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Histological Alternations Of Cardio-Skeletal Myocytes In Broiler Induced by Cypermethrin

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ABSTRACT

In Libya, using of insecticides is not well controlled compared to the developed countries. Therefore, the designed experiment was undertaken to identify the degree of damage caused by cypermethrin (CYP) on broiler cardio-skeletal muscles and to find out safety dose that may minimize toxic residues in chicken meat. 4 groups of broiler (10 each) were administrated water without (control) or mixed with CYP (7mg\kg, 14mg\kg and 21mg\kg body weight) for 2 weeks. After completion experiment period, heart and skeletal muscle samples were taken from breast, thigh and leg, using standard procedure and fixed in 10% buffered formalin saline, followed by immersion the tissues in a series of gradually increasing concentration of alcohol and xylene, then embedded in hard paraffin and sectioned at 3 um. Special stains were used to study histological alternations and examined under light microscope. The gross observations showed pathological changes on heart, whereas no lesions found in skeletal samples. CYP intoxicated broiler chickens showed loss normal architectures of cardio-skeletal myocytes represented in wide intercellular spaces, high amount of collagen and elastic fibers, adipose tissue found between muscle fibers, congested blood vessels and extravasation of blood cells observed in an inter-ventricular septum of cardiac tissue. Histochemical investigations revealed increase glycogen deposition between and within cardio-skeletal myocytes. The study concluded that CYP promote histological alternations in broiler cardio-skeletal myocytes even at low concentration.

التغيرات النسيجية للخلايا العضلية القلبية و الهيكلية في دجاج التسمين الناتجة عن مادة السايبرمثرين

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الكلمات المفتاحية:
الخلايا العضلية
السايبرمترين
القلبية الهيكلية
دجاج التسمين

الملخص

تم تصميم هده الدراسة التجريبية لتحديد درجة الأضرار الناتجة عن استخدام مادة السايبرمثرين على الخلايا العضلية القلبية و الهيكلية لدجاج التسمين. أجريت التجربة على 4 مجموعات من دجاج التسمين، 10 في كل مجموعة ، حيث تم تجريع مجموعة التحكم مياه نقية خالية من مادة السايبرمثرين، بينما تم تقسيم المجموعة التجريبية إلى 3 مجموعات، حيث تم تجريع المجموعة الأولى و الثانية و الثالثة لتراكيز مختلفة من مادة السايبرمثرين (7مليجلرام ، 14 مليجلرام و 21 مليجلرام يوميا) لمدة أسبوعين. بعد انتهاء التجربة تم تجميع عينات كلا من القلب و العضلات الهيكلية من جهة الصدر،الفخذ و الساق و خفضها في مادة الفورمالين ، بعد دلك تم تمرير العينات بتراكيز مختلفة من الكحول و الزايلين و وضعها في قوالب شمعية، قطعت باستخدام جهاز الميكروتوم بحوالي 3 ميكرومتر و صبغها بصبغات مختلفة و فحصها تحت المجهر الضوئي. تم ملاحظة تغيرات مظهرية على عينات القلب بينما لم تسجل أية ملاحظات على عينات اللحوم التي أخدت من الصدر و الفخذ و الساق. أظهرت الفحوصات المعملية تغيرات نسيجية للخلايا العضلية و الهيكلية تمثلت في تقطع و ووجود فراغات بين النسيج العضلي ، بالإضافة إلي زيادة الألياف النسيجية و الذهنية و احتقان الأوعية الدموية و تخلل كرات الدم الحمراء بين خلايا النسيج . أيضا تم ملاحظة زيادة ترسب حبيبات الجلايكوجن بين الخلايا العضلية،

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خاصة في المجموعة الثالثة و الثانية مقارنة بالمجموعة الأولي و مجموعة التحكم على التوالي. مما سبق استنتجت الدراسة أن مادة السايبرمثرين لها تأثير على التركيب النسيجي للخلايا العضلية و الهيكلية حتى بالنسبة للجرعات الأقل تركيزا.

Introduction

The cypermethrin (CYP) is a viscous semi solid, yellowish brown colored, odorless substance with Molecular formula : C22H19Cl2NO3 and chemical structure : [Cyano-(3-phenoxyphenyl) methyl] 3-(2,dichloroethenyl)-2,2-dimethylcyclopropane-1-carboxylate. It is a synthetic pyrethroid insecticide containing 3 chiral centers, giving a racemic mixture of 8 isomers comprising 4 diasterioisomeric pairs. Its structure is based on pyrethrum, a natural insecticide produced from chrysanthemum flowers having higher biological activity and stability than its natural model[1].

