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Estradiol and ascorbic acid alleviate malathion-induced lung damage in albino Wistar rats: A histopathological study

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ABSTRACT

Aim: To assess the modulatory role of estradiol and ascorbic acid in malathion-induced pulmonary toxicity in albino Wistar rats.

Methods: A total of twenty albino Wistar rats were randomly divided into four groups; the control group (group 1) was given corn oil alone, the test group (group 2) received a daily dose of malathion 20 mg/kg in corn oil, treatment group A (group 3) was administered a daily dose of malathion 20 mg/kg in corn oil plus estradiol 40 µg/100 g (gram), and treatment group B (group 4) received a daily dose of malathion 20 mg/kg in corn oil plus ascorbic acid 100 mg/kg. Experimental rats were administered once daily for four weeks. The lungs were examined histopathologically using two staining methods (Hematoxylin and Eosin, Masson Trichrome).

Results: There were significant reductions in degeneration, interstitial pneumonia and interstitial fibrosis for group 3 (treatment group A) compared to group 2 (test group) ($p<0.05$). These reductions were more statistically significant for group 4 (treatment group B) compared to group 2 (test group) ($p<0.01$). Therefore, the damage was less pronounced and injury severity was moderate in group 3 treated with estradiol. Group 4, with ascorbic acid, showed the most improvement with significant tissue repair under microscopic examination and mild injury compared to group 3.

Conclusions: The results of our present study suggest that both estradiol and ascorbic acid have clear protective effects against malathion-induced lung injury. However, ascorbic acid exhibited more pronounced protective effects compared to estradiol. With more comprehensive studies, the positive effects of ascorbic acid and estradiol can be used to prevent lung damage in individuals exposed to malathion.

Keywords: Ascorbic acid, estradiol, inflammation, lung damage, malathion, rats.

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Introduction

In the modern European agricultural system, large land areas are farmed, increasing the need to protect crops with insecticides [1]. Insecticides are widely used with plants to kill insects that harm crops and play a key role in spreading diseases [2]. Continuous use of insecticides over large land areas has resulted in the contamination of all environmental elements with these toxic substances, thereby exposing all living organisms to danger, from microorganisms to large animals [3]. Every year, many people are poisoned by pesticides, with a significant number succumbing to death [4]. The organophosphates are the most frequently used pesticides, and their application results in serious health issues in

most systems [1,5]. Organophosphate toxicity significantly affects rat lungs. The rats complained of shortness of breath and died after about 4.5 days. The examination reveals marked thickening of the interalveolar septum, and a significant increase in lung weight [6]. Soil and food contamination, as well as various food products with organophosphates has been recorded [3]. Thus, in the absence of safety measures, with insecticides, people are exposed to them in three ways: through the skin, orally, and by inhalation [4]. Despite malathion being highly toxic [7], it is still widely used as an organophosphate pesticide to control mosquitoes and other flying insects. Malathion smells like garlic, yet it is colorless [8]. Malathion is widely used in the eradication of insects such as flies and mosquitoes, and in agriculture, as well as for the protection of ornamental plants [9]. It has been used in health management of insect control programs, and is widely used in the United States to control mosquito-borne diseases [10]. However, malathion-induced pulmonary toxicity involves severe inflammation, fibrosis and thickening of the interalveolar septa, as well as hyperemia in the blood vessels [11]. Malathion also causes changes in the respiratory tract epithelium, including a decrease in the number of Clara cells [12]. Malathion toxicity is due to various mechanisms. The first is the inhibition of acetylcholinesterase, leading to the accumulation of acetylcholine and increased cholinergic activity [13]. An important mechanism of malathion toxicity is oxidative stress, as it results to increase in increased formation of unstable oxygen atoms known as free radicals, leading to oxidative stress [14]. All biological macromolecules such as carbohydrates, fats, and proteins are destroyed by oxidative stress [15,16]. Estradiol demonstrated a strong antioxidant effect during pregnancy [17] and its deficiency due to ovariohysterectomy led to high reactive oxygen species production [18]. It has also been suggested a significant antioxidant factor for the fetal brain [19,20]. It may play a significant protective role against malathion toxicity. Vitamin C (ascorbic acid) is a water-soluble vitamin, with a strong antioxidant effect [21]. Vitamin C is crucial for a healthy life, serving as an antioxidant for bodily fluids, in the blood and intercellular tissues. It is also very important in controlling lipid peroxidation in the intracellular organelle and the cell membrane [22]. Our study aimed to examine the histopathological changes in the lungs caused by malathion and the mitigating potential of oral estradiol and ascorbic acid administration on malathion-induced lung damage.

