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COMPARATIVE EFFECT OF INHALATION OF PYRETHROID-CONTAINING MOSQUITO REPELLENT MATS ON THE LIVER OF THE ADULT AND GROWING MALE ALBINO RATS

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INTRODUCTION

Pesticide have been used extensively for agricultural, domestic and industrial purpose (**Hayes and Laws, 1991**). Pyrethroid insecticides are synthetic analogues of the natural pyrethrins contained in flowers of genus Chrysanthemum (**Leahey, 1985**). In recent years, pyrethroids have been used widely due to their good insecticidal activity and low mammalian toxicity (**Hutson and Roberts, 1985**). However, their wide spread use, their high nonselective potency and their considerable stability in the environment make them potentially harmful (**Gassner et al., 1997**). In addition, they are commonly used in mosquito repellents to protect the human population, either by impregnating bednets with pyrethroids (**Huailu et al., 1995**) or in the form of vaporizing mats, coils, scented sticks and liquidators (**Miyamoto and Kearney, 1983; Warui, 1992**). Because these repellants are used routinely overnight, it may allow human to inhale vapors that may be harmful especially to children due to the immature blood - brain barrier (BBB) and metabolic inefficiency compared to adults (**Sheets et al., 1994**). Several experimental studies demonstrated that young mammals were generally more sensitive than adults to the acute toxicity of insecticides (**Pope and Liu, 1997; Moser and Padilla, 1998**). However, very few studies have evaluated age - related differences in response to chronic exposure to lower doses of these insecticides. **Eriksson (1997)** proved that low - dose exposure to both persistent and non-persistent environmental agents, e.g. pyrethroids, nicotine and paraquat, during the neonatal growth spurt period could lead to functional abnormalities of the brain during adulthood. Encephalopathies in children have been reported following the use of insect repellent containing fenvalerate, which is a pyrethroid pesticide (**Garrettson, 1997; Osmitz and Murphy, 1997**). Studies of

Gupta et al. (1999) exhibited a significant effect of pyrethroid pesticides on BBB, liver and kidney functions. Changes in the BBB permeability **(Srinivas et al., 1993)**, free-radical generation and oxidative stress **(Bagchi et al., 1995)** as well as affection of mitochondrial and microsomal function **(Yamano and Morita, 1995)** are among the suggested mechanisms by which these pesticides may exert their toxicity.

The aim of this work is to study the possible histological changes and their reversibility that may occur in the liver of the adult and growing male albino rats following chronic inhalation of commercially available pyrethroid-containing mosquito repellent mats (Ezalo mats).

MATERIAL AND METHODS

Sprague-Dawley albino rats were used in this study. Twenty-four adult males, weighing 190 - 250 gm, and 8 dams with 2 day-old pups (8 pups / dam, no selection of sex at this age) were kept in plastic cages (16.5" x 10.5" x 7.5") in an air-conditioned animal house (temperature 22 ± 2C) with optimal illumination cycle. The animals had free access to drinking water and a pellet diet and were divided into three groups as follows :

Group I (control group) :

This group consisted of 12 adult males and 4 dams with their pups. The animals were reared under normal hygienic conditions as described above. Then three adults and three growing males were killed at a time, at intervals of 3, 6, 9 and 13 weeks.

Group II (pyrethroid-exposed group) :

Consisted of 9 adult males and 3 dams with their pups. According to the design of **Gupta et al. (1999)**, a partition of Perspex sheet with numerous holes was provided in each of the cages (16.5" x 10.5" x 7.5") for the animals of this group. An electric device for vaporizing the pyrethroid from mosquito repellent mats (Ezalo) was put on one side of the partition where one gm of the mat contained 9.5 mg of bioallethrin 93%, a synthetic pyrethroid, and 14.4 mg of pyrethrum 95%, a natural pyrethrin. Each three adult males or a dam with its pups were kept on the other side of the partition. The animals were allowed daily to inhale the vaporizing pyrethroids for 10 hours / day. Three adult and three growing males were killed at a time, at intervals of 3, 6 and 9 weeks.

Group III (pyrethroid withdrawal group) :

Consisted of 3 adult males and 1 dam with its pups. The animals were exposed to pyrethroids, as described in group II, then, withdrawn from the inhalation after 9 weeks of exposure. The adults and three of the growing male rats were killed 4 weeks later to study the reversibility of the possible alterations induced by pyrethroid inhalation.

The animals were killed by over dose of ether and the liver was extracted. The total body weight as well as the liver weight of each animal was calculated. The mean body weight and the mean liver weight of the animals of each group, at the same interval, were measured and the percentage of the mean liver weight to the mean body weight (the relative liver weight) was determined as follows :

% = Mean liver weight (gm) / mean body weight (gm) X 100.

The results were subjected to statistical analysis, and were represented in histograms (Figs. 1 - a & b).

Histological study :

A) Light microscopical study :

After excision of a small piece for ultrastructural study, each liver specimen was fixed in 10% formol saline and processed for paraffin block. Sections of 7 µm in thickness were cut and stained with hematoxylin and eosin and Masson's trichrome **(Masson, 1924)** for light microscopical examination.

B) Electron microscopical study :

The small pieces taken from the liver specimens were immediately fixed in 4% gluteraldehyde solution for 3 hours and then washed in phosphate buffer, post fixed in 1% buffered osmium tetroxide for one hour, dehydrated and finally embedded in epoxy (Epon). Ultrathin sections 50 - 80 nm were contrasted with uranyl acetate **(Watson, 1958)** and lead citrate **(Reynolds, 1963)** and photographed with a Joel S_{100} electron microscope.

Histomorphometric quantification :

Using the binary image of the image-analyzer computer assisted by the software Leica Qwin 500 with a standard measuring frame of 118476.6 μm^2, the proportion of collagen fibers in the frame was calculated as follows :

Area % = area of collagen fibers / total area of the field X 100.

This data was measured in 10 fields of each specimen and the mean values were obtained. The results were subjected to statistical analysis and represented in a table.

