



HISTOPATHOLOGICAL CHANGES IN ALBINO RATS EXPOSED TO METHOMYL

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ABSTRACT

The present study was designed to evaluate the toxic effects induced by different time intervals of methomyl exposure on the histological changes in liver, kidneys, lungs and heart of albino rats. Three treatment groups of rats were employed. The 1st group was served as control, while the 2nd and 3rd groups were orally treated with 1/10 LD₅₀ (3.33 mg a.i./kg b.wt.) and 1/20 LD₅₀ (1.66 mg a.i./kg b.wt.) of methomyl, respectively, for five days. The histology of rat liver treated with methomyl 1/10 LD₅₀ showed portal lymphohistiocytic aggregations, proliferative bile ductules, dilated portal, and central vein with widening of hepatic sinusoids, which found in common. Besides, siderocytes were seen accompanying the inflammatory infiltrates with various types of cell injury of the hepatic cells. Focal interstitial and interasinusoidal infiltrations of inflammatory cells mainly lymphohistiocytic cells and hemosiderosis were observed with 1/20 LD₅₀ and some hepatic cells had acute cell swelling which contains hemosiderosis accompanied hypertrophy of kuffer cells. Histopathological studies showed that degeneration of tubular epithelia were common in kidneys treated with 1/10 LD₅₀ and some tubules contain hyaline and granular casts, as well as other necrotic tubules were seen with lymphocytic infiltration in the renal cortex. Meanwhile, the results of 1/20 LD₅₀ treatment showed small focal tubular necrosis or different forms of acute cell swelling and some tubules contain desquamated cells inside its lumen in kidneys. Lungs were thickened with irregular bronchiolar wall due to leukocytic infiltration, peribronchial lymphoid hyperplasia, and desquamated bronchiolar epithelium with 1/10 LD₅₀ methomyl. Furthermore, focal lymphocytic aggregations replacing the pulmonary tissue with mild perivascular lymphocytic infiltration were observed at same dose. Mild hyperplasia of peribronchial lymphoid tissue including mild thickening of interalveolar septae accompanied with minute perivascular lymphocytic aggregations were the common pulmonary lesions with 1/20 LD₅₀. The heart had severe dilatation and widening of intramuscular blood vessels with partial hyalinization of some myocardial muscle fibers beside intramuscular edema which treated with 1/10 LD₅₀. On the other hand, it was normal cardiac muscle and intramuscular blood vessels at 1/20 LD₅₀ treatment. It could be concluded that the 1/10 LD₅₀ dose of methomyl had a significant histopathological changes in the studied albino rat organs. The pesticide administration caused extensive destruction to the renal tissue. This damage was more pronounced in the higher dose.

Key words: Methomyl, histopathology, albino rats, liver, kidneys, lungs, heart, toxicity.

INTRODUCTION

Pesticides are widely used for agricultural purposes for pest control on field and horticultural crops. The widespread use of pesticides leads to severe environmental pollution and health hazards (Mokhtar *et al.*, 2013).

Pesticides have destructive effects on environment if they are drifted outside the area of intentional application, and if they persist in environment longer than necessary. In fact, most of the chemicals that are used as pesticides are not highly selective, but are generally toxic to many non-targeted organisms including human and other desirable forms of life.

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Generally, a great danger on public health of human, which was produced by pesticides, represents alteration in the physiological and biochemistry condition of living organisms. Also, represents some toxic and adverse effects on physiological function of organs, reproductive system and offspring of humans.

Carbamates are mainly used in agriculture as insecticides, fungicides, herbicides, nematocides and plant growth inhibitors. They are also used for industrial and other applications in household products and for the control of vectors in public health. They are among the most extensively used insecticides comprising the third major group of synthetic insecticides being utilized worldwide for agriculture (West and Marnett, 2006).

Methomyl is an oxime carbamate insecticide that controls a broad spectrum of arthropods (Kidd and James, 1991). Methomyl, a systemic and contact poison is used as a broadspectrum insecticide and acaricide. It is widely using throughout the world since it is effective as "contact insecticide" as well as "systemic insecticide". Various commercial preparations of methomyl *viz.* lannate, methovin, methomex and nudrin are used to control a wide range of insects particularly aphids and lepidoptera, that infest a variety of vegetables, field crops and commercial plantings (Bonatti *et al.*, 1994; Wei *et al.*, 1997). Methomyl acts as a reversible acetyl cholinesterase inhibitor of central nervous system. It is classified as a pesticide of category-1 toxicity and has been accused of causing short-term adverse health effects (Baron, 1991; WHO, 1996).

