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RESEARCH ARTICLE

Histophathological, histochemical and biochemical studies on rat's liver as biomarkers for environmental pollution with insecticide

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Abstract

The present study was planned to evaluate the histopathological and histochemical effects of organophosphorus Chlorpyrifos insecticide on serum and liver of the white rat Rattu, norvergicus. The rats classified into two major groups ,first group was received 1/10 of LD50 for 0,2,7,10,14,21 days, of Chlorpyrifos ,the second group was administered with 1/2 of LD50 for 0,2,4,6 days ,and then left for 15 days for recovery Serum levels of AST,ALT,ALP were significantly increase with both 1/10 and 1/2 LD 50. Histopathological investigation showed that hepatic tissues exhibited dilated and congested portal vessels with perivascular mild lymphocyte infiltration in cases of single and repeated doses .Also, development of focal necrosis in the liver . The histochemical examination indicated depletion of the polysaccharides and protein content of the liver. This study showed that Chlorpyrifos caused increased liver enzymes with histopathological and histochemical effects in liver .

INTRODUCTION

Pesticides are being commonly used by the human beings for their benefits insect control and increase yield of many crops. The use of pesticides has caused severe environmental and health hazards to organisms including human beings ***(Abdollahi et al,2003 and Tuzmen et al,2008)***. Due to their high insecticidal activity, low environmental persistence and moderate toxicity, the organophosphorus compounds are most favoured insecticides.

Organophosphorus (OP) pesticides are extensively used for control of insects around the home and in agricultural practice. However, they can pose a threat to public health. OP pesticides are known to cause millions of acute poisoning cases per year around the world ***(Raheja and Gill, 2007)***.

Chlorpyrifos (ClPF) is a broad-spectrum organophosphate insecticide widely used in agriculture, industry, and at home. Like all organophosphates, it affects the nervous system by inhibiting the enzyme acetylcholinesterase (AChE) ***(Salyha ,2013)***. Liver is the organ where its activation and detoxification takes place. Chlorpyrifosis moderately toxic to human ***EPA, (1989),*** Poisoning from Chlorpyrifos may affect the central nervous system, the cardio vascular system and the respiratory system. It is also a skin and eye irritant ***(Gallo and Lawryx, 1991)***.

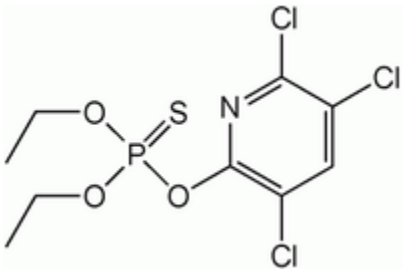
The aim of the present work was to investigate the effect of the organophosphorous insecticide, Chlorpyrifos on the albino rat *Rattus norvegicus* with special reference to its effects on the liver function (AST, ALT, and ALP) with special reference to its effects on the histopathological structure and histochemical components of liver.

Material and Methods

Chemicals:

The tested Insecticide used:

Chlorpyrifos (Trade names include Dursban, Empire, Eradex, Paqeant, Nufos and Lorsban). Its emperical formula is $C_9H_{11}Cl_3NO_3PS$.



Chemical name: O, O - diethyl O- (3, 5, 6 –trichloro – 2- pyridinyl) phosphorothioate.The lethal Does (LD_{50}) (70 mg/kg) body weight for Chlorpyrifos ***(Anonymous, 2004)***.

Chlorpyrifos was purchased from Sigma Chemical Company (St. Louis, Mo, USA).

Animals:

Albino rats were used in this experiment were obtained from the experiment breeding station from National Research Centre, Cairo.The animals were housed in universal galvanized wire cages at room temperature (22 ± 2^{o}C) and in a photoperiod of 12:12 light/dark cycle, 50% ± 5% humidity. The rats were acclimatized for 2 weeks perior to the start of the experiment. Rats were maintained on commercial pellet diet and water ad libitum.

Experiment I

A total of 45 rats were used. The rats were divided into nine groups (5rat/each) injected with 1/10 LD_{50} of Chlorpyrifos at days (0, 2, 7, 10, 14, 21) each to determine the rate of activity of liver function (AST, ALT and ALP) and then leave 15 day without injection to recovery period, the second groups were decapitated at intervals 5,10and 15 day

Experiment (II)

A total of 35 adult rat *Rattus norvegicus* were used. The rats were divided into seven groups (5 rat/each) injection with 1/2 LD_{50} of Chlorpyrifos (0, 2, 4, and 6) each to determine the liver function (AST, ALT and ALP) and then leave 15 day without injection.

Rats were anaesthetized with light ether and venous blood samples were collected by direct heart puncture into sterilized vials.

Blood samples allowed setting to clot at 4^{o}C and centrifuged at 4000 rpm for 5 min. Then 1000 µl aliquots of serum were placed in microfuge tubes and frozen on dry ice.

Labeled bags were placed into a -20^{o}C freezer until the time of the assay.

The histological and histochemical studies:

One hundred and eight rats weighting (100-150 gm.) were used in the present study. They were divided into control (12 rats) and 2 equal main groups (48 rats each).

Rats of 1/10 LD_{50} group were subdivided into six subgroups

Group I 1: rats given daily oral 1/10 LD_{50} of Chloropyrifos for one week.
Group I 2: rats given daily oral 1/10 LD_{50} of Chloropyrifos for two weeks.
Group I 3: rats given daily oral 1/10 LD_{50} of Chloropyrifos for three weeks.
Group I 4: rats given daily oral 1/10 LD_{50} of Chloropyrifos for three weeks and then were left for recovery for 5 days.
Group I 5: rats given daily oral 1/10 LD_{50} of Chloropyrifos for three weeks then left for recovery for 10 days.
Group I 6: rats given daily oral 1/10 LD_{50} of Chloropyrifos for three weeks then left for recovery for 15 days.

Rats of 1/2 LD_{50} group were subdivided into six subgroups

Group II 1: rats given daily oral 1/2 LD_{50} of Chloropyrifos for 2 days.
Group II 2: rats given daily oral 1/2 LD_{50} of Chloropyrifos for 4 days.
Group II 3: rats given daily oral 1/2 LD_{50} of Chloropyrifos for 6 days.
Group II 4: rats given daily oral 1/2 LD_{50} of Chloropyrifos for 2 days and then left for recovery for 4 days.
Group II 5: rats given daily oral 1/2 LD_{50} of Chloropyrifos for 4 days then left for recovery for 6 days.
Group II 6: rats given daily oral 1/2 LD_{50} of Chloropyrifos for 6 days then left for 12 days.