Statistical analysis :

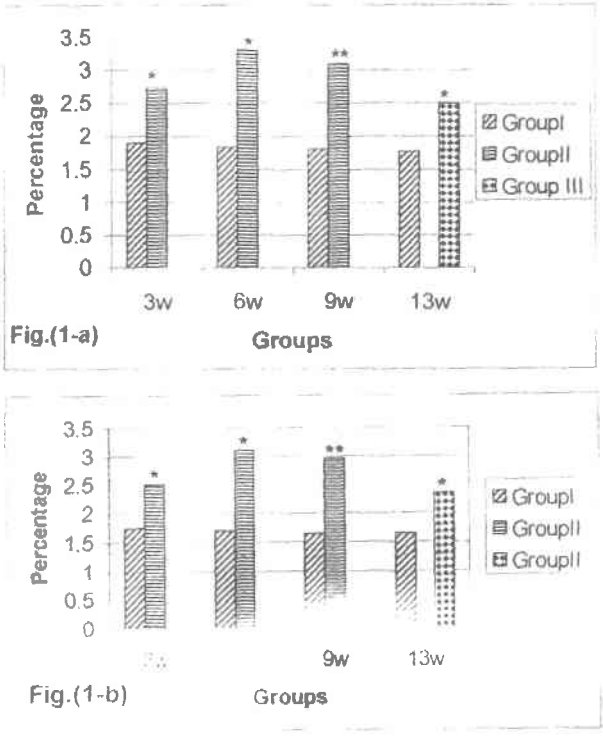
The Statistical Package for the Social Sciences (SPSS version 7.5) was used in data analysis. Data were expressed as mean ± SE. One-way analysis of variance (ANOVA) was used.

RESULTS

A) Morphometric quantification :

It showed statistically significant increase in the relative liver weight, in all pyrethroid exposed groups, compared with those of age-matched controls and such increase was proportional to the duration of pyrethroid exposure. In group III, four weeks after withdrawal of pyrethroid, there was still statistically significant increase in the values of mesurements of relative liver weight compared with the age-matched control values (Table).

B) Histological results :



Figs. (1 - a & b) :Histograms showing percentage of mean liver weight / mean body weight in all experimental groups of growing (a) and adult (b) animals.
* : significant with respect to the matching control group (group I) ($P < 0.05$)
** : highly significant with respect to the matching control group (group I) ($P < 0.01$)

Table : Mean area percent of collagen fibers in liver specimens of all experimental groups.

	Experimental groups of the growing animals				
	Group I (control group)	Group II (pyrethroid- exposed)			Group III (pyrethroid-withdrawal)
		3 w	6 w	9 w	
% ± SE	0.55 ± 0.035	0.68 ± 0.041	0.78 ± 0.049*	0.81 ± 0.061*	1.31 ± 0.065**
	Experimental groups of adult animals				
	Group I (control group)	Group II (pyrethroid- exposed)			Group III (pyrethroid-withdrawal)
		3 w	6 w	9 w	
% ± SE	0.59 ± 0.042	0.69 ± 0.053	0.71 ± 0.063*	0.79 ± 0.055*	0.96 ± 0.046**

W = Week

* : Significant with respect to the control group ($P < 0.05$)

** : Highly significant with respect to the control group ($P < 0.01$)

Group I (control group) :

The light microscopical examination of liver specimens from both adult and growing animals showed normal architecture of hepatic lobules and portal tracts (Fig. 2).

The electron microscopical study of both adult and growing liver specimens of this group demonstrated normal hepatocytes with large vesicular nucleus and many intracellular organelles including rough endoplasmic reticulum and abundant mitochondria (Fig. 3).

Group II (pyrethroid exposed group) :

Three weeks exposure :

The light microscopical study of the hepatic lobules of the growing animals showed cellular infiltration in vicinity of dilated central venules. Dilated sinusoids with prominent Kupffer cells and vacuolation of the cytoplasm of many hepatocytes were demonstrated in some lobules (Fig. 4). Examination of the adult animal hepatic lobules showed similar histological pattern as that of the growing ones but the central venules were less dilated with absence of the cellular infiltration (Fig. 5).

The electron microscopical study of the hepatocytes of growing and adult ani-

mal specimens were nearly similar and revealed focal areas of rarified cytoplasm with some fat droplets as well as giant and irregularly-shaped mitochondria (elongated, horse-shoe and ring-forms) denoting reactivity. Lakes of glycogen accumulation were also noticed (Fig. 6).

Six weeks exposure :

Light microscopical examination of liver specimens of the growing animals revealed some hepatic lobules with focal cellular infiltration and congested sinusoids in regions of degenerated hepatocytes (focal necrosis) as well as scattered fibroblasts between the hepatocytes (Fig. 7 - a). Periportal hepatic cell degeneration (periportal necrosis) with massive cellular infiltration and proliferation of the bile ductules extending into the area of necrosis were also demonstrated (Fig. 7 - b). Some other hepatic lobules showed dilated congested central venules and sinusoids with large fat droplets occupying the cytoplasm of many hepatocytes (fatty degeneration); some cells were completely degenerated (Fig. 8). On the other hand, the light microscopical examination of the adult animal specimens demonstrated massive cellular infiltration in the region of degenerated hepatocytes located mainly close to the area of portal tract (periportal necrosis) with dilated congested portal venules and sinusoids. Proliferated bile ductules lined with cuboidal epithelial cells and extending into the area of cellular infiltration were also seen (Fig. 9).

The ultra structural study of the hepatic cells of growing and adult animal specimens showed mono-or binucleated hepatocytes (more encountered in adult animal specimens) with rarified cytoplasm (focal cytolysis), fat globules and marked accumulation of glycogen displacing most of the organelles. The mitochondria were less in number, compared with the controls, and irregular in shape (Figs. 10). Degenerated mitochondria with loss of cristae as well as those showing division could be noticed (Fig. 11). In different fields, the hepatocytes showed shrinkage of the nucleus (pyknosis), absence of organelles and ill-defined cell boundaries (coagulative necrosis) (Fig. 12).

Nine weeks exposure :

Light microscopical examination of the hepatic lobules of the growing animals showed that some lobules had similar histological patterns as those after 6 weeks of

pyrethroid exposure while other lobules showed degeneration of numerous numbers of hepatocytes with dilated central venules and sinusoids (Fig. 13). A statistically significant increase in fibrous tissue formation in the hepatic lobules compared with the control group was noticed (Table) and was clear in the regions of the portal tracts (Fig. 14). Light microscopical study of the hepatic lobules of the adult animal specimens revealed some inflammatory cell infiltration beside the central venules and dilated and congested sinusoids. Some hepatocytes in the cords surrounding the central venules were degenerated while others showed prominent nucleoli (sign of reactivity), especially those at the periphery of the lobule (Fig. 15). Dilated portal venules with thickened walls (periportal connective tissue formation) and proliferation of the bile ductules surrounded by cellular infiltration were encountered (Fig. 16). There was statistically significant increase in fibrous tissue formation in the hepatic lobules compared with the control group (Table).

The electron microscopical examination of the hepatocytes of growing and adult animal specimens showed ultrastructural findings similar to those obtained after 6 weeks of pyrethroid exposure. However, shrinkage of the nucleus, absence of organelles and ill-defined cell boundaries (coagulative necrosis) became much encountered.

