

A Study of the Immunotoxicity Induced by Short Term Chronic Exposure to Deltamethrin in Adult Male Albino Rats

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ABSTRACT

KEYWORDS

Deltamethrin,
Oxidative stress,
Caspase 3.

Deltamethrin is a widely used type 2 pyrethroid insecticide in home and agriculture because of its high insecticidal activity, environmental stability and relatively low acute toxicity. It was found that deltamethrin alters the immune response signaling pathways, but the mechanism is still under study. Thus, this study aimed to investigate the propensity of deltamethrin to cause immunotoxicity in rats. Random division of rats into three equal groups was done, Group (I): (negative control group): allowed drinking water ad libitum; Group II (positive control group): administered orally with 1mL corn oil /rat/day for 14 weeks: and Group III (deltamethrin treated group): Each rat was given deltamethrin (5mg/kg body weight/ day) dissolved in corn oil for 14 weeks. At the end of the study, blood samples and spleen tissues were collected for estimating malondialdehyde and glutathione peroxidase activities, as well as immuno-histochemistry examination. Results indicated significant increase in blood and splenic tissue malondialdehyde and significant decrease in blood and splenic tissue glutathione peroxidase activity in deltamethrin group when compared with those of negative or positive control groups. Light microscopic examination revealed disruption in splenic histoarchitecture while immuno-histochemical staining showed many positive reacted cells to active caspase 3 in deltamethrin group. In conclusion, this study demonstrated that deltamethrin may have immunotoxic effects on spleen of adult rats.

Introduction

Pyrethroids are synthetic organic compounds that are used extensively as household and commercial insecticides and produced from *chrysanthemum* flowers. The lipophilic keto-alcoholic esters of chrysanthemic and pyrethroic acid are responsible for the insecticidal properties. There are 2 types of pyrethroids; first and second generation pyrethroids. The second generation (Type 1) pyre-

throids are more toxic to mammals than the first generation (Type II) pyrethroids (Rehman et al., 2014). Primarily, pyrethrin compounds have been used to control human lice, cockroaches, flies and mosquitoes. Other pyrethrins are used in poultry pens, on dogs and cats to control lice and fleas and in grain storage (I.P.C.S., 1990).

Generally, pyrethroids are lipophilic and so readily absorbed dermally and orally. After oral administration, deltamethrin is rapidly absorbed from the gastrointestinal tract. Studies on rats found that deltamethrin is rapidly metabolized by liver microsomal oxidases and by tissue esterases, which are widely distributed in the liver and gut wall (He et al., 1991).

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In rodents, the main routes of metabolism include cleavage of the ester link to produce alcohol and acid moieties, oxidation of various parts of the molecule, and conjugation of the products of oxidation with glucuronic acid, sulfuric acid or glycine. Within 2–4 days, metabolites are excreted in faeces and urine with the exception of the cyano group, which is converted to thiocyanate and excreted more slowly (about 20% still retained mainly in skin and stomach after 8 days) and about 13–21% of unchanged deltamethrin is excreted in rat faeces (Rehman et al., 2014).

As humans are exposed to deltamethrin in their day-to-day life, its toxicity is a matter of concern. Deltamethrin induced nephrotoxicity, hepatotoxicity, neurotoxicity, reproductive toxicity, genotoxicity and immunotoxicity depends on many factors such as doses, routes and time of exposure (Sharma et al., 2013).

Spleen is the largest secondary lymphoid organ and it is used to evaluate for enhanced histopathology of the immune system. It contains B and T lymphocytes on which the immunotoxic effects of xenobiotics or their metabolites can be reflected (El-more, 2006).

Years ago, the synthetic pyrethroids such as α -cypermethrin and deltamethrin were studied for immunotoxicity in male F344 rats (Madsen et al., 1996).

