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HISTOPATHOLOGICAL EFFECT OF DIMETHOATE ON LUNG OF RAT AND THE PROTECTIVE ROLE OF *LAUROCERASUS OFFICINALIS* ROEM. (CHERRY LAUREL)FRUIT*

DİMETOAT'IN SIÇAN AKCİĞERİNE OLAN HİSTOPATOLOJİK ETKİSİ VE *LAUROCERASUS OFFICINALIS* ROEM. (KARAYEMİŞ)MEYVESİNİN KORUYUCU ROLÜ*

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ABSTRACT

Dimethoate is one of the most important and widely used organophosphate insecticides on a large number of crops against several pests. The aim of this study was to evaluate the subchronic toxicity of orally administered dimethoate in Wistar albino rat and the ameliorative effect of cherry laurel fruit extract or vitamin C as control, based on the histopathological findings in the lung tissue. For this purpose, animals were divided randomly into six groups of ten rats each and were treated daily by oral gavage for 60 days. Histopathological alterations in the lung tissue section were determined using a light microscope. Dimethoate exposure exhibited severe histopathological changes in the lung sections compared with control group rats. The morphology of the lung seemed to be mostly affected by dimethoate treatment leading to degenerative changes. An increased hemorrhage within the alveoli, necrosis and alveolar edema as well as an infiltration of inflammatory leukocyte were observed. However, pre- and post-treatment with cherry laurel fruit extract or pretreatment with vitamin C to dimethoate administered rats showed an amelioration in the lung injury. In conclusion, treatment with cherry laurel fruit extract or vitamin C may protect against lung toxicity induced by dimethoate exposure.

Keywords: Toxicity, dimethoate, lung, histopathology, cherry laurel fruit.

ÖZ

Dimetoat, çok sayıda mahsul üzerinde bulunan birçok zararlıya karşı en önemli ve yaygın kullanılan organofosfatlı insektisitlerden biridir. Bu çalışmanın amacı, Wistar albino sıçanlarına oral olarak uygulanan dimetoatın subkronik toksisitesini ve taflan meyve ekstresinin veya kontrol olarak vitamin C'nin akciğer dokusundaki histopatolojik bulgulara dayalı iyileştirici etkisini değerlendirmektir. Bu amaçla, hayvanlar rastgele her biri on sıçandan oluşan altı gruba ayrıldı ve 60 gün boyunca her gün oral gavaj ile muamele edildi. Akciğer dokusu kesitindeki histopatolojik değişiklikler ışık mikroskop kullanılarak belirlendi. Dimetoat maruziyeti, kontrol grubu sıçanlara kıyasla akciğer bölümlerinde ciddi histopatolojik değişiklikler gösterdi. Akciğerin morfolojisinin çoğunlukla dejeneratif değişikliklere yol açan dimetoat muamelesi ile etkilendiği görüldü. Alveolde artmış kanama, nekroz ve alveolar ödem ile inflamatuvar lökosit infiltrasyonu gözlendi. Bununla birlikte, dimetoat uygulanan sıçanlara taflan meyve ekstresinin ön- ve sonraki muamelesi veya vitamin C'nin ön muamelesi akciğer hasarında bir iyileşme gösterdi. Sonuç olarak, taflan meyve ekstresi veya vitamin C muamelesi, dimetoat toksisitesinin indüklediği akciğer toksisitesine karşı koruma sağlayabilir.

Anahtar kelimeler: Toksisite, dimetoat, akciğer, histopatoloji, taflan meyvesi.

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INTRODUCTION

Dimethoate is one of the most important and widely used organophosphate insecticides and frequently used in agriculture against a wide range of insects (1). For humans, the main risk groups of dimethoate exposure are its producers, pesticide workers, and farm owners (2). It has been reported the toxicity of dimethoate results in deleterious effects on many organs such as lung, liver, kidney, brain, testes, pancreas of rats (3). The lung is the first vital organ that comes into contact with inhaled and ingested toxic substances. In addition, some studies have reported that organophosphate compounds give rise to pulmonary impairments in mice and rabbits, such as alveolar congestion, hemorrhage, neutrophil infiltration, emphysematous changes (4). One of the toxic effects of dimethoate is to induce oxidative stress through generation of free radicals and induction of lipid peroxidation (5). However, antioxidants as vitamins can prevent the excess formation of free radicals or inhibit their reaction with biological sites (6). There is a growing interest in the role and usage of natural dietary antioxidants as a strategy to amelioration of the various health disorders (7).