Pyrethroids are widely used in many commercial pesticide formulations and constituting about a quarter of total insecticides market. These chemicals are frequently utilize in agriculture, residential areas and public health[2]. Previous studies have been detected pyrethroids in water, fruits and vegetables[3], [4]. The residual toxic of these compounds has been found in different types of crops in some regions of Libya[5].

CYP is primarily absorbed from gastrointestinal tract or by inhalation of spray mists, and because of its lipophilic nature, it has been found to accumulate in adipose tissue, skin, liver, blood, kidneys, adrenal glands, ovaries and brain[6]. Cardiovascular system is also target and mediator of toxicity following chemicals exposure[7]. Mammals are able to metabolize pyrethroids after ingestion in urine forming the main metabolite, 3-phenoxybenzoic acid (3-PBA) [8]. Recently, some studies were detected the bioaccumulation of CYP in meat of chickens, fish and beef as well as chicken eggs[9], [10].

Chickens are vulnerable to pyrethroid toxicity because household species are dusted with pests[11]. Exposure of birds to insecticides causes health hazards and constituting a possible threat to public health as result of presence of toxic residues in poultry meat[12]. This kind of pesticides causes acute and chronic health effects , including respiratory, neurological, gastrointestinal, endocrinal and reproductive effects, and stimulation carcinogenic activity[13], [14].

All animals have three types of muscles : smooth, cardiac and skeletal muscles. Cardiac muscle is the specialized muscle of the heart. Skeletal muscle is the type of muscle responsible for shape and voluntary movement of birds. The poultry meat is classified as skeletal muscle type into two types of meat in chickens; white meat which founds in breast of chickens and referred to as white meat due to minimal activity of these muscles. Dark meat founds in the thigh and leg meat as they sustained activity and because of presence the chemical compound in the muscles called myoglobin, which is important for oxygen transport[15].

The skeletal muscles receive relatively large amounts of administered doses of chemicals as they represent about half of a mammal's body weight. Therefore, it is a target organ for a variety of toxic effects range from minor muscle weakness and slight pain to complete paralysis, and may produce cardiotoxicity by prolongation of cardiac myocytes [16], [17].

However, there is a widely used of different insecticides which CYP is one of them with lack of awareness and inappropriate pest management, this lead to serious

environmental deteriorations that indirectly effect on non-target species. Thus, this study was undertaken to identify the degree of injury caused by CYP intoxication on cardio-skeletal myocytes of broiler and find out safety dose that successfully manage pesticides, and ensure that pesticide residues in chicken meat do not exceed maximum residues levels.

Materials and Methods

1.Ethics

All the chickens were supplied with water and poultry food, handled accordingly as proposed by the guidelines for the care and use of animals for experiment set by W.H.O.

2.Experimental Design and Sitting

An experimental study was conducted at Aboud's Farm, Tripoli – Libya. Department of Histology and Embryology, Faculty of Veterinary Medicine, Department of Histology and Genetics, Faculty of Medicine, University of Tripoli; Tripoli – Libya.

3. Experimental Tools

CYP was purchased from local supermarket in Tripoli city by the trade name of DIPACXON – 39 (CYP 10%), manufactured by CENAVISA company – Spain. The solution was prepared and used immediately by dilution 1ml of concentrated CYP in 1 litter of distilled water according to the manufacturer instructions. The doses of CYP were determine by testing the compound on few chickens and the maximum toxic dose which was used according to the active ingredients of the substance. Forty matured broiler chickens of both sexes were randomly selected for this study. The chickens were healthy and weighed between 1,163 and 1,598 kg, and were allowed to acclimatize for 10 days before start the experiment. They housed in stainless steel cages and maintained under standardized environmental conditions (25 ºC) away from stress with a 12 – hour light\dark cycle and 50% humidity.

4. Experimental Samples

To study the histological alternations of CYP in cardio-skeletal muscle cells, a total of 40 broilers were randomly divided into 4 groups,10 chickens in each group : Control group: chickens received water without any ingredient of CYP; low treated (T_1): received 7mg\kg; intermediate treated (T_2): received 14mg\kg and high treated (T_3): received 21mg\kg body weight of CYP by oral gavages, using disposable syringe after removing the needle daily for 2 weeks. The chickens were weighed daily for adjustment of the daily dose volume.

5. Sample Collection

After completion of treatment period, the chickens were anesthetized using chloroform and sacrificed by cervical dislocation. Heart and skeletal muscle were taken from breast, thigh and leg, using standard procedure and washed thoroughly with 0.9% normal saline to remove any race of blood then fixed immediately in 10 % buffered formalin saline.