Increased NK (natural killer) cells activity and number of splenic cells producing antibody to SRBCs were detected at oral daily doses of 5 and 10 mg/kg body weight for 28 days. With the highest dose, these effects were associated with decreased body weight and increased adrenal weight that suggested generalized toxicity occurring with high doses. It was also reported that non-continuous repeated deltamethrin inhalation in rats resulted in multiple immunotoxic effects (Emara and Draz, 2007).

The present study was undertaken to investigate the propensity of deltamethrin to cause immunotoxicity in adult male albino rats.

Material and Methods

[A] Chemicals

Deltamethrin, product of Sumitomo Chemical Company, was purchased from Sigma –Egypt in the form of technical material of 99% purity, (CAS Number: 52918-63-5): [(S)-Cyano-(3-phenoxyphenyl)methyl](1R,3R)-3-(2,2-dibromoethenyl)-2,2-dimethylcyclopropane - 1-carboxylate (IUPAC).

[B] Animals:

The study was done on 36 healthy adult male albino rats weighing 150-200 g. Rats were obtained from the animal house of Zagazig Faculty of Medicine. The rats received balanced food rich in all stuffs necessary to maintain their health before and during drug administration. It consisted of bread, barley and milk. Water was offered in separate clean containers. The experimental work was done according to the guiding principles for the use and care of experimental animals and was approved by the Institutional Research Board.

[C] Experimental Design:

Rats were randomly divided into three equal groups. **Group I (negative control group):** No intervention was done to rats in this group for adjusting the basic parameters, and allowed drinking water *ad libitum*. **Group II (positive control group):** daily administered 1mL corn oil orally for 14 weeks. **Group III (deltamethrin treated group):** the rats were orally administered deltamethrine at a dose of (5mg/kg body weight) dissolved in corn oil [equivalent to 1/20 of the LD50; based on Yadav et al., 2006]. The pesticide was given daily for 14 consecutive weeks. Twenty-four hours after the end of experimental duration, the rats were subjected to sampling of blood and spleen tissue as the follows:

- 1) **Collection of blood samples;** under light ether anesthesia, venous samples

from the retro-orbital plexus were obtained by capillary glass tubes as described by Schemere (1967), to measure: malondialdehyde (MDA) and glutathione peroxidase (GPx) in sera.

- 2) **Collection of spleen samples;** spleen was dissected and divided into two equal parts; one for estimating splenic MDA and GPx activities. The other part was sent for histopathological examination (light microscope and immunohistochemical staining for active caspase 3).

[D] Biochemical measurements

- 1) **Malondialdehyde (MDA) level:** Serum MDA was assayed colorimetrically guided by the method of Ohkawa et al (1979). In this method; Thiobarbituric acid reactive product was formed from the reaction between MDA and Thiobarbituric acid at temperature of 95°C for 30 min in acidic media. Measuring the resultant product can be done by spectrophotometer at 534 nm. Calculation: serum malondialdehyde = $(A_{\text{Sample}} \div A_{\text{Standard}}) \times 10 \text{ nmol/L}$.

- 2) **Glutathione peroxidase (GPx):** in serum was determined according to Pleban et al. (1982), as follows: 20 μl aliquot of hemolysate was transferred to 1 ml quartz cuvet containing 980 μl of the reaction mixture. The mixture was incubated for 5 minutes at 37°C. The reaction was started by adding 10 μl of H_2O_2 (8.8 mmol/L). After 30 second lag, the decrease in NADPH absorbance was read every minute for 3 minutes at 430 nm by spectrophotometer. The decrease was linear with time. To avoid non-enzymatic reaction, water was used instead of hemolysate for blank. Calculation: A unit of peroxidase is defined as change in absorbance/ minute at 430nm .

- 3) **Splenic malondialdehyde (MDA) level:**

The dissected tissues were put in petri dishes. After washing the tissues with physiological saline (0.9% NaCl), samples were kept at -87°C until analyzed. The tissues were homogenized for 5 min in 50 Mm ice cold KH_2PO_4 buffer solution (pH7.0) 0.5g tissue + 5 ml buffer solution) using a glass-porcelain homogenizer and then centrifuged at 7000 x g for 15 min. All processes were carried out at 4° C. Supernatants were used to determine the MDA concentration (Jain et al., 1989). MDA was assayed colorimetrically following the method of Ohkawa, et al. (1979).

