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Original article

Histopathological Alterations in Liver of Male Rabbits Exposed to Deltamethrin and the ameliorative Effect of Folic Acid

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ABSTRACT
Aims*: Deltamethrin is classified as low mammalian synthetic pyrethroids that is used in a wide range in the agriculture field, animal's husbandry, being applied to public health. However, some reports have shown that it has been implicated in a serious noxious effect on non-targeted species animals and even human. Hence the current study was aimed to demonstrate deleterious effect of deltamethrin on the hepatic tissue of adult male rabbits on long term exposure, along with examine the role of folic acid in minimizing the tissue toxicity.* ***Methods****. Twenty adult male New-Zealand rabbits were divided into four groups: first group served as control. Deltamethrin treated group were administrated 1.28mg/Kg of insecticide orally by gastric gavage for 12 weeks. Folic acid group received folic acid 5mg/kg body weight. Combination group received both Folic acid with deltamethrin.* ***Results.*** *Deltamethrin induced cellular alterations in treated rabbits in which hepatocytes demonstrated hydropic degeneration, cellular features of necrosis including loss of cell membrane integrity, pyknosis and karyolysis. In addition, other the histological changes within portal area in the form of dilated, congested blood vessels and periportal lymphocytic infiltration were also observed. Carbohydrates staining methods (PAS reaction) was confirmed pathological changes such as destruction of cell membrane and a moderate decrease of amount of glycogen in the periportal area. Masson's trichrome stains showed that there was a portal and periportal fibrosis (NASH) stage 1 induced by deltamethrin. Similarly, group treated with insecticides along with giving protective antioxidants folic acid showed marked deposition of collagen in the portal area with presence of fibrous bridges between hepatic lobules.* ***Conclusion****. It can be concluded that deltamethrin induced hepatic damage to the normal liver architectures as well as there was no protective role of folic acid in minimizing toxic effect of deltamethrin.*

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INTRODUCTION

Deltamethrin (DLM) is type of the synthetic pyerthroids (type II) that widely used in agriculture field, animal's husbandry, being applied to public health and home activities as well. It has a beneficial role in killing pests and in improving crops yields [1-3]. Because of its low mammalian toxicity and has a wide range of effect an against harmful pests it becomes the best choices and the most popular synthetic pesticides [1, 4]. Previous studies reported many noxious effect on non-targeted species animals and even human particularly when they are applied in inappropriate amount. It acts on central nervous system of insect causing rapid damage since its effects are irreversible [1, 5]. DLM is lipophilic targeting cell membranes of body cells other than sodium channels and rapidly absorbed after oral administration [2]. It is mainly metabolized in liver by action of liver microsomes (esterase and CYP450) [5, 6]. It will be converted into inactive metabolites (acid and alcohol) by cleavage of ester bond which further undergo of oxidation and conjugation to be easily excreted into urine and feces. Thus upon metabolism reactive oxygen species (ROS) will be produced which are consider a contributing cause of deltamethrin impacts.

Accidental exposure of deltamethrin can cause symptoms range from skin irritation, paresthesia and diarrhea to acute poisoning which have been reported in some workers in the manufactory [1]. Furthermore, Lukowicz et al., reveled deltamethrin can cause suppression of the immune system inducing atrophy of the main cells of thymus gland [7-9]. Others have reported that lowering of testosterone level [10]. Hossian et al reported that type I and type II pyrethroids had effect on releasing acetylcholine (Ach) when injected intraperitoneally in experimental rats [11-13]. However, long term exposure could result in a devastating effect on human tissue particularly hepatic architectures since it is the major site of its metabolism [14, 15]. Antioxidants such as folic acid may play a role in maintaining histological and physiological function of the body some antioxidants like vitamin E ameliorate the negative impact of pesticides as it has role in decrease oxidative stress induced by insecticide exposure [16-18]. Therefore, the present study was aimed to evaluate deleterious effect of deltamethrin at very low dose on hepatic tissue of adult male rabbits on long term exposure a long with examine the role of folic acid in minimizing the tissue toxicity.

METHODS

Chemicals

Commercial formulation of deltamethrin (25% EC), of high purity (98%) product of DuPont, was obtained from Kafr El-Zayat Pesticides & Chemicals Company, Egypt. Folic acid Tablets 5mg was purchased from Sigma Aldrich, India.

