

## EFFECT OF SUPERCYREN PESTICIDE IN THE HEART AND EYE TISSUES OF MICE

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### ABSTRACT

Organophosphorus insecticides constitute a large family of pesticides and they are used widely for controlling pests in the household, agricultural and urban environment, the present study aims to study effect of supercyren pesticide in the heart and eye tissues of mice . about Thirty albino male mice divided into three groups treated orally with supercyren pesticide in dose (ze, o,10, 20 mg /kg). After 30 days of the experiment the troponin protein was measured in blood samples of three groups for one month. For histological study samples of the heart , retina and cornea were collected and prepared with paraffin technique and stained with Hand E stain. The blood samples were negative for troponin protein in the control group and were positive at doses of (10mg and 20mg). The histological sections showed some changes in the heart and eyes with dose (10 and 20 mg/ kg ). The present study concluded harmful effects of various concentrations of supercyren in some body organs.

**Key words:** pesticides, troponin , heart , retina, Organophosphorus

### INTRODUCTION

Organophosphorus insecticides constitute a large family of pesticides and they are used widely for controlling pests in the household, agricultural and urban environment [Kacham *et.al.*, 2006]. Chlorpyrifos is one of organic phosphorous compound used in different aspects throughout life, such such as in agricultural fields it is also used in house pest control throughout the world [Saulsbury *et al.*, 2009]. Chlorprifos has pharmackinetic mode similar to other organic phosphorous compound by inhibited acetylcholine esterase activity [Kaur and Sandhu,2008]. Chlorprifos effected in pregnant rats was inhibited materialfetal foetal brain activihours within 24 hr. [Zheet *altal.*, 2000]. The most powerful effect of chlorpyrifos poisoning is dermal absorption especially in workers with means of plant protection on the other hand, certain pestiside have composed of two or three active ingredients in order to decreased poisoning effect of its [Zheng *et al.*, 2000]. The modern use of insecticides is substantially improving the economic and social well being of the inhabitants of developing world by increased food production and by the effective control of public health vector-borne diseases, blood disorders, brain and nerve damage, cancer infertility (sterility) are often associated with low residual level of pesticides [Hatice and Kalendor , 2011]. In animals , interrupted the estrous cycle, decreased appetite or reproductive problems,

decreased milk production can be correlated with continues low-level exposure of pesticides [Kalender *et al.*, 2010]. The aim of this study was investigated the toxic effects of different pesticide concentrations in troponin level and histological changes in heart and eye (retina and cornea) in adult male mice.

### MATERIALS AND METHODS

Thirty albino male mice weighting 140- 180 gram were housed in separated cages in the animal house at College of Science - Al-Mustansiriyah University under laboratory conditions ( Temperature 20-25 c ) were performed at January, 2017. The mice were divided into three groups each (10 mice) , group one (control ), group two orally administrated supercyren pesticide (10%) a dose (10 mg /kg/ BW) for one month , group three was administrated supercyren pesticide given daily at dose (20 mg/kg/BW).All mice were supplied with feed and water during the period of experenimt ( one month). using ( material active: 500 g /l chlorpyrifos and 50 g / l cypermethrin). Blood samples from each group were collected by direct cardiac puncturethen serum separated by centrifuging (3000 rpm) for (15 minutes) after this it transferred to freezer at( 20c) until analysis using enzyme immunoassay (ELIZA) .

Heart and eyes were dissected out , cleaned with physiological saline solution (0.9 % NaCl ) and fixed with 10% buffered formalin about 7 days

then subsequently tissue samples of heart and eye were dehydrated with different concentrations of upgrading ethanol, cleared in xylol and embedded in paraffin at 58°C. The tissue block was sectioned at 5µm with rotary microtome and stained with hematoxylin and eosin stain [Bancroft and Stevens, 1982]. Tissue sections were examined by (AHBT) research photomicrographic microscope system of Olympus Corporation, U.S.A.

