A Histological Studies of Rats' Lung Subacutely Treated with Fenitrothion

[1]Siti Balkis Budin, [2]Hanis Saimin, [1]Izatus Shima Taib, [1]Putri Ayu Jayusman, [1]Jamaludin Mohamed

[1] Programme of Biomedical Science, School of Diagnostic and Applied Health Sciences, Faculty of Health Sciences, Universiti Kebangsaan Malaysia, Malaysia
[2] Faculty of Health Sciences, Universiti Teknologi MARA (UiTM), Puncak Alam Kampus, Selangor, Malaysia

Corresponding Author: Jamaludin Mohamed at Faculty of Health Sciences, Universiti Kebangsaan Malaysia; Email: jamal3024@yahoo.com

ABSTRACT

Fenitrothion or Sumithion [*o,o*-dimetyl-o-(3-methyl-4-nitrophenyl) phosphorothiote] is an organophosphorus pesticide widely used in agriculture and public health programmes. This experimental study aimed to evaluate the morphological changes in lungs due to ingestion of this pesticide. The study was performed on 20 male Sprague-Dawley rats. Ten rats were used as control group while another 10 received fenitrothion (FNT) by oral gavage for 28 consecutive days. The animals were sacrificed at the end of treatment period and lung was isolated for histopathology purpose. Rats in FNT group exhibit cholinergic signs such as hypoactivity, tremor, lacrimation and piloerection. At the end of the study, the body weight of the FNT group was significantly lower than the control group. However, the difference in the lung weight between control group and FNT group was not significant. Histological examination using light microscope revealed there is disruption of alveolar walls, swollen alveolar cells, inflammation cells and cells necrosis. Terminal bronchiole also showed destruction of its lining. Presence of highly infiltrate MALT was noted in the rats of FNT group. The results suggest that ingestion of FNT could cause damage and injury towards lung tissues as well as lung toxicity in male Sprague-Dawley rats. It is also suggested that this pesticide leads to neurotoxicity and induces the immune system.

Keywords:

Introduction

Organophosphate compound are widely used in agriculture, domestic as well as in public health programs [21]. One of the most common organophosphorus pesticides is fenitrothion [7]. Fenitrothion [*o,o*-dimethyl-*o*-(3-methyl-4-nitrophenyl) phosphotioate] is mainly used for controlling insects on rice, fruits, vegetables and in forest areas. Although FNT have low toxicity in mammals [12], a number of studies on the side effects including liver [3], kidney [11], lung [1-2] and reproductive system [15] have been done.

Organophosphate poisoning have been reported to occur either by occupational, accidental or suicidal. Poisoning of parathion occupationally also been reported to occur in several orange pickers [21]. Most organophosphate poisoning occurs through oral route. Organophosphorus pesticides concentration in poisoning cases showed the highest values are in fat tissues and followed by several organs including lung [12]. Since FNT is widely used in agriculture and public health programmes, it increases the risk of subacute lung toxicity due to ingestion of FNT. This is because subacute toxicity is more likely to occur after continuous ingestion of small quantities of pesticidal chemical through foodstuff [11].

Lung toxicity is a major concern to the public in regard to organophosphorus pesticides especially in aerosolized form. This is because respirable small size spray droplet could accidentally been exposed to human via inhalation [1-2]. Apart from inhalation, human are exposed to organophosphorus pesticides through oral and dermal route. In oral route, ingestion of organophosphorus pesticides may have been occupational, accidental or suicidal. Therefore, the aim of the study was to determine the histopathological changes of lung on male Sprague-Dawley rats induce with FNT.

Materials and Methods

Chemicals and Equipments

Fenitrothion [o,o-dimethyl-o-(4-nitro-m-tolyl) phosphorothioate, CAS 122 – 14 – 5, 99 % purity] was obtained from Supelco Analytical (USA). It was diluted in corn oil for the final test concentration.

Animal & Treatment

Twenty male Sprague-Dawley rats weighting between 200 – 250 g were used animal in this experiment. The rats were obtained from Animal Resource Unit, Faculty of Medicine, Universiti Kebangsaan Malaysia (UKM), Kuala Lumpur. All rats were handled in accordance with the standard guide for the care and use of laboratory animals. After acclimation period, the rats were randomly divided into two groups, control (corn oil) and FNT supplementation group (20 mg/kg). The substances were administered for 28 days continuously through oral gavage. Rats in each group were sacrifices and dissected at the end of treatment. Body weight and lung weight was recorded. Lung was isolated for histopathology study.