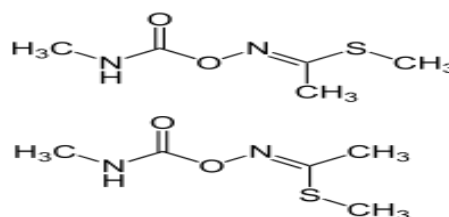
The present investigation aimed to study histological changes in some organs of female albino rats after subchronic exposure to the insecticide methomyl.

MATERIALS AND METHODS

Insecticide Used

Methomyl (Lannate 90% SP)

Chemical name (IUPAC): *S*-methyl (*EZ*) *N*-(methylcarbamoyloxy) thioacetimidate.



Structural formula: $C_5H_{10}N_2O_2S$.

Experimental Animal

Mature albino rats *Rattus norvegicus* Brok were obtained from the Organization of Serum and Vaccina (Helwan farm). Eighty seven, clinically healthy, mature female rats having an average weight of 170 ± 10 g were kept under the normal laboratory conditions in stainless cages for one week at least before being used. The rats were provided with 23% protein diet and water *ad libitum* throughout the study.

Experimental Design

A subchronic toxicity study was designed to investigate histopathological changes in some organs of female albino rats exposed to methomyl.

The rats were divided into three experimental groups as follows:

Group A: rats were kept without any treatments as a normal control. Group B and C: rats were treated with methomyl (90% SP) at 1/10 (3.33 mg a.i./kg b.wt.) and 1/20 of LD_{50} (1.66 mg a.i./kg b.wt.), respectively. They were applied orally administration for five times started from the 7th to the 12th days of the gestation period.

The rats were sacrificed and the liver, kidneys, heart and lungs were removed and fixed in 10% formalin saline.

Histopathological Study

Routine histopathological procedure was done and stained by Hematoxylin and Eosin stain (H&E) according to Suvarna *et al.* (2013). Specimens from the lungs, heart, liver and kidneys of different groups were collected and fixed in 10% buffered neutral formalin solution, dehydrated in gradual ethanol (70-100%), cleared in xylene, and embedded in paraffin. Five-micron thick paraffin sections were prepared and then routinely stained and then examined microscopically.

RESULTS AND DISCUSSION

Pathological Findings in Liver

The results of histological study of rat liver are shown in Plates 1, 2, 3, 4 and 5. Microscopically, apparently normal hepatic parenchyma as control group is shown in Plate 1. Portal lymphohistiocytic aggregations, proliferative bile ductules, dilated portal, and central vein with widening of hepatic sinusoids were common at methomyl 1/10 LD₅₀ (3.33 mg a.i./kg b.wt.) (Plates 2 and 3). Siderocytes were seen accompanying the inflammatory infiltrates with various types of cell injury of the hepatic cells. Focal interstitial and interasinusoidal infiltrations of inflammatory cells mainly lymphohistiocytic cells and hemosiderosis were observed with methomyl 1/20 LD₅₀ (1.66 mg a.i./kg b.wt.) (Plates 4 and 5).

Liver is vulnerable to various environmental toxicants which may cause structural and functional abnormalities (Shyamal *et al.*, 2010). Some hepatic cells had acute cell swelling which contain hemosiderosis accompanied with hypertrophy of kuffer cells. These results were in line with Banerjee *et al.* (1999) who reported that methomyl exposure promoted oxidative damage of liver cells by enhancing peroxidation of membrane lipids and this might enhance apoptosis. There were also midzonal vacuolar degeneration, necrobiotic changes of hepatocytes and focal areas of necrosis. These lesions were similarly reported in other carbamate toxicities. For example, El-Manakhly (1996) and Muthuviveganandavel *et al.* (2008) observed degeneration and multiple necrotic areas of hepatocytes infiltrated with mononuclear cells as a result of carbendazim and carbosul-fan treatment in rats, respectively.

Pathological Findings in Kidneys

Kidneys are responsible for the elimination of metabolic wastes and the control of the amount and composition of the body fluids. Nephrotoxicity can result in systemic toxicity causing: decreased ability to excrete body wastes, inability to maintain body fluid and electrolyte balance and decreased synthesis of essential hormones (*e.g.*, erythropoietin) (Finn, 1977; Laurent *et al.*, 1988).

Microscopically, normal renal tissues are presented in Plate 6 as control group. In rats exposed to methomyl at 1/10 LD₅₀ value (Plate 7), congested renal blood vessels and glomeruli with preglomerular lymphocytes and hypercellularity of some glomeruli beside acute cell swelling and degenerated of tubular epithelia were found in common. Some tubules contain hyaline and granular casts as well as other necrotic tubules were seen associated with lymphocytic infiltrations in the renal cortex.

Small focal tubular necrosis or different forms of acute cell swelling and some tubules contain desquamated cells inside its lumen (cellular and hyaline casts) were seen (Plate 8) with methomyl at the 1/20 LD₅₀. Methomyl administration caused extensive destruction to the renal tissue. This damage was more pronounced in the higher dose, which may cause renal failure (El-Morsy, 2013).