The insecticide was diluted with distilled water to get concentrations that differ according to the dose given. The diluted insecticide was freshly prepared daily. The animals were administrated by using a metallic stomach tube, while animals of the control group were given distilled water in the same manner.

The animals were killed by decapitation 24 hours after giving the last dose of the insecticide. Liver samples were collected from all groups and immediately fixed in neutral buffered formalin fixative.

Liver functions (ALT and AST) in serum:

Serum alanine aminotranseferase (ALT; EC2. 6. 1. 2) and asparatate aminotranseferase (AST; EC. 2. 6. 1. 1) activities were determined using commercials kits obtained from BioM'erieux, France ***(Reitman and Frankel, 1957)***.

Serum alkaline phosphatase: (ALP; EC 3.1. 3.1):

Alkaline phosphatase (ALP) activity was measured at 405 nm ***(Rosalki and Foo, 1993)*** using commercial kits obtained from Bio ADWIC, Egypt.

The histopathological study:

Liver was dissected out and fixed in neutral buffered formalin for 24 hours. The specimens washed in tap water, dehydrated in ascending grades of ethanol, cleared in xylene , embedded in paraffin wax (melting point 55-60C°).Sections of 5 um thickness were prepared and stained with Haematoxylin and eosin according to the method of ***(Drury and Willington,1980).***

The histochemical studies:

Mercuric bromophenol blue method of (***Mazia et al,1955)***was applied for the histochemical demonstration of the total protein. Periodic acid Schiff method of ***(McManus ,1946)*** was applied for detection of the polysaccharides.

Statistical analysis

Data were expressed as the mean ± SE. Data analysis were done using t-test according to ***(Hill, 1971).***

Results

Liver function:

1- Effect of 1/10 $LD_{50}$of Chlorpyrifos:

Treatment with 1/10 LD_{50} ofChlorpyifosrevealed highly significant (P<0.001) increased in the activities of AST, ALT and ALP in serum compared to control animals (Table 1). The present study demonstrates that treatment with 1/10 LD_{50} of Chlorpyrifosfor 21 days was found to be extremely unhealthy to rats. However, control rats remained healthy and there was no mortality throughout the experimental period.

Effect of 1/2 LD_{50} of Chorpyrifos:

Remarkably significant increase (P<0.001) in the levels of AST, ALT and ALP in serum affected by single dose (75mg/kg) body weight in respect to the control group (Table 3).

The highest level of AST, ALT and ALP were found in rats treated withChlorpyrifos for 6 days.

2- Recovery test of AST, ALT and ALP in serum:

After being without treatment and withdrawal of the rats with Chlorpyrifos, the elevation of level of AST, ALT and ALP moved towards the normal levels. Thus, AST started recovery. The elevation of level ALT started recovery faster than AST at the different periods (26, 31 and 36) days and (10, 12 and 18) days respectively (Table 2 and 4).

Table 1: effect of continuous treatments of 1/10 LD_{50} of Chlorpyrifos on the levels of AST, ALT and ALP in serum of male rats at 21 days.

		AST (Umol/L)	ALT (Umol/L)	ALP (Umol/L)
Control	Mean ± SE % change	30.13±2.50 100	45.10±4.32 100	260±5.6 100
2 days	Mean ± SE % change	41.11±2.40* +36.44	55.38±3.30 +22.80	335±6.10*** +28.85
7 days	Mean ± SE % change	60.47±1.80*** +100.70	65.12±5.10* +44.39	400±6.60*** +53.85
10 days	Mean ± SE % change	63.47±2.10* +110.45**	73.11±4.12** +62.11	432±5.50*** +66.15

14 days	Mean ± SE % change	66.32±5.2*** +120.11	86.78±1.9*** +92.42	455±5.16*** +75.00
21 days	Mean ± SE % change	68.11±1.4*** +126.05	90.70±2.2*** +101.10	460±3.90*** +76.92

* P<0.05 ** P<0.01 *** P<0.001 significant as compared with control

Table 2: effect of continuous treatments of 1/10 LD_{50} of Chlorpyrifos on the levels of AST, ALT and ALP in serum of male rats at 21 days, followed by 15 days, recovery.

		AST (Umol/L)	ALT (Umol/L)	ALP (Umol/L)
Control	Mean ± SE % change	30.13±2.50 100	45.10±4.32 100	260±5.6 100
26 days	Mean ± SE % change	50.12±1.2**** +66.35	76.61±3.2*** +69.86	380±2.9*** +46.15
31 days	Mean ± SE % change	46.16±1.3*** +53.20	60.12±1.9* +33.30	360±2.1*** +38.46
36 days	Mean ± SE % change	37.12±1.9 +23.19	51.21±2.2 +13.54	315±3.2*** +21.15

* P<0.05, ** P<0.01, *** P<0.001 significant as compared with control

Table3: effect of continuous treatments of 1/2 LD_{50} of Chlorpyrifos on the level of AST, ALT and ALP in serum of male rats at 6 days.

		AST (Umol/L)	ALT (Umol/L)	ALP (Umol/L)
control	Mean±SE % change	30.13±3.6 100	45.10±3.6 100	260±5.6 100
2 days	Mean±SE % change	53.7±3.4** +78.22	60.2±4.1* +33.48	285±4.2** +9.62
4 days	Mean±SE % change	60.88±4.3*** +102.15	65.3±3.2** +44.79	292±3.1*** +12.30
6 days	Mean±SE % change	67.85±3.1*** +125.19	70.4±2.2*** +56.09	300±1.8*** +15.38

* P<0.05, ** P<0.01, *** P<0.001 significant as compared with control

Table 4:effect of continuous treatments of 1/2 LD_{50} of Chlorpyrifos on the level of AST, ALT and ALP in serum of male rats at 6 days, Following by 12 days recovery.