Group III (drug withdrawal group) :

Light microscopical examination of the hepatic lobules of the growing animal specimens 4 weeks after withdrawal of pyrethroid, have still showed dilated central venules and congested and dilated sinusoids as well as degenerated and necrotic hepatocytes. Binucleated hepatocytes as well as those with prominent nucleoli were also encountered denoting reactivity (Fig. 17). Increases of the connective tissue formation around the central venule and focally between the hepatocytes within the lobules (Figs. 18 - a, b) as well as periportal fibrosis with dilated congested portal venules were revealed (Fig. 19). The inflammatory cellular infiltration completely subsided. Light microscopical study of the adult animal specimens showed similar histological findings to those of the growing animals. However, fibrous tissue formation was seen only in the periportal regions with congested blood vessels and duplicated bile ductules that regressed to the portal area (Fig. 20). There was highly statistically significant increase in the fibrous tissue formation in the hepatic lobules

of the specimens of both growing and adult animals compared with the age-matched controls and such increase was much more in the growing animals than in the adult ones (Table).

The electron microscopical examination of the growing and adult animal specimens showed hepatocytes with signs of reactivity represented in increase the incidence of binucleation with nuclei containing more than one nucleolus as well as irregularly shaped mitochondria (Fig. 21). Hepatocytes with rarified cytoplasm (cytolysis) and those showed complete degeneration with nuclear ghost were also encountered (Fig. 22). The cell boundaries and cell to cell contact became clear (Figs. 21, 22).

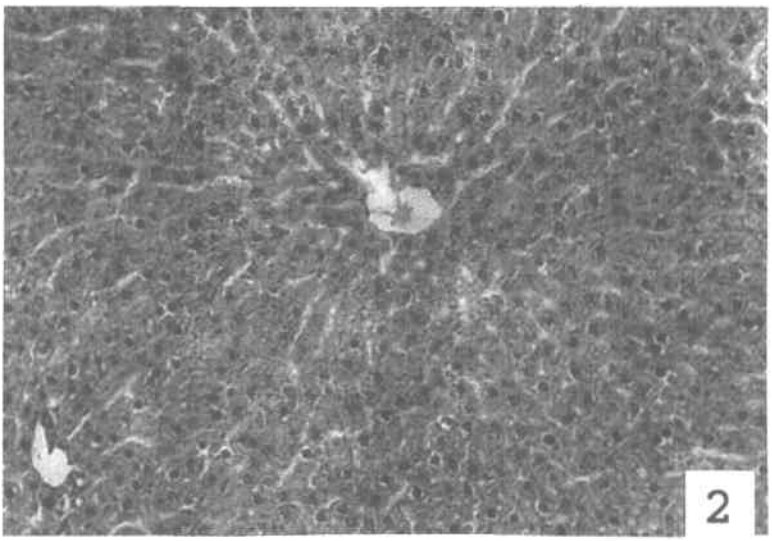


Fig. (2) :A photomicrograph of cross section of liver specimen of an adult albino rat of the control group showing normal architecture of a hepatic lobule and portal tract.
(Hx. & E.; x 200)

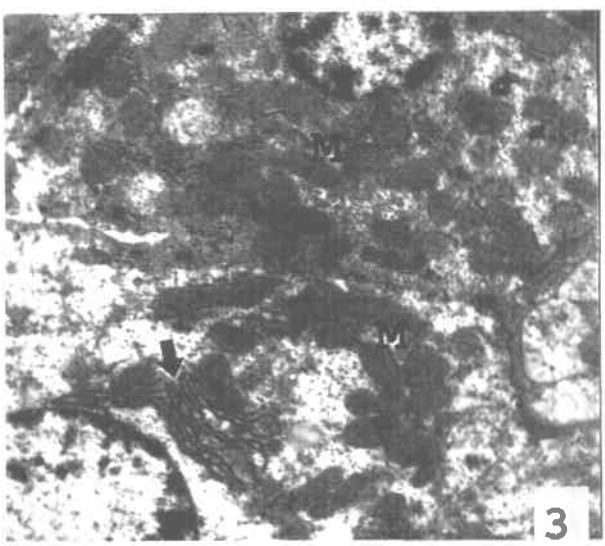
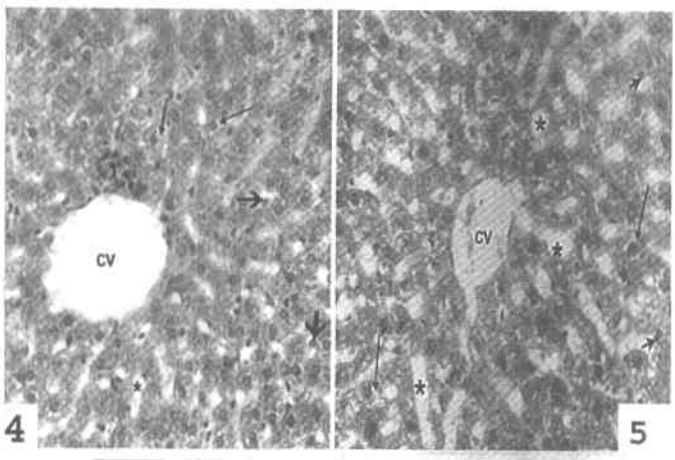


Fig. (3) :An Electron micrograph of a liver specimen of an adult rat of the control group, showing normal hepatocytes with large vesicular nuclei and many intracellular organelles including rough endoplasmic reticulum (arrow) and abundant mitochondria (M)..

(x 3600)



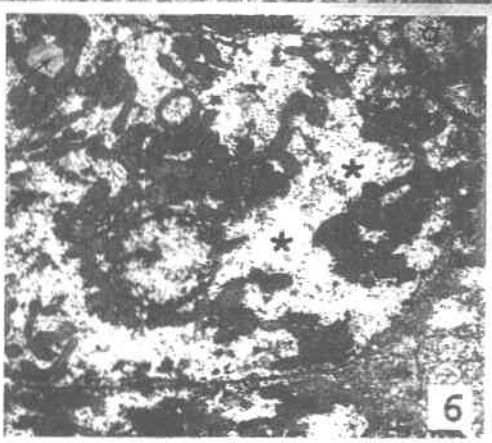


Fig. (4) :A photomicrograph of cross section of liver specimen of a growing rat of group II, following three weeks of pyrethroid inhalation, showing cellular infiltration (I) in vicinity of dilated central venule (CV) of a hepatic lobule. Dilated sinusoids (*) with prominent Kupffer cells (short arrows) and vacuolation of the cytoplasm of some hepatocytes (long arrows) can be seen.