Histopathological examination

For histologic studies, the lung were dissected and fixed in 10% formalin. The fixed tissues were dehydrated in ascending series of ethanol, cleared in toluene and embedded in paraffin wax. The paraffin block were sectioned at 4-5 μ using a microtome and stained with hematoxylin and eosin (H&E). The stained tissues were observed using OLYMPUS BX41 camera microscope.

Statistical method

Normality test was done. Independent t-test was used to assess statistical significance between the two groups and dependent t-test for within group. The difference is considered to be significant at $P< 0.05$. All values were expressed as means ± SEM.

RESULT

Signs of toxicity

Signs of toxicity such as hypoactivity, tremor, lacrimation and piloerection could be seen in rats of FNT group. All rats in FNT group showed piloerection of fur while six suffered from tremor. Lacrimation only presence in two of the rats in the treatment group. All signs of toxicity start to occur after 2 hours of oral gavage. Hypoactivity and lacrimation end after 2 days while tremor and piloerection persists during the treatment period, 28 days.

Mean body weight, absolute and relative lung weight

Figure 1 showed body weight between control and FNT group weekly for 4 weeks. FNT caused significant reduction in body weight by week as compared to control group. Figure 2 and 3 showed absolute and relative lung weight respectively between control and FNT group. Induction of FNT did not cause any significant difference in both absolute and relative lung weight.

Discussion

Ingestion of 20 mg/kg bw of FNT resulted a few cholinergic sign in rats. Lacrimation and piloerection are most prominent signs indicating disturbance of autonomic nervous systems. Tremor in the other hand is a common sign indicating neurotoxicity [14]. FNT can cause neurotoxicity by inhibiting acetylcholinesterase (AChE) in the nervous system. Acetylcholinesterase is an enzyme that degrade or hydrolyse the acethylcholine (ACh), a neurotransmitter [7,8,16,19]. ACh is mainly found at the neuromuscular junctions and cholinergic nervous system. It serves to terminate synaptic transmission.

Rats in FNT group showed significant reduced weight gain when compared to control group at the end of treatment period. This result is consistent with previous study [12-14]. High energy is required in biotransformation of FNT into its metabolites such as fenitrooxon and 3-methyl-4-nitrophenol [5]. Penetration of FNT and its metabolites from bloodstream into the lung also requires energy. These energy is obtained through breakdown of carbohydrates (food), thus

resulting it to be used rather than being stored as fat. Hence, resulting the rats in FNT group to have lowered weight gain compared to control group.

FNT group have lowered lung weight compared to control group, however, statistical analysis reveals no significant difference. Previous study [6] suggested direct correlation of body weight loss with morphological alteration in terminal bronchiolar epithelium by trialkyl phosphorothiotes, an impurity in organophosphorus pesticides. This may explain the relation on body weight loss, lung weight loss and morphological changes in the lung. Morphological changes such as destruction of terminal bronchiole lining and necrosis of cells may result in weight loss of the lung. Loss of the cells' weight itself leads to the loss of lung weight as cell is basic unit. Decreased in lung weight will also contribute to loss of body weight.

In the present study, only male Sprague-Dawley rats are used. This is because male animal are generally more sensitive to the acute effect of FNT compared to female animal [14]. FNT was given orally by oral gavage rather than in the diet in order to control its daily intake independently from the food requirement of the animals. This will avoid nutritional problems created by the unpleasant taste of FNT in the food. Thus, allowing the treatment with sufficient dose to be carried out [20].

Oral administration of 20mg/kg bw of FNT resulted in several morphological changes in rats' lung. Destruction of the bronchiole lining at the terminal bronchiole was never been reported in previous studies. Study [6] on *O,O,S*-Trimethyl Phosphorothioate, an impurity found in FNT only highlighted the extensive amount of cellular debris in the terminal bronchiole. Ingestion of FNT also resulted in swollen of alveolar cells. This result is similar to those reported in pulmonary effect of deltamethrin through inhalation route [4-6,13]. A slightly disruption of alveolar walls as well as necrosis of cells is seen although lung is not the vital organ which come in contact through ingestion route. These results could be due to penetration of FNT and its metabolites such as fenitrooxon and 3-metyl-4-nitrophenol into lung tissues through bloodstream since alveolus is surrounded by a network of capillaries.