Materials and methods

Chemical products

Malathion (Purity = 99.9%) was sourced from Biogenic Company., Ltd (*USA*) and purchased from Alhewar Altibi Company for pesticides. Estradiol was sourced from NexGen Pharmaceuticals and ascorbic acid was obtained from Almarkazia-Libya.

Animals and experimental groups

The experimental protocol of this study adhered to the ethical standards for experimental animal care of the Faculty of Pharmacy, Alasmarya Islamic University, Libya. Ethical approval for this study was granted under code number AIUS-2020/02. Our study was conducted on twenty female white rats weighing 250 -300g with an average age of about 10 weeks at the Faculty of Technology, Misurata University, Libya. Animals were housed under standard laboratory conditions, at 25°C ± 2°C and 55% ± 5% relative humidity. Animals were housed in polypropylene cages, with free access to a standard laboratory diet and water ad libitum. The rats were randomly divided into four groups as shown in table 1.

Table 1. Description of the experimental groups.

Groups	Treatment and doses
Group 1 (Control, n=5)	0.5 ml corn oil for each rat (Orally).
Group 2 (Test, n=5)	0.5 ml corn oil with malathion 20 mg/kg body weight (Orally).
Group 3 (Treatment A, n=5)	0.5 ml corn oil with malathion 20 mg/kg body weight (Orally) + 40 µg/100g estradiol body weight (Subcutaneously).
Group 4 (Treatment B, n=5)	0.5 ml corn oil with malathion 20 mg/kg body weight (Orally) + 100 mg/kg ascorbic acid body weight (Intraperitoneally).

The control group (group 1) was orally administered corn oil, the test group (group 2) received a daily dose of malathion 20 mg/kg in corn oil, treatment group A (group 3) was given a daily dose of malathion 20 mg/kg in corn oil plus estradiol 40 µg/100g (gram) and treatment group B (group 4) received a daily dose of malathion 20 mg/kg in corn oil plus ascorbic acid 100 mg/kg. Experimental rats were administered once daily for four weeks. The doses used in our study were determined based on previous studies [23-27]. Table 1 shows the treatment groups along with dosage and administration details. This experiment lasted 28 days, a duration based on a previous study [27]. After completing our research program, the rats were euthanized by cervical dislocation following sedation with xylazine (5 mg/kg intraperitoneal) dose. Afterward, that their lungs were examined histopathologically using two staining methods (Hematoxylin and Eosin, Masson's Trichrome).

Histopathological examination

Lungs were collected for investigation of potential histopathological changes. Lung tissue samples were fixed in 10% formaldehyde solution for 48 hours. After fixation, these tissues underwent a series of graduated alcohols and xylene treatments and were embedded in paraffin blocks. 5 µm thick sections from paraffin blocks were serially cut at 50 - 100 micrometer intervals. Histopathological changes were assessed by hematoxylin and eosin staining of the sections. Sections were stained with Masson's Trichrome according to the kit's procedure to assess fibrosis in these tissues. Sections were rated as absent (−), mild (+), moderate (++) and severe (+++) based on the severity of lesions.

Statistical analysis

In the histopathological exam, the Kruskal–Wallis test, a nonparametric test, was used to analyze differences between groups for semiquantitative data, while the Mann–Whitney U test was used for paired group comparisons. The GraphPad Prism software was used for these statistical analyses.

Results

Histopathological findings are summarized in table 2. Generally, based on the statistical analysis shown in figure 1, there were significant reductions in degeneration, interstitial pneumonia and interstitial fibrosis for group 3 (Treatment group A) compared to group 2 (Test group) (p<0.05). These reductions were more statistically significant for group 4 (Treatment group B) compared to group 2 (Test group) (p<0.01).

Control group: It was observed that the pleura and lung parenchyma tissues had normal histological structure (Figure 2).

Test group: Severe degeneration in bronchial epithelial cells, severe inflammation in interstitial spaces, hyperemia in vessels and severe fibrosis by Masson's Trichrome staining were observed in the lung tissues of experimental rats (Figure 2).

Treatment group A: Moderate degeneration in bronchial epithelium, inflammatory cell infiltration in interstitial spaces, and moderate fibrosis observed by Masson's Trichrome staining (Figure 2).

Treatment group B: Cellular infiltration-induced thickening in interstitial spaces, hyperemia in vessels and slight connective tissue increase observed with Masson's Trichrome staining (Figure 2).