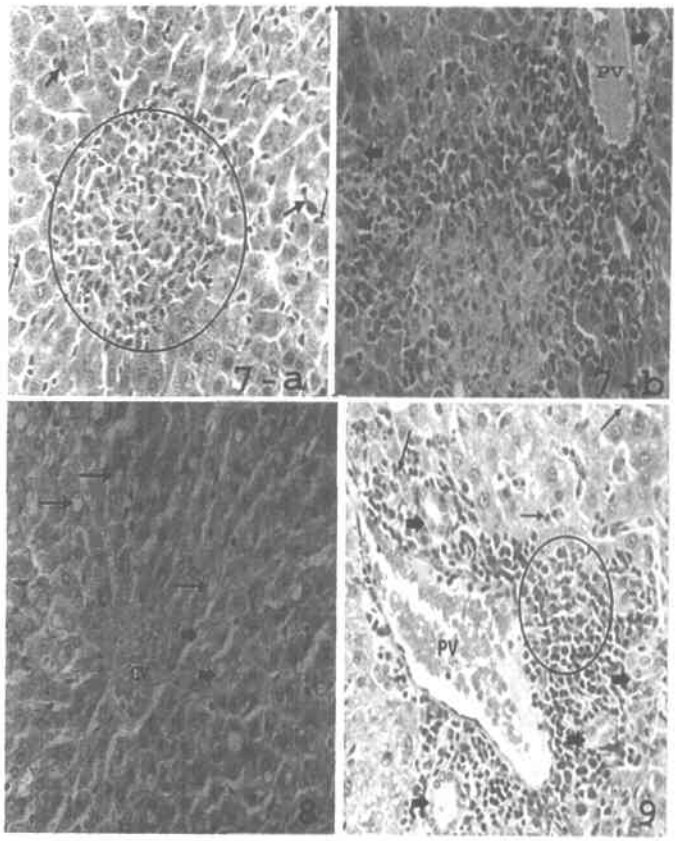
(Hx. & E.; x 400)

Fig. (5) :A photomicrograph of cross section of liver specimen of an adult rat of group II, following three weeks of pyrethroid inhalation, showing slightly dilated central venule of a hepatic lobule (CV). Kupffer cells (short arrows) lined the wall of dilated sinusoids (*) as well as vacuolation of the cytoplasm of some hepatocytes (long arrows) are noticed.

(Hx. & E.; x 400)

Fig. (6) :An electron micrograph of a liver specimen of a growing rat of group II, following three weeks of pyrethroid inhalation, showing a hepatocyte with focal areas of rarified cytoplasm (*) and giant and irregularly-shaped mitochondria (elongated, horseshoe and ring-forms). Note the parts of the hepatocytes in the upper part of the picture showing a fat droplet (arrow) and lakes of glycogen accumulation (g).

(x 1800)



Figs. (7 - a, b) :Photomicrographs of cross sections of liver specimens of growing rats of group II, following six weeks of pyrethroid inhalation showing :
a) Focal cellular infiltration and congested sinusoids in a region of degenerated hepatocytes (circle) as well as scattered fibroblasts between the hepatocytes (thick arrows). Note the prominent Koupffer cells (thin arrows) lining the wall of the sinusoids.
(Hx. & E.; x 400)
b) Periportal hepatic cell degeneration with massive cellular infiltration and proliferation of the bile ductules (arrows) extending into the area of necrosis. Note the part of congested portal venule (PV).
(Hx. & E.; x 400)

Fig. (8) :A photomicrograph of a cross section of liver specimen of a growing rat of group II, following six weeks of pyrethroid inhalation, showing a hepatic lobule with dilated congested central venule (CV) and sinusoids (*). Many hepatocytes show large fat droplets in their cytoplasm (long arrows), some cells are completely degenerated (short arrows).
(Hx. & E.; x 400)

Fig. (9) :A photomicrograph of a cross section of a liver specimen of an adult rat of group II, following six weeks of pyrethroid inhalation, showing massive periportal cellular infiltration (circle) in the region of degenerated hepatocytes with dilated congested portal venule (PV) and sinusoids (thin arrows). Note the proliferated bile ductules lined with cuboidal epithelial cells (thick arrows) and extending into the area of necrosis.
(Hx. & E.; x 400)

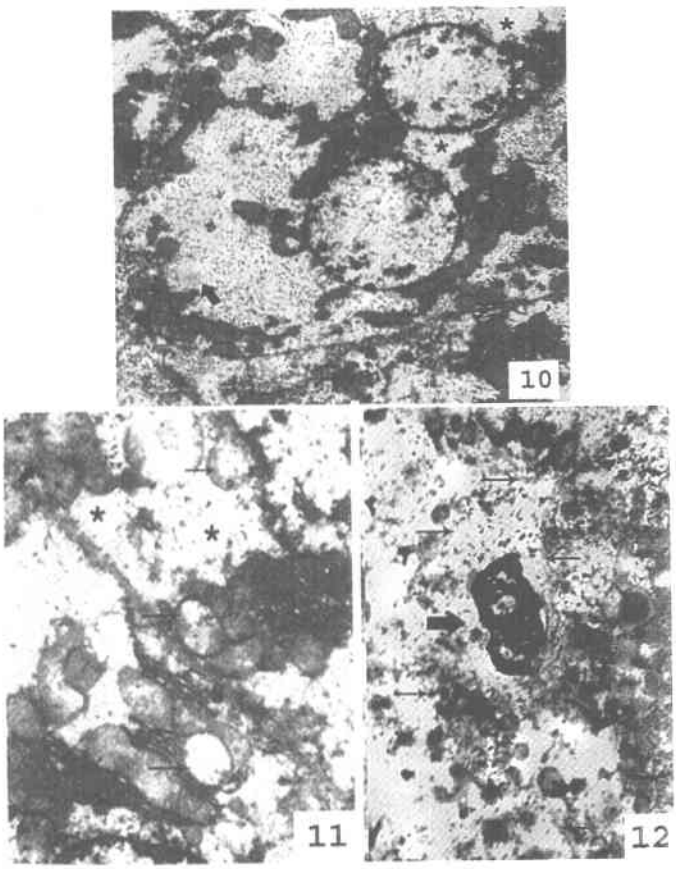


Fig. (10) :An electron micrograph of a liver specimen of an adult rat of group II, following six weeks of pyrethroid inhalation, showing binucleated hepatocyte with rarified cytoplasm (*), fat globule (arrow) and marked accumulation of glycogen displacing most of the organelles. Note that the mitochondria are less in number and irregular in shape.

