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The Injury in Pulmonary Tissue Following Fipronil Exposure in Male Albino Rats and The Protective Role of Vitamin E (Histological and Immune-Histochemical Study)

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ABSTRACT

Background: The extensive use of fipronil exposes mammals to this hazardous environmental toxin. It is transformed into many components, which affects GABA-gated chloride channels in mammals. Vitamin E (Vit E) is one of the fat-soluble vitamins and is abundant in vegetable oils, margarine, nuts, and cereal and has an antioxidant effect.
Aim of the work: Evaluate the effects of fipronil on the lung tissue of rats and the possible protective effects of Vit E.
Material and methods: Thirty adult male albino rats, 200 - 250 gm, were partitioned into:- group I (control): formed of 10 rats taking regular diet and water, group II (fipronil treated): 10 rats took fipronil at a dose of 20 mg/kg, orally once daily for 5 days only, and group III (fipronil and Vit E treated): 10 rats got Vit E at a portion of 1000 mg/kg orally once daily for 2 weeks then fipronil given as the previous dose orally once daily during the last 5 days.
Results: In the group treated with fipronil there was destruction in the bronchi and alveoli present in alveolar septa, excessive collagen deposition presented around a bronchiole and the alveolar wall using Masson trichome stain, and positive expression of (TNF-α and caspas-3). Vit E had a moderate effect in a damaged cell but a sign of inflammation was still present in the cell. **Conclusion:** Fipronil causes damage to the tissue of the lung and vitamin E has a weak effect on the protection of the lung against fipronil.
Keywords: Fipronil, Lung, Vitamin E, Caspas-3.

INTRODUCTION

Around 4.6 million pesticides are used in recent year to avoid or damage pest in agricultural settings. The danger of insecticides is present in their persistence for long time in the environment [1].

Fipronil (FPN)= is member in the phenyl pyrazole family, which is used in different areas as anti-flea and tick sprays and for agriculture [2]. It affects GABA-gated chloride channels, so it is more effective in insects compared to other, while in mammals its effect through production of substance (sulfone, sulfide, and disulfonyl), which affect these channels[3-4].

It focuses on gamma amino butyric acid receptor (GABAR) receptors as an antagonist; these receptors are expressed in the lungs of both humans and animals making them vulnerable to damage [5]. It can enter the body either through inhalation where the farmers spray insecticide without taking precautions, or oral pathway through contamination of food obtained from animals and plants [6].

The extensive use of FPN in the environment leads to its precipitation in the soil and water, which exposes mammals to this dangerous environmental toxin [7-8]. It has been reported that fipronil applies dangerous impacts on vital organs through suppress of mitochondrial respiratory chain and calcium homeostasis, and its harm towards nucleic acids and proteins [9].

Vitamin E (Vit E) is one of the fat-soluble vitamins and is abundant in vegetable oils, margarine, nuts, and cereal [10]. It is antioxidant organically present in biologic systems by the overproduction of ROS and nitrogen species. It produces protection to the outer layer of the cell against lipid peroxidation [11]. Also, it can attack free radicals, which begin the oxidation of the lipid chain directly or indirectly, so it is considered an antioxidant [12]. Several research revealed that use of vitamins with other antioxidants strengthen their effects[13].

AIM OF THE WORK

Evaluate effects of fipronil on the lung tissue of rats and possible protective effects of VIT. E.

MATERIAL AND METHODS

Drugs:

Fipronil, FPN: introduced as (cockroach killing bait powder) applied from local market of pesticides.
Vit E :1000 mg brought from Pharco, Pharmaceuticals Industries.

Animals:

Thirty adult male albino rats, 200 - 250 gm each. The animals were obtained from the Animal House, Faculty of Medicine, Assiut University, and were housed in the Animal Facility at Faculty of Medicine, Sohag University, Egypt.
Rats were divided into 3 groups:
1-Group I (control): formed of 10 adult albino rats fed normal diet and water.
2-Group II (fipronil treated): 10 adult albino rats received fipronil at a dose of 20 mg/kg, 1/5 the LD50 (where LD 50=97mg /kg), orally once daily for 5 days only[14].
3- Group III (fipronil and Vit E treated): 10 adult albino rats received Vit E at a portion of 1000 mg/kg orally once daily for 2 weeks then during the last 5 days fipronil was given as the previous dose; orally once daily [15].

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After 24 hours from the last dose, the rats were anesthetized, sacrificed, and dissected for extraction of the lung.

Histological examination:
The specimens were prepared for examination by light microscope: The specimens were fixed in 10% neutral buffered formalin, then were washed under water and immersed in alcohol at serial dilutions then in paraffin, then cut at 5-7 µm thickness and subjected to staining with hematoxylin and eosin (H&E), Masson trichome, and immunohistochemical stains [16].

Immunohistochemical examination:
Immunohistochemical staining is done by the avidin biotin peroxidase complex method.
The specimens from the lungs were used for biochemical examination of caspase-3, The reaction appeared brownish either cytoplasmic or nuclear. Sections were then counterstained with Mayer's hematoxylin (HX), dehydrated, cleared, and mounted. [17]

Paraffin sections were stained with avidin-biotin-peroxidase for determination of cells immunoreactive to TNF-α and counterstained with HX. TNF-α expression was a cytoplasmic immune positive reaction [18].