		AST (Umol/L)	ALT (Umol/L)	ALP (Umol/L)
Control	Mean±SE % change	30.13±3.6 100	45.10±3.6100	260±5.6 100
10 days	Mean±SE % change	51.71±3.4**+71. 62	61.3±1.2**+35.9 2	290 ±3.2** +11.53
12 days	Mean±SE % change	46.33±2.9 +53.76**	50.2±1.9 +11.30	281±3.5* +8.07
18 days	Mean±SE % change	39.11±1.9* +29.80	48.10±2.6*** +6.65	272±3.9 +4.61

* P<0.05, ** P<0.01, *** P<0.001 significant as compared with control

Histopatholological Results

The liver of the Control groups

The hepatic lobules are the structural units of the liver; each is formed of cords of hepatocytes and blood sinusoids in- between (**Fig**.1). The hepatocytes are polyhedral cells with one or rarely two spherical nuclei and abundant cytoplasm. The cytoplasm of such cells is granular and strongly eosinophilic. The nuclei of the hepatocytes are large with peripherally dispersed chromatin and prominent nucleoli. Hepatocytes are oriented in cords composed of a single row of cells separated from vascular sinusoids by endothelial cells. The wall of these sinusoids also contains phagocytic irregular cells with multiple processes known as Von Kupffer. The sinusoids run radially, converging at the center of the hepatic lobule to form the central or centrolobular vein. The central vein has thin walls consisting only of endothelial cells supported by a sparse population of collagen fiber.

The Insecticide-treated Animals:

Daily administration of oral dose of Chlorpyrifos equivalent to 1/10 LD_{50} for one week caused venous congestion in the liver. Focal necrosis of hepatocytes and lymphocytic infiltration were encountered (**Fig. 2).**

Examination of liver sections of rats given daily oral dose equivalent to 1/10 LD_{50} of Chlorpyrifos for two weeks showed disturbance of the hepatic lobules, Cell necrosis, and dilated blood sinusoids were present (**Fig. 3).**

Examination of liver sections of rats received oral dose equal to 1/10 LD_{50} of Chlorpyrifos for three weeks showed micro and macro vesicular fatty change. Some nuclei showed pyknosis (**Fig. 4).**

Administration of oral dose equivalent to 1/10 LD_{50} of Chlorpyrifos for 3 weeks and then were left for recovery for 5 days caused hydropic degeneration of the hepatocytes. Necrosis of some hepatocytes, presence of cell debris in the blood sinusoids and pyknosis of some nuclei of hepatocytes **(Fig. 5)**.

Histopathological examination of liver sections of rats given daily oral dose equivalent to 1/10 LD_{50} of Chlorpyrifos for 3 weeks then left for recovery for 10 days revealed focal necrosis and many of the hepatocytes appear as normal one and dilatation of the blood sinusoids **(Fig.6).**

Examination of liver sections of rats given daily oral 1/10 LD_{50} of Chlorpyrifos for three week then were left for recovery for 15 days showed normal architecture of the hepatocytes with the exception of some focal necrosis and inflammatory infiltration beside the central vein **(Fig. 7)**.

Daily administration of oral dose equivalent to 1/2 LD_{50} of Chlorpyrifos for 2 days caused slight haemorrhage and pyknotic nuclei of some hepatocytes (**Fig. 8).**

Examination of liver sections of rats given daily oral dose equivalent to 1/2 LD_{50} of Chlorpyrifos for 4 days demonstrated disturbance of hepatic lobules, periportal necrosis of the hepatocytes that surround the portal area and inflammatory infiltration **(Fig. 9).**

Examination of liver sections of rats received daily oral dose equal to 1/2 LD_{50} of Chlorpyrifos for 6 days showed the presence of vacuolated hepatocytes and focal necrosis **(Fig. 10).**

Daily administration of oral dose equivalent to 1/2 LD_{50} of Chlorpyrifos for 6 days and then were left for recovery for 4 days showed normal structure but a necrotic area still detected **(Fig. 11).**

Examination of liver sections of rats given daily oral dose equivalent to 1/2 LD_{50} of Chlorpyrifos for 6 days then were left for recovery for 6 days demonstrated fatty changes and dilated blood sinusoids **(Fig. 12)**.

Examination of liver sections of rats received oral dose equal to 1/2 LD_{50} of Chlorpyrifos for 6 days then were left for 12 days showed slight haemorrhage, Some liver cell nuclei were pyknotic and some cells are necrotic **(Fig. 13).**

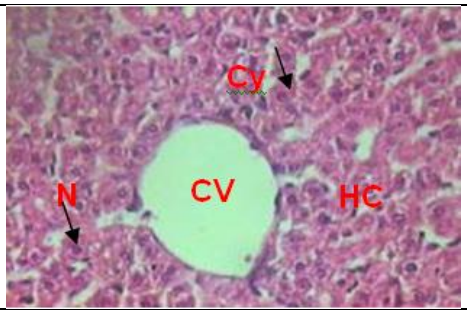


Fig.**1**: photomicrograph of a section of control liver shows the architecture of a hepatic lobule. The central vein (CV) lies at the centre of the lobule surrounded by the hepatocytes (HC) with strongly eosinophilic granulated cytoplasm (CY) and distinct nuclei (N). Between the strands of hepatocytes, the hepatic sinusoids are shown (HS)

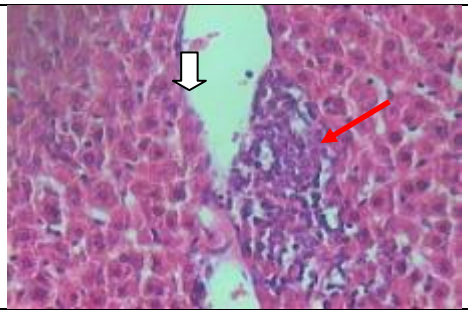


Fig. 2: photomicrograph of a section of liver of rat given oral dose equivalent to 1/10 LD_{50} of Chlorpyrifos for 7 successive days shows focal necrosis (arrows) with inflammatory infiltration. Notice the venous congestion (arrowheads) **(H & E - X 300)**.

(H & E -X 300).	