Animals

Twenty adult male New-Zealand rabbits were used in this study and their average weight was (1.891 ± 27.6 kg). These animals were divided into four groups with five animals each. All animals were housed in a separate cages and they acclimatized for five days before experiment under providing a standard laboratory condition with free access to the food and water. Cleanliness and hygiene of the rabbits were checked regularly and cages were also cleaned from animal's waste daily.

Experimental design

Animals were divided into four groups as follow; Control group received only distilled water, Deltamethrin treated group in which rabbits were administrated 1.28mg/Kg body weight (BW) of insecticide orally by gastric gavage [19-21]. While, folic acid group which received only folic acid 5mg/kg BW. Combination group was treated with deltamethrin (1.28kg/mg BW) plus folic acid (5mg/Kg BW).

Treatment was applied once daily in the morning after food supplement for 12 weeks. At the end of the experiment, animals were anesthetized by chloroform and sacrificed, and livers were removed from each animal, then fixed by 10% buffered formalin and processed by routine histopathological technique. The prepared slides were stained by Harris Hematoxyline and Eosin (H&E), Periodic acid Schiff's (PAS) stain and Masson's trichrome.

RESULTS

Light microscopic examination of liver sections obtained from control group stained with H&E showed normal histological features in term of central vein, surrounding hepatocytes and lumen of sinusoidal capillaries (Fig. 1 A). In addition, control of liver tissue with PAS staining showed normal amount of glycogen inside hepatocytes (Fig. 2 A) as well as with Mason's trichrome liver appeared with normal deposition of collagen around portal area (Fig. 3A).

In comparison to treated rabbit, deltamethrin induced cellular alterations within hepatocytes in the form hydropic degeneration, cytoplasmic and nuclear signs of necrosis including loss of cell membrane integrity, pyknosis and karyolysis (Fig. 1 B). Meanwhile, other the histological changes within portal area in the form of dilated, congested blood vessels, and periportal lymphocytic infiltration were also observed (Fig. 1C).

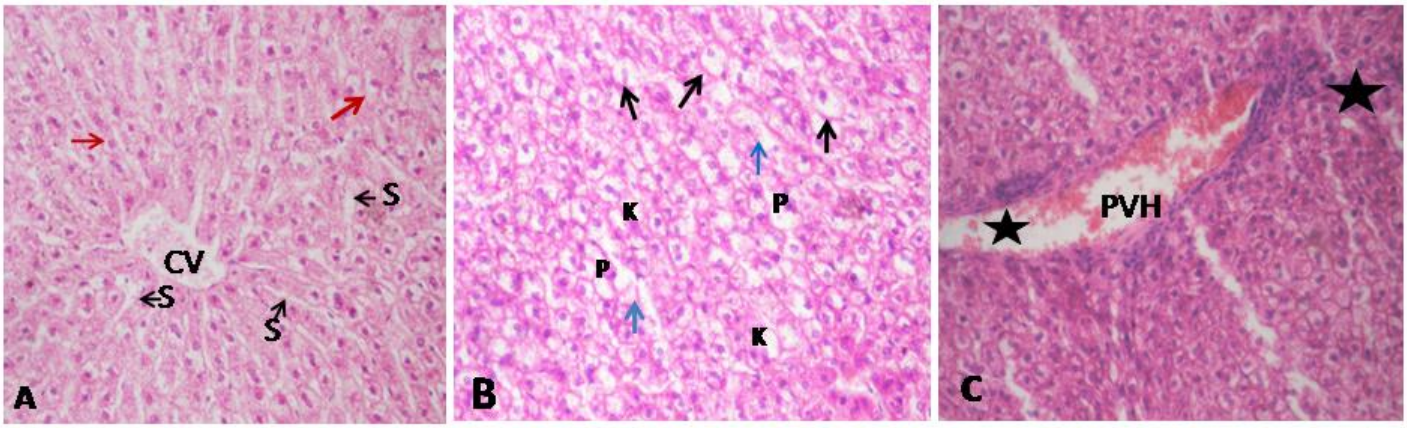

Fig 1. *Light micrographs of liver section of control and treated rabbits showing: A). Normal hepatic architecture with normal parenchymal hepatocytes (black arrow) and normal sinusoidal lumens (S). B). Histopathological changes upon deltamethrin exposure reveals degenerative features, like vacuolated cytoplasm (black arrow) and signs of necrosis including karyolysis (K), pyknotic nuclei (P) and loss of membrane integrity (blue arrow). C). dilatation and congestion of portal veins (PVH) as well as presence of leukocytic infiltration(star). (Mic Mag x200 (A) x400(B&C) H&E)*

Carbohydrates staining technique (PAS reaction) was confirm pathological changes that seen in rabbit's liver treated with insecticide such as destruction of cell membrane and a moderate decrease of amount of glycogen in periportal area (zone II & III) indicating of degenerative changes while hepatocytes in the zone I was lesser effect than others. (Fig. 2: B&D).