Cherry laurel (*Laurocerasus officinalis* Roem.) is locally called "Taflan" or "Karayemiş" and grown as a native fruit in the coasts of the Black Sea region of Turkey (8). It was found that cherry laurel fruit is a rich source of protective antioxidant compounds such as phenolics and ascorbic acid (9,10). Some studies indicated that cherry laurel fruit has antioxidant effect by scavenging superoxide and 2,2-diphenyl-1-picrylhydrazyl radicals (11).

The aim of this study was to evaluate the toxicity of dimethoate in rat and the ameliorative effect of cherry laurel fruit extract or vitamin C as control, based on the histopathological findings in the lung tissue.

MATERIALS AND METHODS

Chemicals

Formulation grade dimethoate (Korumagor 40 EC, 40%, Koruma Agriculture, Turkey) was used. It was in the form of an emulsion dissolved and diluted in saline (0.9% NaCl) in order to obtain an effective concentration of body weight (bw) of the rat. The test concentration of dimethoate was calculated from the percentage of the active ingredients. All solutions were freshly prepared before use. All other reagents used in this study were analytical grade and obtained from Sigma Chemical Co. (St. Louis, MO, USA) and Merck (Darmstadt, Germany).

Preparation of Fruit Extract

Cherry laurel fruits were collected from Akçaabat, Trabzon and voucher specimen is deposited in the herbarium of Pharmacy Faculty, Ankara University, Turkey (AEF 26257). 20 g of pulp were macerated with 200 mL of MeOH for 8 h at room temperature with magnetic stirrer and the extracts were filtered by Whatman No. 1 filter paper. The collected filtrates were dried under vacuum using a rotary evaporator at 40°C and they were lyophilized.

Animals

Sixty adult male Wistar albino rats weighing around 200 -250 g were obtained from the Experimental and Clinical Research Centre of Erciyes University, Kayseri, Turkey. All procedures performed on animals were in accordance with the European Union Directive 2010/63/EU for care and use of laboratory animals. The protocol for the use of experimental animals was approved by the Ethical Committee for Animal Research at Erciyes University (Approval date:15.08.2012; no:12/82).

Experimental Procedure

The rats were divided randomly into six groups consisting of ten rats each and were treated daily by oral gavage for 60 days as follow: Group I served as control received only saline, Group II was treated with dimethoate in saline, Group III was given fruit extract, Group IV was treated with fruit extract 30 min prior to dimethoate administration, Group V was given vitamin C 30 min before dimethoate administration, Group VI received the daily dimethoate for the first month, during the second month the rats were treated with dimethoate 30 min prior to administration of fruit extract.

The dose of dimethoate used in this study represents 1/50 of the LD50 (380 mg/kg), which has been applied previously by another study (2) since it is toxic but not lethal to rats. The dose of vitamin C used as an antioxidant (100 mg/kg/day) provides protection against toxicity. Dose of fruit extract was 4 mg/kg/day, contains effective antioxidant compounds such as phenolics which give good protection against the toxicity (2).

Sample Preparation

At the end of the experimental period, the rats were euthanized with xylazine/ketamine anesthesia by cervical dislocation. Lung tissues were removed immediately, washed with ice-cold physiologic saline solution, and blotted. Small representative slices of lung tissue were fixed in 10% formalin for routine histopathology evaluation.

Histopathologic Evaluation

The lung pieces fixed in formalin were dehydrated in a graded ethanol series, cleared in xylene and embedded in paraffin. 5 µm thick paraffin sections were cut from each specimen. All sections were stained with hematoxylin and eosin dye and examined by using a microscope (Olympus BX-51, Japan) equipped with a high-resolution camera (Olympus DP-71, Japan).

RESULTS

In the current study, light microscopic examination indicated a normal structure of the lung alveoli and interstitial tissue in the control group presented in Figure 1A. The normal alveoli were lined with the normal squamous cell with some macrophages.