Ethical approval:

The experiment was performed according to guidelines of Sohag University Committee for Animal Care with approval certificate number 5-12-2022-4. The experiment conformed to "Guide for the care and use of Laboratory Animals" for the use and welfare of experimental animals, published by the US National Institutes of Health (NIH publication No. 85–23, 1996).

Morphometric and statistical analysis:
The following measures were taken:
A. The percent of collagen area in Mallory's trichrome-stained sections [19].
B. Area percent of caspase-3 immunoreaction.
10 nonoverlapping fields for each section were taken. This was done using Image J software (version 1.51k, Wayne Rasband, National Institutes of Health, USA). From each variable, the mean± SD (standard deviation of the mean) was measured using SPSS program version 16. One-way analysis of variance (ANOVA) and a post-hoc test were used to find the statistical difference between the groups when ANOVA was statistically significant (P value ≤0.05) [20].

RESULT
Histological result:
Control group (I):
Examination of these groups showed a normal appearance of alveoli with some open into alveolar sacs and normal interalveolar septa, bronchiole, and blood vessels **(figure 1).** when using Masson trichome stain minimal collagen deposition presented around blood vessels, bronchioles, and the alveolar wall **(figure 4).**

Fipronil treated group (I1):
Showed dilation in the bronchi with the area of epithelial destruction, blood vessels appeared congested, there was hemorrhage in the alveoli, thinning in the alveolar septa, and area of destruction present in alveolar septa **(figure 2)**. Masson trichome showed excessive collagen deposition presented around a bronchiole and the alveolar wall **(figure 5).**

Fipronil and vitamin E treated group (III):
Lung had mild epithelial destruction in the bronchi with thinning of its epithelial wall, blood vessels appeared normal, alveoli and alveolar sinus appeared intact, but an area of hemorrhage presented in the interalveolar septa and area of cellular infiltration appeared **(figure3)**. Masson trichome showed moderate collagen deposition around a bronchiole and the alveolar wall; also, hemorrhage appeared inside alveoli and interalveolar septa **(figure 6).**

In immunohistochemistry:
With caspase -3: in control rat showed minimal reaction for caspase-3 **(figure 7)**, in II treated group massive caspase-3-positive cells (brown stain) were observed at the walls of bronchi **(figure 8).** There was significant difference compared to control, while in III treated group there was moderate reaction compared to control group **(figure 9).**

In TNF- α: control group showed a negative reaction **(figure 10)** while in II treated group, positive reaction was observed in the alveolar inter septal region and muscle cells surrounding the bronchi **(figure 11)**, group III showed a moderate reaction compared to control group **(figure 12).**

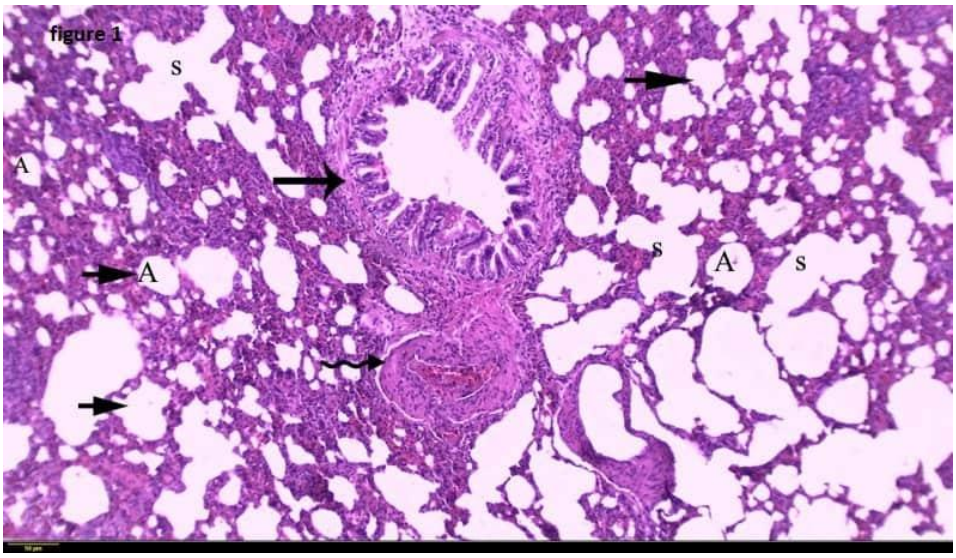


Figure 1: A photomicrograph of lung tissue in control rat shows the normal appearance of alveoli **(A)** open into alveolar sacs **(S)** and interalveolar septa appear intact **(short arrow),** bronchiole **(B**), and blood vessel **(BL). H&Ex100**.

