An investigation on lung tissue damage and morphological changes in newborns of pregnant rats exposed to methidathion

Protective effects of caffeic acid phenethyl ester

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ABSTRACT

الأهداف: التحقق من التغيرات النسيجية في الرئة والتغيرات الشكلية التي تطرأ على الجرذان حديثي الولادة وذلك بعد تعريض الأمهات إلى مادة الميثيداثيون خلال فترة الحمل، بالإضافة إلى معرفة مدى تأثير إستر فينيثيل حمض الكافييك على هذه التغيرات.

الطريقة: أجريت هذه الدراسة بكلية الطب في جامعة سليمان ديميريل، أسبرطة، تركيا وذلك خلال الفترة من مايو 2007م إلى يونيو 2007م. شملت الدراسة 50 جرذياً أنثى من فصيلة ألبينو ويستر، وقد تم تقسيمها عشوائياً إلى المجموعات الخمسة التالية: المجموعة (1) وهي مجموعة التحكم (العدد=10)، المجموعة (2) وأُعطيت مادة الميثيداثيون بمقدار 5 ملجرام/كجرام يومياً وذلك في الأيام السبعة الأولى من الحمل (العدد=10)، المجموعة (3) وأُعطيت نفس الكمية من الميثيداثيون يومياً مع إستر فينيثيل حمض الكافييك بمقدار 10 ميكرومول/ كجرام يومياً في الأيام السبعة الأولى من الحمل (العدد=10)، المجموعة (4) وأعطيت الميثيداثيون بنفس الكمية يومياً ولكن في الأيام السبعة الأخيرة من الحمل (العدد=10)، المجموعة (5) وأعطيت نفس الكمية من الميثيداثيون مع نفس الكمية من إستر فينيثيل حمض الكافييك يومياً ولكن في الأيام السبعة الأخيرة من الحمل. لقد تم وضع الميثيداثيون في زيت الذرة ومن ثم أُعطي للجرذان بواسطة التغذية القسرية عن طريق الفم، أما إستر الفينيثيل فتم حقنه داخل الصفاق.

النتائج: لقد أُصيبت الجرذان الحوامل بكل من الرعاش، والهياج، وتشنج الأطراف وذلك بعد إعطاؤها الميثيداثيون. أشار فحص الأنسجة الرئوية إلى وجود كلاً من: التهاب في محيط القصبة، ونزيف نسخي مع نزيف نسخي قصبي، واحتقان وعائي داخل المتن مع تخثر، تدمر جدران الأنساخ، وارتشاح داخل المتن.

خاتمة: تسبب مادة الميثيداثيون نقصاً في اكتساب الوزن خلال فترة الحمل بين الجرذان الحوامل بالإضافة إلى موت الأمهات والأجنة داخل الرحم. وتساهم هذه المادة في نقص أوزان الأجنة بعد الولادة، كما أنها تؤدي إلى تغيير الأنسجة في رئات الأجنة. ويساعد إستر فينيثيل حمض الكافييك على التخفيف من تغيير هذه المادة للأنسجة الرئوية.

Objectives: To investigate histopathological changes in the lungs and morphological changes of newborn rats whose mothers are exposed to methidathion (MD) during their pregnancy, and also the effects of caffeic acid phenethyl ester (CAPE) on these changes.

Methods: The study was conducted in the Faculty of Medicine, Suleyman Demirel University, Isparta, Turkey between May and June 2007. Fifty female Wistar albino rats were randomly divided into 5 groups, as follows: Group I (n=10): control group, Group II (n=10): 5mg/kg/day MD treated group in the first 7 days of pregnancy, Group III (n=10): 5 mg/kg/day MD + 10 µmol/kg/day CAPE treated group in the first 7 days of pregnancy, Group IV (n=10): 5mg/kg/day MD treated group in the last 7 days of pregnancy, and Group V (n=10): 5 mg/kg/day MD + 10 µmol/kg/day CAPE treated group in the last 7 days of pregnancy. The MD was administrated by oral gavage in corn oil, and the CAPE was administrated intraperitoneally.

