Histological and Enzyme Histochemical Changes of Liver Induced By the Action of Maxxthor Insecticide in Male Albino Rats

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Abstract

The present study aimed to investigate the histological, enzyme histochemical changes and liver function effects of Maxxthor insecticide on albino rats in liver. The experiment included 20 rat which were divided into four groups, the first group 5 rats were considered the control animals and the others were divided equally into three groups with a dose of 0.01, 0.1and 1 mg / kg of body weight, respectively for a period 40 days. The animals given each 48 hours via oral route Maxxthor by tube dosage after dissolved with distilled water. Microscopic examination of liver showed inflammatory cell aggregation around vessels, congestion and dilation of sinusoids hepatocytes hypertrophy with severs inflammatory cells infiltration, kupffer cells proliferation and hydropic degeneration. Enzyme histochemical study of liver showed weak expression of ALP activity in hepatocyte in low dose, sever expression in middle dose, and mild expression in high dose. There was significantly increase in serum aminotransferases (ALT, AST) and alkaline phosphatase (ALP) level in treated group of rats as compared with control group.

Key words: *Maxxthor, Histopathology, Enzyme histochemical,liver function*

Introduction

Pesticide is any substance or mixture of substance intended to preventing, destroying or controlling any pest, including vectors of human disease or animal species, unwanted plants or animals that cause damage [(1)]. Pesticides spread within the ecosystem elements due to its extensive and random application, and its resist to physical and chemical breakdown and metabolic degradation [(2)]. Pesticides residues accumulation in the environment components over time lead emergence of several ecological and health setbacks [(3)]. Pyrthroid the important new class of synthetic insecticides of the past three decades are rapidly replacing other insecticides due to low mammalian toxicity and an unusually fast biodegradation [(4)]. Pyrethroid act by enhancing sodium channel activity by shifting activation to more negative membrane potentials as well as slowing channel activation [(5)]. Maxxthor is pesticides belong to pyrethroid insecticides type I. The active ingredient in Maxxthor product is bifenthrin , a second generation of synthetic pyrethroid [(6)]. Maxxthor use against termites and labeled for the long term control of wide range of pests including insect pests, vectors, spiders, ticks, and turf insects [(7)]. The neurotoxicity of Maxxthor depend on the affinity to the voltage sodium channels in insects and mammals, where Maxxthor bind to the sodium channel transiently and stop the closing of the channel permanently, leading to permanently depolarize of the membrane, no further action potential can be generated because the resting potential will not be restored [(8)]. pyrthroid induce oxidative stress as mechanism of their toxic action in the body leading to generation of free radicals and influence the activity of antioxidant enzymes in tissues [(9)]. Our goal was to studied the histological changes, ALP activity expression and alternation in liver enzyme induced by the action of Maxxthor .

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Materials and Method

Experimental animals and Study Design

The animals examined in this study were mature male Sprague-Dawley albino rats (*Rattus norvegicus*) with about 8-10 weeks age obtained from biotechnology research center / Al-Nahrian university with an average weight of (200-250 gm) and kept under climate controlled conditions of the animal house with temperature 22-25 C^{o} , good ventilation , regular 12 hours light duration . Rats were randomly divided into four groups five rats for each group and treated as follow: G1 was administrated with 1 ml of normal saline, while G2, G3 and G4 were administrated with1ml of (0.01, 0.1 and 1 mg/kg b.w.) respectively of maxxthor orally once each 48 hours for 40 days.

Histopathological and Enzyme histochemical study

We began sacrificing the animals at the end of the experiment; Liver was quickly removed. First portion of organ was dissected and fixed in 10 % neutral formalin, dehydrated in ascending grades of alcohol and imbedded in paraffin wax. Paraffin sections (5µm thick) were stained for routine histological study using Hematoxylin and Eosin stain (H&E) [10].

Second portion of organ fixed and dehydrated in a mix of absolute alcohol and cold acetone for different periods and imbedded in paraffin wax. ALP demonstrated in paraffin sections (5µm thick) as described by [11].

Liver Function

Serum sample was collected for the estimation of liver function (ALT, AST, ALP) by using Linear kit.