6. Sample Preparation

All the samples were washed with distilled water, Followed by dehydration by immersing the tissue in a series of gradually increasing concentrations of alcohol (50%, 70%, 80%, 95% and absolute alcohol) and xylene, then immersing in soft paraffin overnight in oven at 60 ºC then embedded into hard paraffin for making blocks and sectioned at 3 µm as serial sections. In order to show the histological alternations of CYP on cardiac and skeletal muscles , Hematoxylin & Eosin (H&E), Masson Trichrome and Periodic-acid Schiff (PAS) stains were used and examined under light microscope[18].

Results

After 2 weeks of the experiment, a post mortem organs were examined and found heart appeared as a dark brown in color with black dots scattered on its apex, whereas accumulation of fatty tissue was observed on its base. In contrast, no gross lesions found in skeletal muscle specimens.

Control Groups :

1. Cardiac Myocytes

The histological investigations revealed normal architecture of cardiac muscle cells with regular short branched fibers with spindle vesicular nuclei and eosinophilic cytoplasm (Fig.1a). Normal distribution of adipose tissue, collagen and elastic fibers were observed between cardiac muscle cells (Fig.1b). Histochemical changes showed normal deposition of glycogen granules within and between cardiac myocytes (Fig.1c).

2. Skeletal Myocytes

Normal structure of muscle fascicle having multinucleated peripheral nuclei exhibited with regular arrangement of muscle fibrils separated by narrow spaces from each other (Fig.2a). Normal distribution of collagen and elastic fibers were observed between skeletal myofibrils (Fig.2b).

Histochemical changes showed normal deposition of glycogen granules within and between muscle cells (Fig.2c).

Treatment Groups :

1. Cardiac Myocytes

Findings with H&E stain representative dystrophic muscle fibers with hypereosinophilic myocytes, deeply stained nuclei and loss normal arrangement of intercalated discs. More accumulation of adipose tissue found between cardiac myocytes (Fig.3a), and atrium myocytes in T_3, in contrast to T_2 and T_1 respectively. Congested blood vessels with extravasation was observed between cardiac myocytes of interventricular septum of T_3 (Fig.3b).

Another important findings, were abundant collagen fibers found between cardiac myocytes in T_3 (Fig.4c –d), compared to T_2 (Fig.4a –b), and T_1, and the histochemical changes of heard specimens of T_3 and T_2 (Fig. 6b) and T_1 (Fig.6a), that showed increased glycogen deposition between cardiac muscle cells.

2. Skeletal Myocytes

Necrotic muscle cells were detected without nuclei in T_3 . In comparison to T_1 and control, wide intercellular spaces with high amount of adipose tissue and dilated blood vessels T_3 (Fig.3 c – d). Dense extensive connective tissue fibers surrounding muscle bundles and between skeletal myocytes were observed in T_3 (Fig.5c – d), compared to T_2 (Fig. 5b) and T_1 (Fig.5a) respectively. High glycogen deposition within myocytes as well as between muscle myofibrils was noticed in T_2 and T_3 (Fig.6c – d).