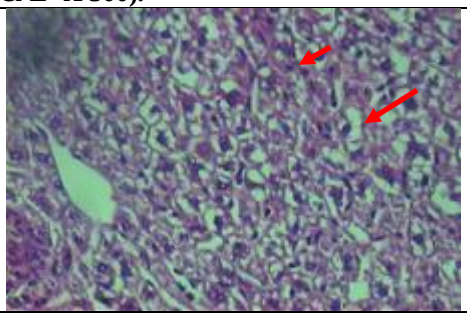
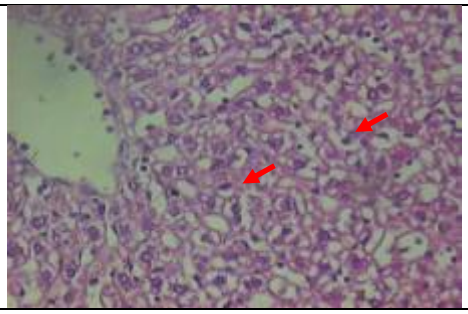

	

Fig.3: photomicrograph of a section of liver of rat daily given an oral dose equivalent to 1/10 LD_{50} of Chlorpyrifos for 14 successive days showing disturbance of the hepatic lobules. Cell necrosis and dilated blood sinusoids (short arrow) **(H & E -X 300)**.	**Fig.4**: photomicrograph of a section of liver of rat daily given an oral dose equivalent to 1/10 LD_{50} of chlorpyrifos for 21 successive days showing micro and macro vesicular fatty changes. Notice, some nuclei are pykontic (arrowhead) **(H & E -X 300).**

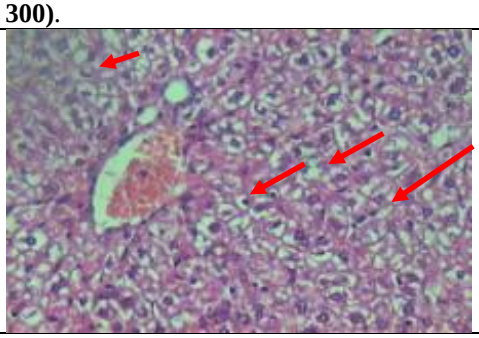
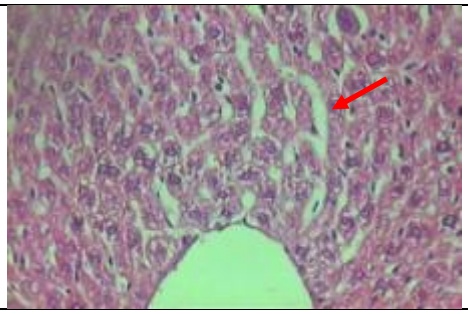

	

Fig.5: photomicrograph of a section of liver of rat daily treated with oral dose equivalent to 1/10 LD_{50} of Chlorpyrifos for 3 weeks then were left for recovery for 5 days showing hydropic degeneration of the hepatocytes (arrows). Notice, necrosis of some hepatocytes (short arrow), presence of cell debris in the blood sinusoids (arrowhead) and pyknosis of some nuclei (long arrow), hemolysed blood cells in the congested central vein **(H& E - X 300)**.	**Fig. (6)**: A section of liver of rat given daily oral dose equivalent to 1/10 LD_{50} of Chlorpyrifos for 3 weeks then were left for recovery for 10 days showing focal necrosis and normal appearance of some hepatocytes but others contain karyolysedneuclei. Notice the dilatation of the blood sinusoides (arrowhead) **(H & E -X 300)**.

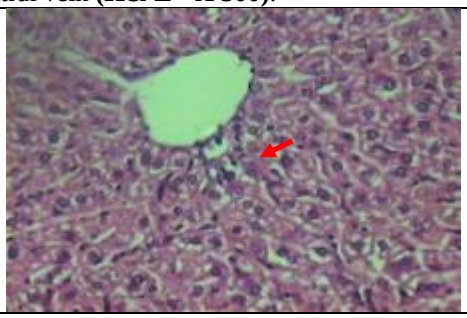
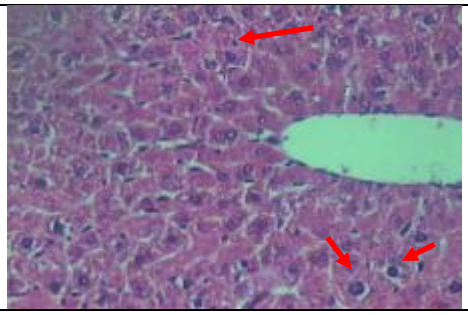

	

Fig. 7: photomicrograph of a section of liver of rat orally administrated with a single dose equivalent to the 1/10 LD_{50} of Chlorpyrifos for 3 weeks and left for recovery for 15 days showing normal structure of the hepatocytes with the exception of some focal necrosis and inflammatory infiltration beside the central vein (arrow) **(H & E -X 300)**.	**Fig.8**: photomicrograph of a section of liver of rat after oral administration of the 1/2 LD_{50} of Chlorpyrifos for 2 successive days showing slight haemorrhage (arrows),pyknoticneuclei in some hepatocytes (arrowhead), and some cells are necrotic (short arrow) **(H & E, X 300).**

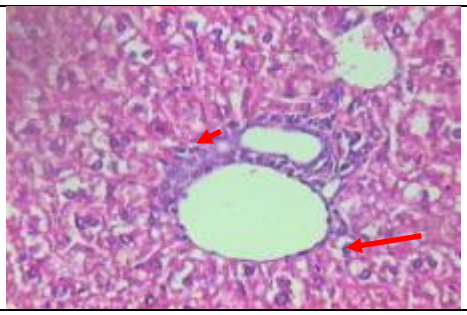


Fig. 9: photomicrograph of a section of liver of rat orally administrated with a single dose equivalent to the 1/2 LD_{50} of Chlorpyrifos for 4 successive days showing disturbed hepatic lobules,periportal necrosis of the hepatocytes that surround the portal area (long arrow), with inflammatory infiltration (arrowhead) **(H & E -X 300)**.