- 4) **Glutathione peroxidase (GPx) activity in splenic tissue:** perfusion of the tissues was done using 0.9 % NaCl containing 0.16 mg / ml heparin. Tissues were washed and minced in ice-cold 0.25 M sucrose, then homogenized, diluted and centrifuged at 4000 rpm and 4°C for two minutes. The supernatant was used to measure Gpx activity following Paglia and Valentine (1967) according to pamphlet of Bio-diagnostic kits using calorimetric method. Principle: The addition of hydrogen peroxide initiated the enzyme reaction in the tube which contains B-nicotinamide Adenine Dinucleotide Phosphate (B-NADPH, reduced form), Glutathione Peroxidase and a sample or a standard. Monitoring the change in the absorbance spectrophotometrically at 430nm was done. A standard curve was plotted for each assay. Calculation: Change in absorbance/ minute at 430 nm define one unit of peroxidase.

[E] **Histopathological changes;** spleen was examined by:

1) Light microscopic examination: The spleen was fixed in 10% formalin saline then the tissues were embedded in blocks of paraffin and 5 μ thickness were prepared. These sections were subjected to Hematoxylin and Eosin (H&E) stains (Horobin and Bancroft, 1998) and then examined by light microscope (400X).

2) Immuno-histochemistry for caspase 3; According to Ramos-Vara (2005). The site where antigen was present was clearly revealed by brown colouration in the cytoplasm. Collected results were tabulated and statistically analyzed using appropriate methods by Statistical Package of Social Science (SPSS), software version 22.0 (SPSS Inc., 2013). *P* value <0.05 was considered statistically significant.

Histological studies were presented as photomicrographs and scientifically interpreted.

Results

1) Biochemical studies:

There were no significant differences in results between negative and positive control groups using unpaired t-test (Table 1).

There were significant increases in blood and splenic tissue MDA in deltamethrine group

when compared with negative and positive control groups by unpaired t-test (Table 2). Also, there were significant decrease in blood and splenic tissue for GPx activity in deltamethrine group when compared with those of negative and positive control groups by unpaired t-test (Table 2).

2) Histopathological results:

Light microscopic examination of H&E stained sections from the spleen of the control groups revealed the red hematogenous pulp and the white lymphoid pulp of the spleen. The white pulp was located around a central arteriole and composed of the marginal zone (B-cell area), the adjacent follicles (B-cell area) and periarteriolar lymphoid sheath (PALS, T-cell area) (Figure 1a). The marginal zone lies between the red and white pulp and the junction between the marginal zone and red pulp is not always clear. The marginal zone contains specific populations of macrophages in addition to lymphocytes. Meanwhile caspase immune-staining showed positive reaction in few cells (Figure 1b).

In deltamethrin group, the white pulp increased in the size and had more fibroblasts and low lymphocyte densities. The red pulp was congested with many red blood cells, more bundles such as trabeculae, and some pyknotic lymphocytes (Figure 2 a, b & c). However, caspase immune-staining showed many positive reacted cells to active caspase 3 (Figure 3).

Table (1): Statistical comparison among the negative and positive control groups as regard to MDA and GPx (in blood and splenic tissue) along the periods of the study by unpaired t test.

Parameter \ Group	Negative control group(I) Mean \pm SD	Positive control group (II);corn oil group Mean \pm SD	T	p
MDA in blood (nmol/ml)	0.22 \pm 0.04	0.23 \pm 0.03	0.6325	>0.05
MDA in spleen (nmol/gm tissue)	21.42 \pm 3.36	21.46 \pm 3.52	0.0260	>0.05
Blood GPx (ng/ml)	29.63 \pm 2.49	29.22 \pm 2.72	0.3516	>0.05
Splenic tissue GPx (ng/ mgm tissue)	168.48 \pm 4.10	167.86 \pm 4.55	0.3201	>0.05

Number of sacrificed rats for each group was 12 rats, MDA: malondialdehyde, GPx: Glutathione peroxidase.