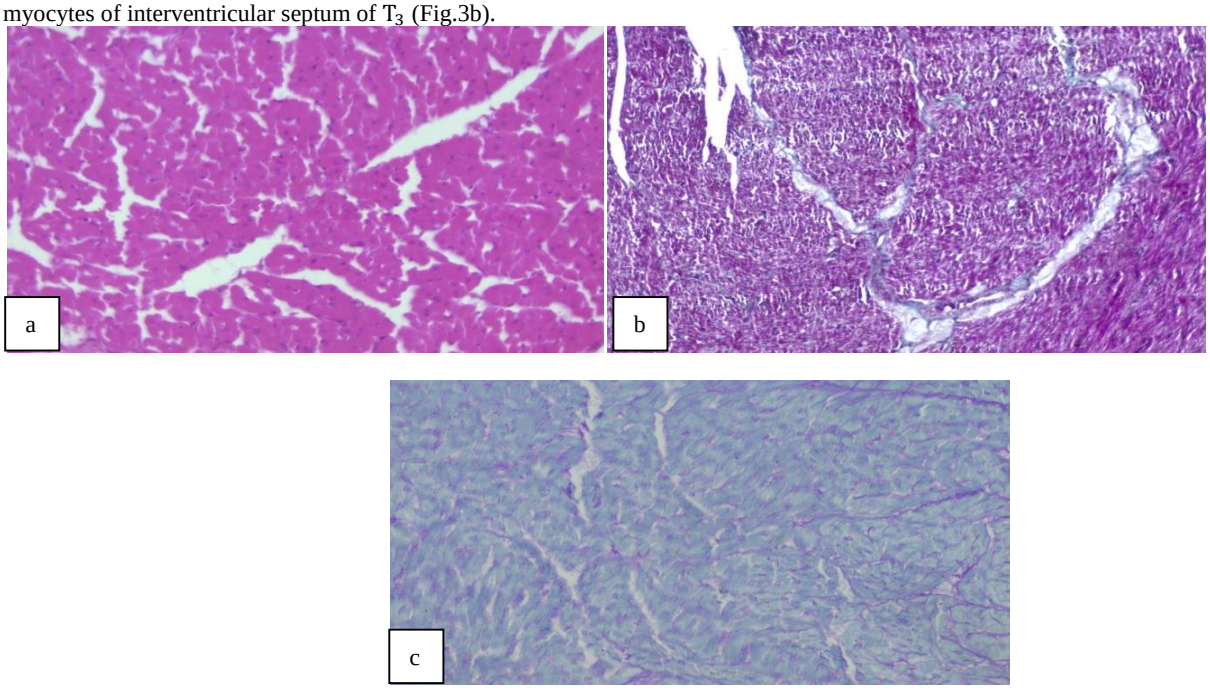

Figure1 : Photomicrographs of normal cardiac muscle showing: (a) Regular short branched fibers with spindle vesicular nuclei and eosinophilic cytoplasm, H&E (×40). (b) Normal distribution of collagen between cardiac muscle cells (**green colour**), Masson Trichrome (×20) (c) Normal deposition of glycogen granules within and between cardiac myocytes(**purple colour**), PAS (×20).

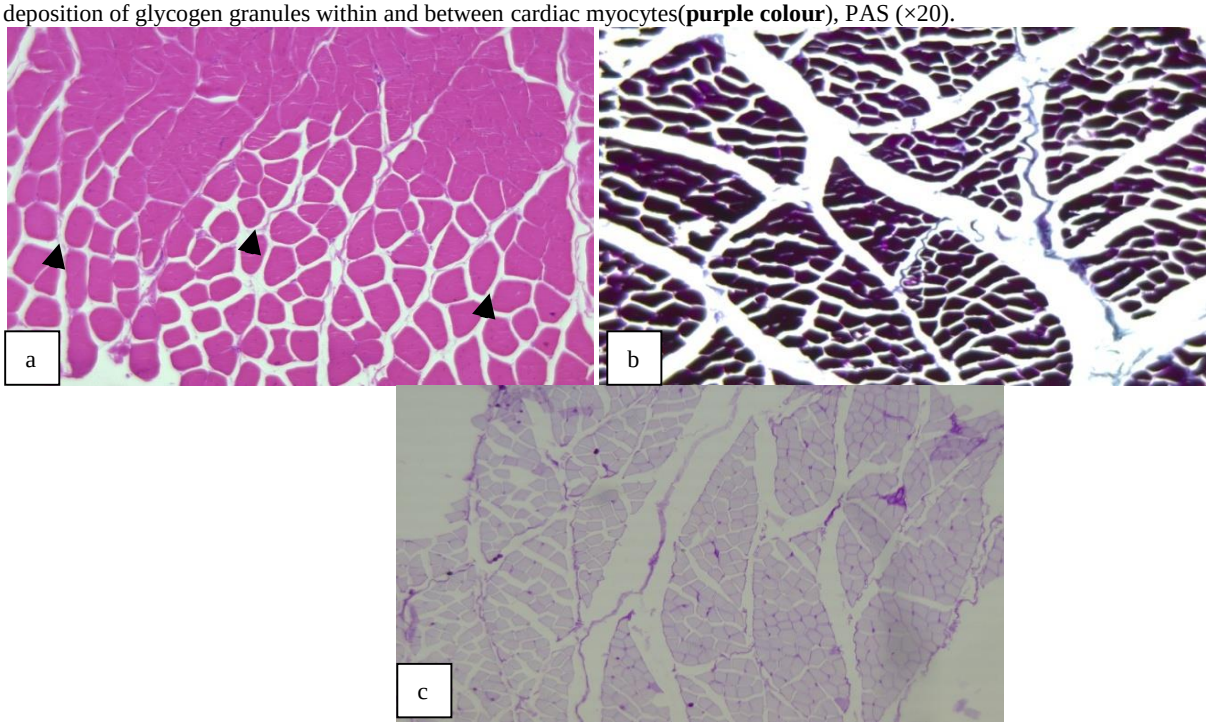

Figure2 : Photomicrograph of normal skeletal muscle fascicle showing : (a) Multinucleated peripheral nuclei separated by narrow spaces from each other (**arrows**) H&E (×10). (b) Normal distribution of collagen fibers between skeletal myofibrils (**green colour**), Masson Trichrome (×20). (c) Normal deposition of glycogen granules within and between muscle cells (**purple colour**), PAS (×10)