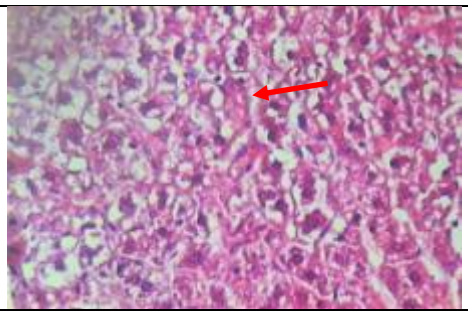


Fig.10: photomicrograph of a section of liver of rat after oral administration of the 1/2 LD_{50} of Chlorpyrifos for 6 successive days showing slight haemorrhage (arrows). Vacuolated hepatocytes with focal necrosis **(H & E, X 300)**.

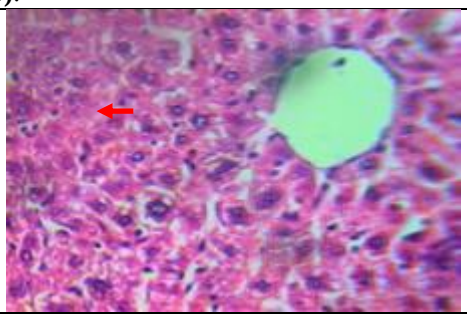


Fig. 11: photomicrograph of a section of liver of rat orally administrated with a single dose equivalent to the 1/2 LD_{50} of Chlorpyrifos for 6 days and left for 4 days showing normal structure except some focal necrosis (arrowhead) **(H & E - X 300)**.

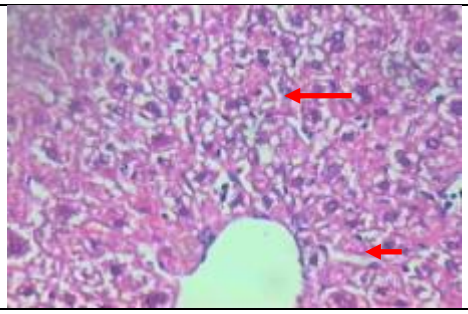


Fig 12: photomicrograph of a section of liver of rat after oral administration of the 1/2 LD_{50} of Chlorpyrifos for 6 days and left for 6 days showing fatty change (arrow) and dilated blood sinusoids (arrowhead)vacuolation , degeneration with karyolitic nuclei**(H & E - X 300)**.

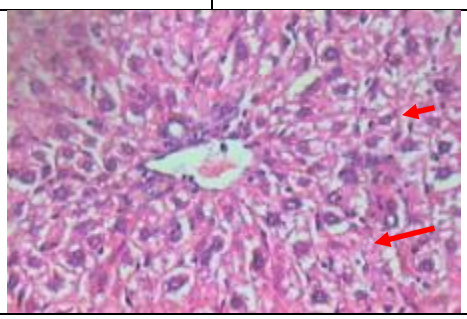


Fig.13: photomicrograph of a section of liver of rat after 2 days of oral administration of the 1/2 LD_{50} of Chlorpyrifos for 6 days and left for 12 days showed slight haemorrhage, highly vacuolated hepatocytes some contain pyknotic nuclei (arrowhead), and some cells are necrotic (short arrow) **(H & E-X 300)**.

The histochemical results

I- Total proteins:

Examination of sections of liver of the control rats displayed the proteinic inclusions in the hepatocytes as grayish blue irregular particles of various sizes against weakly to moderately stained ground cytoplasm. The nuclear chromatin and the nucleoli are densely stained indicating their rich content of proteinic constituents (**Fig**. 14).

Daily treatment with an oral dose equivalent to 1/10 LD_{50} of Chlorpyrifos for 7 successive days revealed relative diffused staining affinity of the proteinic inclusions of the hepatocytes **(Fig. 15).**

In rats administrated with oral dose equivalent to 1/10 LD_{50} of Chlorpyrifos for 14 successive days, marked diminution of the proteinic inclusions was detected un the hepatocytes **(Fig. 16).**

In animals received daily oral dose of 1/10 LD_{50} of Chlorpyrifos for 21 successive days, no changes in most hepatocytes **(Fig. 17)**.

Moderate diminution of the proteinic contents in the liver of rats daily received an oral dose of 1/2 LD_{50} of Chlorpyrifos for 4 successive days was showed **(Fig. 18)**.

Liver of rat daily received an oral dose of 1/2 LD_{50} of Chlorpyrifos for 6 successive days showed poorly stained proteinic inclusions of the hepatocytes (**Fig. 19)**.

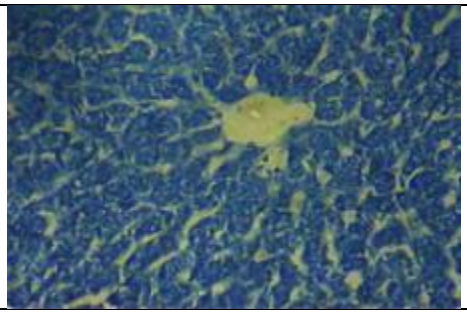


Fig.14:photomicrograph of a section of liver of control rats induced decrease in the proteinic content of the liver in animals treated with any of the used doses (1/10 LD50, 1/2 LD50) until after recovery. section of liver of control rat showing the proteinic contents. Notice the irregular particles of various sizes that are equally- distributed in the cytoplasm of the liver cells. The nucleoli are intensely stained while the ground cytoplasm and nucleoplasm faintly stained**(Mercuric bromophenol blue reaction- X 300)**.

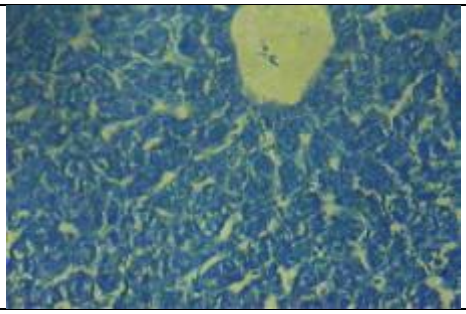


Fig. (15): photomicrograph of a section of liver of rat given oral dose equivalent to 1/10 LD_{50} of Chlorpyrifos for 7 successive days showing the staining affinity of total protein relatively diffused in both the cytoplasm and nucleus **(Mercuric bromophenol blue reaction- X 300).**