## RESULTS AND DISCUSSION

The results were revealed marked changes in troponin activity in the blood between three groups, the second and third group showed positive results compared to control group (Tab.1). The present result suggests that the troponin has been released in the blood when the heart muscle contracted and damaged such result is supported by Anjaneyulu and Chopra, (2003) whom reported that the troponin I and troponin T are proteins with the microstructure of the myocardium and elaborated into serum after myocardium damage or any cardiac myopathy. A high troponin elevation may indicate some degree of damage to the heart. In people with angina, an elevated troponin may indicate that their condition is worsening and they are at increased risk of a heart attack [Gifford *et al.*, 2006]

Table 1 : Shows the serum troponin test in different concentrations of pesticide. (concentration please or mention method used for detection troponin)

Concentration of supercyren pesticide	Troponin test
Control	-tive
10 mg / kg / day	+tive
20 mg / kg / day	+tive

These results of troponin came identical with heart tissues that have been taken for three groups of supercyren pesticide (Zero, 10, 20 mg/kg/day) observed that there does not in texture heart in control tissue heart but had the effect in heart

tissue when the concentration of (10 mg) and (20 mg) but was greatest in fluence upon to a (20 mg). The sub chronic and chronic poisoning with of organic phosphorus insecticide may related with many cases showed pathological changes in various body organs [Yavus *et al.*, 2004], [Kalender *et al.*, 2010] in liver, [Karaoz *et al.*, 2002] lung, [Yehia, *et al.*, 2007] kidney, [Uzunhisarcikli *et al.*, 2007] and tests, also many studies showed that the pesticides have adverse effects in heart tissue [Yavua, *et al.*, 2004], [Thomaz *et al.*, 2009]. On the other hand pesticides has same effects to the pro-oxidants which recorded in various body organs [Limonpacheco and Gonsebatt, 2009]. Oxidative effect and damage by pesticides have initially occurred through elaboration of reactive oxygen species and can distract fats, amino acids, nucleotides and cellular DNA, oxidative cellular damage may lead to loss of cellular enzymes activity and integrity consequently activate inflammatory processes [Ozyurt *et al.*, 2004]. The present result revealed that the chlorpyrifos led to disorganization and degeneration of myocardial fibers, cytoplasmic vacuolization in cardiac muscle cells, edema in connective tissue, degenerative changes in cardiac muscle cells were detected in heart tissues. The present result suggests that the positive troponin test was due to the increase of intra-cellular reactive oxygen species in heart tissues (Fig. 1,2,3). Many studies recorded that the use of chlorpyrifos compound induced cardiotoxicity in rats, and serum malondialdehyde concentration is increased in the case of myocardial damage in rats while in chlorpyrifos treated rats, there is an increase in MDA concentration related to the induction of oxidative stress in rats treated with chlorpyrifos [Hatice and Kalender, 2011].

On other hand the treatment for four weeks led to disorganization and degeneration in myocardial fibers, cytoplasmic vacuolization in cardiac muscle cells, edema in connective tissue, degenerative changes in cardiac muscle cells were detected in heart tissues, these changes could be related from an increase in reaction oxygen activities in heart tissue. The pathological changes in the antioxidant treated groups were mild, such result suggest that a dose of chlorpyrifos lead to heart toxicity and decreased glutathione peroxidase and glutathione-S-transferase (GST)

activities in the heart compared to the control then induced histopathological alterations in heart [Hatice and Kalendor, 2011].

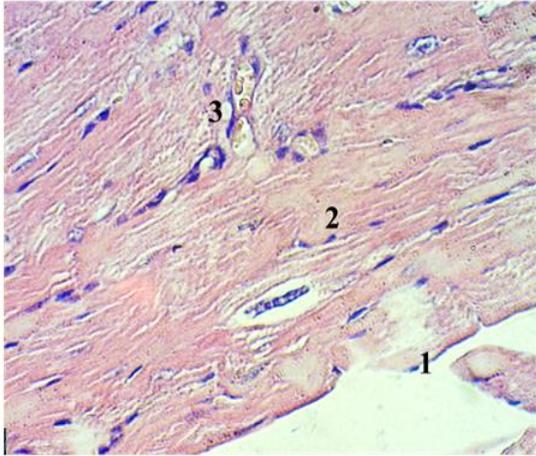


Figure 1: Section of heart from mice control group showing Endocardium a thin layer of connective tissue lined by simple squamous endothelium (1) . Between the endocardium and pericardium a layer of variable thickness called the myocardium (2) containing small nerves, blood vessel (3) and the ventricles conducting (Purkinje) fibers these fibers are cardiac muscle cells specialized for impulse conduction, purkinje fibers intermingling with contractile fibers within the myocardium. (HandE, 400X)