Once in the lung, the FNT will undergo biotransformation as lung also possess metabolizing enzyme, cytochrome P450 mixed function oxidases and flavin-containing monooxygenase. Both cytochrome P450 mixed function oxidases and flavin-containing monooxygenase utilize NADPH and oxygen to peform oxidative desulfuration which yield oxon of the parent compound. Apart from these enzymes, rats' lung also possesses glucuronosyl transferase in a medium amount compare to liver and kidney. Glucuronosyl transferase involves in glucosylation which transfers glucose from uridine diphosphate glucose to FNT [18]. Biotransformation process will yield a highly active metabolite, fenitrooxon and other inactive metabolites. These metabolites as well as FNT itself may accumulate in lung tissue if ingest continuously [5].

MALT is a diffuse lymphatic tissue because of its association with mucus membrane that exposed to the external environment. MALT is located to intercept antigens and initiate immune system. Thus, presence of MALT in control group is considered normal as respiratory passage is exposed to external environment. Once it is in contact with antigen, they will travel to the regional lymph nodes where they undergo proliferation and differentiation. Progeny of these cells return as effector of B and T lymphocytes [9,17]. At this point, MALT which consists of numerous lymphocytes may infiltrate the epitelium. Plasma cells, mast cells, eosinophils and fibroblast may also present. In this study, presence of highly infiltrated MALT in the FNT group indicates FNT or its metabolites act as an antigen that initiate immune response. Presence of inflammation cells is also consistent with the study done [10,13]. It is suggested that in this present

study, mix of acute and chronic inflammation cells are present. However, it is hard to differentiate whether the inflammation cells are mainly neutrophils and/or lymphocytes.

Conclusion

Several changes in lung morphology could be seen in FNT group such as disruption in terminal bronchiole lining and alveolar wall, swollen of alveolar cells and necrosis of cells. These findings seem to indicate that FNT caused damage and injury to lung tissue. Thus, suggesting that FNT may cause lung toxicity through oral route. Presences of inflammation cells and highly infiltrated MALT in FNT group suggested that FNT also act as antigen that induces the immune system. As a conclusion, FNT caused morphological alterations of lung in male Sprague-Dawley rats.

Conflict of Interest: None declared. (Or mention here if any)

References

1. Breckenridge C, Pesant M, Durham HD & Ecobichon, DJ. A 30-day toxicity study of inhaled fenitrothion in the albino rat. Toxicology and Applied Pharmacology. 1982. 32-43.
2. Chevalier G., Henin JP, Vannier H, Canevet C, Cote MG & Le Bouffant L. Pulmonary toxicity of aerosolized oil-formulated fenitrothion in rats. Toxicology and Applied Pharmacology. 1984. 349-355.
3. El-Shenawy NS. Effects of insecticides fenitrothion, endosulfan and abamectin on antioxidant parameters of isolated rat hepatocytes. Toxicology in Vitro. 2010. 1148-1157.
4. Erdogan S, Handan ZE, Mustafa E, Ozlem A & Derya G. Pulmonary effects of deltamethrin inhalation : an experimental study in rats. Ecotoxicology and Environmental Safety. 2006. 318-323.
5. Franklin MR & Yost GS. Biotransformation : A Balance between Bioactivation and Detoxification. In R. S. Phillip L. Willis, Principles of Toxicology Environmental and Industrial Applications (pp. 57-59, 62). John Wiley & Sons, Inc. 2000.
6. Gandy J & Imamura T. Cellular responses to O,O,S-Trimethyl Phosphorothiote-Induced Pulmonary Injury in Rats . Toxicology and Applied Pharmacology. 1985. 51-57.
7. Goel A & Aggarwal P. Pesticide poisoning. The National Medical Journal of India. 2007. 182-191.
8. Hoffmann U & Papendorf T. Organophosphate poisonings with parathion and dimethoate. Intensive Care Medicine. 2006.
9. Junqueira LC & Carniero J. Basic Histology Text & Atlas. McGraw-Hill Companies. 2003.
10. Karaoz E, Gultekin F, Akdogan M, Oncu M & Gokcimen A. Protective role of melatonin and a combination of vitamiin C and vitamin E on lung toxicity induced by chlorpyrifos-ethyl in rats. Exp Toxic Pathol. 2002. 97-108.
11. Mahboob M & Siddiqui MKJ. Long-term effects of a novel phosphorothionate (RPR-ll) on detoxifying enzymes in brain, lung and kidney rats. Ecotoxicology and Environmental Safety. 2002. 355-360.