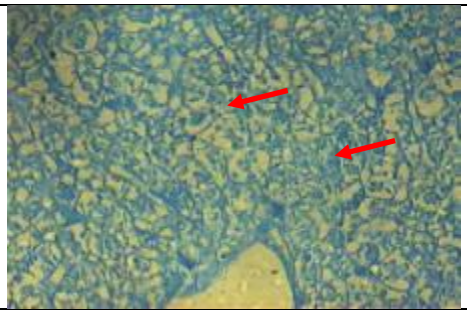


Fig.16: photomicrograph of a section of liver of rat daily received an oral dose of equivalent to 1/10 LD_{50} of Chlorpyrifos for 14 successive days showing marked diminution of the proteinic inclusions in many liver cells (arrows). **(Mercuric bromophenol blue reaction- X 300).**

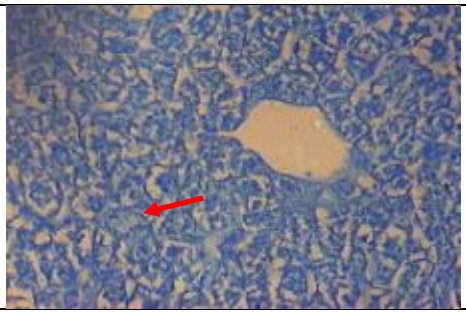


Fig. 17: photomicrograph of a section of liver of rat after administration of a single dose of 1/10 LD_{50} of Chlorpyrifos for 21 successive days showing reduced proteinic inclusions. **(Mercuric bromophenol blue reaction- X 300).**

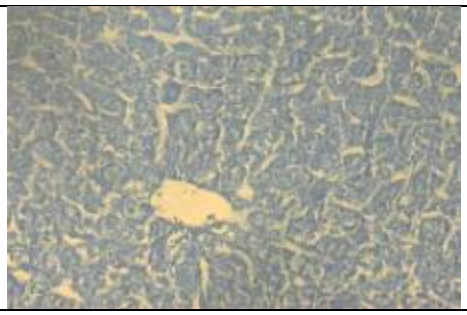


Fig.18: photomicrograph of a section of liver of rat daily received an oral dose of 1/2 LD_{50} of Chlorpyrifos for 4 successive days showing moderate diminution of the proteinic contents **(Mercuric bromophenol blue reaction- X 300).**

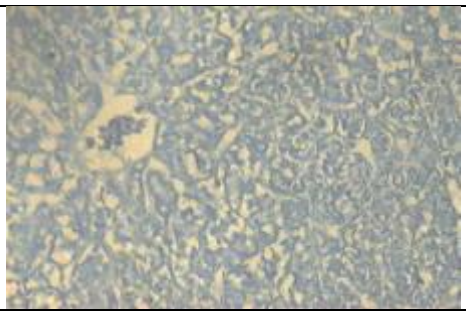


Fig19: photomicrograph of a section of liver of rat daily received an oral dose of 1/2 LD_{50} of Chlorpyrifos for 6 successive days showing poorly stained proteinic inclusions of the hepatocytes. The stainability is relatively diffused in both the cytoplasm and nucleus **(Mercuric bromophenol blue reaction- X 300).**

Polysaccharides:

Examination of liver thin sections of control rats stained by Periodic Acid Schiff`s reaction (PAS) showed the abundance of polysaccharide materials in the hepatocytes. The nuclei of the hepatocytes give negative Periodic Acid Schiff`s reaction indicating the absence of polysaccharides (**Fig**.20). The poly saccharide particles appear accumulated at one side of the cytoplasm leaving the other side almost devoid of such material.

Histochemical examination of liver of rat given oral dose equivalent to 1/10 LD_{50} of Chlorpyrifos for 7 successive days showed diffuse staining affinity of polysaccharides inclusions. A few number of the hepatocytes display dense staining affinity than the others **(Fig. 21).**

In rats given oral dose equivalent to 1/10 LD_{50} of Chlorpyrifos for 14 successive days, the polysaccharides inclusions in liver displayed diffuse staining affinity. A number of the hepatocytes display dense staining affinity than the others **(Fig.** 2**2).**

Sections of liver of rats given oral dose equivalent to 1/10 LD_{50} of Chlorpyrifos for 21 successive days showed the polysaccharide inclusions that displayed diffuse stainability. **(Fig. 23**).

Examination of liver of rat given oral dose equivalent to 1/2 LD_{50} of Chlorpyrifos for 2 successive days showed that the polysaccharides inclusions displayed diffuse staining affinity. A few number of the hepatocytes display dense staining affinity than the others **(Fig. 24).**

The polysaccharides inclusions of liver of rats given oral dose equivalent to 1/2 LD_{50} of Chlorpyrifos for 4 successive days displayed severe depletion. A few number of the hepatocytes display dense staining affinity than the others **(Fig. 25).**

Liver of rats given oral dose equivalent to 1/2 LD_{50} of Chlorpyrifos for 6 successive days showed moderate depletion of the polysaccharides inclusions **(Fig. 26).**

The above results revealed that Chlorpyrifos caused reduction in the polysaccharide materials in the liver of the treated rats. The degree of this reduction increased progressively according to the amount of the given dose of the insecticide. On the other hand, until after recovery these inclusions remained in the reduction form.

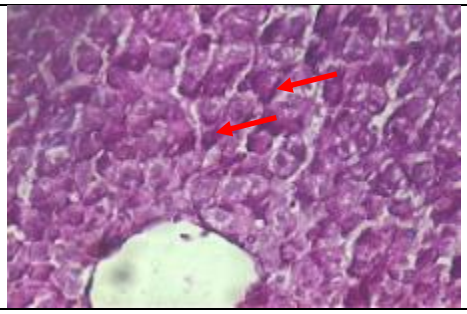


Fig.**20**: photomicrograph of a section of liver of control

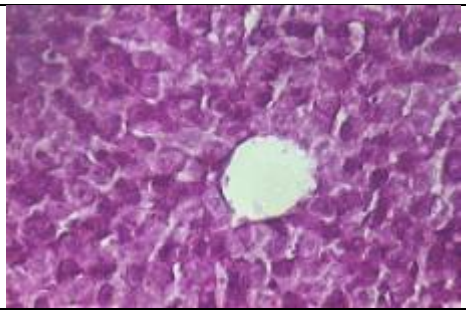
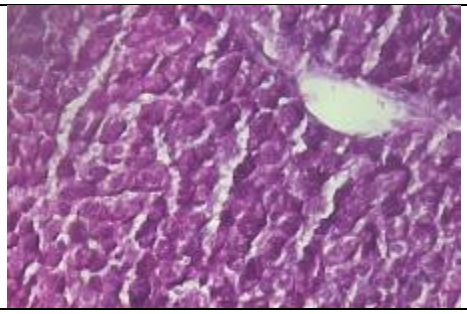