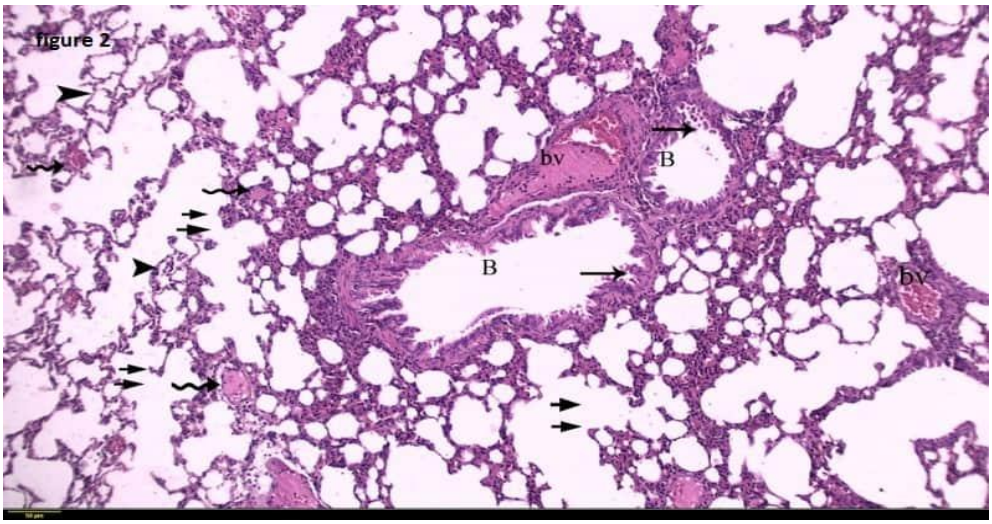


Figure 2: A photomicrograph of lung tissue in fipronil-treated rat shows dilation in the bronchi **(B)** with an area of epithelial destruction, congested blood vessel **(bv),** there is hemorrhage in the alveoli **(irregular arrow),** thinning in the alveolar septa **(arrowhead)** also there is an area of destructed alveolar septa **(double arrow). H&E x100.**

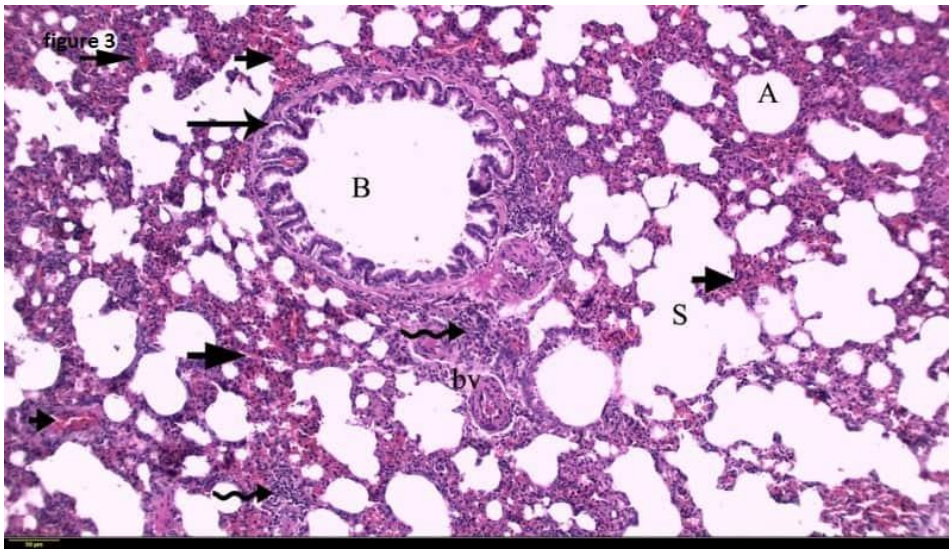
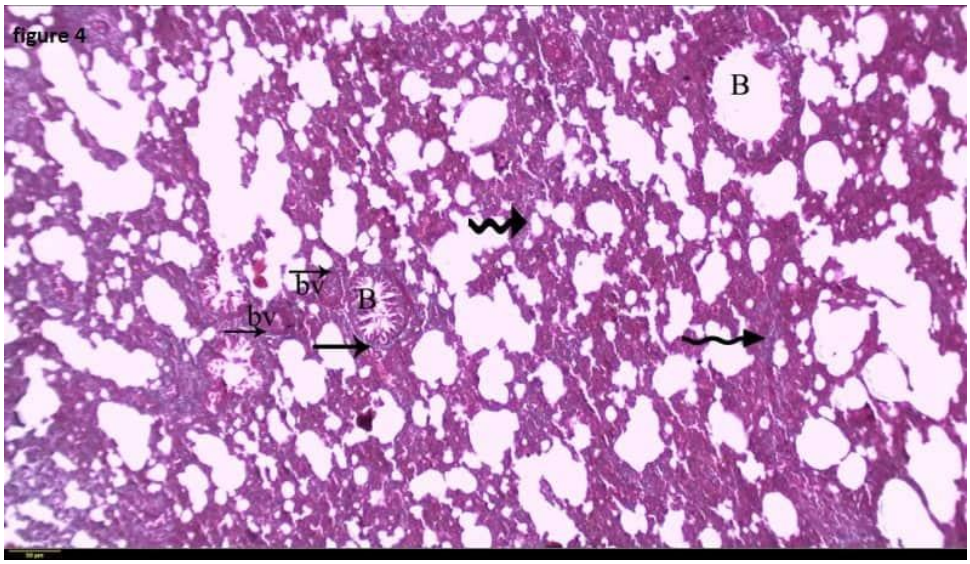