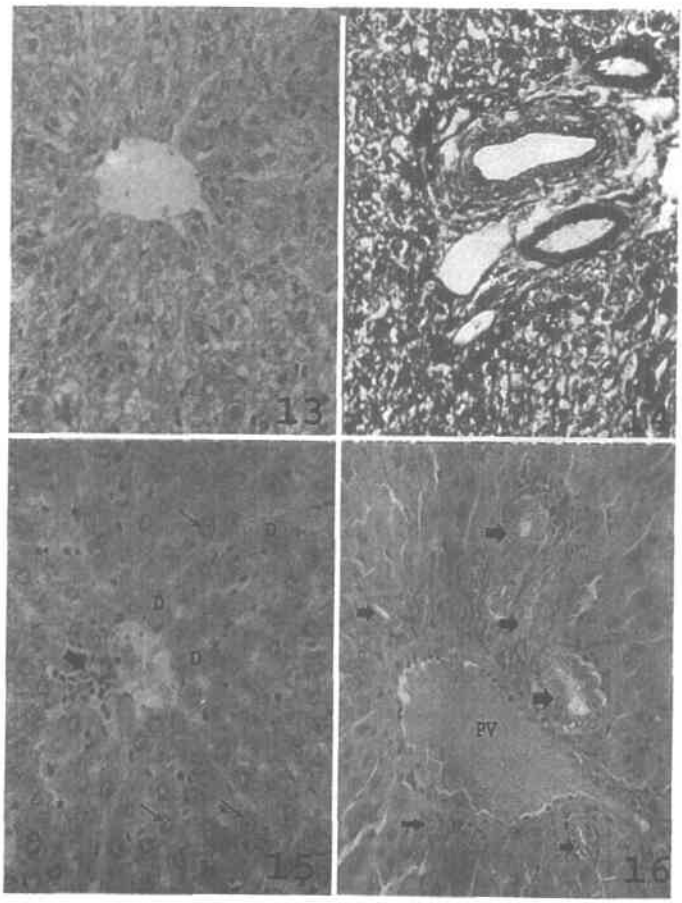
(x 1,800)

Fig. (11) :An electron micrograph of a liver specimen of a growing rat of group II, following six weeks of pyrethroid inhalation, showing degenerated mitochondria with loss of cristae (thin arrows) as well as those undergoing division (thick arrow). Note the rarified cytoplasm (*).

(x 5,400)

Fig. (12) :An electron micrograph of a liver specimen of a growing rat of group II, following six weeks of pyrethroid inhalation, showing a hepatocyte (thick arrow) with shrunken nucleus and absence of organelles. Note the ill-defined cell boundaries between the hepatocytes (thin arrows).

(x 3,150)



Figs. (13 & 14) :Photomicrographs of cross sections of a liver specimen of a growing rats of group II, following nine weeks of pyrethroid inhalation, showing :
13) Dilated central venules and sinusoids of a hepatic lobule with degeneration of numerous hepatocytes.

(Hx. & E.; x 400)

14) Fibrosis at the region of the portal tract. Note the cytoplasmic vacuolation of most of the hepatocytes.

(Masson's trichrome; x 300)

Figs. (15 & 16) : Photomicrographs of different fields of a cross section of a liver specimen of an adult rats of group II, following nine weeks of pyrethroid inhalation, showing :
15) Some inflammatory cell infiltration (arrow) beside the central venule of hepatic lobule with dilated and congested sinusoids (*). Some hepatocytes are degenerated (D) while others showed prominent nucleoli (arrows), especially those at the periphery of the lobule.

(Hx. & E.; x 400)

16) A dilated congested portal venule (PV) with thickened wall as well as duplication of the bile ductules (arrows) surrounded by inflammatory cells.

(Hx. & E.; x 400)

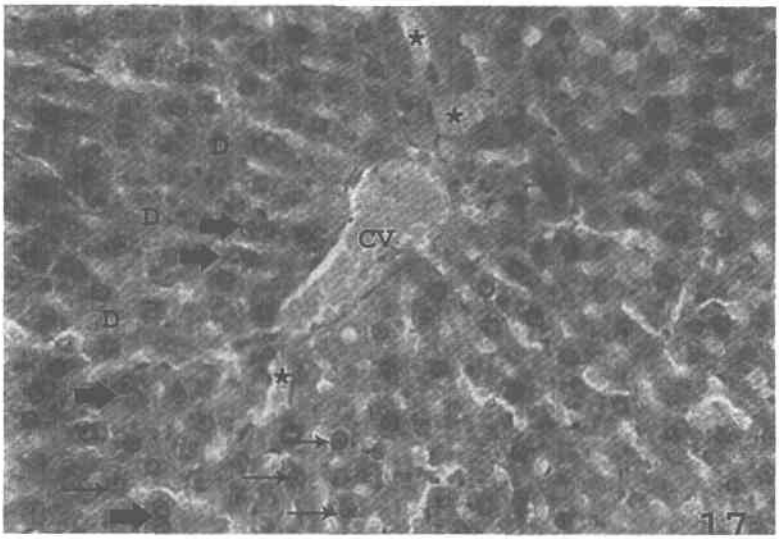
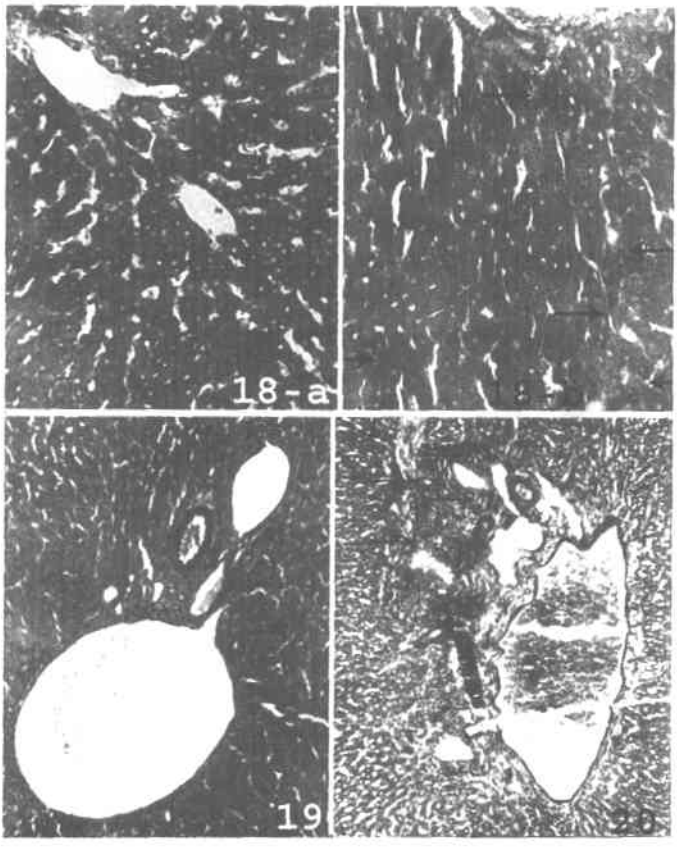


