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Histological Changes Induced by Organophosphate Chlorpyrifos in Liver Tissue of Adult Male Rabbits

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الملخص:

المقدمة: مادة الكلوربرفوس هي أحد اهم المبيدات الحشرية التي يكثر استخدمها في حماية المزارع والحظائر الحيوانات لما لها من دور في القضاء على الحشرات ولكن تأثيرها قد يكون ضـــار على حياة الإنســـان لذلك هدفت الدراســـة لمعرفة تأثير الكلوربرفوس على الأنســـجة الكبدية للأرانب البالغة على المدى الطويل من التعرض وكذلك معرفة الدور الوقائي لفيتامين هاء في حماية خلايا النسيج.

المواد والطرق: عشرون أرنب بالغ تم تقسيمها الى أربع مجموعات: -المجموعة الأولى ضـابطة تم تجريعها فقط ماء المقطر اما المجموعة الثانية فهي المجموعة المعالجة تم تجرعيها 33.3م/كم من الكلوربرفوس، والمجموعة الثالثة تم تجريعها فيتامين هاء فقط 100م/كم والمجموعة الرابعة تم اعطاءها المبيد مع جرعة فيتامين هاء، جرعة واحدة صباحية لمدة 12اسبوع، وبعدها تم تخدير الحيوانات بالكلوروفورم وإزالة الأكباد ووضـعها في الفورمالين ومن ثم معالجتها وصـبغها بصبغة الهمياتوكسلين والأيوسين وصبغة ماسون ترايكروم.

النتائج: التجربة بينت أن المبيد له عدة تأثيرات على نسيج الكبد حيث سبب تغيرات في مكونات السيتوبلازم للخلايا الكبدية وظهور علامات التغيرات الدهنية على النسيج، كذلك سبب احتقان الأوعية الدموية وتوسع جدارها بالإضـافة الى ارتشـاح بالخلايا الالتهابية وتبين انها عند تجريع الحيوانات بمضـادات الأكسدة فيتامين (هاء)كان هناك تحسـن في تقليل ارتشـاح الخلايا الالتهابية وكذلك كمية الاحتقان وأصـبحت الخلايا مقاربة لشـكل الطبيعي فضـلا عن بعض الخلايا المتأثرة. يمكن الاستنتاج ان الكلوربرفوس له تأثير سمي على النسيج الكبدي ولكن يمكن تخفيف هذا التأثير باستخدام مضادات الاكسدة مثل فيتامين هاء.

الكلمات المفتاحية: الكلوربرفوس، فيتامين هاء، المبيدات الحشرية، الأنسجة الكبدية الأرانب.

Abstract

Background: Chlorpyrifos (CPF) is one of the most common pesticides that is extensively applied in agriculture and animal houses. Several experimental studies on rats demonstrated that exposure to CPF elicits a group of deleterious effects including hepatic dysfunction, immunological defect and neurotoxicity. Moreover, there is concern regarding their deleterious effects on human life. This study investigated the influence of chlorpyrifos on the hepatic tissue of adult male rabbits in long-term exposure and evaluated the protective role of vitamin E in ameliorating toxic impacts.

Methods: Twenty adult male rabbits were used in the study. Their average weight was (27.6 ±1.891) Kg. They were divided into four groups, five animals in each. The control group received only distilled water. The CPF-treated group was administrated 33.3mg/Kg of insecticide orally by gastric gavage. The vitamin E group received only Vit E; 150mg/kg body weight. Animals in the combination group were treated with CPF and given Vit E at the same time. The treatment was applied once daily in the morning after a food supplement for 12 weeks. Animals were then anesthetized with chloroform, their livers were removed, placed in formalin, processed and stained with hematoxylin and eosin, and with Masson's trichrome

Results: Histopathological changes showed that CPF caused degenerative changes in hepatocytes in terms of vacuolization of cytoplasm, congestion, dilatation of the sinusoidal lumen, and leukocytic infiltration of liver parenchyma, as well as the presence of signs of fibrosis manifested by trichrome stain indicating the chronic effect of pesticides. In the combination group, the majority of hepatocytes presented with normal structures, and the amount of fibrosis was diminished around the portal area. Additionally, congestion appeared to a milder degree than in the experimental group, suggesting the antioxidant role of vitamin E in reducing toxic effects. Conclusion: The study concluded that CPF could cause hepatocellular damage to the rabbits' tissue which can be alleviated by applying antioxidants such as vitamin E.