Table 2. Scoring of histopathological findings in lung tissues.

	Group 1	Group 2	Group 3	Group 4
Degeneration in bronchial/ bronchiole epithelium	−	+++	++	+
Interstitial pneumonia	−	+++	++	+
Hyperemia in vessels	−	+++	+++	+
Interstitial fibrosis	−	+++	++	+

Group 1 (Control group), Group 2 (Test group): The group treated with malathion, Group 3 (Treatment group A): The group treated with malathion plus estradiol, Group 4 (Treatment group B): The group treated with malathion plus ascorbic acid. Sections examined under the light microscope were evaulated based on the severity of lesions as no (−), mild (+), moderate (++) and severe (+++).

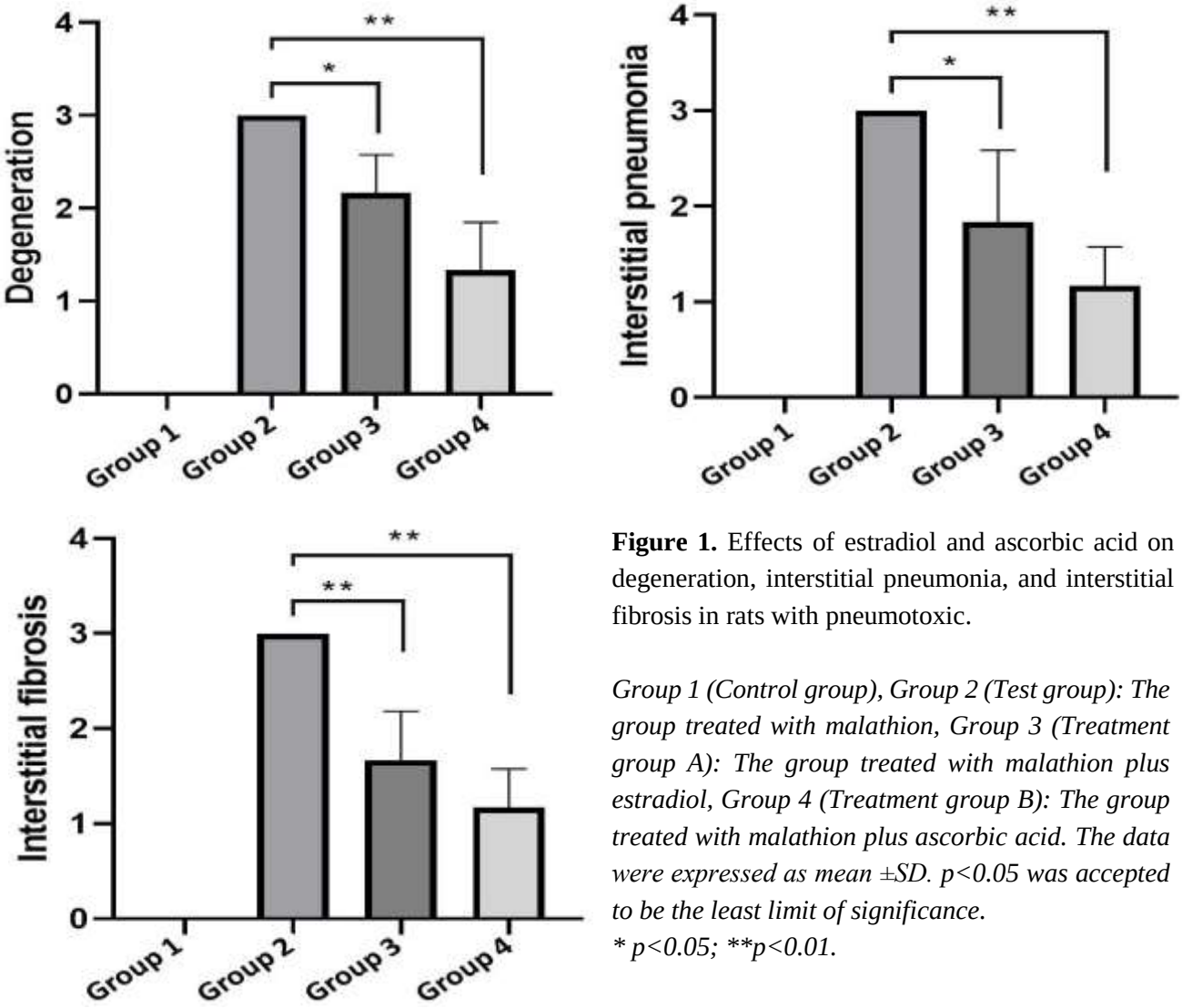

Figure 1. Effects of estradiol and ascorbic acid on degeneration, interstitial pneumonia, and interstitial fibrosis in rats with pneumotoxic.