Fig. (17) :A photomicrograph of a cross section of a liver specimen of a growing rat, four weeks after pyrethroid withdrawal (group III), showing dilated congested central venule (CV) and sinusoids (*). Degenerated hepatocytes can be seen (D). Note the binucleated hepatocytes (thick arrows) and those with prominent nucleoli (thin arrows).
(Hx. & E.; x 400)

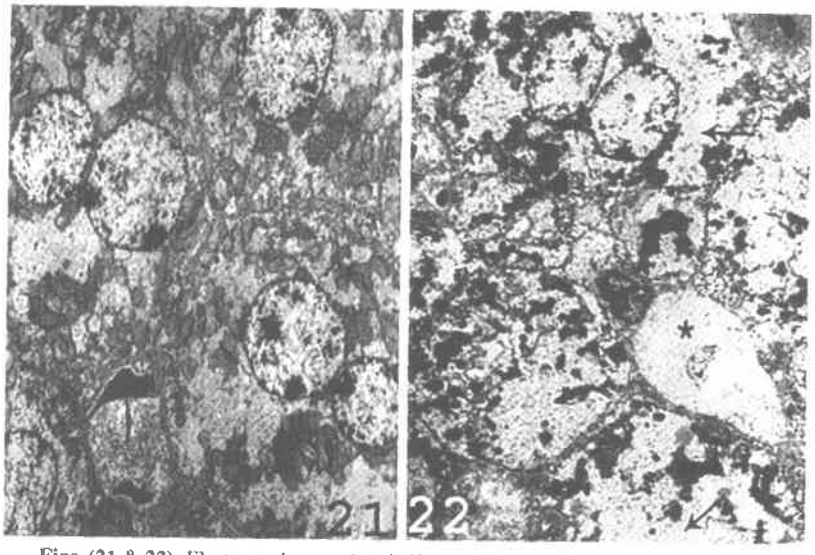


Figs. (18 - a, b) :Photomicrographs of different fields of a cross section of a liver specimen of a growing rat, four weeks after pyrethroid withdrawal (group III), showing increased connective tissue formation around the central venule (a) and focally between the hepatocytes (arrows) within the lobules (b).

(Masson's trichrome; x 200)

Figs. (19 & 20) :Photomicrographs of cross sections of liver specimens of a growing (19) and an adult (20) rats, four weeks after pyrethroid withdrawal (group III), showing periportal fibrosis with dilated congested blood vessels. Note the duplicated bile ductules (arrows) confining to the portal area (Fig. 20).

(Masson's trichrome; x 100)



Figs. (21 & 22) :Electron micrographs of different fields of a liver specimen of an adult rat, four weeks after pyrethroid withdrawal (group III), showing :
21) Binucleated hepatocytes where the nuclei contain more than one nucleolus as well as irregularly shaped mitochondria. Note the clear cell boundaries.

(x 1350)

22) Hepatocytes with focal areas of cytoplasmic vacuolation (arrows) and irregularly shaped mitochondria. Note the clear cell to cell contact and the completely degenerated hepatocyte with a nuclear ghost (*).

(x 900)

DISCUSSION

The present study revealed that the inhaled pyrethroid induced light and electron microscopic alterations of the liver specimens of both growing and adult animals. Similarly, histopathological changes were recorded in rat liver following chronic adlinistration of pyrethroid compounds applied either orally (**Shakoori et al., 1988; El-Toukhy and Girgis, 1993; Kostka et al., 2000; Luty et al., 2000**), through intraperitoneal injection (**Aldana et al., 1998 & 2001**), by inhalation (**Gupta et al., 1999**) or dermally (**Luty et al., 1998**). In the present work, the earliest response to pyrethroid inhalation, in both growing and adult animals, was liver hyperemia represented in dilatation and congestion of the central and portal venules as well as the sinusoids accompanied by massive cellular infiltration. This inflammatory reaction which persisted along the whole duration of pyrethroid exposure explains the significant increase of the relative liver weight of the pyrethroid-exposed animals compared with the age-matched controls. Comparable findings were presented by **Luty et al. (2000)** who demonstrated, as well, increased phagocytic activity of neutrophils and significantly higher numbers of monocytes and lymphocytes in the blood of male mice received orally sublethal doses of alpha-cypermethrin, a pyrethroid compound.

In the present work, cellular infiltration started earlier in the pyrethroid-exposed growing animals and was more extensive than in the corresponding adult ones. In the growing animals, it was located in the pericentral and periportal areas as well as focally within the hepatic lobules at sites of degenerated hepatocytes while in the adult animals it was located mainly in the periportal areas. The distribution of the inflammatory cellular infiltration and the areas of cellular necrosis, in cases of liver toxicity, have been explained by **Haschek and Rousseaux (1991)**, according to whether the toxic agent is inherently toxic or it becomes so after being metabolized by the hepatic cells. In the first condition, the periportal hepatocytes become more sensitive to toxic injury, compared with the central lobular ones, since they receive blood-borne toxins first and presumably in the highest concentration. In the second condition, the central lobular hepatocytes are more affected, compared with periportal ones, as they have a much higher concentration of cytochrome P - 450 and associated enzymes that metabolize and thereby activate xenobiotics (**Haschek and Rousseaux, 1991**). According to this view, the hepatocytes of the growing animals, of this study, seem to be sensitive to both pyrethroid and its metabolites while the sensitivity of those of the adults is mainly to the pyrethroid only.

One of the interesting finding of the present work was the proliferation of the bile ductules and their extension into periportal areas of degenerated hepatocytes, followed by their regression after pyrethroid withdrwal. Such phenomenon was discussed by **Haschek and Rousseaux (1991)** who attributed the proliferation of the bile ductules and their extension into the areas of degenerated cells, in cases of hepatotoxicity, to their participation in the process of repair. They added that with additional time after the toxic insult, the bile ductules usually regress so the periportal area is once again composed of normal-appearing hepatocytes.

Moreover, the current experiment revealed that inhalation of pyrethroid resulted in marked ultrastructural alterations of the hepatocytes represented in rarified cytoplasm, appearance of intracytoplasmic fat globules, accumulation of glycogen and giant, irregularly shaped as well as degenerated mitochondria. These findings partially match those represented by **Shakoori et al. (1988) and Aldana et al. (1998 & 2001)** following exposure of albino and Wistar rats, respectively, to cypermethrin (pyrthroid). The former authors added that the hepatic cells became hypertrophied as result of accumulation of glycogen. Moreover, **Aldana et al. (1998)** attributed the presence of lipid droplets, the accumulated glycogen and the appearance of giant mitochondria to the translated but not secreted apo A - 1 and B mRNA, which are molecular marker of liver damage, resulting in alteration of metabolism of lipids and proteins in rat liver.

