologically.

RESULTS

In normal liver of *G. affinis*,the hepatocytes are arranged around hepatic venules in the form of distinct cords (Fig. 1. 1). Each hepatocyte is hexagonal in shape with a distinct centrally

placed nucleus and homogeneous cytoplasm. The hepatic tissue contains numerous sinusoids. The intracellular bile canaliculi are also visible among hepatocytes.

The histopathological lesions commenced at the first day of exposure showing cellular degeneration and necrosis in some parts of the liver. The hepatocytes lost their cord-like formation. Some of the hepatocytes exhibited rupturing in their membranes (Fig. 1.2).

After 2 days of exposure to malathion , the histological examination of the liver revealed that there was an extreme cellular degeneration and necrosis. Some of the hepatocytes lost their polygonal shape and their basophilia, also there was a liver cord disarray and rupturing of the membranes. Some hepatocytes were completely vacuolated with nuclei coming out of the cells. In some of the partially vacuolated cells, the nuclei were seen deeply stained and pushed towards the periphery. The sinusoidal lumens were very narrow and could not be identified (Fig. 1.3).

The damage of liver was more pronounced after the 3rd day of exposure. It was characterized by many histopathological symptoms. Most of the hepatocytes were completely vacuolated , losing their arrangement and were free from any cytoplasmic materials. In few of the partial vacuolated cells, the nuclei were deeply stained and pressed towards the periphery. Necrotic areas were observed among the hepatic cells. The hepatic sinusoids showed signs of disintegration and could not be seen. The blood vessels appeared empty of blood cells (Fig. 1.4).

Exposure of fishes to high dose of malathion for 4days resulted in severe damage in the liver tissue.where the malformation and shrinkage were

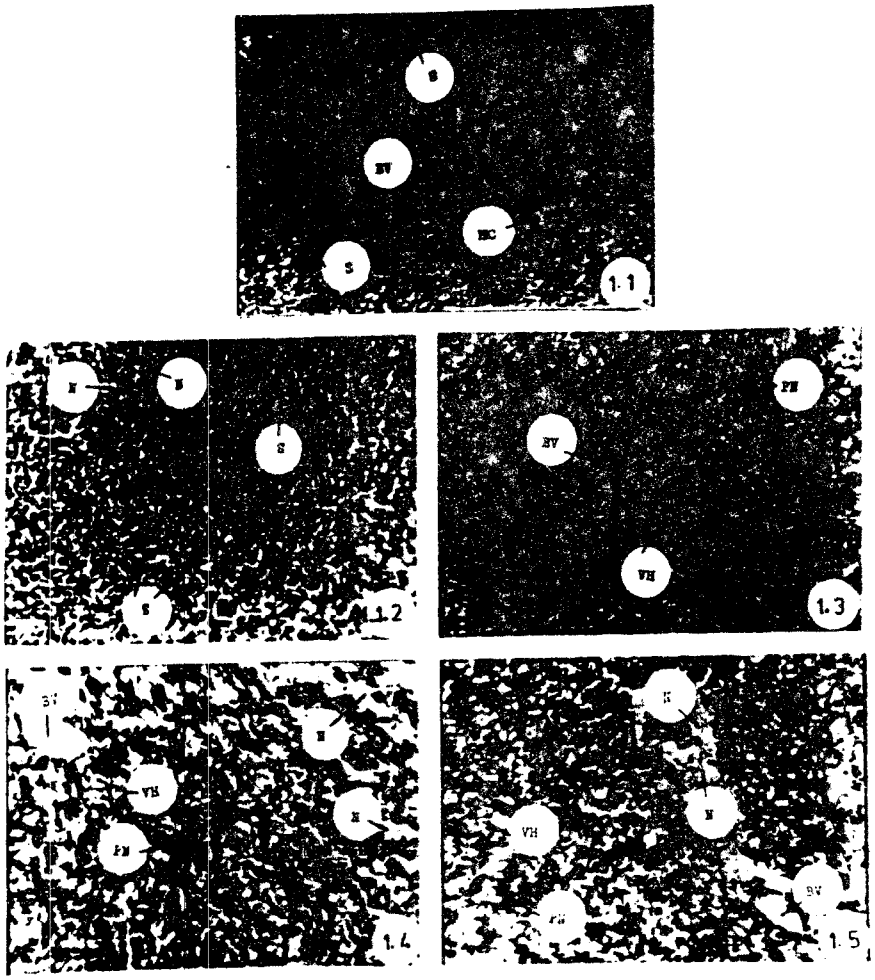


Fig. 1 : Histopathological changes induced by acute treatment with malathion in the liver of ***G. affinis***.