Statistical analysis

The Statistical Analysis System- SAS (2012) program was used to detect the effect of difference groups in study parameters. Least significant difference –LSD test was used to significant compare between means in this study.

Results and Discussion

Histological effects

Microscopic examination of liver of control rat showed normal structure of the central vein, hepatocytes and blood sinusoids (Figure 1A). Histological changes investigation in sections of liver rat treated with 0.01 mg / kg b.w. of Maxxthor pesticide showed hydropic degeneration in the hepatocytes figure (1B) and dilation in the sinusoids (1C). Aggregation of inflammatory cells near central vein and congestion with dilation of sinusoids were seen in liver section of rat treated with 0.1 mg/kg b.w. of Maxxthor pesticide after 40 days of treatment as shown in figure (2A,B) and proliferation of hepatocyte and hemorrhage with proliferation of kupffer cells figure (2C,D). Hydropic degeneration in hepatocytes with degradation of chromatins cells and aggregation of inflammatory cells figure (3A,B) was in liver of rats treated with 1 mg\kg body weight.

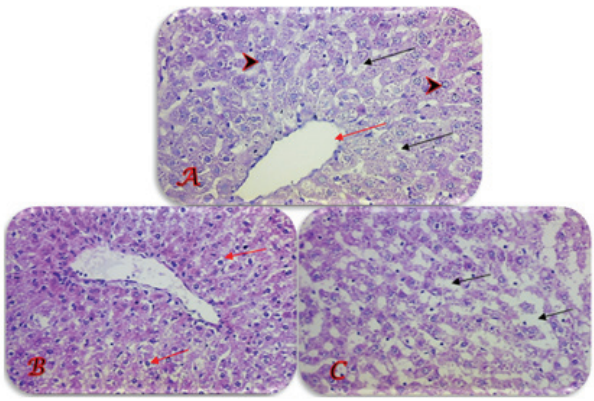

Figure (1): Crosse section in liver of rat detected by H&E staining A: normal liver of rat showing hepatocytes (head arrows), central vein (red arrow) and sinusoids (black arrows),B: treated rat with 0.01 mg/kg body weight of Maxxthor showing (A): hydropic degeneration in the hepatocytes (red arrows) ,C: dilation in the sinusoids (black arrows) (A,B and C, X10) .