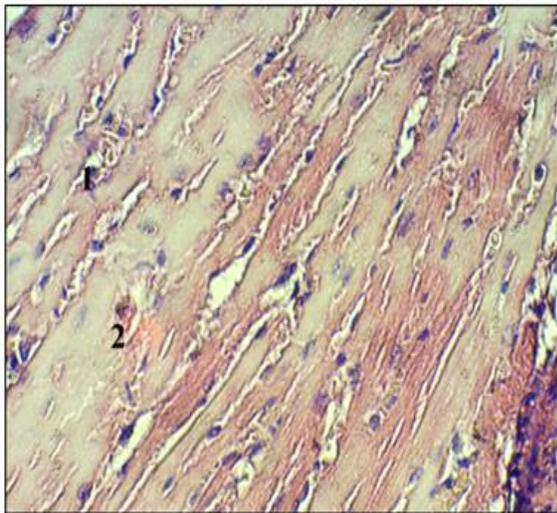


Figure (2) Section of heart from mice groups treated with (10 mg/kg/day) of supercyren pesticide for one month showing infarction of the myocardium is caused by acute ischemia , the result generally of sudden narrowing of a coronary artery , the artery is blocked by occlusive thrombus which has formed on the surface of an athromatous plaque (1) The necrotic muscle fibers . The nuclei no longer stain and are not visible necrotic muscle fibers are separated by a richly cellular vascular tissue ( 2 ) which contains many polymorphs ,

macrophages and fibroblastis would have formed a fibrous scars . (H and E , 400X )

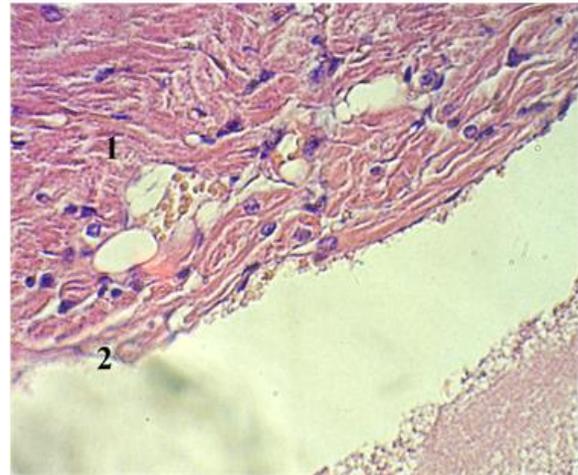


Figure (3) Section of heart from mice groups treated with (20mg/kg/day) of supercyren pesiticide for one month showing infacts confined for no obvious reason to the right ventricle. In this part of the infarct ,the myocardial muscle fibers are elongated , necrotic and aligned in parallel ( 1) . the nuclei in the muscle fibers are not visible,the fibers are more deeply eosinophilic (2) than normal myocardial fibers , in addition to some nuclear fragments , macrophages which have migrated into the sheet of dead muscle . (Hand E , 400X ) .

Histopathological results revealed marked degeneration of the sensory cells of the retina with marked abnormalities in the pigmented epithelial cells and outer plexiform layer of the retina. This result confirmed that the pesticides lead to disorganized of retinal structure . The pesticides have an environmental risk factor which associated with retinal degeneration [ Geller , 2002].

The present histopathological results of the control group were presented in (Fig.4) the tissue sections of normal in the sclera , choroid and retina while in ( Fig.5,6) represented mice groups treated with (10 , 20 mg/kg/day ) of supercyren for one month we showing section of abnormalities in the pigment epithelium cells and separation of outer plexiform layer in retina forming synape with dendrites of the neurons in the inner nuclear layer. Clorpyrifos produce oxidative of lipid pe-oxidative products in different organs of rats [ Joshi, *et al.*, 2007]. Furthermore, no changes in the section of cornea in mice that we shown as in (Fig.7,8,9)