Results: Tremors, agitation, and spasm of extremities were observed in pregnant rats after administration of MD. Histopathological examination of lung tissues revealed peribronchial inflammation, alveolar and bronchoalveolar hemorrhage, intraparenchymal vascular congestion and thrombosis, alveolar destruction, and intraparenchymal infiltration.

Conclusions: Methidathion causes low weight gain and deaths among pregnant rats, increases intrauterine fetus deaths, causes low birth weights in the newborns, and histopathological changes in the lung tissues of newborn rats. The CAPE has an ameliorating effect on these histopathological alterations.

Saudi Med J 2010; Vol. 31 (10): 1095-1100

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Received 17th February 2010. Accepted 18th September 2010.

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Organophosphate (OP) compounds are a diverse group of chemicals used for both domestic and agricultural purposes.[1] Organophosphates often show their effects by inhibiting acetylcholinesterase activity.[2] They are rapidly absorbed by the respiratory and gastrointestinal system, and through the skin as they are lipofilic.[3] A study carried out in the USA investigated pesticide residues on children's diets (fresh vegetables, fruit, fruit juice, and milk products), and determined 6 different pesticide residues including methidathion (MD), methyl parathion, chlorpyrifos, malathion, azinphosmethyl, and phosmet.[4] Blasco et al[5] investigated pesticide residues in honey sold in Spanish and Portuguese markets, and determined OP residues in 22%, and MD residues in 4% of 50 samples. These studies show that OPs can be taken both directly or via food residues. Farag et al[6] gave dimethoate (which is one of the OP) to pregnant rats and observed some symptoms such as tremors, weakness, salivation, decrease in food consumption, and weight gain. They also determined an increase in intrauterine fetus deaths, and a decrease in live birth and fetal weight. Dabrowski et al[7] determined low birth weight in newborns of women who work in agricultural fields, and therefore exposed to OP insecticides. Similarly, Breslin et al[8] determined low birth weight and an increase in deaths in infants of pregnant rats exposed to chlorpyrifos. Methidathion is one of the OP insecticides used against pests of grape, nut, olive, and fruit trees, and in rose fields. Methidathion is widely used in the Isparta region (Agricultural Ministry Office of Isparta Province) of Turkey. Previous studies also determined that MD causes histopathological changes in kidney, liver, and blood vessels of adult rats and increases lipid peroxidation in rat liver.[9-11] It is possible for pregnant women who live in the area, or working in agricultural fields to be exposed to OPs. Previous studies determined that OPs are harmful during pregnancy as they pass through the placenta.[12] Therefore, the newborns of MD exposed pregnant women are also affected by MD. Caffeic acid phenethyl ester (CAPE) is an active component of honeybee propolis. It has been stated that CAPE has no harmful effects on normal cells.[13] However, it is well known that CAPE has anti-inflammatory,[14] anticarcinogenic,[15] and antioxidant properties.[16,17] Reviewing related literature showed that the harmful effects of MD on lung tissues of adults, pregnant, and newborns have not yet been thoroughly investigated. This study, therefore, aims to investigate changes in the lungs of newborn rats whose mothers are exposed to MD during their pregnancy, and also the beneficial effects of CAPE on these changes.