Table (2): Statistical comparison among the negative control and deltamethrin treated groups as regard MDA and GPx (in blood and splenic tissue) along the periods of the study by unpaired t test.

Group \ Parameter	Negative control group (I) Mean \pm SD	Deltamethrin group (III) Mean \pm SD	T	p
MDA in blood (nmol/ml)	0.22 \pm 0.04	1.31 \pm 0.03	68.9377	<0.0001***
MDA in spleen (nmol/gm tissue)	21.42 \pm 3.36	233.54 \pm 4.35	122.0369	<0.0001***
Blood GPx (ng/ml)	29.63 \pm 2.49	11.76 \pm 1.78	18.4625	<0.0001***
Splenic tissue GPx (ng/ mgm tissue)	168.48 \pm 4.10	10.62 \pm 1.6	113.4246	<0.0001***

Number of sacrificed rats for each group was 12 rats, MDA: malondialdehyde, GPx: Glutathione peroxidase.

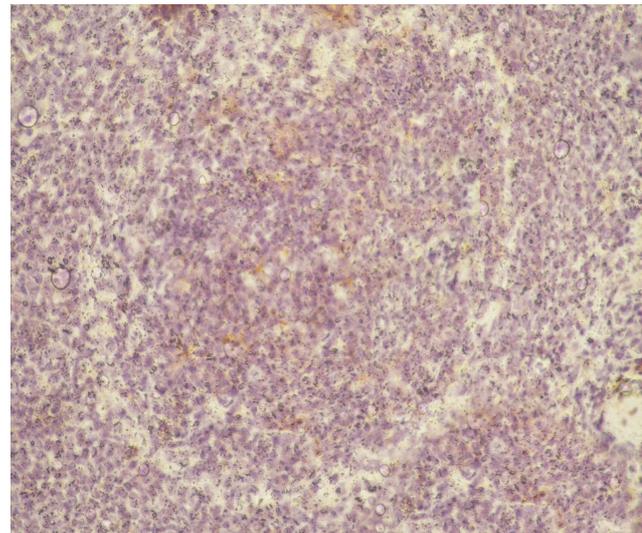
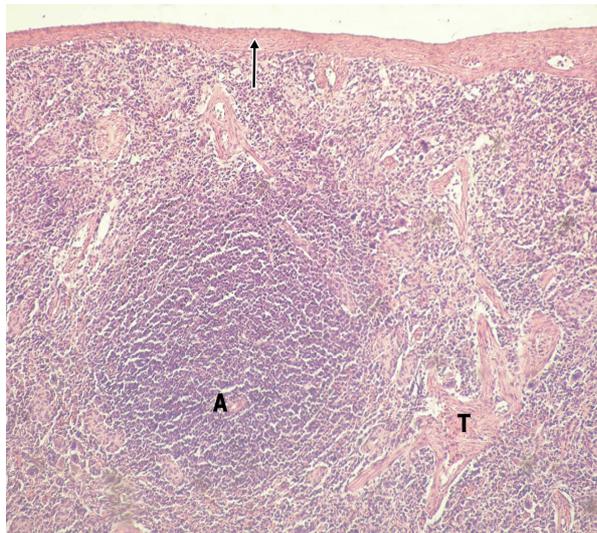


Fig. (1 a) A photomicrograph of a section in splenic tissue from control group showing thin capsule (arrow, trabeculae (T) scattered in the red pulp, central arteriole in the white pulp (A) (Hx & E X 100).

Fig. (1 b) A photomicrograph of a section in splenic tissue of control group showing scanty reaction to caspase 3 (caspase-3 immunostaining X 400).