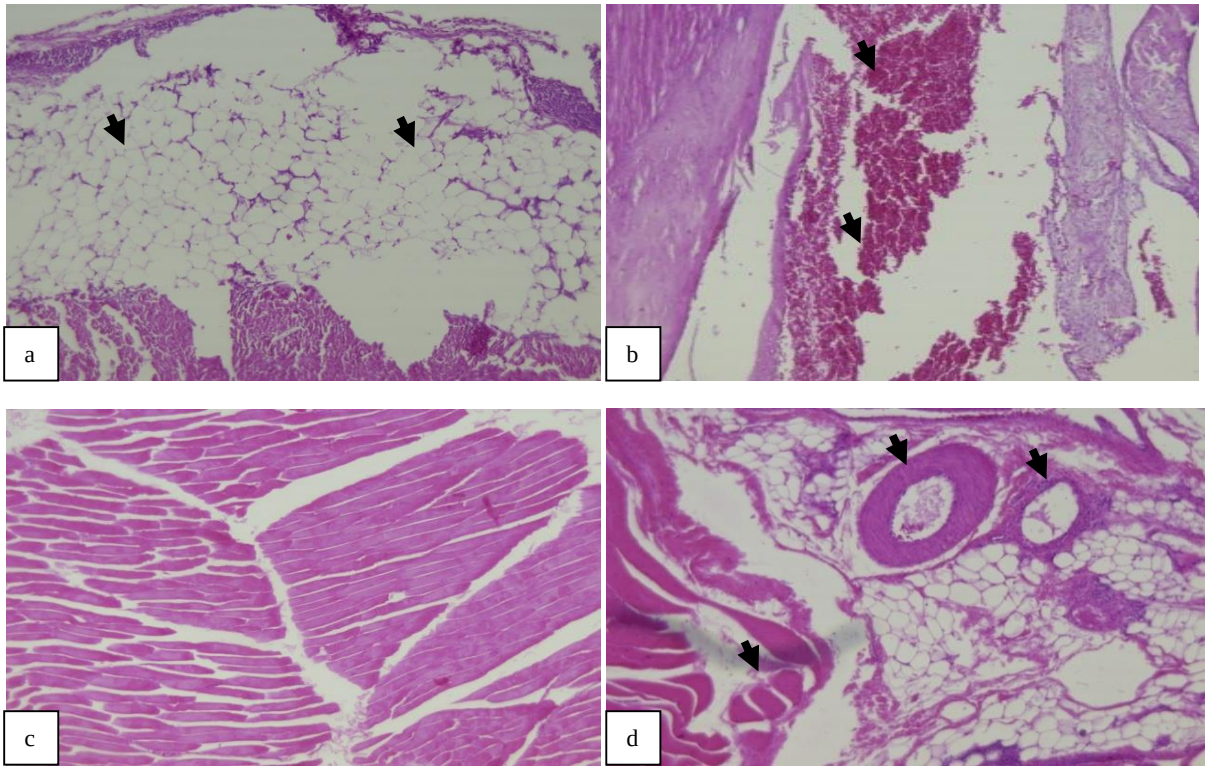

Figure3 : Photomicrographs of cardiac muscle of broiler administrated CYP (21mg\kg BW); showing : (a) High accumulation of adipose tissue surrounding epicardium layer (**arrows**). (b) Congested blood vessels resulting in extravasation in interventricular septum (**arrows**), H&E (×20). Photomicrograph of skeletal muscle of broiler administrated CYP, taken from wing and breast (21mg\kg BW) showing: (c) Necrotic muscle cells showing completely loss nuclei. (d) High amount of fatty tissue with congested dilated blood vessels and destruction of muscle fibers (**arrows**),H&E(×10).