In the current experiment, signs of reactivity of the hepatocytes were represented by the irregularly shaped mitochondria, increase in binucleated hepatocytes and the presence of more than one nucleolus within the hepatocytic nuclei. Matching observation was represented by **Kostka et al. (2000)** who emphasized that the pyrethroid permethrin affected DNA synthesis and increased binuclear hepatocytes but did not increase the number of mitotic figures suggesting that permethrin might inhibit phase G2 in the cell cycle and consequently it could suppress the cell entering into the stage of mitosis (M-phase). This suggestion is consistent with ultrastructural findings of the present work which did not record any mitotic figure in the hepatocytes apart from occasional division of the mitochondria.

From the results of the present work, it is obvious that the hepatotoxic effect of pyrethroid increased with prolongation of the duration of exposure where the histological and ultrastructural changes of the rat liver were mild at the 3rd week and became marked by the 9th week of exposure. Equivalent observation was reported by **El - Tawil and Abdel - Rahman (1997 & 2001)** who deduced that the pyrethroid cypermethrin had toxic effects on rat hepatocytes in a dose - and time - dependent

manner. **El - Tawil and Abdel - Rahman (1997)** added that female rat hepatocytes could be more sensitive to the toxic effects of cypermethrin than male cells.

The underlying mechanisms of the hepatotoxic effects of pyrethroids have been discussed by many authors. Pyrethroids have been reported to exert their toxic effect through induction of free radical generation oxidatively damaging hepatic tissues of the experimental animals (**Bagchi et al., 1995; Piotrowski et al., 1996; Gupta et al., 1999; Giray et al., 2001; El Demerdash et al., 2003**). **Giray et al. (2001)** revealed the increased oxidatively damaged end-products of lipids, measured as lipid hydroperoxides in cerebral and hepatic tissues of rat following daily oral doses of cypermethrin Additionally, **Gupta et al. (1999)** demonstrated increased oxidative product of protein, measured as protein carbonyls as well as oxidative modification of the cellular proteins in liver and kidney of developing rats following pyrethroid inhalation. Moreover, **El Demerdash et al. (2003)** reported reduction of liver enzymes including aminotransferase and alkaline phosphatase associated with the free radical generation in cypermethrin treated animals. The oxidative stress-mediated mechanism of pyrethroids in production of cellular damage was proved by the preventive effect of antioxidants including alpha-tocopherol (**Aldana et al., 2001**), vitamin E and allopurinol (**Girary et al., 2001**), isoflavone (**El Demerdash et al., 2003**) as well as vitamin C which was considered as a primary antioxidant and hepatoprotector modulating up to 90% of the hepatic cell damage caused by cypermethrin (**Barja et al., 1994**). The appearance of fat globules as well as accumulation of glycogen in the hepatocytes of both adult and growing pyrethroid-exposed animals of the present experiment are in favor of the assumption of free radical generation associated with affection of liver enzymes as a mechanism for the pyrethroid induced hepatotoxicity. However, the preventive effect of the antioxidants needs further study.

Furthermore, disturbance of mitochondrial respiratory chain by pyrethroids could provide a new explanation for some of the symptoms of pyrethroid intoxication as revealed by **Yamano and Morita (1995) Gassner et al. (1997).** The authors demonstrated potent inhibitory effect of pyrethroids (tralomethrin, permethrin and cyhalothrin) on complex I and uncoupling state 3 respiration of the hepatic cell mitochondria suggesting the possibility of mitochondrial dysfunction. Such explanation is compatible with the ultrastructural findings of the present work, which demonstrated irregularly shaped as well as degenerated mitochondria in the hepatocytes of both adult and growing pyrethroid-exposed animals. Moreover, other biochemical interactions of pyrethroids have been described in the literature including inhibition of the ATPase activity in liver tissues resulting inhibition of active transport of metal

ions and oxidative phosphorylation of hepatic cells (**El-Toukhy and Girgis, 1993**), alteration of the sodium channel kinetics (**Tatebayashi and Narahashi, 1994**) and inhibition of Ca^{++} channels (**Kadous et al., 1994**). However, whether any of these interactions are responsible for the toxicological effects of pyrethriods in higher animals remains unclear.

The current work showed that 4 weeks following pyrethroid withdrawal, resulted in partial recovery of the hepatic tissue of both adult, and growing animals represented mainly in subsidence of the inflammatory reaction, and increased reactivity of the hepatic cells. However, healing by fibrous tissue formation around the portal tract, in all pyrethroid-exposed animals, around the central venules and sporadically within the hepatic lobules, in pyrethroid-exposed growing animals, implied that restoration of the normal appearance of the hepatic lobules could not be achieved, even with longer periods of pyrethroid withdrawal. This leads to the assumption that, prolonged exposure to pyrethroid leads to irreversible changes in the hepatic lobules with more affection of the growing animals than adult ones. In agreement, **El-Toukhy and Girgis (1993)** revealed irreversible histopathological changes in rat liver following chronic exposure to cypermethrin, while **Eriksson and Fredriksson (1991)** detected permanent changes in the cholinergic system of both adult and neonatal rats following daily exposure to bioallethrin or deltamethrin (pyrethroids). On the other hand, **Gupta et al. (1999)** declared that inhalation of pyrethroid-based liquid mosquito repellent by developing rats for a short duration (8 days) could exert some toxic effects on the liver that were non-persistent in nature and could recover soon after cessation of exposure. However, the authors warned against using these repellents for younger individuals on long-term exposure.

In the present study, though the ultrastructural changes of the hepatocytes of the pyrethroid-exposed growing and adult animals were similar, yet the early appearance of cellular infiltration, the more extensive inflammatory reaction and the more spread of the fibrous tissue formation in the hepatic lobules of the growing animals, indicates their more sensitivity than adults to the hepatotoxic effects of pyrethroids. More sensitivity of developing animals than adults was also reported in the neurotoxic effects of chronic exposure to sublethal doses of pyrethroids and organophosphorus insecticides (**Eriksson, 1997; Liu et al., 1999**). **Eriksson (1997)** attributed that to the immaturity of body organs, both structurally and functionally, as well as to the incomplete development of enzymes which catalyze the metabolism of pyrethroids in liver of young animals. Furthermore, **Atterberry et al. (1997)** ascribed the age related differences in sensitivity to pesticides to the metabolic

differences in their distribution and excretion. In contradiction to the present finding, **Sheets et al. (1994)** and **Sheets (2000)** concluded that young rats were more sensitive than adults to a lethal dose of pyrethroid (cypermethrin and permethrin) but not to chronic lower doses where the young animals were protected by existing tolerances.

In conclusion, long-term exposure to pyrethroid containing mosquito repellents is hepatotoxic to both growing and adult animals with more affection of the growing ones. Withdrawal of pyrethroid following prolonged exposure results in partial recovery of the hepatic lobules with persistent changes represented mainly in increased fibrous tissue formation. This draws the attention to the importance of using these repellents in a very narrow scale with a short period of exposure especially for younger individuals. The preventive effect of the antioxidants needs further study.