Figure 3: A photomicrograph of lung tissue in fipronil and vitamin E-treated rats shows mild epithelial destruction in the bronchi **(B)** with thinning of its wall **(long arrow),** normal blood vessel **(bv),** there is hemorrhage in the interalveolar septa **(short arrow),** the alveoli **(A)** and alveolar sinus **(s)** appear intact also, there is an area of cellular infiltration **(irregular arrow). H&E x100.**



- **Figure 4:** A photomicrographic section of lung tissue in control rat sections shows minimal collagen deposition **(arrow)** around blood vessels **(Bv),** a bronchiole **(B**), and the alveolar wall **(curved arrow). Masson trichrome, x 100.**

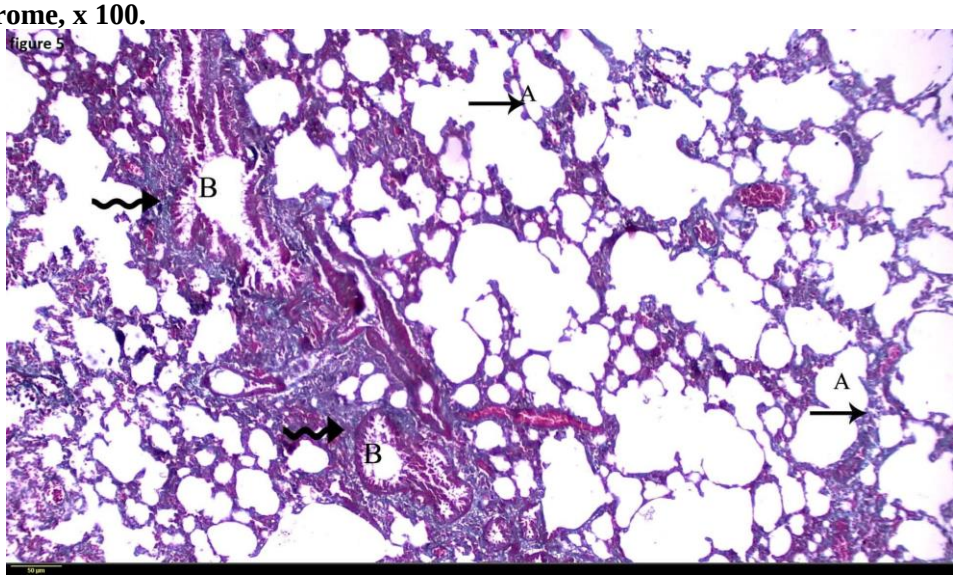


Figure 5: A photomicrographic section of lung tissue in fipronil treated rat shows excessive collagen deposition **(irregular arrow)** around a bronchiole **(B)** and the alveolar wall **(A) (long arrow)**.
Masson trichrome, x 100.

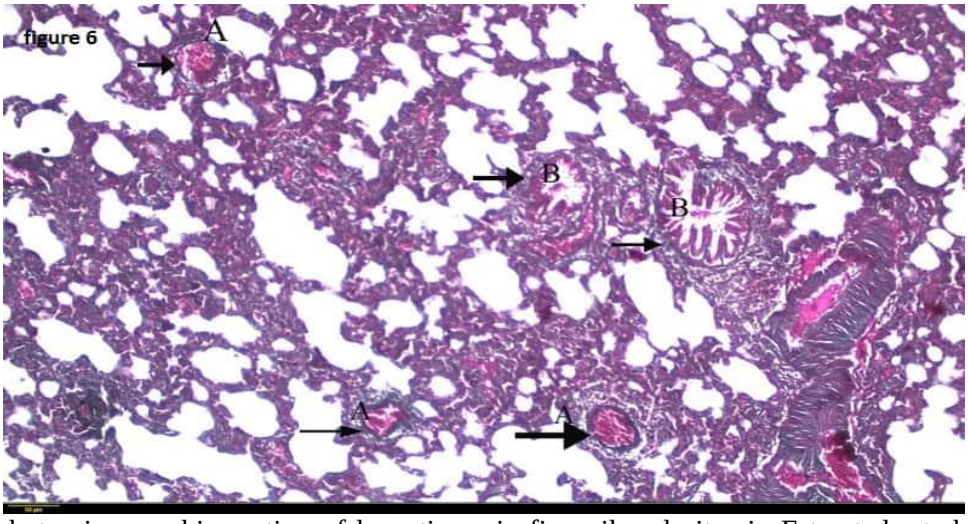
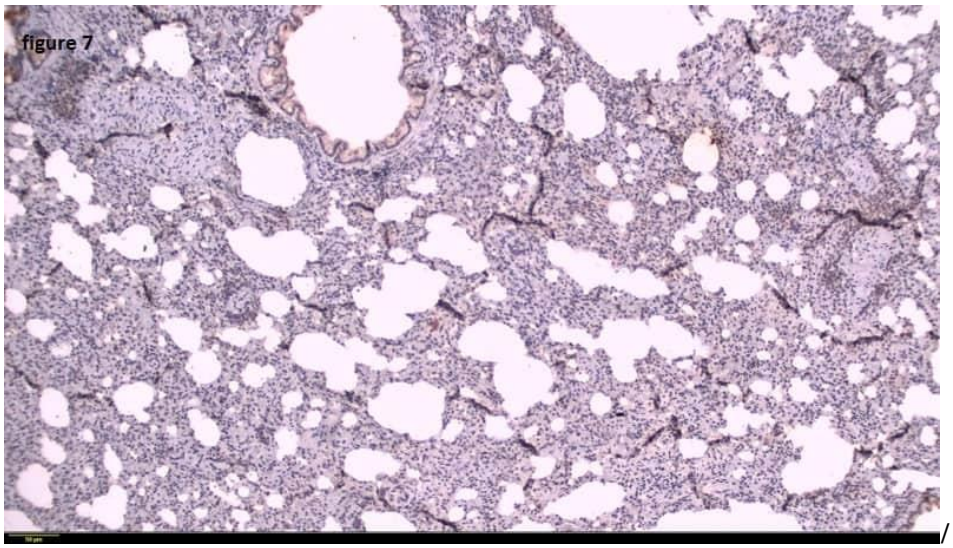