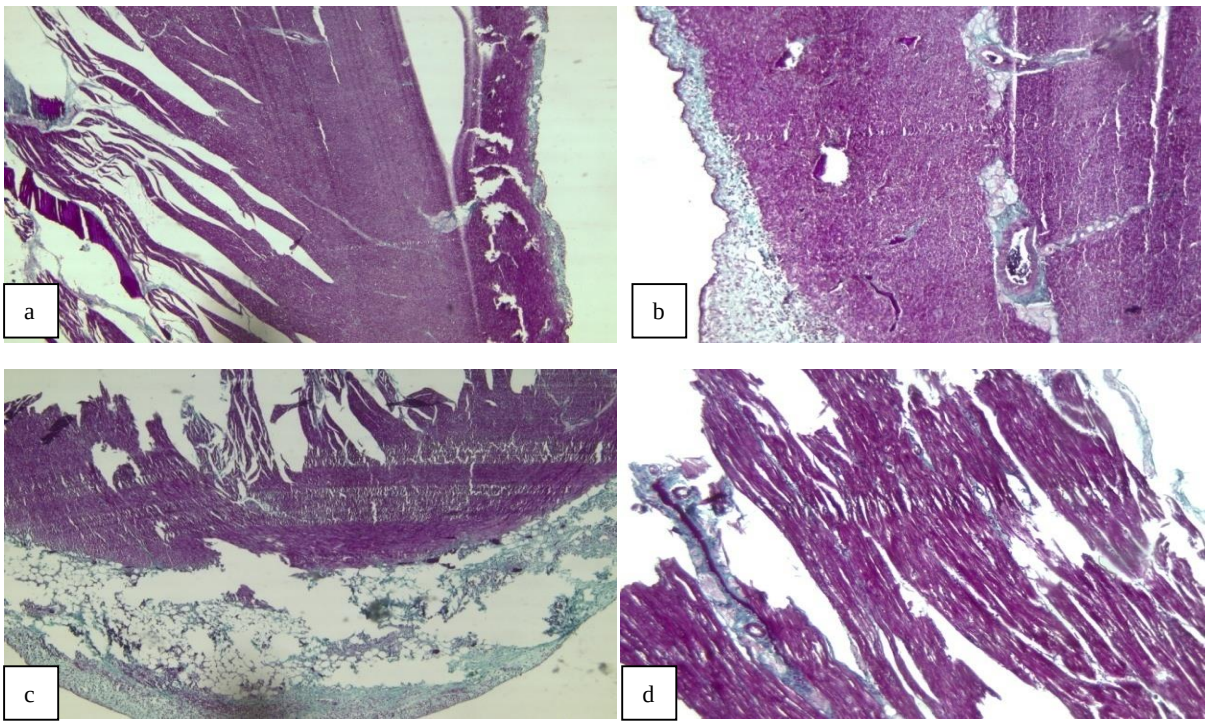

Figure4 : Photomicrographs of cardiac muscle of broiler administrated CYP (14mg\kg BW) showing : (a – b) Moderate collagen fibers found between cardiac myocytes and pericardium layer. Photomicrographs of cardiac muscle of broiler administrated CYP (21mg\kg BW) showing : (c – d) Abundant collagen fibers found between cardiac myocytes and pericardium layer (**green color**), Masson Trichrome (×4).