Fig.**21**: photomicrograph of a section of liver of

rat showing the abundance of polysaccharides of hepatocytes, they appear accumulated at one side of the cytoplasm leaving the other side almost devoid of such material **(PAS- X 300)**.

rat given oral dose equivalent to 1/10 LD_{50} of Chlorpyrifos for 7 successive days showing diffused staining affinity of polysaccharides inclusions. Notice that such inclusions displayed diffuse stainability. A few number of the hepatocytes display dense stainability than the others. **(PAS - X 300).**



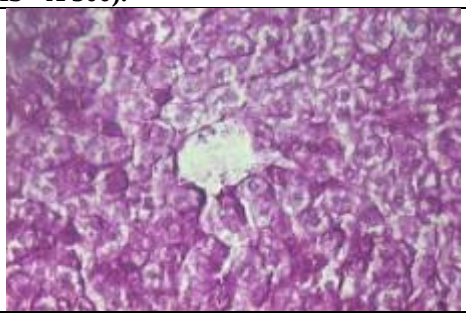
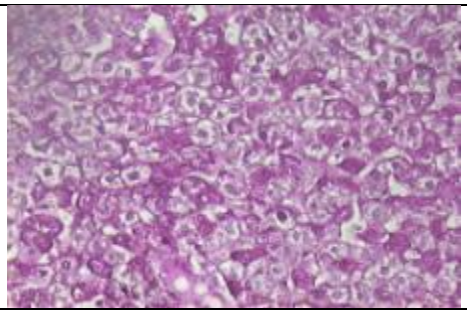


Fig.22: photomicrograph of a section of liver of rat given oral dose equivalent to 1/10 LD_{50} of Chlorpyrifos for 14 successive days showing diffused staining affinity of the polysaccharides inclusions. **(PAS - X 300).**

Fig.23: photomicrograph of a section of liver of rat given oral dose equivalent to 1/10 LD_{50} of Chlorpyrifos for 21 successive days showing the polysaccharide inclusions. Notice that such inclusions displayed diffuse stainability.**(PAS- X 300)**.



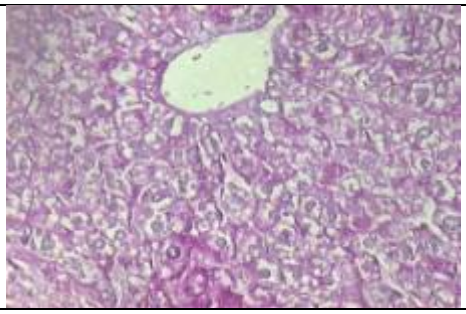


Fig.**24**: photomicrograph of a section of liver of rat given oral dose equivalent to 1/2 LD_{50} of Chlorpyrifos for 2 successive days showing highly reduced of the polysaccharides inclusions. **(PAS- X 300).**

Fig.25: photomicrograph of a section of liver of rat given oral dose equivalent to 1/2 LD_{50} of Chlorpyrifos for 4 successive days showing severe depletion of the polysaccharides inclusions. **(PAS- X 300).**

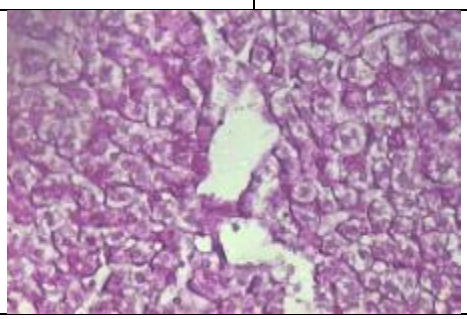


Fig.**26**: photomicrograph of a section of liver of rat given oral dose equivalent to 1/2 LD_{50} of Chlorpyrifos for 6 successive days showing depletion of the polysaccharides inclusions **(PAS- X 300).**

Discussion

Chlorpyrifos (ClPF) is a broad-spectrum organophosphate insecticide widely used in agriculture, industry, and at home. ***(Salyha ,2013)***.

Organophosphates are some of the most widely used pesticides in the world. They are used in agriculture, homes, gardens and veterinary practices replacing the same uses as the organochlorines, many of which have been banned for years. In general, they are not persistent in the environment as they break down quickly. Because of their relatively fast rate of degradation, they have been a suitable replacement for the more persistent organochlorines ***(Frederick,2005).*** Pesticide use in public health protection and agricultural programs is pervasive and growing and serious adverse health effects on animal populations and on humans are widespread and common ***(Orabi et al ,2013)***.

The data showed that level of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) and alkaline phosphatase (ALP) were subjected to marked increase in the serum of rats after treatment with chlorpyrifos.This increase may be attributed to several pathological conditions as damage of liver, and remarkable histological damage**.**The proposed hypothesis is based on the fact that, the repeat exposure of the hepatocytes to the toxic compounds might cause severe histological alterations in these cells including rupture of the hepatocytes membranes, causing the release of these enzymes from the cytoplasm of the damaged cells into the blood stream which explain the elevation of these enzymes ***(Rahman and Siddiqui, 2005).***

Impaired liver functions and changes of cellular permeability which may cause release of these enzymes into the circulation as a result of necrosis of the tissues ***(Rahman and siddiqui, 2004).***

Chlorpyrifos treatment to normal rats indicated a marked increase in the liver marker enzymes including AST, ALT and ALP ***(Orabi et al ,2013)***.

Liver is a major site for metabolism of exogenous chemicals (pesticides, drugs,and metals), resulting in the formation of metabolites which may be more or less toxic than the parent compound***(Orabi et al ,2013)***.