Methods. ***Animals and treatment.*** This study was conducted in the Faculty of Medicine, Suleyman Demirel University, Isparta, Turkey between May and June 2007. Fifty adult (4 months old) female Wistar albino rats (mean weight between 140-200 gr) obtained from the Laboratory of Animal Production Unit at Suleyman Demirel University (Isparta, Turkey), were used in the experiment. This study was approved by the Suleyman Demirel University Animal Research Ethics Committee (approval date 23/12/2005 and number 12/09). This experiment was carried out in accordance with the National Guidelines for the Care and Use of Laboratory Animals. Temperature was maintained at 23±2°C, and a relative humidity of approximately 50%, with a 12 hour light/dark photo period. Animals were housed in a plastic cage individually and had free access to standard rat chow and tap water. Fifty rats were randomly divided into 5 groups, as follows: Group I: control (n=10), Group II: MD-treated group in the first 7 days of pregnancy (5mg/kg/day, by oral gavage in corn oil) (n=10), Group III: MD + CAPE-treated group in the first 7 days of pregnancy (5 mg/kg/day, by oral gavage in corn oil and 10 µmol /kg/day intraperitoneally [i.p.]) (n=10), Group IV: MD treated in the last 7 days of pregnancy (5mg/kg/day, by oral gavage in corn oil) (n=10), Group V: MD + CAPE treated group in the last 7 days of pregnancy (5 mg/kg/day, by oral gavage in corn oil and 10 µmol/kg/day, i.p.) (n=10). Fifty females (10 in each group) were paired with a male (1:1) overnight, and were examined for the presence of a vaginal plug the following morning. The day on which a vaginal plug was observed was considered day one of gestation. The MD (Sygenta, Zurich, Switzerland) was administered by oral gavage in corn oil at a dose of 5mg/kg body weight. The CAPE (Sigma Chemical Co., St. Louis, MO, USA) was co-administered i.p at a dose of 10 µmol/kg/day. Corn oil was given by oral gavage at a dose of 5mg/kg body weight to the control group. The pregnant rats were examined following the administration of MD for signs of toxicity. Maternal body weight was recorded on gestational days of 1, 7, 14, and 20. Newborn weights were recorded at the end of the pregnancy period. Newborns were examined for any macroscopic anomaly and malformations. The lungs were examined for any macroscopic anomaly and pathology.

Histological examination. Ten lung tissue samples were selected from each group by a systematic random sampling method. Lung tissue samples were initially fixed in 10% neutral buffered formaldehyde solution. After dehydration procedures, the samples were blocked in paraffin. Four µm sections were cut and stained with hematoxylin-eosin. Mounted slides were examined under a light microscope (Nikon microscope ECLIPSE E600W, Tokyo, Japan) and photographed using a digital camera (Microscope Digital Camera DP70, Tokyo, Japan). Evaluation of the lung histological

damage was accomplished by scoring its degree of severity according to previously published methods.[18,19] In the criteria for evaluation of the peribronchial inflammation (PI), alveolar hemorrhage (AH) and bronchoalveolar hemorrhage (BAH), intraparenchymal vascular congestion and thrombosis, alveolar destruction (AD), and intraparenchymal infiltration (II) changes were based on intensity and diffusion of staining in the samples. Intensity and diffusion of these observations were separately numbered 0 to 3 + A semiquantitatively. Following this procedure, the counted values were summed in each section, recombining the values of intensity and diffusion (called the 'degree of staining') as follows: 0: no changes; 1+: minimal; 2+: low; 3+ moderate; 4+: strong; 5+: heavy; and 6+: most heavy.[18,19]

Statistical analysis. Data were presented as mean ± standard deviation (SD). As the data of histopathological changes were considered to be non-parametric, Kruskal-Wallis and Man-Whitney U tests were performed for statistical analyses. A level of 5% ($p<0.05$) was considered statistically significant. The maternal weight gain and the newborn weights were analyzed using one-way ANOVA test. Data were analyzed using SPSS Statistical Package Version 15.0 (SPSS Inc, Chigago, IL, USA).