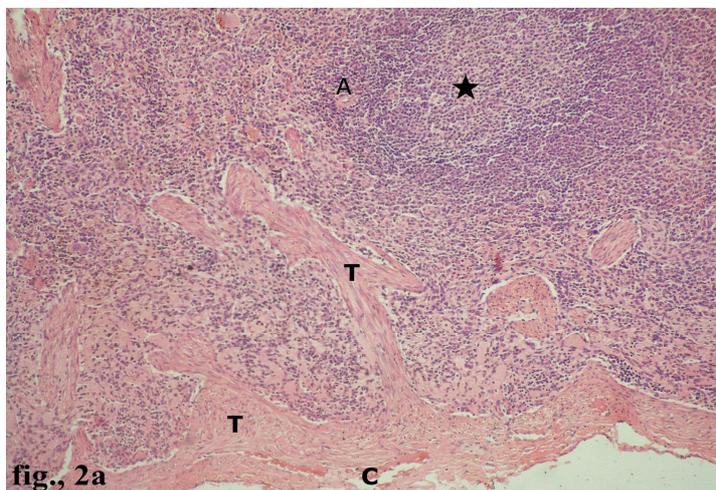


Fig. (2 a): A photomicrograph of a section in splenic tissue of deltamethrine group showing thick congested capsule (C), white pulp with low lymphocytic density in germinal center (*) and adjacent central arteriole (A), thick trabeculae (T) (2a Hx & E X 100).

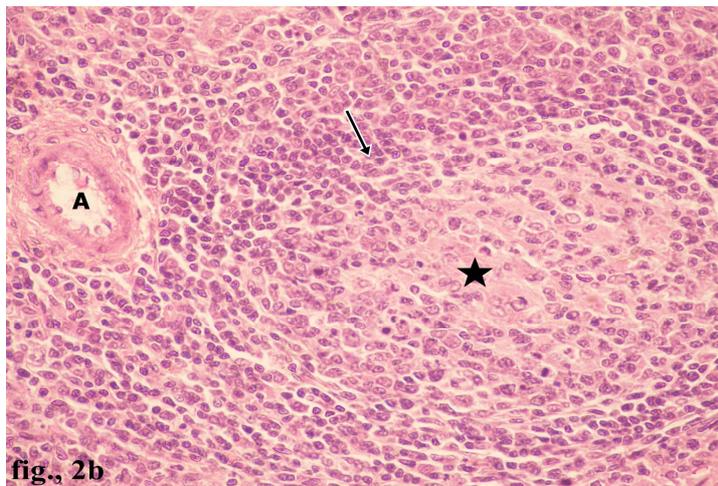


Fig. (2 b): A photomicrograph of a section in splenic tissue of deltamethrine group showing white pulp with some pyknotic lymphocytes (arrow) with low lymphocytic density in germinal center (*) (Hx & E X 400).

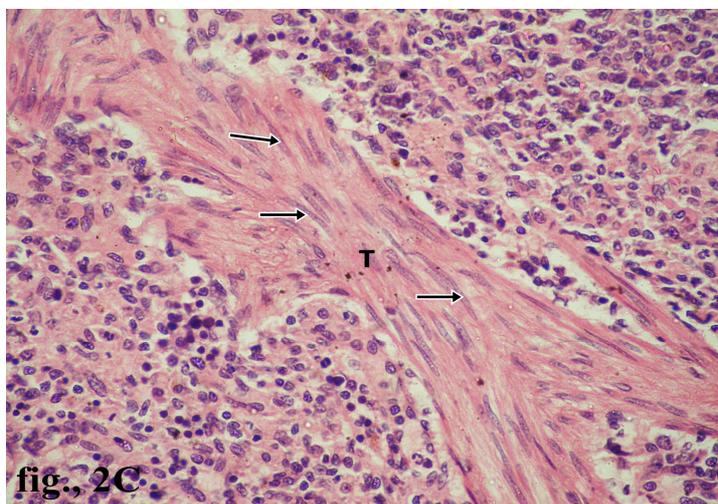


Fig. (2 c): A photomicrograph of a section in splenic tissue of deltamethrine group showing thick trabeculae (T) with many fibroblasts (arrows) (Hx & E X 400).