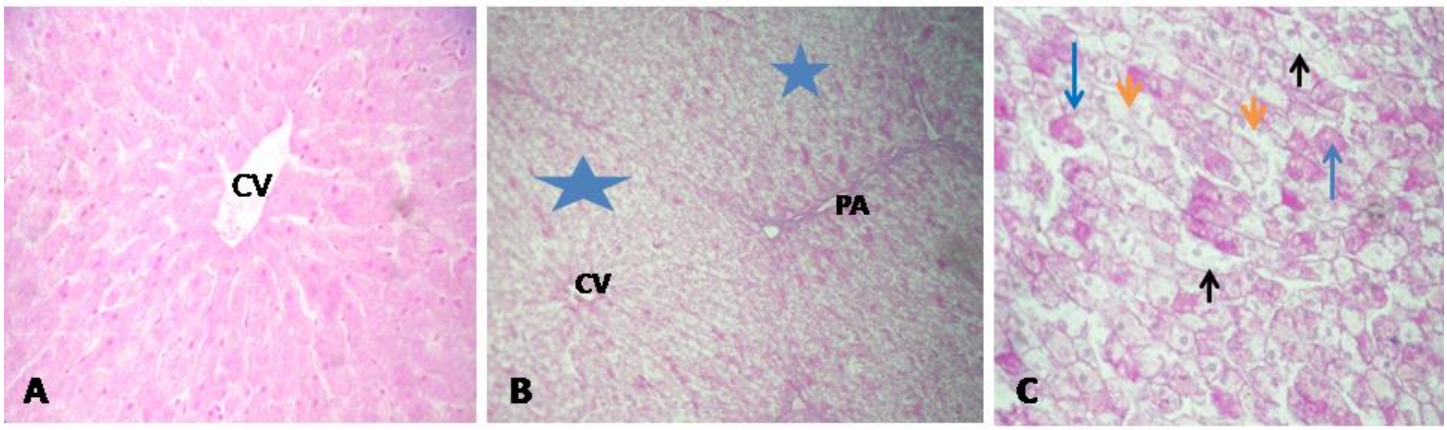

Fig 2. *Liver sections of rabbit stained with PAS showing: A) Control liver with normal glycogen content as intense red color inside hepatocytes. B) liver sections of deltamethrin treated animals revealed a moderate decrease in amount of glycogen(star). C) It shows figure B at higher magnification in which majority of hepatocytes are completely loss of glycogen (black arrow) while some appeared non affected (blue arrow) also destruction of cell membrane is very obvious (orange arrow head). (Mic Mag x400 (A&C) x200(B). PAS).*

Masson's trichrome stains showed that there was a portal and periportal fibrosis (NASH) stage 1 induced by deltamethrin (Fig. 3B), while in group 4 (rabbit treated with insecticides and folic acid) there was a marked deposition of collagen in the portal area (stage 3) with presence of fibrous bridges between hepatic lobules (Fig. 3C &D). Similarly, these animals revealed a hepatocellular damage and also presence of lobular infiltration and congested portal vein, indicating no protective role of folic acid on the tissue cells (Fig. 4 A&B). As well as with PAS stains, there was a moderate loss of glycogen contents inside hepatic cells (Figure 4C).