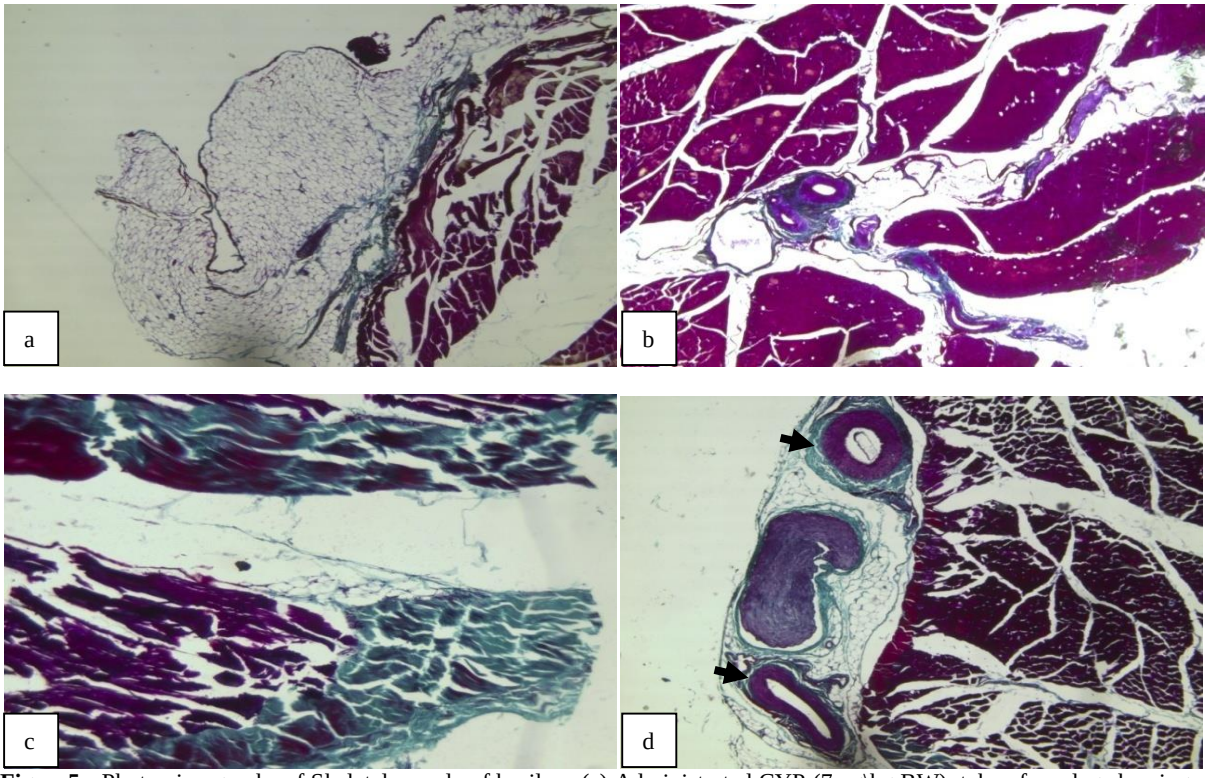

Figure5 : Photomicrographs of Skeletal muscle of broiler : (a) Administrated CYP (7mg\kg BW), taken from leg showing; mid collagen fibers surrounding cardiac myofibrils. (b) Administrated CYP (14mg\kg BW), taken from leg showing; moderate collagen fibers found between muscle bundles with congested dilated blood vessels. (c – d) Administrated CYP (21mg\kg BW), taken from breast showing; abundant collagen fibers found between muscle bundles (**green color)**, with highly congested dilated blood vessels (**arrows)**, Masson Trichrome (×4, ×10).