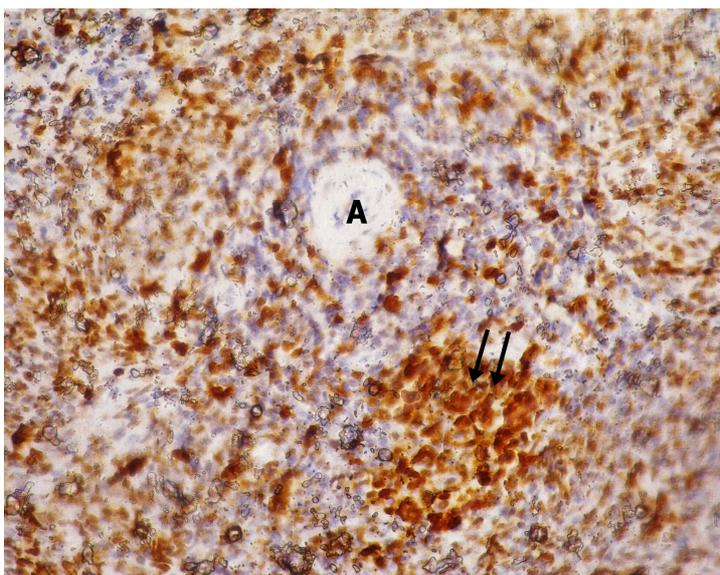


Fig. (3) : A photomicrograph of a section in splenic tissue of deltamethrine group showing many positive reactions to caspase3 (arrows) (caspase-3 immunostaining X 400).

Discussion:

United States Environmental Protection Agency (2010) reported that deltamethrin is a broad-spectrum synthetic pyrethroid insecticide that is registered for direct application to a wide variety of food/feed crops, for use in food/feed handling establishments, and for use on stored grains. Many studies on the side effects of deltamethrin were established, including immuno-suppression, neurotoxicity, decreased testosterone levels, hypertension and allergy (Issam et al., 2012). Therefore, this study was carried out to explore the immunotoxicity induced by short term chronic exposure to deltamethrin in adult male albino rats.

Regarding control groups (negative and positive control groups); rats of the control groups showed no abnormal findings as regards to biochemical studies. There was no significant difference between the negative and the positive control groups as regard to all these parameters. Also, there were no abnormal histopathological or immune-histochemical changes in the spleen specimens of the adult male albino rats of these groups all over the periods of the study.

Regarding deltamethrin group; deltamethrin had induced a significant increase in the mean values of blood and splenic MDA when compared with the control groups. Also there was significant decrease in GPx activity in blood and spleen tissue when compared with control groups.

These results were matched with Tuzmen et al. (2008) who found that deltamethrin induced toxicity in mammals depended mainly on the oxidative stress; in which there was imbalance between the reactive oxygen species production and antioxidant enzyme system.

Also, Aydin (2011) reported that deltamethrin treatment 3mg/kg (*in vivo*) resulted

in increased the lipid peroxidation and decreased the antioxidant enzymes levels in rats.

Recently, Kumar et al. (2015) found that apoptogenic signalling pathways in both splenocytes and thymocytes were activated by deltamethrin induced reactive oxygen species.

Histopathological examination in the present study showed that white pulp were greater in size with more fibroblasts and low lymphocytic densities. The red pulp was congested with more bundles such as trabeculae and some pyknotic lymphocytes. Also immune-histochemical studies showed many positive reacted cells to active caspase 3.

These results were parallel with Adams and Cory (2001) who stated that programmed cell death (apoptosis) was mediated by caspases. Oxidative stress resulted in up regulation of pre apoptotic (Bad, Bax) and down regulation of anti-apoptotic (bcl-2) proteins. Deltamethrin induced oxidative stress also caused up regulation of Bax and down regulation of Bcl-2.