Figure 6: A photomicrographic section of lung tissue in fipronil and vitamin E treated rat shows moderate collagen deposition **(long arrow)** around a bronchiole **(B)** and the alveolar wall **(A), also** hemorrhage appears inside alveoli and interalveolar septa**.** **Masson trichrome, x 100.**



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- **Figure 7:** Transverse section of the lung tissue in control rat shows minimal positive reaction for caspase-3 in the wall of bronchi and alveoli**. Caspase-3× 100.**

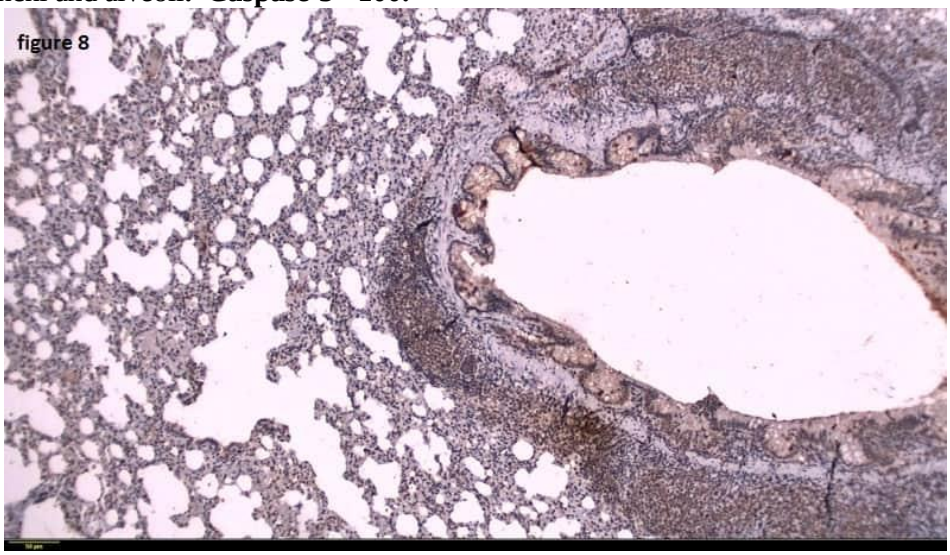


Figure 8: Transverse section of the lung tissue in fipronil-treated rat shows massive caspase-3-positive cells are observed at the walls of bronchi, **Caspase-3× 100.**

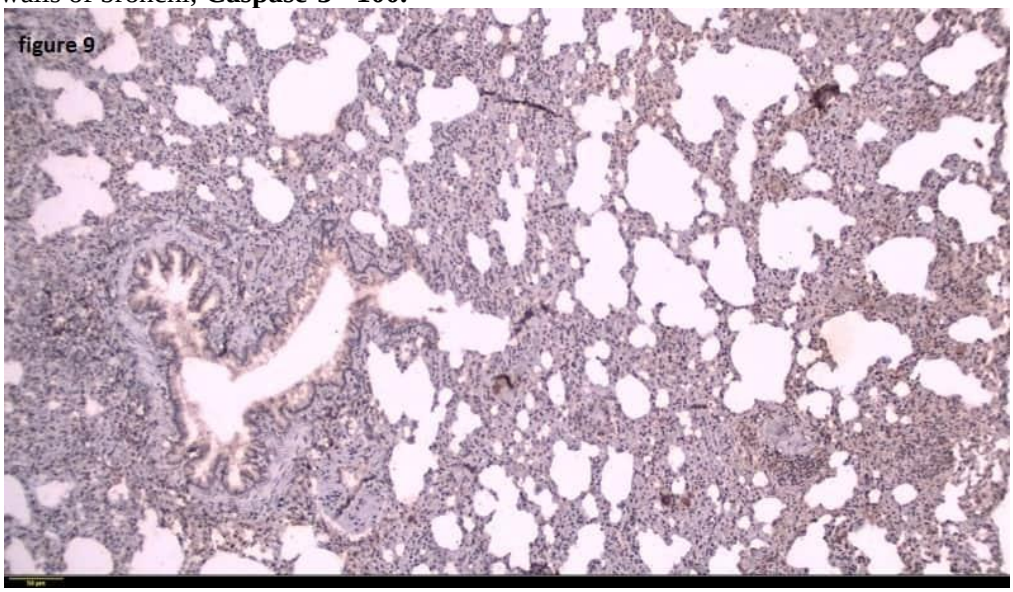


Figure 9: Transverse section of the lung tissue in fipronil and vitamin E-treated rats showing moderate reaction to caspase-3 observe at the walls of the bronchi and alveoli. **Caspase-3× 100.**