Keywords: Chlorpyrifos, Vit E, pesticides, rabbit's liver.

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1. INTRODUCTION

Chlorpyrifos (CPF) is one of the most popular types of chlorinated organophosphate pesticides (1) that have an insecticidal effect against pests and is vastly applied in a wide field including agriculture, animal husbandry, gardens, and even households (2). CPF is a cholinesterase inhibitor that is commonly used (3). The mechanism of action of cholinesterase inhibitors is the same in both therapeutic and toxic uses. Inhibition of acetylcholinestrase (Ach E) disturbs the dynamic interaction between acetylcholine synthesis, release, and degradation. This causes an accumulation of synaptic ACh levels with prolonged stimulation of cholinergic receptors on postsynaptic cells. This comparative rise in cholinergic signaling leads to practical signs and symptoms of cholinergic toxicity (4). However, the range of cholinergic signs can be diverse with different inhibitors, the same inhibitor in different classes, or the same inhibitor with different ways of exposure (5). The general dysfunction seems due to inhibition, revealing the primary toxic effect on the endocrine system but it can also affect other organs such as the liver and kidneys (1). Chlorpyrifos is categorized as a moderately toxic pesticide in rats and mice with a lethal toxic dose (LD50) between 50-500 mg/kg body weight. Additionally, it shows moderate toxicity in sheep, guinea pigs and pigs (5, 6).

Several experimental studies on rats demonstrated that exposure to CPF elicits a group of deleterious effects including hepatic dysfunction, immunological defect and neurotoxicity (7). Acute and chronic toxicity of CPF is associated with an increased production of reactive oxygen species (ROS), which has been anticipated as a mechanism to create oxidative stress (8, 9). Antioxidants are molecules that can decrease or stop the oxidation of other molecules by ROS(10). Vitamin E is a non-enzymatic antioxidant, which is a main membrane-bound antioxidant employed by the cell (11). It has been reported that vitamin E has an antioxidant role against lipid peroxidation of cell membranes of mammals and has a role in maintaining the activities of mitochondrial enzymes from ROS (12).

The present study aims to investigate the influence of chlorpyrifos on the hepatic tissue of adult male rabbits on long-term exposure. It is also intended to evaluate the protective role of vitamin E in ameliorating toxic impacts.

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2. METHODS

Chemicals

Commercial formulation of chlorpyrifos (50% EC) of high purity (500g/L) product of Pesticides & Chemicals Company, China. Vitamin E Tablets 100mg were purchased from Sigma Aldrich, India.

Animals

The study was conducted in the Histology Department at the Faculty of Medicine of Omer AL-Mukhtar University. Twenty adult male New Zealander rabbits were used in this study and their average weight was (27.6 ±1.891) Kg. These animals were divided into four groups with five animals in each. They were housed in cages in the experimental animal house of the Chemistry Department of the Science Faculty at Benghazi University and they acclimatized for five days before the experiment under providing standard laboratory conditions with free access to food and water. The cleanliness and hygiene of the rabbits were checked regularly and cages were also cleaned of animal waste daily.

Experimental design

Animals were divided into four groups as follows;

- The control group received only distilled water.
- The CPF-treated group were administrated 33.3mg/Kg of insecticide orally by gastric gavage (13).
- The vitamin E group received only Vit E 100mg/kg body weight.
- The combination group those rabbits were given both CPF (33.3mg/Kg) and Vit E (100mg/Kg).

The treatment was applied once daily in the morning after a food supplement for 12 weeks. At the end of the experiment, animals were anesthetized by chloroform and sacrificed. The liver was removed from each animal and fixed by 10% neutral buffered formalin, washed, dehydrated, cleared, and embedded in paraffin. The prepared sections of 5 µm thickness were stained by Harris Hematoxyline and Eosin (H&E) and Masson's trichrome for histopathological microscopy examination(14).

3. RESULTS

Histological features of control liver sections of rabbits revealed normal hepatic architectures; the central vein (CV) in the center of the lobule and normal sinusoidal lumens (Fig. 1 B). Hexagonal hepatocytes with clear and well-defined nuclei were regularly arranged in the lobule (Fig. 1 B).