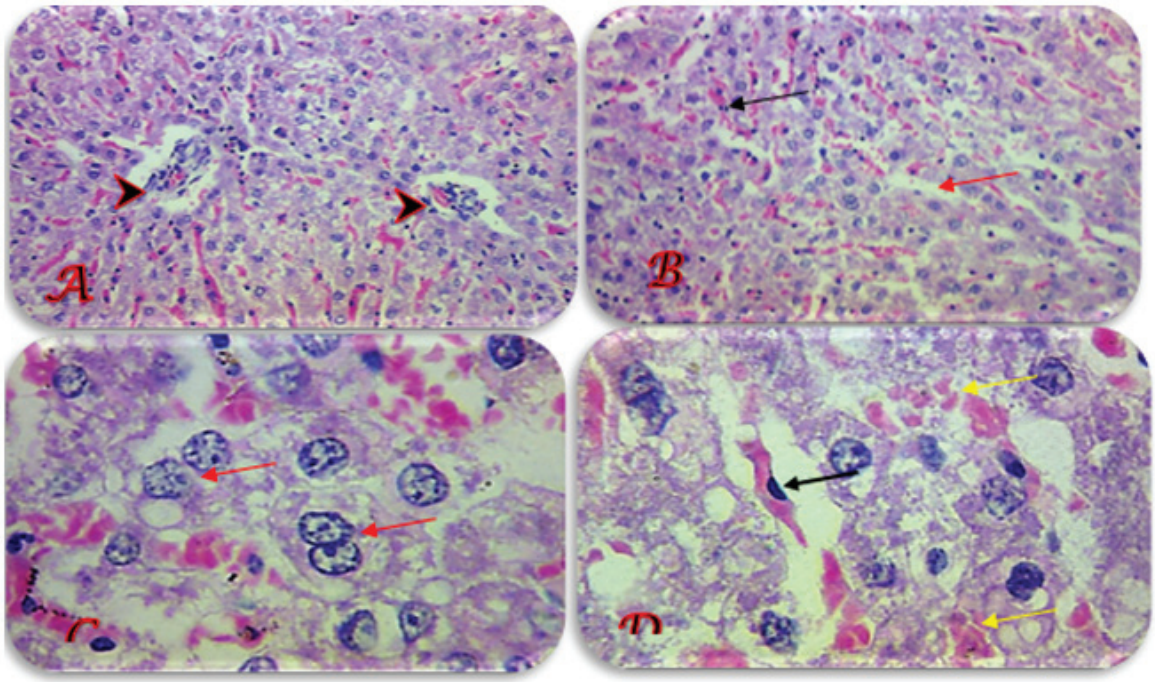

Figure (2): Cross section in liver of rat treated with 0.1 mg/kg body weight of Maxxthor detected by H&E staining: A. aggregation of inflammatory cells near central vein (head arrows) (X10). B: and congestion (black arrow) with dilation of sinusoids (red arrow) (X10), C: proliferation of hepatocytes (red arrows) (X40), D: hemorrhage (yellow arrows) with proliferation of kupffer cells (black arrow) (X40).

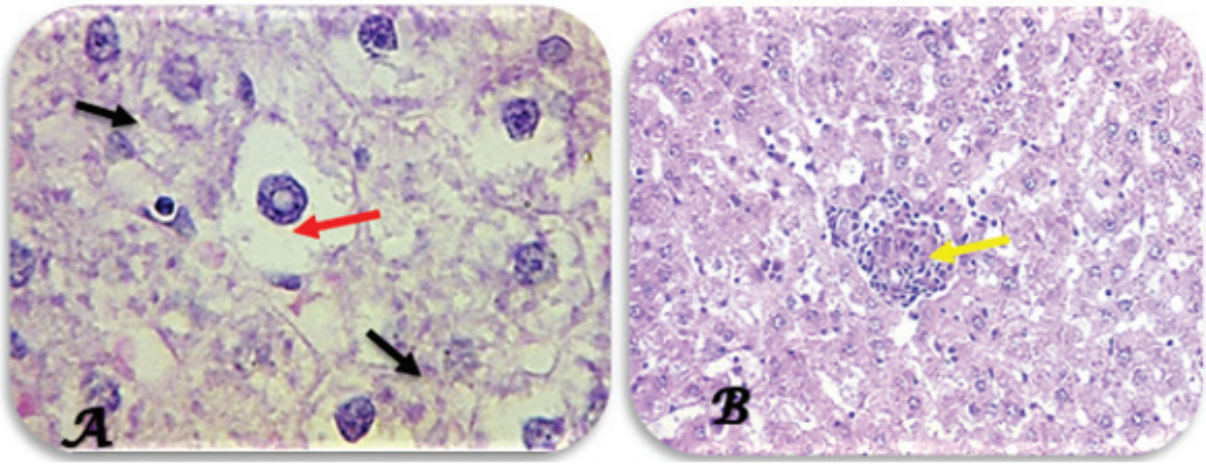

Figure (3): Crosse section in liver of rats treated with 1 mg/kg body weight of Maxxthor showing A: hydropic degeneration in hepatocytes (red arrows) with degradation of chromatins cells (head arrows) B: showing granuloma consist of aggregation of inflammatory cells (yellow arrow) [H &E (A)X10 & (B) X40].

This result agreement with [12] who confirmed the damage caused by cypermethrin pesticide in the liver through degeneration of hepatocytes, infiltration of inflammatory cells, necrosis and mitochondrial swelling. Vascular degeneration in the hepatocytes as well as congestion in the central vein and sinusoid in rat liver when treated with deltamethrin was recorded by [13]. These changes in liver tissue could be explained by understanding that bifenthrin is metabolized in the liver by cytochrome P450 through hydrolytic ester cleavage and the oxidative route to produce acid and alcohol moieties [14]. Congestion and hemorrhage could be as a result to increased blood flow to the inflamed area due to toxic substance of the pesticide. Proliferation

and hyperplasia of the hepatocytes indicate the role of pesticide with its toxic effect in causing important change, that may be the first signs of the occurness of cancer. Granuloma from aggregation of inflammatory cells to the toxic effect of the pesticide that cause inflammatory reaction in liver tissue, which resulted in the attraction of neutrophil and mononuclear cells of the site of injury to remove damaged tissue [15]. Intoxication of rats with pesticide led to generation of ROS, the over production of ROS metabolites can creates lethal chain reaction, that involve oxidation and damage to structures which are crucial for integrity of cell and these free radical lead to membrane and macromolecule damage [16 , 17] .