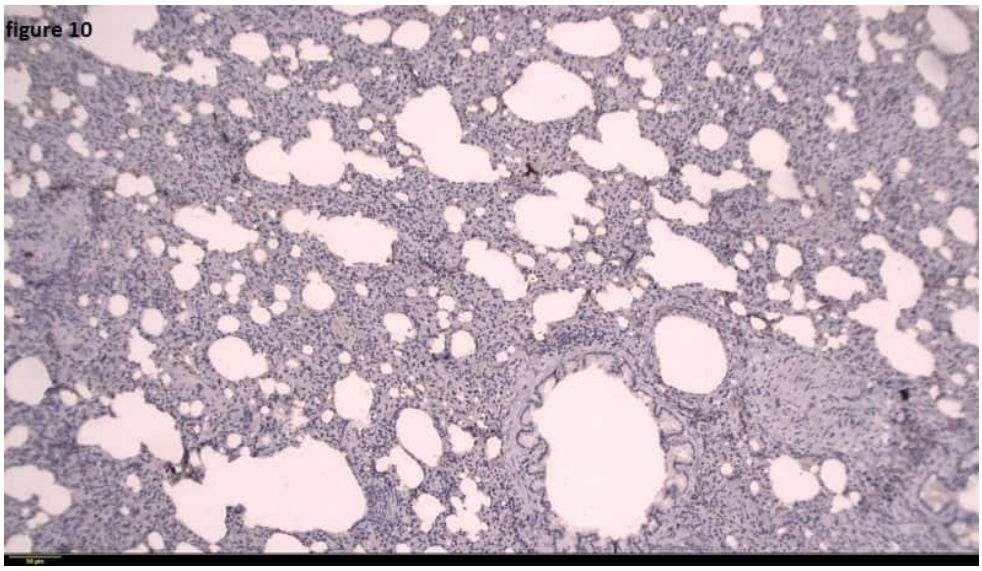
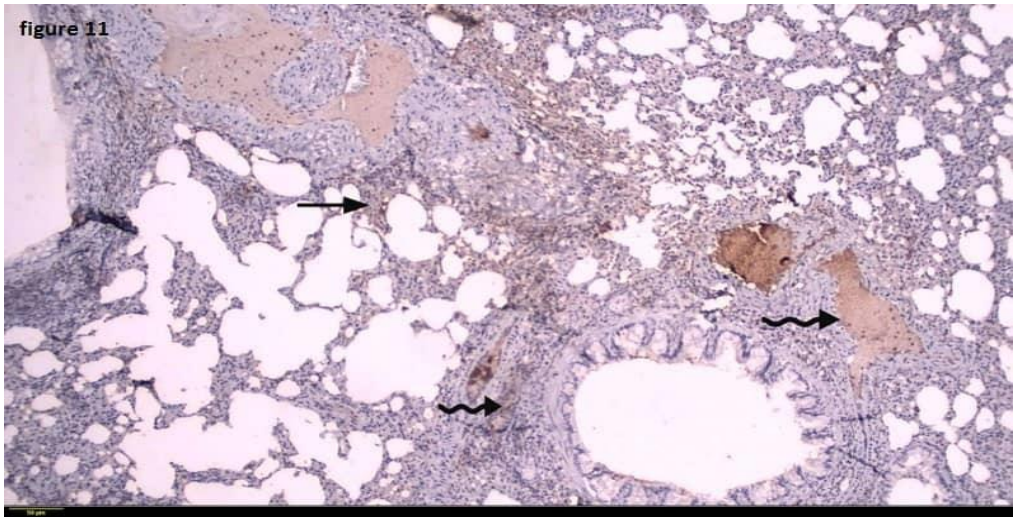


Figure10: Transverse section of the lung tissue of control rat shows negative reaction for TNF- α between lung tissues. **TNF-α × 100.**



- **Figure11:** Transverse section of the lung tissue of fipronil-treated rats shows positive reaction to TNF-α observed in the alveolar inter septal region **(arrows)**, muscle cells surrounding the bronchi **(irregular arrow)**. **TNF-α × 100.**