common in the whole structure of the liver (Fig. 1.5). The vacuolation of the hepatocytes was more pronounced than the former three days. The liver on the whole, showed necrosis and the cell membranes in the majority of the hepatic cells were ruptured. The deeply stained nuclei were noticed at the periphery of the hepatocytes. The hepatic sinusoids were completely disintegrated and the blood vessels appeared free from blood cells.

During chronic treatment of *G. affinis* with low dose of malathion , the pesticide induced histopathological changes in the liver comparing with the control group. Light microscopical observations of the liver after 5 days of treatment with malathion showed that some of the hepatocytes lost their polygonal shape and their normal basophilia. The cell membranes , in some cells were ruptured.The normal liver cord orientation was somewhat altered. The nuclei appeared to be pushed to one side of some hepatocytes. The blood sinusoids were very narrow (Fig. 2.1).

After 10 days of treatment with malathion, it was cleared that the normal liver cord was altered and degeneration of hepatocytes was observed. This degeneration was characterized by the presence of necrotic areas. Most of the nuclei appear to be pushed towards the periphery. The lumens of blood sinusoids were narrow and many of them were observed empty of blood cells (Fig. 2.2).

The histopathological lesions observed at the end of treatment period (15 days) can be summarized in the followings , the hepatocytes appeared slightly enlarged losing their polygonal shape and some of them were observed free from any protoplasmic materials. Therefore, they appeared look like vacuoles. The present nuclei were pushed to one side of the hepatocyte. The lumens of blood sinusoids were very narrow and