Group 1 (Control group), Group 2 (Test group): The group treated with malathion, Group 3 (Treatment group A): The group treated with malathion plus estradiol, Group 4 (Treatment group B): The group treated with malathion plus ascorbic acid. The data were expressed as mean ±SD. p<0.05 was accepted to be the least limit of significance.
** p<0.05; **p<0.01.*

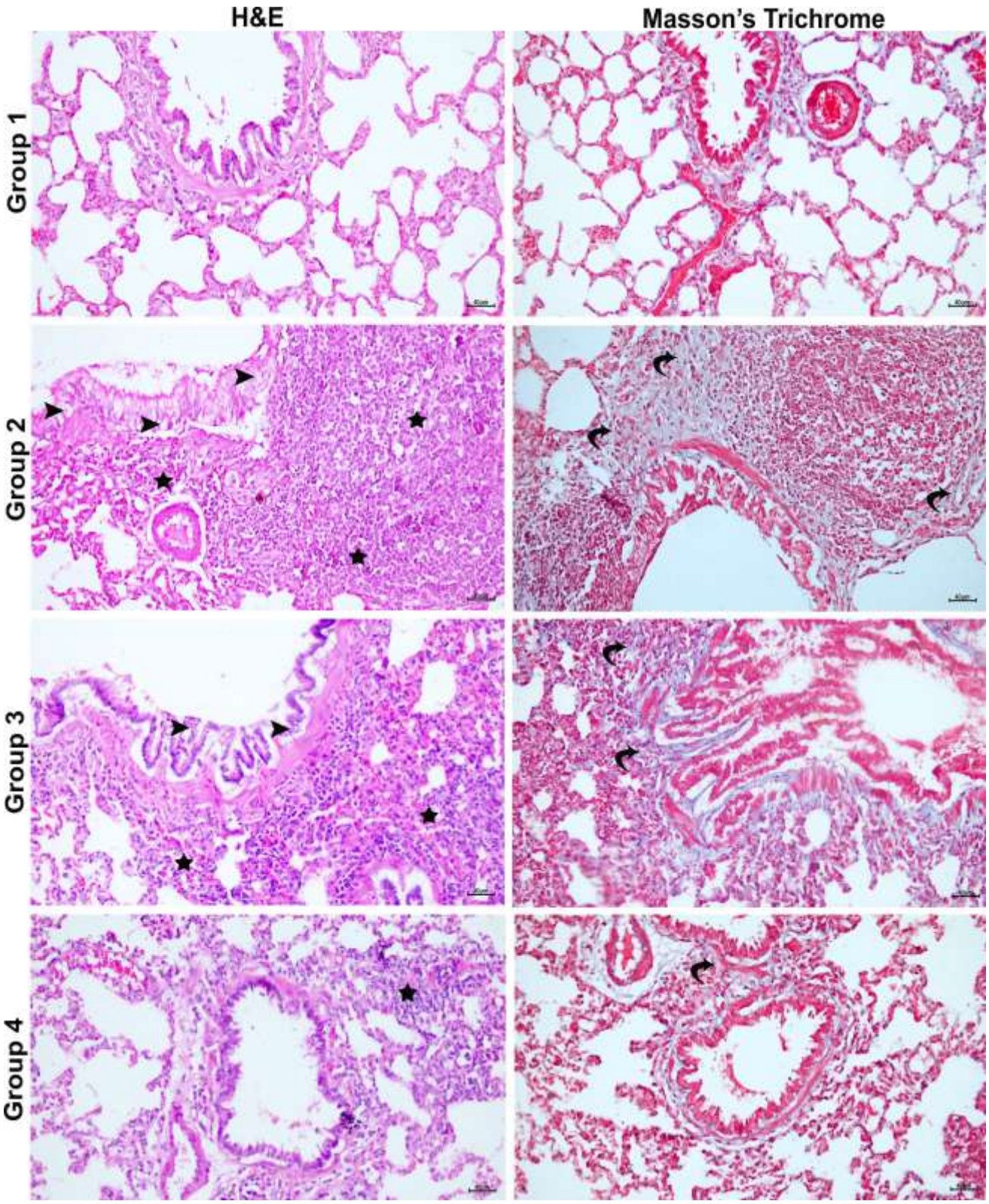

Figure 2. Photomicrography of the lung sections. Group 1 (Control group), Group 2 (Test group): The group treated with malathion, Group 3 (Treatment group A): The group treated with malathion plus estradiol, Group 4 (Treatment group B): The group treated with malathion plus ascorbic acid. Degeneration in bronchial/bronchiole epithelium (arrowheads), interstitial pneumonia (star), hyperemia in vessels, H&E: Hematoxylin and Eosin. Proliferation in interstitial connective tissue (curved arrows), Masson's Trichrome, Bar: 20µm.