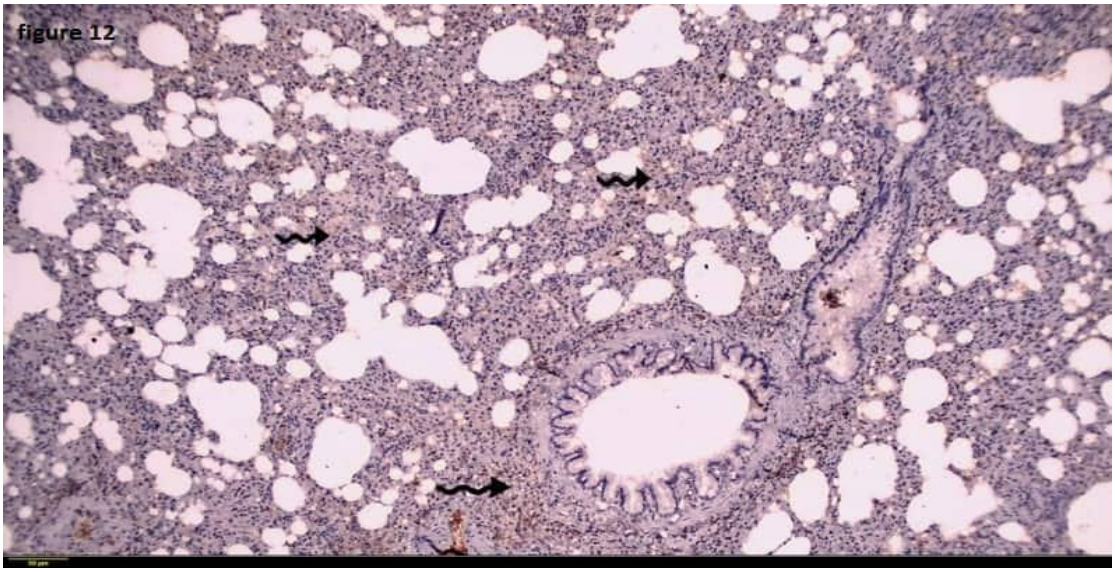
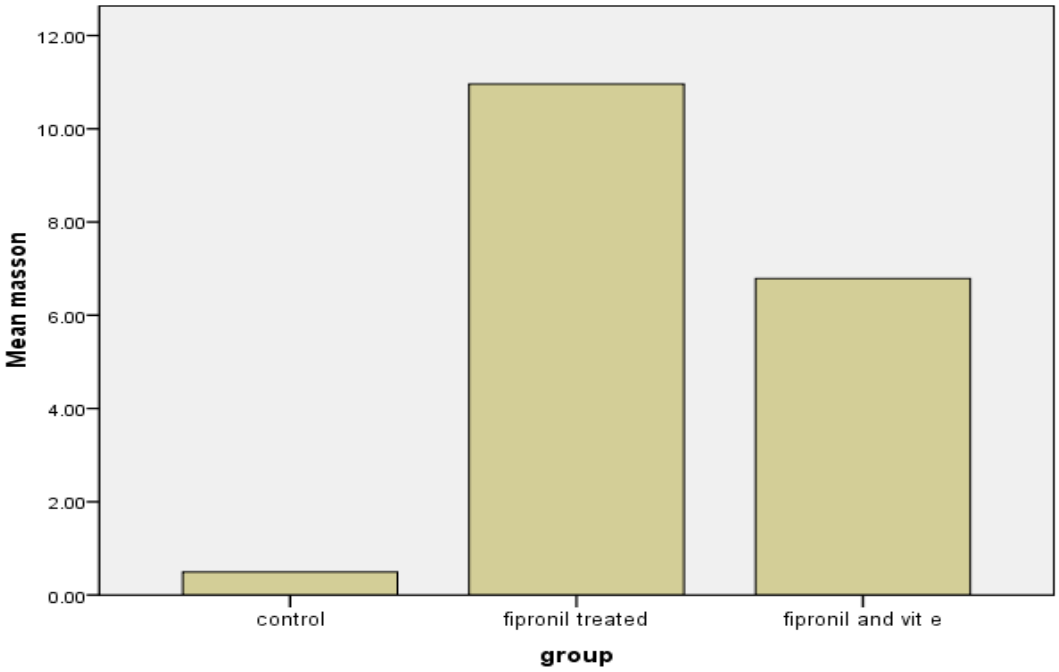


Figure12: Transverse section of the lung tissue of fipronil and vitamin E-treated rat shows a moderate reaction to TNF-α observe in the alveolar inter septal region (**irregular arrows**), muscle cells surrounding the bronchi (**irregular arrow**). **TNF-α × 100.**

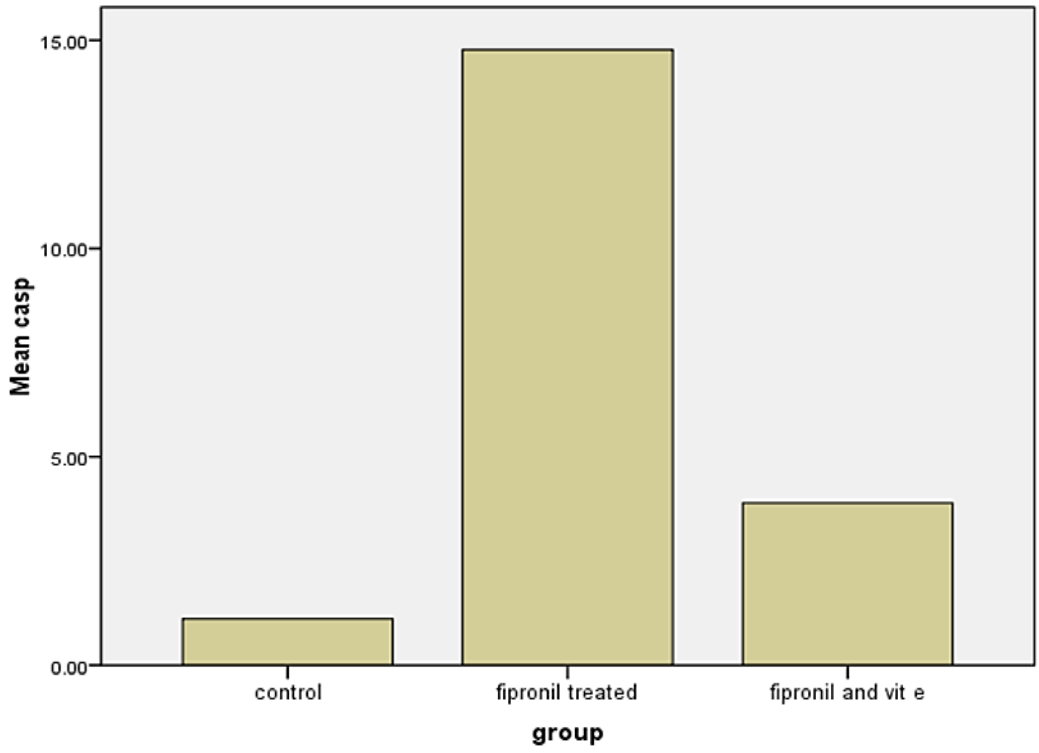
Morphometric Results:

1-The mean value of percentage collagen area in control group was(0.49) while in fipronil treated group was (10.96) which was very highly significantly increased compared to control, while in fipronil and vitamin E treated group it was (6.79) which very highly significantly different from fipronil treated and control group **(histogram 1).**

2-The mean value of Percentage of an area of caspase expression in control group was (1.11) ,while in fipronil treated group was (14.77) which very highly significantly increased compared to the control, while in fipronil and vitamin E treated group it was (4.1) which very highly significantly different from fipronil treated and control group **(histogram 2).**



Histogram 1: Comparison of the examined groups according to the percentage of collagen expressed in the lung



Histogram 2: Comparison of the examined groups according to the caspase expression in the lung.

DISCUSSION

Fipronil is an insecticide that attacks pests such as fleas, ticks, and lice. It produces its action through gamma amino butyric acid receptor (GABAR) in humans and animals. These receptors are present in the lungs so they are more exposed to damage [21].

The exposure to fipronil occurred through dermal contact with the product and treated material i.e., vegetation or animal or by spray inhalation [6].

Vitamin E is one of the fat-soluble vitamins present in vegetable oils, nuts, and cereal. Many researches approved that Vit E produces protection against pesticide-induced oxidative damage where it can trap free radicals, which start the oxidation of the lipid chain [23-24].

In our study the lung tissue in the fipronil treated group showed dilation in the bronchi with damage of its epithelium, alveolar septa appeared thin, and an area of hemorrhage was present, and also blood vessels appeared congested. Masson's trichrome showed excessive collagen deposition presented around a bronchiole and the alveolar wall.

This agreed with **Khalil *et al.*** [24] who observed that fipronil induced effect in both heart and lung tissues through the increase in the synthesis of NO and expression of genes related to inflammatory cytokines. Also, **Merkowsky *et al.*** [6] reported that fipronil shows lung inflammation following oral and intranasal administration of fipronil. Also, these results agree with **Yehia** [25] who said that fipronil is transformed into fipronil sulfone, which is more toxic than fipronil, and it accumulated in the lungs, liver, kidney, and brain.

In this study, there was a positive reaction to both TNF-α and caspase-3 in rats who received fipronil. This comes with **Pandit *et al.*** [26] who approved that exposure to fipronil for a long period changes the lung histoarchitecture. **Khalil *et al.*** [24] reported that fipronil enhances inflammation in both heart and lung tissues through stimulating the manufacture of nitric oxide and the expression of genes encoding inflammatory cytokines (TNF-α, IL-6, and NF-κB) in the heart and lung.

In our study, the lung tissue in the group that received fipronil and vitamin E showed mild epithelial destruction in the bronchi with thinning of its epithelial wall, the alveoli and alveolar sinus appeared normal, but an area of hemorrhage presented in the interalveolar septa and area of cellular infiltration also appeared. Regarding Masson trichome stain, there was moderate collagen deposition around a bronchiole and the alveolar. Also, there was a moderate reaction to both TNF-α and caspase-3.

Bayrak *et al.* [27] approved that the antioxidant vitamin as vitamin C and vitamin E reduced pulmonary edema and tissue damage by inducing antioxidant enzyme activities in D-galactosamine treated rats, but it did not affect the avoidance of inflammation in these rats. **Mohammed *et al.*** [19] observed that vitamin E decreases fibrosis of the lung induced by bisphenol A, which is done by down-regulation of pro-fibrotic and pro-inflammatory genes expression.

Matsunaga *et al.* [28] proved that g-tocotrienol (type of vitamin E) reduces TNF-a and IL-6 production by preventing NF-κ B activation. At the same time, **Hassani *et al.*** [12] found that curcumin and Vit E can attenuate chlorpyrifos-induced pulmonary damage.

Abdel-Daim and Abdeen [14] found that fipronil induces damage in the liver and kidney and using Vit E in combination with rosuvastatin reduced damage induced by fipronil.

CONCLUSION

fipronil produces a damaging effect on lung alveoli and use of vitamin E with fipronil has a little protective effect.

Conflict of interest: The authors declare no conflict of interest.

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