Enzyme histochemical study

Cross section in control liver rat showing no expression of ALP activity represented in figure (4A). While treated rat with 0.01 mg\kg b.w. of maxxthor showing weak expression of ALP activity in hepatocyte figure (4B), but figure (4C) show sever expression of ALP activity in hepatocyte and figure (4D) show mild expression of ALP activity in hepatocytes.

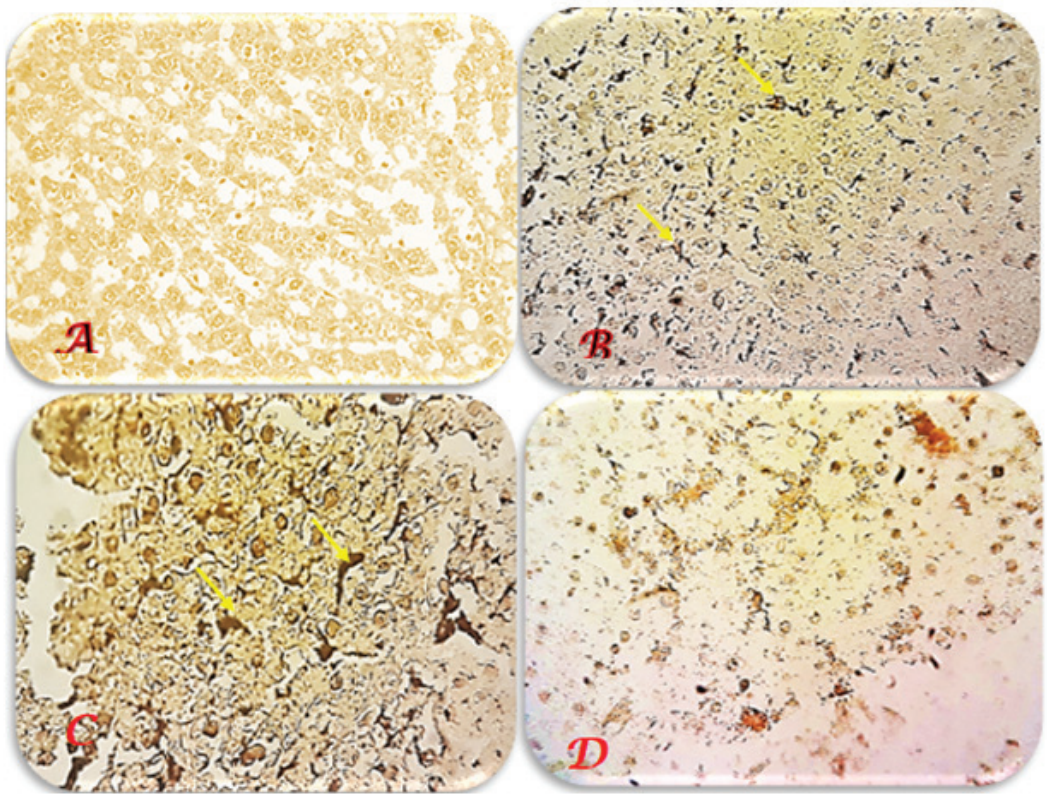

Figure (4): Cross section in liver of rat detected: A. control group showing no expression of ALP activity (X10).B: treated rat with 0.01 mg/k b.w. Maxxthor showing weak expression of ALP activity in hepatocytes (arrows) (X40)., C: the treated rat with 0.1 mg/k b. w. Maxxthor showing sever expression of ALP activity in hepatocytes (arrows)(X10), D: treated dosage 1 mg/k b.w. Maxxthor showing mild expression of ALP activity in hepatocytes (X10).