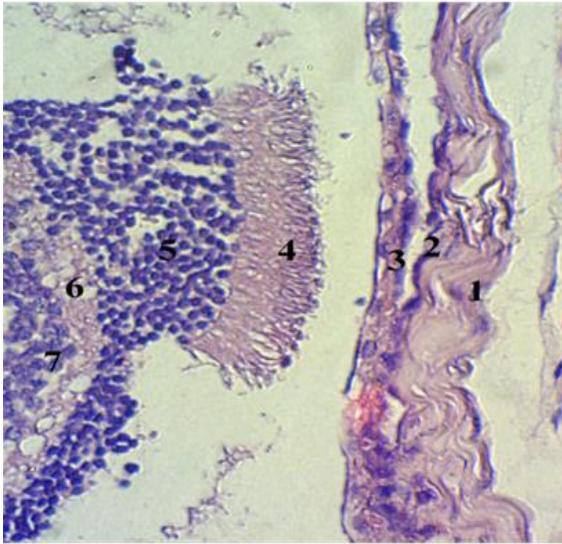


Figure 4: Section of sclera , choroid and retina in mice control group. The outer layer of the eye shows the dense connective tissue of the sclera (1) and the loose vascular connective tissue of the choroid (2 ). The choroid's inner region , the choroidocapillary lamina has a rich microvasculature which helps provide O<sub>2</sub> and nutrients to the adjacent retina . The outer layer of the retina is the pigmented layer of cuboidal epithelium containing melanin (3) . Adjacent to this are the packed photoreceptor components of the rods and cones (4) , the cellbodies of which make up the outer nuclear layer (5) . Axons of the rods and cones extend into the outer plexiform layer (6) forming synapse there with dendrites of the neurons in the inner nuclear layer (7) . These neurons send axons into the inner plexiform layer .( Hand E , 400X )

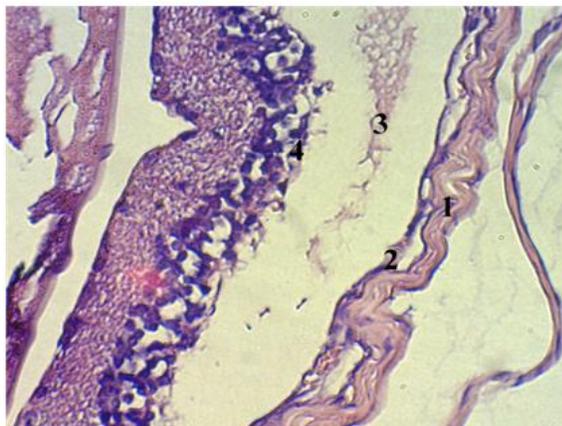


Figure 5: Section of sclera(1) , choroid (2)and retina from mice group treated with (10 mg / kg / day ) of supercyren pesticide for onemonth showing abnormalities in the pigment epithelium cells and separation of outer plexiform layer ( 3 ) who forming synapse with dendrites of the neurons in the inner nuclear layer(4). ( H and E , 400X )

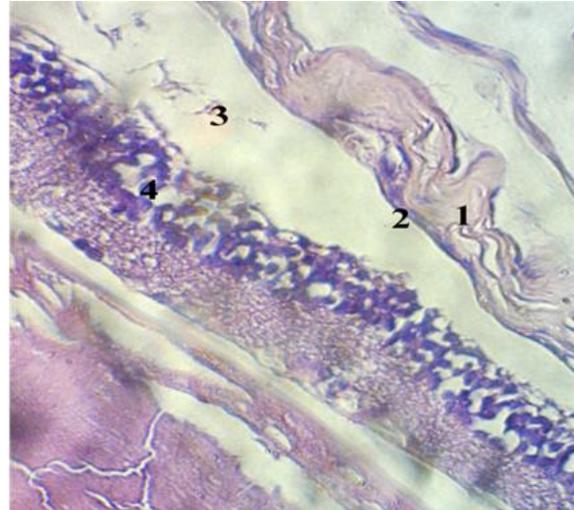


Figure 6: Section of sclera (1) , choroid (2) and retina from the mice group treated with (20 mg / kg / day ) of supercyren pesticide for one months showing abnormalities in the pigment epithelium cells and separation of outer plexiform layer ( 3 ) who forming synapses with dendrites of the neurons in the inner nuclear layer (4) . ( HandE , 400X)