SUMMARY

Twenty-four adult males and 8 dams with 2 day-old pups were used in this study. The animals were divided into three groups; Group I (control group) reared under normal hygienic conditions, and killed at intervals of 3, 6, 9 and 13 weeks, group II exposed daily to inhalation of pyrethroids vaporized from mosquito repellent mats (Ezalo) and killed at intervals of 3, 6 and 9 weeks and group III exposed to inhalation of pyrethroid for 9 weeks then killed 4 weeks after its withdrawal. Three adults and three male pups, of each group, were killed at each interval of those mentioned above. The percentage of the mean liver weight to the mean body weight (relative liver weight) was calculated, in each killed animal then the liver specimens were prepared for light and electron microscopical studies.

The morphometric quantification showed statistically significant increase in the relative liver weight, in all pyrethroid exposed groups, compared with the age-matched controls and such increase was proportional to the duration of pyrethroid exposure. Four weeks after withdrawal of pyrethroid was not enough for the measurements of the relative liver weight to return to their age-matched control values.

The light microscopical study revealed that pyrethroids had an adverse effect on hepatic lobules represented in cellular infiltration, dilatation and congestion of the central and portal venules as well as of the hepatic sinusoids, degeneration of the hepatocytes and increased fibrous tissue formation which was periportal, in both growing and adult animals, pericentral and sporadical within the hepatic lobules, in the growing animals only. The electron microscopical study of the hepatocytes

revealed ill-defined cell boundaries, intracytoplasmic fat droplets, accumulation of glycogen, irregularly-shaped as well as degenerated mitochondria and more frequent binucleation with nuclei containing more than one nucleolus. Complete degeneration of the hepatocytes with disappearance of all cytoplasmic organelles was also demonstrated. Withdrawal of pyrethroids resulted in subsidence of the inflammatory reaction and much increase of the connective tissue formation as well as increase of the hepatocytes reactivity with clear cell boundaries.

In coclusion, long-term exposure to pyrethroids is hepatotoxic to both growing and adult rats with more affection of the growing ones, and its Withdrawal, after prolonged use results in partial recovery of the hepatic lobules with increase in fibrous tissue formation. This draws the attention to the importance of restricted use of pyrethroids as mosquito repellent especially for younger individuals.

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الملخص العربى

التاثير المقارن لاستنشاق الأقراص الطاردة للبعوض المحتوية لمادة البيريثرويد على كبد ذكور الفئران البيضاء البالغة وتلك التى فى طور النمو

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أستخدم فى هذا البحث ٢٤ فأر ذكر بالغ وثمانية فئران من الأمهات مع صغارهم البالغين يومين من العمر ، وقد تم تقسيم الحيوانات إلى ثلاثة مجموعات الأولى (المجموعة الضابطة) وقد تعرضت لظروف صحية طبيعية وتم قتل الحيوانات فيها على فترات بعد ٣ و٦ و٩ و١٣ اسبوع والمجموعة الثانية وقد تعرضت يوميا لاستنشاق مادة البيريثرويد المتصاعدة من الأقراص الطاردة للبعوض (الايزالو) وقد تم قتل حيوانات هذه المجموعة على فترات بعد ٣ و٦ و٩ اسابيع والمجموعة الثالثة وقد تعرضت يوميا لاستنشاق مادة البيريثرويد لمدة ٩ اسابيع ثم تم قتل الحيوانات بعد أربعة أسابيع من توقف تعرضهم لهذه المادة . وفى كل فترة ، من السابق ذكرهم ، تم قتل ثلاثة من الفئران البالغين وثلاثة من صغار الفئران الذكور وقد احتسبت النسبة المئوية لمتوسط وزن الكبد لمتوسط وزن الجسم (وزن الكبد النسبى) لكل فأر تم قتله ثم تم تحضير عينات الكبد للدراسة بالميكرسكوب الضوئى والالكترونى .

وقد أظهرت القياسات المرفومترية زيادة ذات دلالة إحصائية فى الوزن النسبى للكبد فى كل المجاميع المعرضة لمادة البيريثرويد بالمقارنة بالمجموعة الضابطة لنفس العمر . وقد تناسبت هذه الزيادة تناسبا طرديا مع طول فترة التعرض لمادة البيريثرويد وأن أربعة أسابيع بعد توقف التعرض لهذه المادة لم تكن كافية لعودة قياسات الوزن النسبى للكبد إلى قيم قياسات المجموعة الضابطة لنفس العمر .

وقد أوضحت الدراسة بالميكروسكوب الضوئى أن مادة البيريثرويد لها تأثير ضار على الفصيصات الكبدية متمثلة فى ارتشاح خلوى ، والذى يبدأ مبكرا فى الحيوانات النامية وكذلك تمدد واحتقان الوريدات المركزية والبابية والمنحنيات الجيبية الكبدية وانحلال الخلايا الكبدية وزيادة فى تكوين الأنسجة الليفية حول المنطقة البابية ، فى كل من الحيوانات النامية والبالغة ، وحول المنطقة المركزية وكذلك فى مناطق متفرقة داخل الفصيصات الكبدية فى الحيوانات النامية فقط . وقد أظهرت الدراسة بالميكروسكوب الالكترونى للخلايا الكبدية عدم وضوح جدران الخلايا وظهور قطيرات دهنية ببروتوبلازما الخلية وتراكم الجليكوجين بها ووجود أشكال غير منتظمة للميتوكوندريا وانحلال بعضها وكذلك كثرة الخلايا ثنائية النواة والتى تحتوى على أكثر من نوية . وقد أظهر أيضا الفحص بالميكروسكوب الالكترونى الانحلال التام لخلايا كبدية مع اختفاء كل العضيوات البروتوبلازمية بها ، وقد أدى توقف التعرض للبيريثرويد إلى خمود التفاعل الالتهابى وزيادة شديدة فى تكوين الأنسجة الليفية وكذلك زيادة فى استرجاع نشاط الخلايا الكبدية مع وضوح جدرانها .

ونستخلص من هذه الدراسة أن التعرض طويل الأمد لمادة البروثرويد سام للخلايا الكبدية لكل من الفئران التى فى طور النمو والبالغة مع زيادة تأثر الأولى وأن توقف التعرض لهذه المادة بعد اطالة استخدامها أدى إلى شفاء جزئى للفصيصات الكبدية مع زيادة فى تكوين الأنسجة الليفية بها . هذا يجذب الانتباه إلى أهمية الاستخدام المحدود لمادة البيريثرويد كطارد للناموس خاصة مع الأشخاص صغيرة السن .