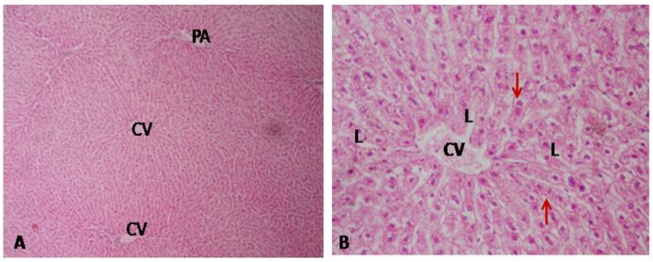


Figure 1: Liver H&E sections of the control group showing: A). (x100)Normal hepatic architecture with the CV in the center of the hepatic lobule and portal area in the corners. Section B (x400) presents normal sinusoidal lumens (L), normal arrangement and structure of hepatocytes (red arrow).

The most frequent histopathological changes in the livers of chlorpyrifos-treated rabbits were cytoplasmic vacuolization of the hepatocytes at the three zones of the liver, which are the first signs of fatty changes (Fig. 2, B), often accompanied by sinusoidal dilatation (Fig. 2, D). Additionally, congestion of the CV has been clearly noticed (Fig. 2 C), with infiltration of mononuclear cells also found especially around CV and PA (Fig. 2 A and C).

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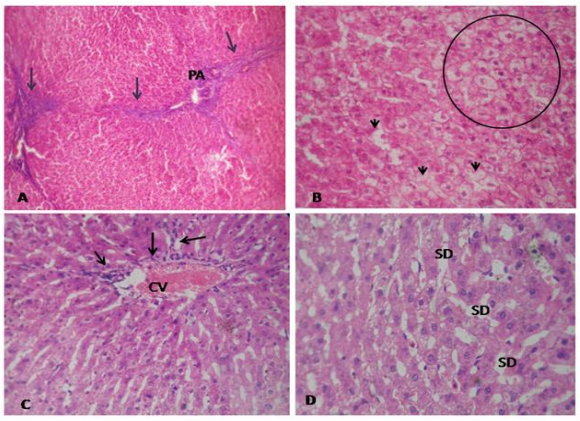


Figure 2: Light micrographs of the liver sections of CPF-treated rabbits showing:- A and B mononuclear cell infiltration around PA (black arrow) and hepatocytes with cytoplasmic vacuolations (circle). C and D congestion of the central vein (CV) and sinusoidal dilatation (SD). (Mic Mag x100 (A) x400(B) H&E).

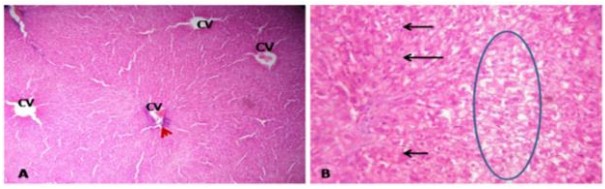


Figure 3: Light micrographs of the livers of rabbits of the combination group showing:- A). Marked reduction in inflammatory cells around CV in liver tissue receiving CPF with Vit E (arrowhead). B). Cytoplasmic vacuolation of hepatocytes was milder than of CPF-treated rabbits without Vit E (circle). (Mic Mag x100 (A) x400(B) H&E).

Microscopic examination of liver tissues of rabbits receiving CPF with Vitamin E shows a reasonable reduction in the inflammatory cells around the CV and PA (Fig. 3A). In addition, the cytoplasmic vacuolation of hepatocytes was milder than that noticed in CPF-treated rabbits (Fig. 3B).

Microscopic examination of liver tissues of rabbits treated with CPF with trichome stain showed collagen deposition around PA, which may indicate an early stage of fibrosis (Fig. 4 B) that is virtually invisible on routine hematoxylin and eosin (H&E) stains. However, in samples from rabbits in the combination group, the amount of collagen deposition reduced around PA as shown in figure 4 C.