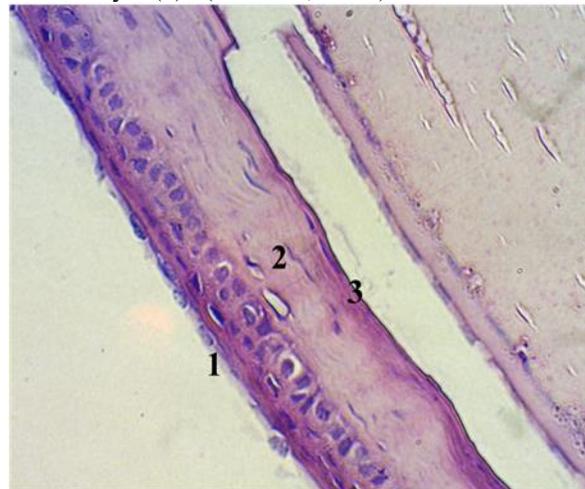


Figure 7: Section of cornea in mice control group showing the interior structure of the eye , the cornea has three layers , the corneal epithelium (1) rests firmly on they thick homogeneous Bowman's membrane . The stroma (2) is completely avascular and nutrient reaches the keratocytes and epithelial cells by diffusion from the surrounding limbs and aqueous humor behind the cornea , the posterior surface of the cornea is covered by simple squamous epithelium (3 ) that rests on another thick strong layer of collagen and other extracellular material called Descemet's membrane . (Hand E , 400X )

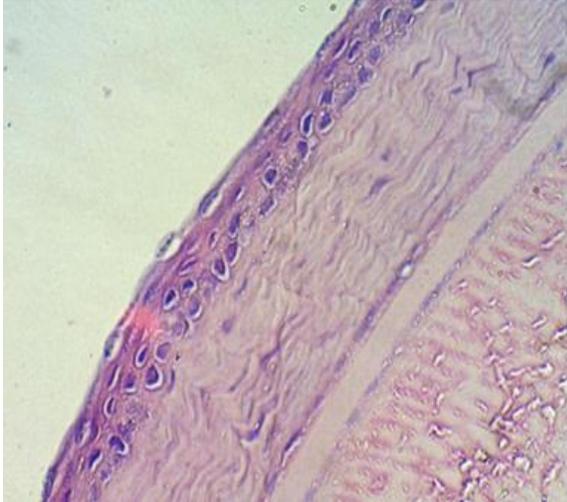


Figure 8: Section of cornea from mice group treated with (10 mg / kg / day ) of supercyren pesticide for one month showing no change from normal cornea section in mice control . ( Hand E , 400X)

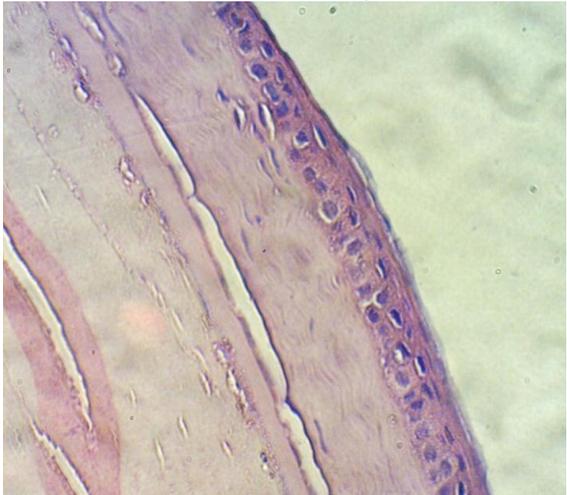


Figure 9: Section of cornea from mice group treated with (20 mg / kg / day ) of supercyren pesticide for one month showing no change from normal cornea section in mice control . ( Hand E , 400X)

### Conclusions

The present study showed significant histomorphological changes in the heart and eye of mice, those orally administered with chlorpyrifos. The chlorpyrifos treatment led to disorganization and degeneration in myocardial fibers, cytoplasmic vacuolization in cardiac muscle cells, edema in connective tissue, degenerative changes in cardiac muscle cells were detected in heart tissues . In eye tissue degeneration of the sensory retina and marked abnormalities in the pigmented epithelial cells and separation of outer plexiform layer in retina .

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