Discussion

Malathion is a commonly used organophosphate for protecting various plants and controlling mosquitoes and other flying insects. At the same time, it was found to be pneumotoxic. Our study is the first to assess the modulating effects of both ascorbic acid and estradiol on the toxic effects of malathion in albino rat lung tissue. In the current study, group 2 (test group) showed severe degeneration of the bronchial and bronchiolar epithelium compared to group 1 (control group). These findings align with Alegre et al., 2022 [28], who reported that malathion induced respiratory epithelium degeneration due to inflammation, with significant cilia loss. Also, malathion caused severe necrosis in the bronchial tree epithelial lining [29]. The group 2 (test group) also showed severe interstitial pneumonia compared to group 1 (control group). These findings are consistent with Abdo et al., 2021 [30], who mentioned that inhalation of malathion caused severe inflammation, pneumonia, and damage to blood vessels. Malathion caused inflammation characterized by an increase in various inflammatory cells and fibrous tissue growth [31]. Malathion toxicity led to severe inflammation of lung tissue with degeneration of the bronchial tree lining epithelium and alveoli. There was also a thickening of the septa between the alveoli and degeneration of the vascular lining endothelium [32,33]. These results are explained by Lasram et al., 2014 [34], who mentioned that inflammation in malathion toxicity is due to free radicals and cytokines. These cytokines can increase fibrosis by activating fibroblasts [35-37]. In the absence of other inflammatory stimuli, the overexpression of certain cytokines can lead to marked fibrosis in the respiratory airways of rats [38]. Malathion also causes oxidative stress due to the formation of reactive oxygen species [39,40]. Inflammation and degeneration of lung tissues were reduced and became moderate after using malathion plus estradiol in this study. This is due to the strong antioxidant effect of estrogens, which play significan role in regulating antioxidant enzymes [41]. Estrogens belong to a group of antioxidants known as phenolic antioxidants. This group can remove free radicals either directly or via its derivatives, thereby protecting tissues from damage [42]. Estradiol reduces peroxide formation by increasing the efficiency of the respiratory chain and also prevents the release of cytochrome c from mitochondria [41]. Estrogens may enhance antioxidant enzyme activity while simultaneously decreasing oxidative proteins [43]. We believe that estradiol, with its hydroxyl group, plays a key role in mitigating malathion toxicity, by donating a hydrogen atom from the hydroxyl group to free radicals, thus transferring the free radicals from highly reactive to a stable state. Therefore estradiol effectively contributes to the elimination of these free radicals and the reduction of their toxic effects. Inflammation and degeneration of lung tissues were significantly reduced, becoming mild, when using malathion plus ascorbic acid in this study. These findings align with those of Taherdehi et al., 2019 [44], who found that ascorbic acid lessened malathion toxicity through its antioxidant properties in testicular tissues. Vitamins are generally important antioxidants that shield the body's organs from harmful substances. Vitamin C is crucial in protecting all cellular components from damage by reactive oxygen species, thereby guarding against various acute and chronic disorders [45]. Ascorbic acid is a potent dietary antioxidant, which explains its ability to reduce the toxic effects of malathion [46]. The significant effects of ascorbic acid in reversing malathion-induced lung damage can be ascribed to the ascorbic acid's antioxidant ability to

rebalance the oxidant/antioxidant system in lung tissue. Furthermore, ascorbic acid plays a key role in restoring the antioxidant properties of oxidized tocopherol. The limitation of this study is the number of animals used.

Conclusions

The results of our study demonstrate that malathion induces hyperemia in the vessels of lungs and inflammation and damage in the lung tissues. Estradiol treatment mitigates the adverse changes caused by malathion, while ascorbic acid treatment more effectively reverses these alterations. Consequently, compared to estradiol, ascorbic acid exhibits more pronounced protective effects. Further investigations in this regard may evaluate the use of ascorbic acid and estradiol to diminish the detrimental effects on the lungs of individuals exposed to malathion, yielding supportive results for potential therapeutic interventions.

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