Ravagnan et al. (2002) and Bras et al., (2005) reported that Bax and Bad were important proteins for the outer mitochondrial membrane permeability. They resulted in release of cytochrome *c* which induced loss of mitochondrial membrane potential, formation of apoptosome and subsequent activation of caspase-9. Caspase 9 activation stimulated the activation of execution caspases.

Kumar et al. (2013) and Kumar et al. (2014) also observed that deltamethrin induced apoptosis in thymus and splenic tissues through activation of caspase dependent signalling pathways in mitochondria.

Conclusion:

Deltamethrin resulted in immunotoxicity through altering variable immune signaling pathways that resulted in immunosuppression. Immunosuppression can be explained by activated oxidative stress signaling pathways and caspase dependent pathways which induced apoptosis.

Recommendation:

More studies on the mechanism of immunotoxicity induced by deltamethrin are recommended. Also follow up investigations are required in farmers and workers exposed to deltamethrin. It is also recommended to study the role of antioxidants in limitation of deltamethrin induced immunotoxicity.

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دراسة التسمم المناعي الناتج عن التعرض المزمن القصير المدى للدلتامثرين في ذكور الجرذان البيضاء البالغة

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تستخدم المبيدات الحشرية البيروثرويدية على نطاق واسع فى الزراعة والاغراض المنزلية وذلك لانها اقل سمية حادة عن المبيدات الاخرى وتنقسم لنوعين ويعد الدلتامثرين من النوع الثانى لهذه المركبات البيروثرويدية .ويهدف البحث الى دراسة التسمم المناعى الناشئ عن التعرض المزمن قصير المدى للدلتامثرين. وقد تم استخدام ٣٦ جرد من ذكور الجرذان البيضاء البالغة مقسمة بالتساوى الى ٣ مجموعات: المجموعة الأولى (مجموعة ضابطة سالبة):-تم اعطاء الجرذان الطعام والشراب بدون أى علاج لمدة ١٤ أسبوع لقياس المعايير الأساسية.المجموعه الثانيه (مجموعه ضابطه موجبه):-تم اعطاء كل جرد (١ مل) من زيت الذره (كمذيب للدلتامثرين) عن طريق الفم مره واحده يوميا لمدة ١٤ أسبوع والمجموعه الثالثه (مجموعه الدلتامثرين):-تم اعطاء الجرذان الدلتامثرين مذاب فى زيت الذره (٥ مجم/كجم) عن طريق الفم مره واحده يوميا لمدة ١٤ أسبوع. تم استخدام فئران كل المجموعات فى نهاية مدة البحث لقياس المألوندايالدهيد والجلوتاثيون بيروكسيداز فى كل من الدم ونسيج الطحال الى جانب فحص نسيج الطحال عن طريق الميكروسكوب الضوئى و والدراسة هستوكيميائيا باستخدام التفاعل لكاسباز ٣. أظهرت النتائج زيادة فى نسبة المألونداى الدهيد فى كل من الدم ونسيج الطحال مع نقص فى نشاط الجلوتاثيون بيروكسيداز فى كل من الدم ونسيج الطحال فى المجموعة المعالجة بالدلتامثرين مقارنة مع المجموعات الضابطة. و أوضح الفحص المجهرى الضوئى باستخدام صبغة الهيماتوكسلين والأيوسين لشرائح الطحال لذكور الجرذان البيضاء فى مجموعة الدلتامثرين وجود تغيرات هستوباثولوجية واضحة الى جانب وجود الكثير من الخلايا الايجابية التفاعل لكاسباز ٣. نستنتج أن التعرض المزمن القصير المدى للدلتامثرين له آثار سمية على الجهاز المناعى عن طريق التأثير على الطحال. ونوصى بدراسة دور الاكسدة فى الاثار السمية لمادة الدلتامثرين ودور مضادات الاكسدة فى الحد من هذه الاثار.