As a result of dimethoate exposure, severe histopathological changes in the lung sections were seen compared with control group rats as shown in Figure 1B.

The morphology of the lung seemed to be mostly affected by dimethoate treatment alone. Dimethoate caused degenerative changes in the lung. Necrosis and alveolar edema as well as an infiltration of inflammatory leukocyte were observed. Increased hemorrhage was observed in within the alveoli.

The histological pattern of the lung tissue was normal in

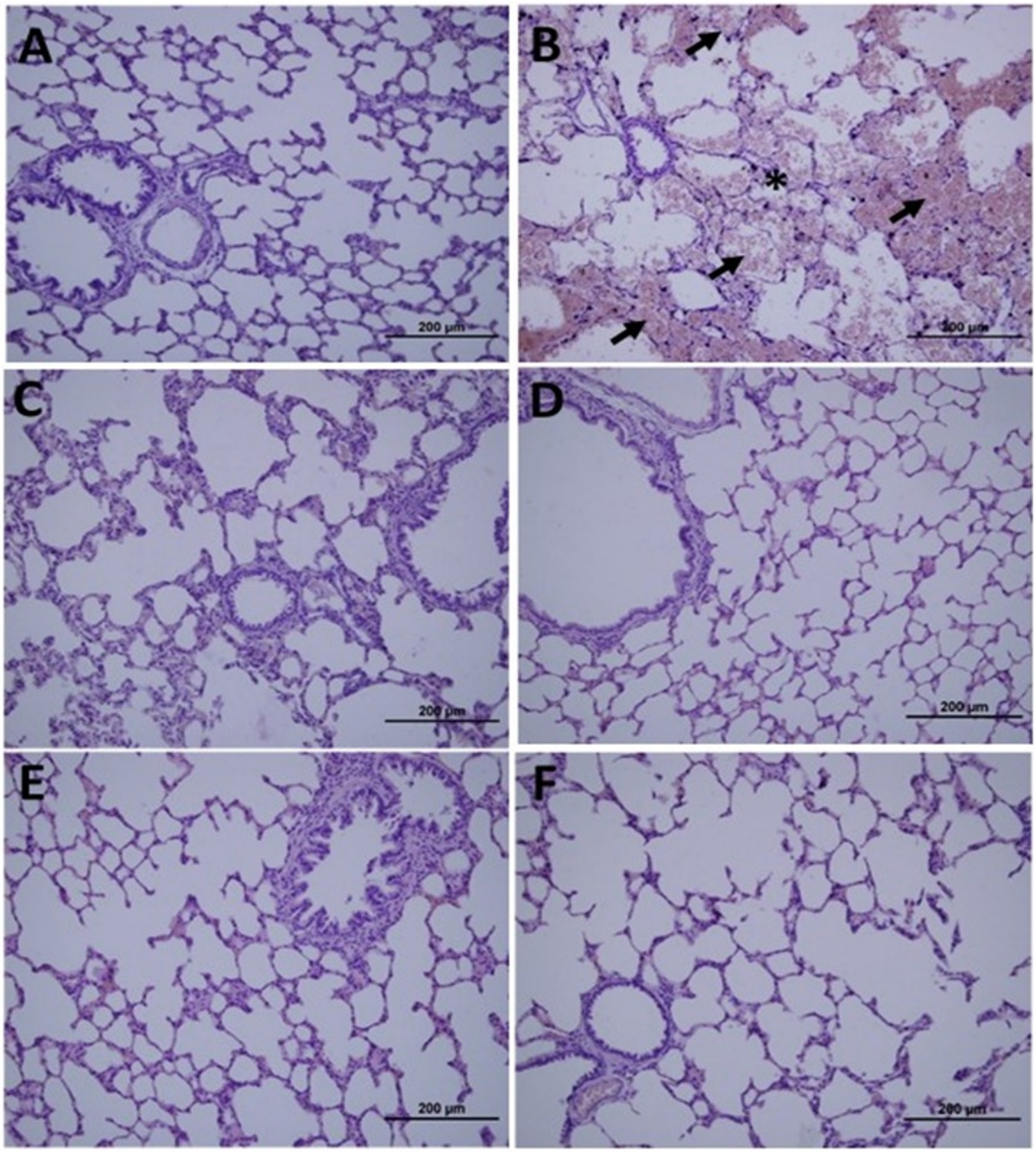

Figure 1: Histological findings in the lung tissue of rats from the experimental groups. **(A)** Control group showed normal alveoli and interstitial tissue. **(B)** Dimethoate-treated group showed histopathologic findings such as hemorrhages (**arrow**) and alveolar edema (**star**). **(C)** Cherry laurel fruit extract-treated group showed normal histological architecture. **(D)** Histological damage decreased in the group of pretreated with cherry laurel fruit extract when compared with only dimethoate-treated group. **(E)** The group of pretreated with vitamin C showed normal lung tissue without any pathological changes. **(F)** Histological picture showed a significant decrease in the lung damages in the group of post-treated with cherry laurel fruit extract (H&E, x20).

the cherry laurel fruit extract-treated group as demonstrated in Figure 1C.

Lung sections in group of pretreated with cherry laurel fruit extract (Figure 1D) showed an amelioration in the injury with little pathological alterations such as lesser hemorrhage, interstitial edema when compared with only dimethoate-treated group (Figure 1B). It was observed that vitamin C administration to dimethoate-treated rats (Figure 1E) showed normal lung tissue similar to the control group (Figure 1A). Post-treatment with cherry laurel fruit extract after exposure to dimethoate (Figure 1F) exhibited an improvement of lung morphology except for hemorrhage and interstitial edema in comparison to the only dimethoate-treated group (Figure 1B).

DISCUSSION

Dimethoate is one of the most important organophosphate insecticides and frequently used in agriculture against a wide range of insects (6). It was reported that