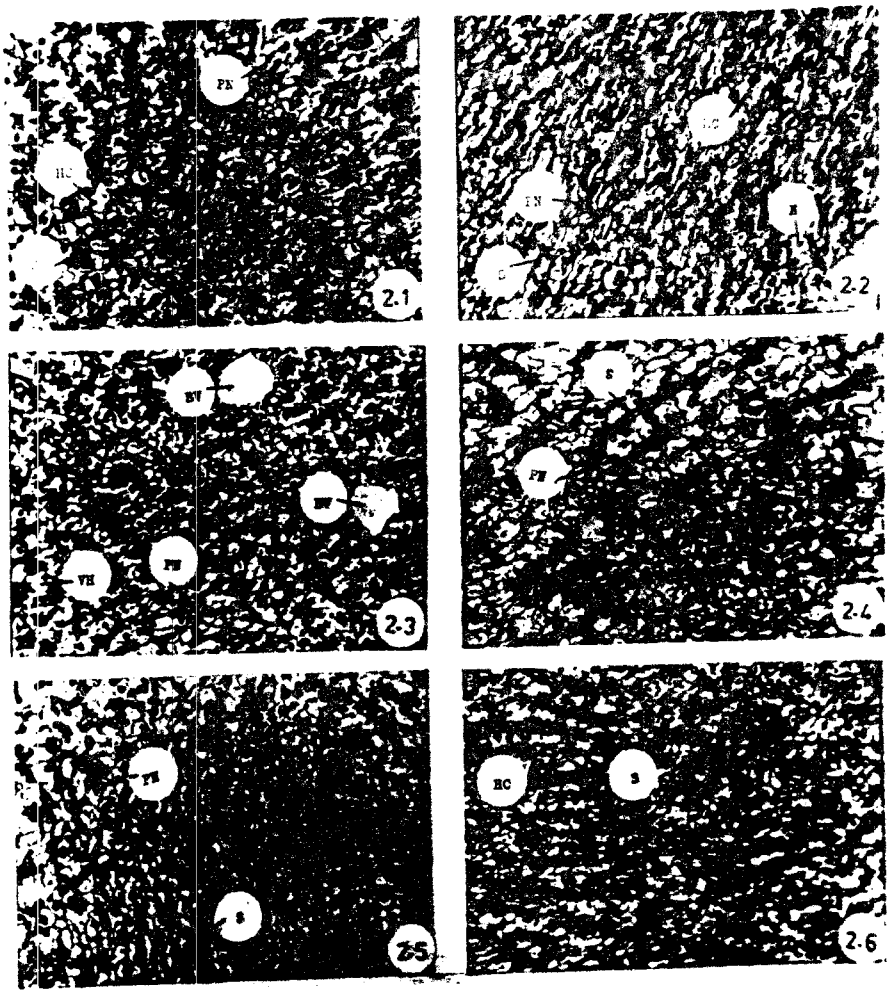


Fig. 2 : Histopathological changes induced by chronic treatment with malathion in the liver of *G. affinis*.

the blood vessels were observed empty of blood cells (Fig. 2.3).

By stopping the treatment of *G. affinis* with malathion , the liver was returned gradually to its normal structure. Thus, after 5 days of discontinuation of treatment,it was found that some hepatocytes returned to the polygonal shape and some nuclei were normal but the others continued pushed to one side of the hepatocyte. The sinusoidal lumens were very narrow (Fig. 2.4).

At the end of 10 days of the recovery period, most of the hepatocytes were polygonal in shape but still slightly enlarged and most of the nuclei were normal but some of them continued disintegrated. The endothelium of the hepatic sinusoids was diffused with the parenchymal cells and it was difficult to differentiate between them. The lumen of the hepatic sinusoids was filled with blood cells but still narrow to some extent (Fig. 2.5).

At the end of 15 days of the recovery period, it was noticed that the liver began to return to its normal structure to some extent. Most hepatocytes returned polygonal and arranged in cord like formation with normal nuclei but some of them still enlarged and free from nuclei. The lumens of the hepatic sinusoids were regular, filled with blood cells and their endothelium could be identified (Fig. 2.6).

DISCUSSION

The present results confirmed certain histopathological changes in the liver of *G. affinis* after acute or chronic treatment with malathion. The liver exhibited liver cord disarray , necrosis , nuclear disarray , vacuolation of the hepatocytes , loss of their polygonal shape and

destruction of the cytoplasmic materials. Slight enlargement of hepatocytes and disintegration of blood materials and blood sinusoids were also observed. A gradual increase in the damage was noticed with higher concentration of malathion as well as by longer exposure period.Eller(1971) reported that exposure of ***Salmo clarki*** to endrin resulted in liver cord disarray , connective tissue damage, enlargement of hepatocytes and their nuclei, phagocytic invation and necrosis. Dubale and Shah (1979) studied the effect of malathion on liver of ***Channa punctatus***. They reported that the histopathological symptoms were characterized by destruction of cytoplasmic and nuclear material, nuclear disarray and shrinkage leading to damage of the protoplasmic material , consequent vacuolization of the cells and necrosis. They added that a gradual increase in the damage was noticed with higher concentrations of this toxicant or by longer period of exposure.

Desai et al.(1984) investigated the histopathological lesions in liver of ***Tilapia mossambica*** treated with Monocrotophos. They observed necrosis and vacuolation of the hepatocytes. They suggested that the necrosis could be either due to direct effect of the compound on the cells or to an accumulation of acetylcholine in the tissues. Also , the results of the present study showed that hepatic tissue was found to be returned gradually to its normal picture after transportation of the fishes to fresh untreated water. However , the liver of treated fishes did not completely returned to their normal structure till 15 days of recovery stage. This slow trend of recovery might be due to the severe hazard effects of chronic sublethal dose of toxicant and/or to the susceptibility of *G. **affinis*** to the insecticide used. *G. **affinis*** has been found to be susceptible to many pesticides and resistant and/or tolerant to others (Scales and Yarbrough, 1975 and Hunsinger et al.,1979). Normalization of liver and kidney of ***Tilapia***

nilotica treated with sublethal single dose of Diazinon were reached after 2 days exposure in fresh untreated water (Gabr, 1986). Also, Dubale and Shah (1979) noticed normalization in the liver of *Channa punctatus* exposed to cadmium nitrate but when the same fish was treated with Methylparathion, no such recovery was observed. This suggests that the recovery is dependent on the susceptibility of fish, sex , dose and type of the insecticide used.