Results. Twenty-eight pregnant rats (4 in controls, 8 in group II, 5 in group III, 6 in group IV, and 5 in group V) were included in the experiment. Following the administration of MD tremors, agitation, and spasm of extremities were observed in pregnant rats in 1-2 minutes. Some of the rats in the experimental groups died during their pregnancy (**Table 1**). When the uteri of these rats were opened, it is observed that all fetuses were resorbed. No significant differences were determined for pregnancy periods between experiment and controls. The pregnant rats were labored between the 21st and 22nd day of their pregnancy. When weight gains during the pregnancy were compared between groups, it is determined that mean weight gains of rats in the experimental groups were significantly lower than mean weight gain of rats in the control group (**Table 1**). **Table 1** shows newborn rat numbers and their mean weight by groups. The mean weight of newborn rats in group II and group III was compared to the controls and no significant difference was determined ($p>005$). However, the mean weight of newborn rats in group IV and group V was significantly lower than that in the control group ($p<0.05$). When group IV and group V were compared, it is determined that the mean weight of newborn rats in group IV was lower than that in group V ($p<0.05$). No anomalies or pathologies were observed in the morphological examinations of newborn rats. Three days after the end of pregnancy, newborn rats were decapitated and their lungs dissected. In macroscopic examination of lungs, some bleeding points were observed. When the control group was compared to the experimental groups for histopathological changes, PI, AH, and BAH, intraparenchymal vascular congestion and thrombosis, AD, and II were determined in the experimental groups (**Figure 1**). Comparisons of these histopathological changes by groups are presented in **Table 2**. When group III (MD and CAPE administered together during the first period of pregnancy) was compared to controls, it was observed that all histopathological changes were significantly recovered except AH and BAH (**Table 2**). When Group V (MD and CAPE administered together during the last period of pregnancy) was compared to controls, significant recoveries were only observed on intraparenchymal vascular congestion and thrombosis (**Table 2**).

Discussion. This study investigated fetal toxicity in pregnant rats exposed to MD in the first and last period of their pregnancy and the beneficial effects of CAPE on these changes. In our study, tremors, agitation, and spasms of extremities were observed in a short time following the administration of MD to pregnant rats. Breslin et al[8] determined tremors and salivation in pregnant rats exposed to chlorpyrifos

Table 1 - Variables of rats used in the experiment.

Variables	Group I	Group II	Group III	Group IV	Group V
Total number of rats	10	10	10	10	10
Pregnant	4	8	5	6	5
Labored	4	4	3	3	4
Died during their pregnancy	0	4	2	3	1
Newborn rats	38	26	27	20	26
Mean weights of newborn rats (g) ± standard deviation	5.88 ± 0.72	5.67 ± 0.50	5.91 ± 0.45	4.70 ± 0.61	5.34 ± 0.75
Mean weight gains of pregnant rats, (g) ± standard deviation	83.25 ± 11.3	57.75 ± 8.1	60.33 ± 2.0	49.33 ± 11.5	66.0 ± 9.4