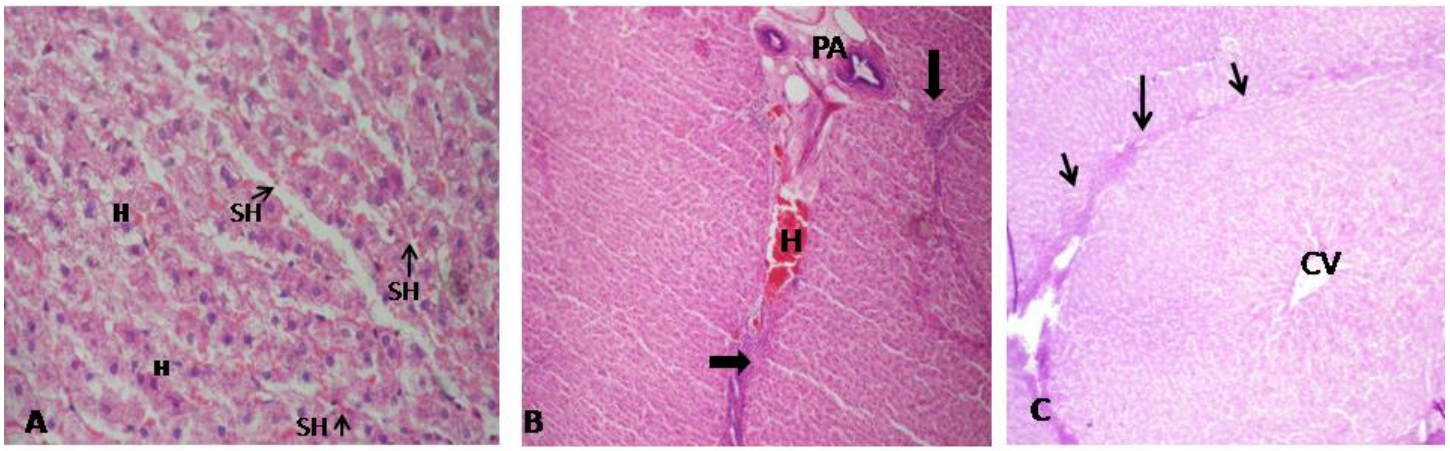

Fig 3. *Light Micrographs of liver's rabbit stained with Masson's trichrome presenting: - A) control liver appeared with normal collagen distribution around portal area. B) deltamethrin treated groups showed a slight increase in collagen fibers (stage1 fibrosis). C) Animals treated with deltamethrin+ folic acid showed that increased deposition of collagen around portal area indicating fibrosis stage 3 (black arrow). Mic Mag x100(A, B&C), and x400(D). Masson's trichrome.*

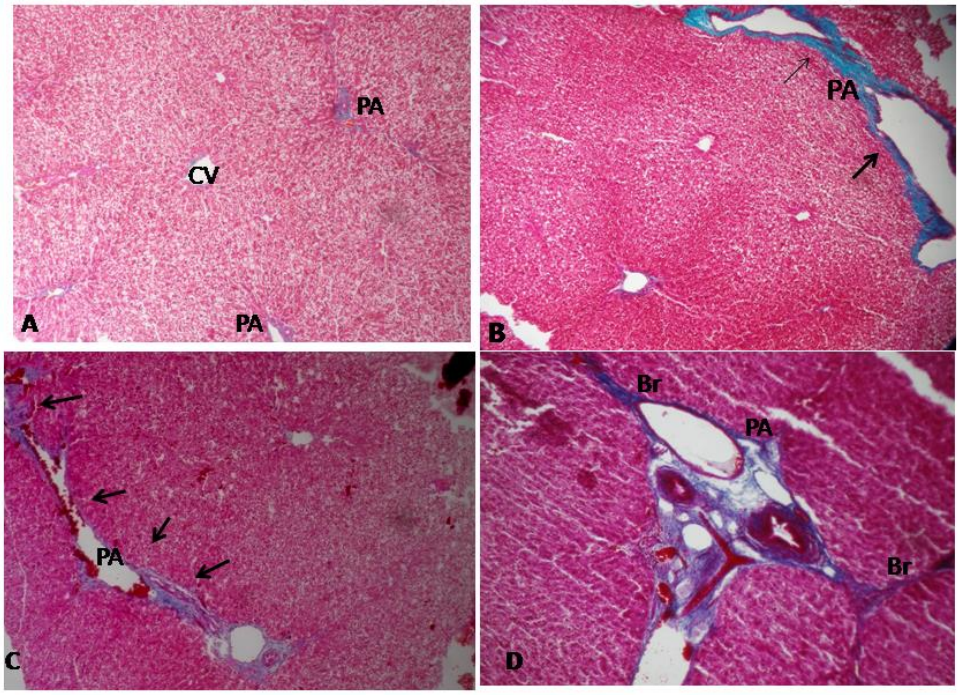

Fig 4. *Histological changes in liver tissue of deltamethrin + folic acid treated rabbit showing. A). degenerative changes including dilatation and hemorrhage of sinusoidal lumens (SH) and necrotic hepatocytes (H). B). Perioral infiltration (arrows) and venous congestion (H). C). signs of chronic toxic effect of pesticide appeared as fibrosis (fibrous bridging) by PAS staining (arrows). (Mic Mag x400(A), x100(B&C) H&E and PAS (C).*