The ALP is eliminated by bile secretion, any increase or accumulation in ALP amount in liver cells indicate a disturbance mechanism of secrtion [18]. Toxic lesion, hepatitis, and congested liver lead to accumulation of ALP in cell, that accumulation indicate a function disturbance of the liver in biobsy or autoptic lived tissue [19]. Increase activity in ALP can be attributed initially to some pathophysiological condation in liver as a result of pesticide intoxication, may be due to damage in membrane permeability of hepatocytes, resulting in leakage of this enzyme, that

mean maxxthor capable of altering normal hepatocytes architecture [20, 21]. Another possibility for the increase in the activity of alkaline phosphatase may be related to the destruction of the hepatic smooth endoplasmic reticulum membrane in the insecticide intoxicated animals [22]. Sublethal concentration of chlorpyrifos to Gambusia fish lead to increase expression of ALP enzyme activity in liver and kidney tissue indicate that pesticide disturpe the chemical constituents of the fish leading to cell damages and finally mortality of the fish [23]. The increment in activity of hepatic ALP activity due to the cellular damages caused by hepatotoxin or a response to overcome toxicity of pesticide [24].

Liver function study

Table (1) summarized the results of Maxxthor effect on liver function in rats after 40 days of treated with (0.01,0.1, 1) mg/kg b.w. Maxxthor treatment has highly significant ($p<0.01$) increased in the serum AST, ALT and ALP in all treated groups as compared with control group.

Table 1: Effect of Maxxthor (0.01, 0.1 and 1 mg/gk. b.wt.) on liver function of rats

Group	Mean ± SE		
	AST U/L	ALT U/L	ALP U/L
G1:Control	56.28 ± 2.14 d	24.99 ± 0.96 d	102.81 ± 2.76 d
G2: treated with 0.01 mg/kg/b.wt. of Maxxthor	69.26 ± 3.07 c	32.21 ± 1.89 c	141.98 ± 2.57 c
G3: treated with 0. 1 mg/kg/b.wt. of Maxxthor	92.44 ± 2.40 b	44.79 ± 2.54 b	185.65 ± 2.97 b
G4: treated with 1 mg/kg/b.wt. of Maxxthor	146.68 ± 4.56 a	70.10 ± 3.27 a	322.46 ± 12.71 a
LSD value	9.147 **	6.667 **	19.488 **

Means having with the different letters in same column differed significantly, ** ($P<0.01$).
AST = Aspartate aminotransferase , ALT = Alanine aminotransferase , ALP = Alkaline phosphtase

This results agreement with Shakoori *et al*. [25] when treated female rabbits' high doses of bifenthrin pesticides within 4 days. Mansour *et al*. [26] study **the revealed that amounts of AST and ALT enzymes were elevated following intoxication of male rats with the insecticides and their mixture**. Exposure to pesticides lead to liver damage and leakage of cytosolic enzymes from hepatocytes and other body organs into blood [27].In fact, transaminase (AST, ALT) are important and critical enzyme in the biological processes. These enzyme are involved in the breakdown of amino acids into α keto-acids and responsible for detoxification process, metabolism and biosynthesis of energetic macromolecules for different essential functions. Increased amount of these enzymes are indices on cellular smashing and loss of functional integrity of cell membranes in the liver [28]. **Mansour and Mossa [29] found that the activities of transaminases were increased in animals after exposure to pesticides.** Increase in ALP activity in serum can be attributed to some pathophysiological changes in liver as a consequence of pesticides intoxication probaplly due to damage in membrane permeability of hepatocytes, resulting in leakage of this enzyme and pesticide can alter normal hepatocellular architecture [30]. Liver enzymes Elevation may also be due to increased gene expression after long term requirement of detoxification of pesticides [31].

Conclusion

Based on the overall findings from present investigation, it obvious that Maxxthor is toxic to liver following oral treatment of rats at the selected doses. Maxxthor induce pathological changes in the liver tissues and elevated ALP activity in liver tissues. Oral treatment of Maxxthor increased the serum AST, ALT and ALP in male albino rats.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: Non

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