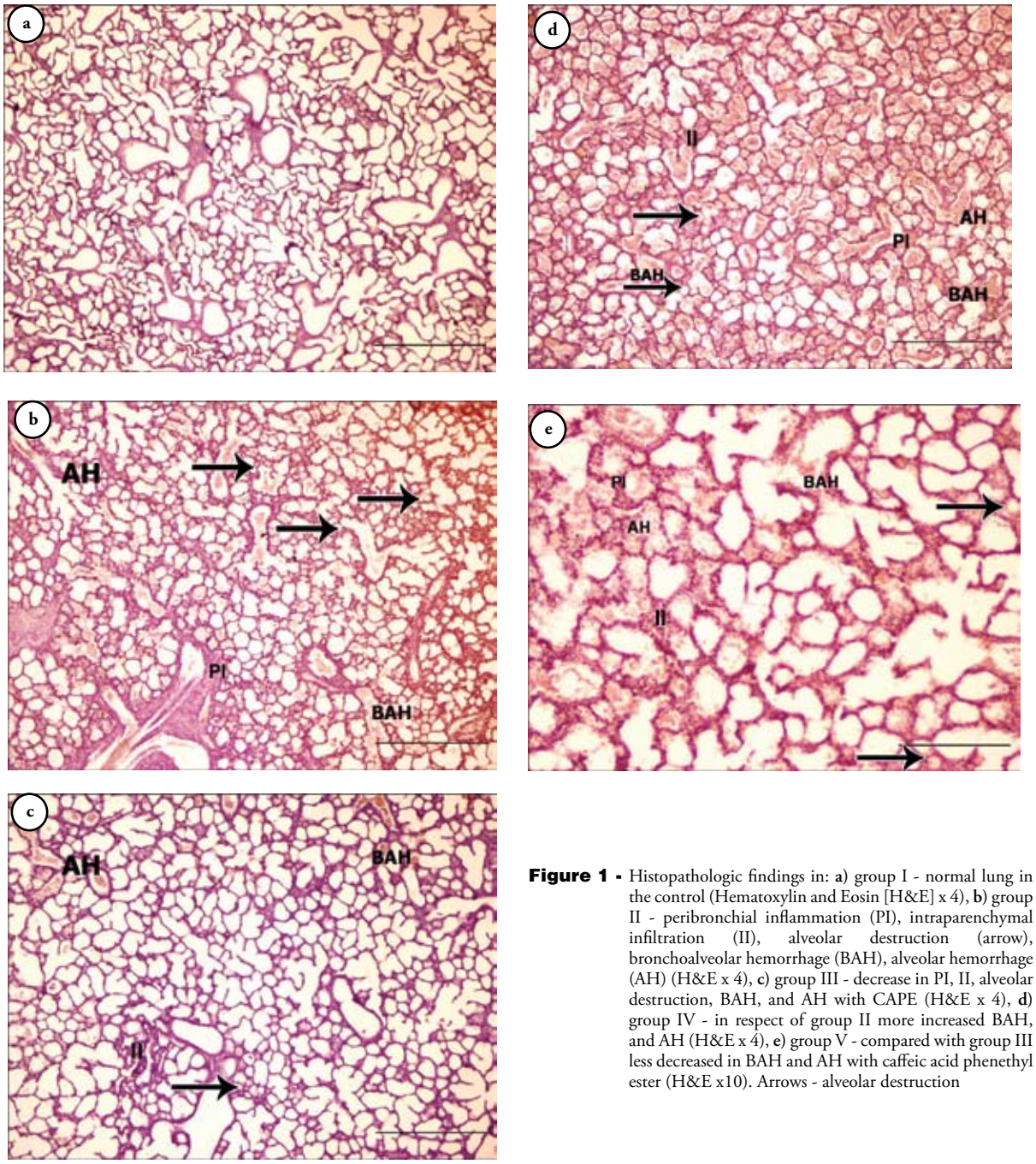

Figure 1 - Histopathologic findings in: **a)** group I - normal lung in the control (Hematoxylin and Eosin [H&E] x 4), **b)** group II - peribronchial inflammation (PI), intraparenchymal infiltration (II), alveolar destruction (arrow), bronchoalveolar hemorrhage (BAH), alveolar hemorrhage (AH) (H&E x 4), **c)** group III - decrease in PI, II, alveolar destruction, BAH, and AH with CAPE (H&E x 4), **d)** group IV - in respect of group II more increased BAH, and AH (H&E x 4), **e)** group V - compared with group III less decreased in BAH and AH with caffeic acid phenethyl ester (H&E x10). Arrows - alveolar destruction

Table 2 - Mean values and standard deviations of histopathological changes in lung tissues by groups.

Hispathological changes	Group I	Group II	Group III	Group IV	Group V
Peribronchial inflammation	0.2 ± 0.63	2.6 ± 0.51*	1.0 ± 1.05*	2.7 ± 0.48*	1.4 ± 0.96*
Alveolar hemorrhage and bronchoalveolar hemorrhage	0.00 ± 0.00	4.5 ± 0.84*	1.1 ± 1.19*	5.4 ± 0.51*	2.3 ± 0.48*
Intraparenchymal vascular congestion and thrombosis	0.2 ± 0.42	2.7 ± 0.48*	0.2 ± 0.63*	2.8 ± 0.42*	1.0 ± 1.05*
Alveolar destruction	0.00 ± 0.00	2.5 ± 0.52*	0.6 ± 0.96*	2.9 ± 0.31*	0.8 ± 1.03*