12. Misu Y, Segawa T, Kuruma I, Kojima M & Takagi H. Subacute toxicity of o,o-dimethyl o-(3-menthyl-4-nitrophenyl) phosphorothiote (Sumithion) in the rats. Toxicology and Applied Pharmacology. 1966. 17-26.
13. Morowati M. Inhalation toxicity studies of Thimet (Phorate) in male swiss albino mouse, Mus musculus: ll. Lung histopathology, pseudocholinesterase level and haematological studies. Environmental Pollution. 1998. 309-315.
14. Mueller U. Pesticide residues in food 2000: Fenitrothion. FAO and WHO. 2001.
15. Okahashi N, Sano M, Miyata K, Tamano S, Higuchi H, Kamita Y & Seki T. Lack of evidence for endocrine disrupting effects in rats exposed to fenitrothion in utero and from weaning to maturation. Toxicology. 2005. 17-31.
16. Padilla S. Regulatory and research issues related to cholinesterase inhibition. Toxicology. 1995. 215-220.
17. Ross MH, Kaye GI & Pawlina W. Histology: A Text and Atlas 4th Edition. Lippincott Williams & Wilkins. 2003.
18. Tang J, Rose RL, Janice E & Chambers. Metabolism of Organopgosphorus and Carbamate Pesticides. In R. Gupta, Toxicology of organophosphate and carbamate compound (pp. 127-134). Elsevier Inc. 2006.
19. Timchalk C. Physiologically Based Pharmacokinetic Modeling of Organophosphorus and Carbamate Pesticides. In R. C.Gupta, Toxicology of organophosphate and carbamate compound (pp. 103-104). Elsevier Inc. 2006.
20. Trottier B, Fraser AR, Planet G & Ecobichon DJ. Subacute toxicity of technical fenitrothion in male rats. Toxicology. 1980. 29-38.
21. WHO. Organophosphorus insectides: A general introduction. Geneva: WHO. 1986.

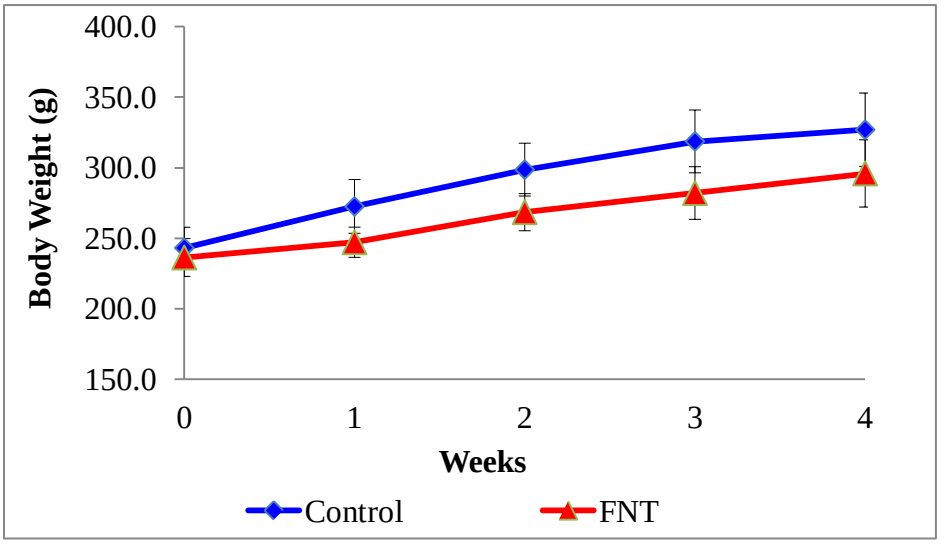

Figure 1. Graph showed the body weight of control and FNT group. FNT caused significantly decreased in body weight ($p<0.05$).