dimethoate leads to tissue damage such as liver (3,6,7,12-15), brain (12), kidney (2,13,16) in animal studies and the principal mechanism that may be responsible for the toxicity of dimethoate involves oxidative stress through generation of reactive oxygen species. In addition, Khogali et al. observed dimethoate-induced histopathological changes in the liver, kidney, stomach and intestine of the mice (17). To our knowledge, there is little information available about dimethoate toxicity on lung tissue in animal studies. However, it was found that an induction of oxidative stress in lung tissue of rats after dimethoate exposure evidenced by histopathological evaluation and biochemical parameters (18).

Histopathological findings of the present study suggested that exposure to dimethoate exhibited severe changes in the lung including an increased hemorrhage within the alveoli, necrosis, alveolar edema, and an infiltration of inflammatory leukocyte compared with control group rats. Our results indicate that treatment with cherry laurel fruit extract or vitamin C to dimethoate applied rats showed an amelioration in the lung toxicity when compared with only dimethoate-treated group. At a dose of 4 mg/kg extract showed an improvement on the histopathological changes in the lung section of rats. This may be explained by its sufficient concentration and antioxidant capacity to defend adequately against free radicals generated by dimethoate exposure.

Our results were in accordance with those obtained by Amara et al. (18) that a 30-day exposure of adult rats to dimethoate at dose of 0.2 g/L caused histopathological alterations in lung tissue such as emphysema, hemorrhages and hemosiderin depositits. However, co-treatment with selenium (0.5 mg/kg) or vitamin E (100 mg/kg) to the diet of dimethoate administered rats alleviated the histological impairments of lung. Karaoz et al. (19) also determined that rats treated with chlorpyriphos ethyl (an organophosphorus insecticide) for sixth day lead to remarkable changes in the histomorphology of the lung. These were infiltration of mononuclear cells, hyperplasia of type II pneumocyte, and thickened and increased connective tissue. However, treatment with vitamin C, vitamin E, and melatonin considerably reduces the toxic effect of chlorpyriphos ethyl on lung tissue in rats.

In conclusion, our findings suggest that subchronic exposure to dimethoate induces histopathological changes in the lungs of rats. However, the accurate mechanism is not yet clear and should be clarified by biochemical parameters. On the basis of this study, it should be taken into consideration that the fruit of cherry laurel or vitamin C might act as a protective agent against lung toxicity induced by dimethoate exposure.

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