Orabi et al ,2013 reported that chlorpyrifos treatment to normal rats indicated a marked increase in the liver marker enzymes including AST, ALT and ALP.This result is an indicator of liver injury, when the liver cell membrane is damaged; varieties of enzymes

normally located on the cytosol (cellular enzymes) are released into the blood stream ***(Singh et al,2011).*** Also, the elevation in alkaline phosphatase level suggests an increase in lysosomal mobilization and cell necrosis due to pesticide toxicity. ***(Singh et al,2011).***

Kalender et al.(2005) and Etim et al.(2006) reported that increase of alkaline phosphatase level after Diazinon and Lindane induced hepatotoxicity.

In the present study, the marked elevation in the serum AST and ALT after treatment with the doses (1/10 LD_{50}) 7 mg/kg and (1/2 LD_{50}) 35 mg/kg body weight respectively of Chlorpyrifos indicate liver dysfunction which is correlated with the histological damage in the liver induced by its toxic components.

However, these results are in agreement with those of ***Rahman et al., (2001) and Rahman and Siddique (2004)***, who observed that the activities of AST, ALP, ACP and ALP in serum were, recovered to normal conditions after 28 days of post – treatment with Vepacide in different tissues of male and female albino wistar rats. Also, these results are in agreement with the present results as the recovery of AST, ALT and ALP after 36 day and 18 day of post – treatment with two doses of Chlorpyrifos in liver of male rats.

Furthermore, reduced values of AST, ALT and ALP were observed in insect pupae after treatment with *azadirachtin* ***(Bream,2008).***

It has been observed in the present investigation that the organophosphorous insecticide, Chloropyrifos, caused histopathological and histochimcal changes in the liver of the white rat.

The present study revealed that oral treatment of rats with chloropyrifos showed different pathological lesions in the liver tissue, which were relevant to the amount of dose given. Nevertheless, it is clear that liver tissues are markedly responded to the adverse effect of the insecticide; it displayed marked histological changes even with the lowest dose and after recovery period.

The present experimental material has revealed that hepatic tissues exhibited dilated and congested portal vessels with perivascular mild lymphocyte infiltration in cases of single and repeated doses .Also, development of focal necrosis in the liver under the effect of chloropyrifos.

Rather similar results were obtained by ***Owoeye et al,2012*** who reported that the microanatomical structure of the liver was altered showing diffuse vacuolar degeneration of hepatocytes with necrotic hepatocytes as well as moderate peri-portal cellular infiltration by mononuclear cells in rats exposed to Dichlorvos (2, 2-dichlorovinyl dimethyl phosphate), an organophosphorus compound for 1week. Also,there was a moderate to severe vacuolar degeneration and necrosis of hepatocytes (diffuse) in rats exposed for 2 weeks , while rats treated for 3 weeks showed loss of hepatocyte outline. In rats treated with dichlorvos for 4 weeks, the microanatomical alterations

included: portal triads and vessels which were completely obscured and appeared circumscribed by fibrous connective tissue, necrotic plaques, periportal cellular infiltration, and diffuse necrosis .

Examination of the liver sections of chloropyrifos - intoxicated rats revealed a microvesicular pattern of fatty change. Fatty change of the mammalian liver has already been reported by earlier authors in cases of exposure to other pesticides. This change was reported by ***Chu et. al., (1986)*** *and* ***Rashwanet. al., (1992)*** for toxaphene and kepone respectively in mice . The mechanism of fatty change is complex; the intracytoplasmic fat can be due to a number of factors, e.g., organelles injury, metabolic disorders and/or\deficiency of essential lipotropic factors. Such change appears in the hepatocyte cytoplasm as macrovesicular and / or microvesicular patterns ***(Gopinath et. . al., 1987).***

In the present investigation, the liver blood sinusoids became dilated under the effect of chloropyrifos. Such lesion was also reported by ***Guzelianet. al.,(1982)*** in human poisoned with chlordecone (kepone) and in human and experimental animals poisoned by chlorodecone. A similar observation has been described by ***Rashwanet. al., (1992)*** who found that kepone injection to mice produced dilation of the liver blood sinusoids. Also the same results was reported by ***Farrag and Shalby,2007*** ,they showed that liver sections of rats received an oral dose of the insect growth regulator, lufenuron and the organophosphorus insecticide, profenophos day after day for two months showed cell necrosis, lymphocyte infiltration and dilation of blood sinusoids of liver then stayed without treatment for another month demonstrated mild lymphocyte infiltration with dilated and congested veins together with dilated sinusoids.

Examination of liver sections of rats received profenofos showed periportal necrosis of section of liver of rat given day after day oral doses for two months then stayed without treatment for another month In case of lufenuron showing disruption of liver structure that associated with focal necrosis and developed vacuoles and in case of profenofos showing dilated sinusoids (***Farrag and Shalby,2007)***

Histochemical identification of total proteins of the liver of rats treated with chloropyrifos showed that there is a weak reactivity of the proteinic contents in these organs. Such change indicates the reduction in the total proteins of the tested organ under the effect of the used insecticide.

A similar observation has been described by ***Sakr et al ,(2002)*** recorded that examination of rat liver after 9 days post tetramethrine treatment, showed some hepatocytes appeared with a slight decrease in the protein materials .After 12 and 15 days, there were obvious decrease in the protein contents. Some cells especially in 15 days treated animals were completely devoid of proteins and their remnants were mainly located at the peripheries of the cell which showed cytoplasmic vacuolation

In the present study, the histochemical investigation of polysaccharides in liver of chloropyrifos-treated rats showed weak and heterogeneous stainability, which indicate depletion of these inclusions.

Several authors using different insecticides introduced almost similar findings. A repeated oral administration of lufenuron or profenofos for two months showed a

severe depletion in the polysaccharides content of the hepatocytes are significantly decrease as compared to control ***(Farrag and Shalby,2007).***

Sakr et al ,(2002) showed that the carbohydrates content of the liver of treated animals with tetramethrine for 9 days showed a moderate depletion of the carbohydrate inclusions. After 12 days of treatment, most of the hepatic cells contained scarce amount of carbohydrate except few ones which showed moderate amount .A marked reduction of carbohydrates was detected after 15 days of treatment .

Conclusion

The uncontrolled use of organophosphorus compound chloropyrifos insecticide leads to marked effect on the histoarchtecture of liver represented as vacuolar, pyknosis and necrotic changes with marked depletion of protienic and polysaccharide contents even after a period of recovery the effect still obvious and clear , so the use of these compounds must to be under control.

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