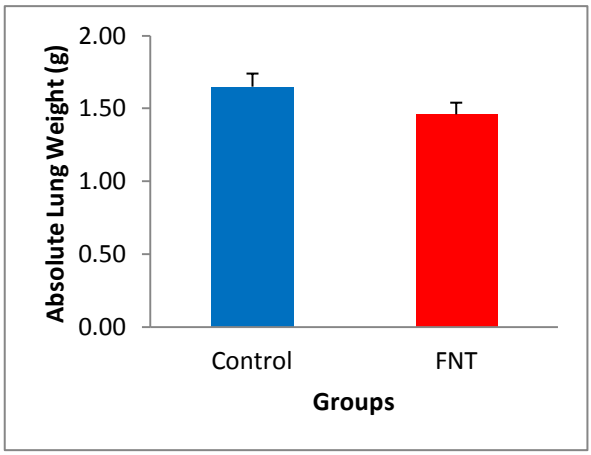

Figure 2. Graph showed the absolute lung weight of control and FNT group.

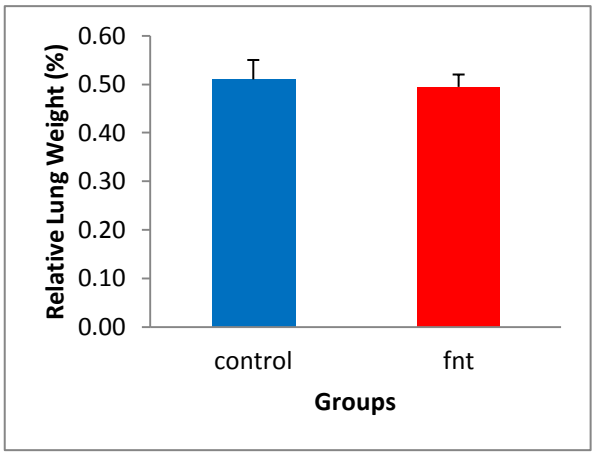

Figure 3. Graph showed the relative lung weight of control and FNT group.

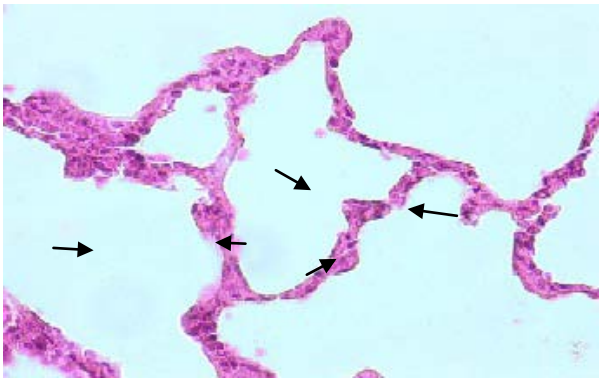

Figure 4. The lung of control rat showed normal alveoli structure (H&E, 400X).

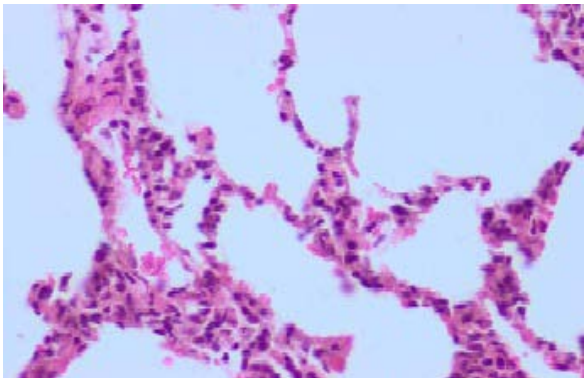

Figure 5. The lung of fenitrothion group showed alveolar cells are swollen. The cells show necrosis and alveolar walls are slightly disrupted (H&E, 400X).

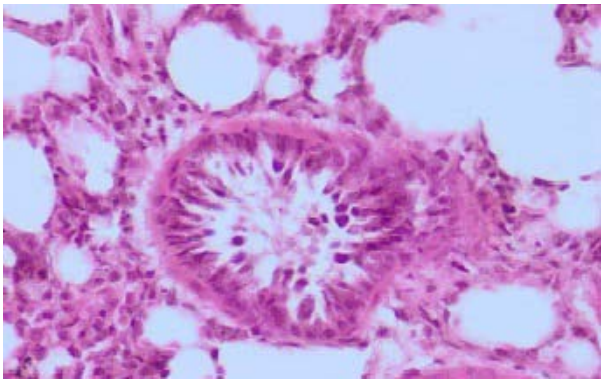

Figure 6. The terminal bronchiole of control rat (H&E, 400X).