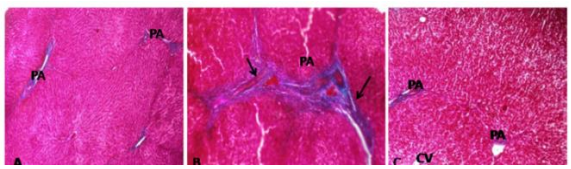


Figure 4: Light micrographs of liver stained with Masson trichrome showing:- A). Control section of liver tissue with a normal amount of collagen around portal area B). Livers of chlorpyrifos-treated rabbits demonstrated an increased amount of collagen and congestion around the portal triad (arrow). C). The combination group showed improved tissue in terms of reducing collagen fibers in the portal area by giving antioxidants.

4. DISCUSSION

The main site of metabolism and activation of CPF is the liver, through the cytochrome P450 enzyme (15). Various previous studies have shown pathological alterations in the liver tissue of animals after exposure to CPF(16, 17). Thus, this experiment was focused on determining the toxic effect of CPF on the liver tissue of adult male rabbits.

Our results showed congestion of the central vein and dilation of the sinusoidal lumens in all liver specimens of CPF-treated rabbits. These pathological changes are probably due to the toxic effect of CPF. These results are in accordance with some investigators (18, 19). Additionally, vacuolation in the hepatocytes was the main histopathological change in all liver samples that received CPF (20). This could be due to an alteration in lipid metabolism in the liver. This result agreed with an experiment on male rats after CPF exposure, where they found a significant elevation in plasma levels of cholesterol and triglycerides (21). Similar pathological changes in liver tissue were reported in previous studies on CPF (22, 23). Moreover, the presence of monocyte infiltration due to CPF intoxication around the CV and PA is shown in **Figure 2 B** and was also highlighted after organophosphate s (OP) exposure in a previous study on male rats (1). A similar finding was presented by EL-Demerdash et al who observed that rats treated with CPF had elevated liver enzymes (AST&ALT) indicating intoxication of hepatic tissue, which subsequently leads to enzyme leakage(24). Thus, the release of enzymes provokes inflammatory reactions and liver necrosis as well. Vitamin E (a-tocopherol) is a lipid-soluble antioxidant (25), which prevents chain initiation and propagation of free radical reaction and lipid peroxidation in the cell membrane (26). It also affects the cellular response to oxidative stress through modulation of the signal-transduction pathway and is considered a membrane stabilizer (27-29). The toxic effects of organophosphates (OP) can be restrained by the oral supplementation of antioxidants such as vitamin E (30). Vitamin E has been shown to have an ameliorating effect against the insecticide's toxicity and plays a role in inhibiting the pathological mechanisms of oxidative stress induced by cypermethrin (31, 32).

The present study found that the severity of hepatocellular damage was minimized in CPF-treated rabbits when they were given vitamin E along with insecticides, indicating that vitamin E ameliorates the toxic effect of CPF. This result is in agreement with the previous work investigating the antioxidant effect of vitamin E against the histopathological changes of CPF treating Kunming mice (33). In addition, other researchers have observed that vitamin E is effective in reducing inflammatory reactions and has a role in the protection of hepatocytes from oxidative stress injury induced by OP in rat tissue(12, 34). Moreover, vitamin E has had an effective role in the prevention of oxidative damage to the lung tissue of CPF-treated animals(35).

In our study, Masson's trichrome stain revealed moderate liver fibrosis between hepatic lobules and around the portal area in the CPF-treated group as shown in Figure 4C; a similar observation has been reported by previous findings(36). This fibrosis is considered the end-stage hepatic injury that usually appeared upon chronic exposure and is consistent with our experiment period. The mechanism of developing liver fibrosis is sophisticated but it is mainly participated by increased reactive oxygen species (ROS) and inhibiting antioxidants of the

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biological system (35, 37). Interestingly, in this experiment, the amount of fibrosis was reduced in rabbit tissue by giving vitamin E with insecticides as compared with the CPF-treated group.

5. CONCLUSION:

It can be concluded that CPF could have a destructive effect on liver tissue and has the risk of being harmful to human life. However, this hepatic damage can be modulated and diminished by using vitamin E as dietary supplementation.

Study limitations

A possible limitation of the current research study is the absence of serum liver enzymes that could have contributed to the valuation of functional liver involvement in CPF intoxication.

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