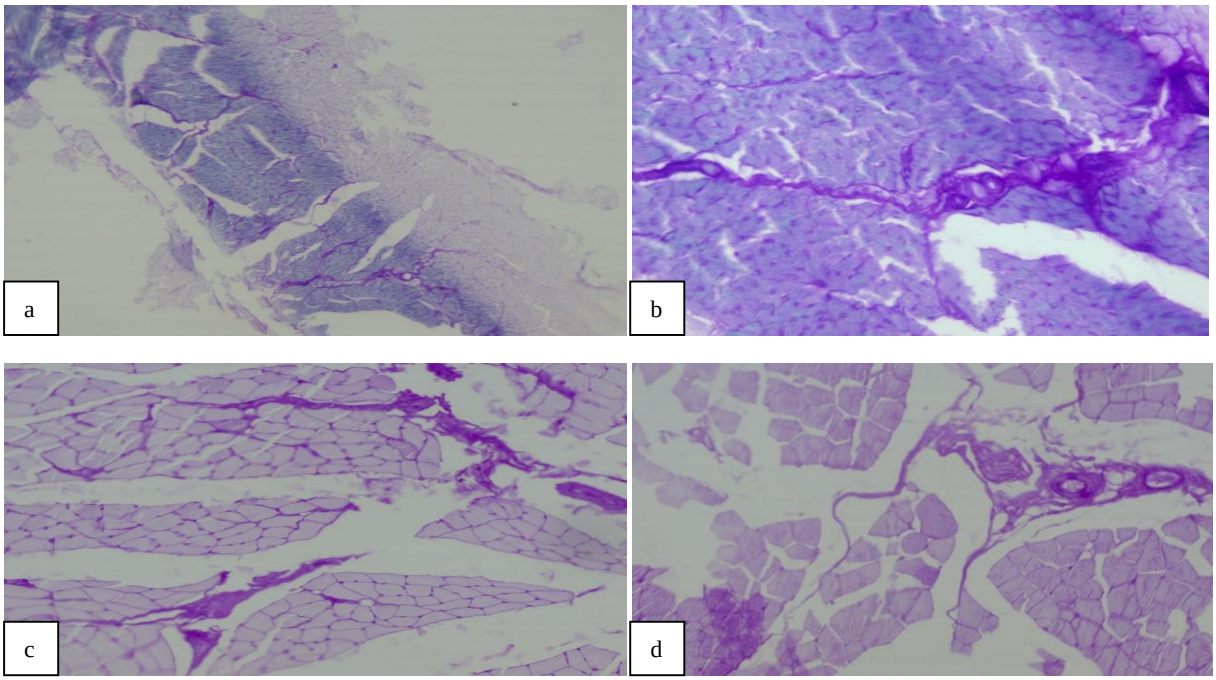

Figure6 : Photomicrographs of cardiac muscle of broiler: (a) Administrated CYP (7mg\kg BW), taken from breast showing; mild to moderate **PAS** reaction; (b) Administrated CYP (14mg\kg BW), taken from breast showing; strong **PAS** reaction. Photomicrographs of skeletal muscle of broiler: (c) Administrated CYP (14mg\kg BW), taken from wing; showing; strong **PAS** reaction. (d) Administrated CYP (21mg\kg BW), taken from leg showing; strong **PAS** reaction within and between myocytes (**purple color**), PAS(×20,×40).

Discussion

In Libya, chicken meat is a major source of protein which widely consume by human population. Although the residual toxic of CYP found in different types of crops in some regions of Libya, but there is no available data on histological changes of muscular tissue resulting from CYP residues in poultry.

CYP had a wide using in veterinary medicine by dipping, spraying, pour-on and spot-on methods [19],[20]. Some studies showed that it has an important uses in agriculture and plant protection due to high insecticidal activity with a low poultry and mammalian toxicity [21],[22]. Practically CYP is non – toxic to birds, but it found highly toxic to aquatic invertebrates and fishes as it is eliminated and metabolized more slowly by fishes than birds and mammals [23], some studies found that CYP has adverse toxic effect on the humans and animals depending on its toxic level, nature and extent [24].

The gross observations of high treated groups showed heart specimens with a dark brown in colour, black dots and increased fatty tissue on outer surface of organs. This may be related to cardiovascular congestion as result of the stress factors. In contrast, no gross lesions found in skeletal muscle samples at the end of the experiment.

The first noticeable histological changes as result of CYP toxicity in

high and intermediate treated groups, was destruction and discontinuation of cardio-skeletal muscle fibers and nuclear proliferation and completely loss in some sections. This may be due to toxic contents causing damage to cardio-myofilaments; pronounced interacellular space was also observed, indicated intramuscular edema associated atrophic changes. Earlier studies found structural changes in muscle tissues

exposed to various concentration of CYP in some fish species [25], [26].

Cardiomyocytes with abundant adipocyte tissue was noticed in intermediate and high treated groups as compared to low treated and control respectively. The CYP has lipophilic nature as many pesticides, responsible for increasing oxidative stress that induces cell membrane deterioration by increasing lipid peroxidation [27]. This in line with other results that showed focal appearance of fat cells between myocardial muscle cells[28]. Congestion of blood vessels was demonstrated between cardiomyocytes in high treated broilers. Some experiments, revealed that CYP can induce oxidative stress in blood cells and plasma membrane caused deterioration of membrane structure[29], [30]. These findings are in agreement with [31], [32]; who found that repeated administration of CYP produced hemorrhages within myocardium. There was excessive amount of collagen fibers accumulated between muscle cells with marked depletion observed between skeletal myofibrils replaced by edema in high and intermediate treated groups. This may explain the CYP toxicity that interacts with proteins and enzymes in muscular tissue. Other researchers reported that chemical components of pesticides induced toxicity and reduced the mass of the heart and other organs, suggesting its adverse effects through degradation of proteins and lipids resulting in organ atrophy[33], [34].

Histochemical changes of myocytes showed that PAS activity for glycogen was intense within skeletal muscle cells with marked increased glycogen granules in intercellular space of cardio-skeletal myocytes. This may due to CYP toxicity that induction glycogen phosphorylase, resulting in degradation of glycogen-protein complex and free glycogen to compensated energy demands [35]. These results are inconsistent with other findings [36], [37], [38]; that found a reduction in glycogen contents in different tissues of freshwater fishes exposed to CYP. This support an assertion of other observations, which observed that different types of muscle fibers are respond differently to chemical toxicity [16].

Conclusion

The experimental study revealed that CYP promotes histological alternations to cardio-skeletal myocytes of broiler chickens even at low concentration dosage.

Recommendation

Utilization of pyrethroids such as CYP should be minimized and alternative techniques should be replaced.

Conflicts of Interest

The authors declare that there is no conflict of interest.

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