*significant differences at 0.05 level

between the sixth and fifteenth days of their pregnancy. Farag et al[6] gave dimethoate to pregnant rats between the sixth and fifteenth of their pregnancy and observed tremors, weakness, salivation, and decreasing food consumption, and weight gain. In our study, tremors, agitation, and spasms of extremities were observed following MD administration, consistent with the results of Breslin et al,[8] and Farag et al.[6] However, in contrast to the studies of Breslin et al[8] and Farag et al,[6] salivation was not observed in this study. Furthermore, our study determined decreasing maternal weight gain in the experimental groups, which is consistent with the studies of Farag et al.[6] Therefore, it is believed that MD causes maternal toxicity leading to decreased appetite and weight loss.

In the present study, 6 rats in the first period of pregnancy, and 4 rats in the last period of pregnancy died. Moreover, the numbers of newborn rats in the experimental groups were less than the control group. Birth weights of newborn rats in the experimental groups were also significantly lower. During the literature review, we did not come across any study suggesting that OPs can cause death in pregnant rats. However, previous studies reported that OPs cause a decrease in live fetus numbers, and an increase in intrauterine resorptions in the early period of the pregnancy.[6,8] In our study, we observed deaths among pregnant rats exposed to MD during the first and the last periods of pregnancy. Systemic maternal toxicity of MD leading to feeding disorders in pregnant rats, and direct toxicity leading to resorptions in the first period may cause a decrease in live fetus number.

This study determined low birth weights in newborn rats whose mothers were exposed to MD in the last period (groups IV and V) of their pregnancy (**Table 1**). We observed that the toxic effects of MD were high in newborns of pregnant rats exposed to MD in group IV. Weights of newborns in group V, in which MD and CAPE were administered together in the last period of the pregnancy, were higher than those in group IV in which only MD was administered. These results suggest that CAPE reduces the harmful effects caused by MD in the last period of pregnancy. Previous studies also determined low birth weights in newborn rats whose mothers were exposed to chlorpyrifos and dimethoate.[6,8] Whyatt et al[12] determined low birth weights in newborns of pregnant women exposed to OPs, and Dobrowski et al[7] revealed low birth weight in newborns of pregnant farmers exposed to OPs. In this study, we did not observe any organ or skeleton anomalies in the morphological examinations of newborn rats. Farag et al[6] did not observe any external, visceral, or skeleton anomalies in newborn rats whose mothers were exposed to low doses of dimethoate. The present study used low doses of MD and did not determine any morphological pathology and/or anomaly. Therefore, it is believed that OPs cause dose-dependent pathology or anomalies in newborn rats.

Limitation of our study: MD is one of the OP insecticides that caused some deaths in pregnant rats during the experiment.

In reviewing related literature, we did not find other histopathological studies on newborn and pregnant rats exposed to MD. However, previous studies determined that one of the OPs, chlorpyrifos-ethyl, caused mononuclear cell infiltration in peribronchial and perivascular areas of the lungs, and thickened and increased connective tissues in adult rats.[20] Caffeic acid phenethyl ester has reduced peribronchial and intraparenchymal lymphocyte and macrophage infiltration in lungs of rats administered with lithium.[19] Another study carried out by Ozyurt et al[21] showed that CAPE reduced pulmonary fibrosis of lungs caused by bleomycin. In line with the above studies, our results also showed that MD causes the above mentioned histopathological changes in the lungs of newborn rats, and CAPE, which has antioxidant and anti-inflammatory effects ameliorates these histopathological changes.

In the light of these findings, we concluded that MD causes an increase in intrauterine fetal deaths, causes low birth weights in newborns, and histopathological changes in the lung tissues of newborn rats. We also conclude that CAPE has ameliorating effects on these histopathological alterations. We hope that present results can be considered as providing useful findings for future studies.

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