DISCUSSION

The current results showed that liver architecture was destroyed in rabbits treated with deltamethrin, some of these changes are presence signs of necrosis include cell's hypertrophy, cytoplasmic vacuolization and nuclear pyknosis. This finding was in agreement with the previous works who found that upon deltamethrin exposure level of lipid peroxidation increased in liver and kidney tissue indicating oxidation of fatty acid in the cell membrane and generation of reactive oxygen species (ROS) which consequently leads to loss of membrane function and structure [22-25]. Some reports have mentioned that deltamethrin's toxicity is considered low in mammals comparing to other pyrethroids. However, hepatotoxicity was in marked degree in our result and this extensive hepatic distortion are probably related to insecticidal metabolism which is mainly occurred in liver that is the master organ responsible for biotransformation and detoxification of noxious compounds. Another report has mentioned that deltamethrin decreased normal body antioxidants glutathione (GSH) or even enzymes related to its metabolism, GSH are normally synthesized by almost human cells and play role in cellular protection against free radicals, thus in absence of GSH, eventually hepatocellular damage will be occurred. This was consistent with Abdel-Daim et al who reported that DLM induced toxicity through increased lipid peroxidation and decreased the hepatic antioxidants (superoxide dismutase, catalase, GSH) [14, 26, 27].

Hemorrhage was observed in the sinusoidal spaces and portal veins of liver parenychyma which is related to decrease in prostaglandin vasodilator as a result of pesticide inhibition mechanism. Reduction in prostaglandin leads in turns to vasoconstriction resulting in rupture of blood vessels. Others said that hepatic damage leads to increased blood flow which further cause blocking to sinusoids lumen and finally obligate all vascular walls to dilate [28]. Infiltration of liver's interstitium with mononuclear cells suggesting that insecticides provokes inflammatory reaction mediated by interleukins IL-6 ,IL-1β and TNFα ends by migration of leukocytic cells to inflamed tissues [19]. These findings were coinciding with the results of other investigators [29, 30].

Hepatic fibrosis has been manifested in our result and it refers to a chronic liver damage due to insecticide which are in parallel to previous researches that revealed oxidative stress promotes fibroblast proliferation and subsequently expanding extracellular matrix [31]. Chinese study on the molecular mechanisms of DLM have recorded that liver fibrosis is induced through Nrf2/TGF-β1/Smad3 signaling pathway which stimulating hyperplasia of fibrous connective tissue septa [30, 32].

By PAS staining there was a depletion in glycogen contents inside hepatocytes as well as destruction of cell membrane of hepatic cells with loss of intercellular borders suggesting the lipophilic nature of deltamethrin, and their effects on lipid components of cell membrane leading to disturbance of membrane structure [28]. This was reinforced by Poonam Sharma et alwho demonstrated that deltamethrin causes an increase in lipid peroxidation in liver tissue, this is consider the source of H2O2, which further leads to the production of Malondialdehyde (MDA) responsible for peroxidation of unsaturated fatty acids in the membrane[14]. Results of the present observations indicated that supplementation of folic acid with deltamethrin did not ameliorate toxic effects of pesticides on hepatic rabbit tissue, and most of animals in this group presented with a marked deposition of collagen and even fibrous bridges between lobules. Despite to other findings which documented that pryethroids toxicity on organs were improved by administrated of ascorbic acids and vitamin E in experimental mice [33].

CONCLUSION

It can be concluded that chronic exposure to deltamethrin have deleterious effects on the hepatic architectures even with low doses, perhaps the oxidative stress and generation of ROS are the main mechanisms of insecticide hepatotoxicity leading to reduce cell viability and promoting liver fibrosis, and using of antioxidants dose not reduce severity of insecticide impacts.

Disclaimer

The article has not been previously presented or published, and is not part of a thesis project.

Conflict of Interest

There are no financial, personal, or professional conflicts of interest to declare.

Ethical approval

All authors hereby declare that "principles of laboratory animal care" (nih publication no. 85- 23, revised 1985) were followed, as well as specific national laws where applicable.

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