Thus on the basis of result of this investigation, it can be concluded that malathion, even in small concentration , is capable of inducing hepatic histopathological alteration which may cause severe physiological dysfunction leading to death. The histopathological effect of malathion are proportionately greater in fishes exposed to higher concentrations and/or to longer periods. A relatively long period of time was required to recover liver structure to near pre-exposure structure.

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EXPLANATION OF FIGURES

Fig.1.1 Photomicrograph of a control liver showing blood vessel(BV), sinusoids(S) and hepatic cords (HC). X 320

Fig.1.2 Photomicrograph of liver exposed to 2.2 mg / L Malathion for one day.Note cellular degeneration and necrosis (N) , the narrow sinusoids (S) and the condensed cells. X 320

Fig.1.3 Photomicrograph of liver exposed to 2.2 mg / L Malathion for two days showing vacuolated hepatocytes(VH), peripherally located nuclei(PN) and degenerated blood materials within blood vessels (BV). X 320

Fig.1.4 Photomicrograph of liver exposed to 2.2 mg / L Malathion for three days showing necrosis (N), empty blood vessel (BV) , vacuolated hepatocytes (VH) and peripherally located nuclei(PN). X 320

Fig.1.5 Photomicrograph of liver exposed to 2.2 mg / L Malathion for four days showing necrosis (N) , empty blood vessel (BV) , peripherally located nuclei(PN) and vacuolated hepatocytes(VH). X 320

Fig.2.1 Photomicrograph of liver exposed to 0.044 mg / L Malathion for 5days showing peripherally located nuclei (PN),narrow sinusoids (S) and disarray of hepatic cords (HC). X 320

Fig.2.2 Photomicrograph of liver exposed to 0.044 mg / L Malathion for 10 days.Note cellular degeneration and necrosis (N) , the narrow sinusoids (S) and the condensed cells. X 320

Fig.2.3 Photomicrograph of liver exposed to 0.044 mg / L Malathion for 15 days showing vacuolated hepatocytes (VH) , peripherally located nuclei (PN) and empty blood vessels (BV). X 320

Fig.2.4 Photomicrograph of liver after 5 days recovery showing narrow sinusoids (S) and the major still peripherally located nuclei (PN). X 320

Fig.2.5 Photomicrograph of liver after 10 days recovery showing the minor peripherally located nuclei (PN) and the somewhat narrow sinusoids(S). X 320

Fig.2.6 Photomicrograph of liver after 15 days recovery showing arranged hepatic cords (HC) and regular sinusoids (S). X 320

الملخص العربي

تاثير الملاشيون على التغيرات الهستوباثولوجية في كبد جامبوزيا افينيس

تم دراسة التغيرات الهستوباثولوجية في كبد سمكة المياه العذبة "جامبوزيا افينيس" بعد تعرضها لجرعات حادة ومزمنة من الملاشيون. نتج عن المعاملة الحادة بجرعة عالية (٣ر٢ مج/لتر) من اعراض مرضية في الكبد، فملت هذه الاعراض عدم انتظام خلايا الكبد في اقرطة ، تنكرزات مع ظهور فجوات داخل الخلايا، مما ادى الى كبر حجمها ودفع بالنواة الى حافة الغشاء الخارجي للخلايا ، لوحظ ان الخلايا الكبدية فقدت شكلها المضلع كما لوحظ تحلل مكونات الدم داخل الاوعية الدموية واختفاء الجيوب الكبدية وقد زادت هذه التغيرات بزيادة فترة التعرض للمبيد.

خلايا فترة التعرض المزمن لجرعات صغيرة (٤٤٠ر٠ مج/لتر) من المبيد اظهرت التجارب ان التغيرات الهستوباثولوجية كانت مشابهة لتلك التغيرات التي لوحظت في حالة التاثير الحاد ولكنها بدرجة اقل. وقد لوحظ ان الكبد بدأ يعود الى شكله الطبيعي نسبيا بعد ١٥ يوم من فترة الاستعادة.