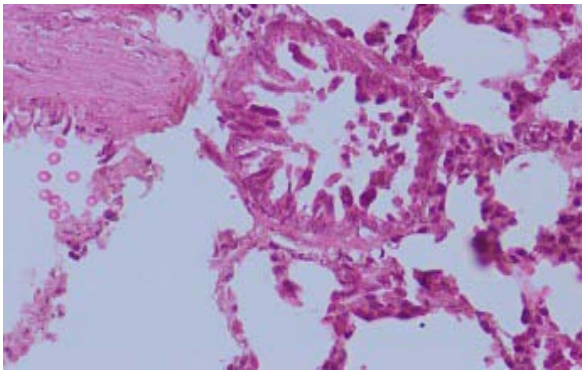

Figure 7. The terminal bronchiole of fenitrothion group showed destruction of bronchiole lining (H&E, 400X).

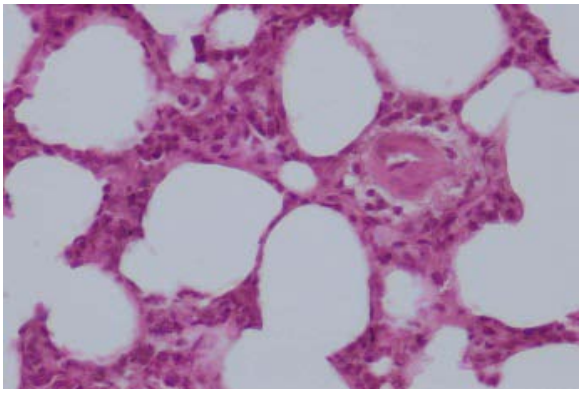

Figure 8. Presence of MALTs in a rat from control group (H&E, 400X).

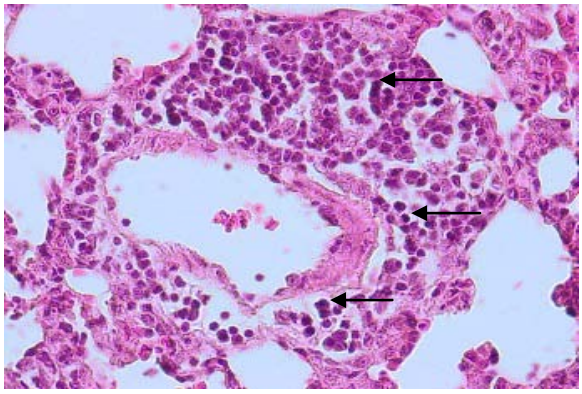

Figure 9. A focus of MALT in a rat from fenitrothion group. It is highly infiltrate with lymphocytes (↑) which is identified by small, round, dark-staining nucleus (H&E, 400X).

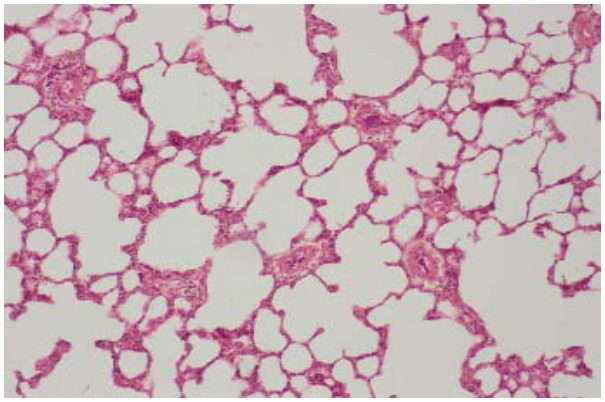

Figure 10. The control rats showed normal lung structure (H&E, 100X).

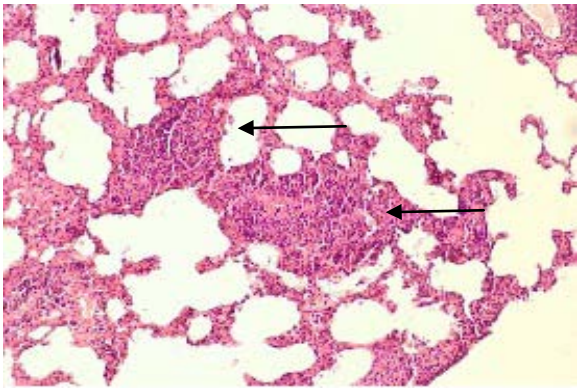

Figure 11. The lung of fenitrothion group showed inflammation cells (H&E, 100X).