The histological disturbances in the renal tissue in this study come with in accordance with the results obtained from Radad *et al.* (2009). In the kidneys, methomyl treatment damaged the glomeruli, the tubules and the interstitium. Similarly, Nariman *et al.* (1995) and Selmanoglu *et al.* (2001) observed proliferation and swelling of glomerular endothelial cells and tubular degeneration, mononuclear cell infiltration and fibrosis in thiodicarb and carbendazim treated rats, respectively.

Pathological Findings in Lungs

Apparently normal lung tissues as a control treatment is visualized in Plate 9. Lungs were thickened with irregular bronchiolar wall due to leukocytic infiltration, peribronchial lymphoid hyperplasia and desquamated bronchiolar epithelium were seen at methomyl 1/10 LD₅₀ (Plate 10). On the other hand, focal lymphocytic aggregations replacing the pulmonary tissue with mild perivascular lymphocytic infiltration were observed as shown in Plate 11. Other bronchioles revealed hyperplastic epithelium and thickened interalveolar septae (Plate 12). The latter due to proliferative pneumocytes and vasculitis with perivascular intense lymphocyte

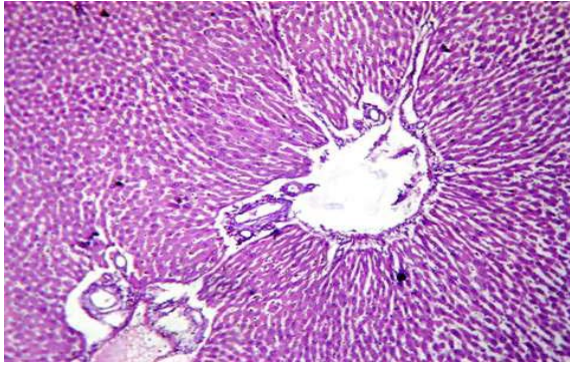


Plate 1. Liver of rat (control) showing normal hepatic parenchyma, H and E (X300)

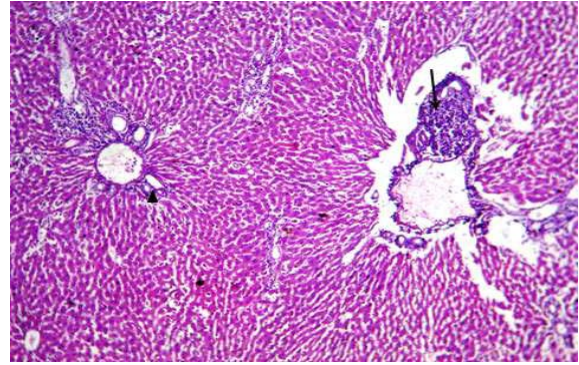


Plate 2. Liver of rat (methomyl 3.33 mg a.i./kg b.wt.) showing portal lymphocytic aggregations (arrow) and proliferative bile ductules (arrow head), H and E (X 300)

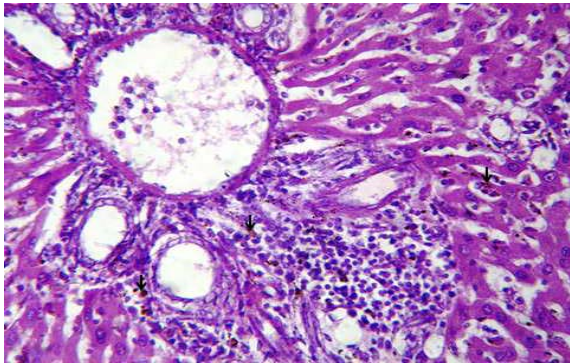


Plate 3. High magnification of the previous picture to show lymphocytes, siderocytes (arrows) and numerous bile ductules with widening of sinusoids, H and E (X 1200)

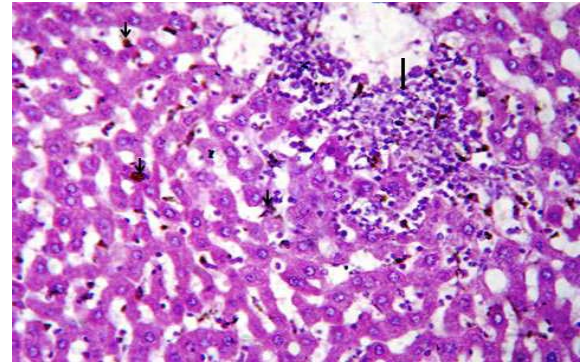


Plate 4. Liver of rat (methomyl 1.66 mg a.i./kg b.wt.) showing hemosiderosis (thin arrows) and lymphocytic infiltrations (thick arrow), H and E (X 1200)

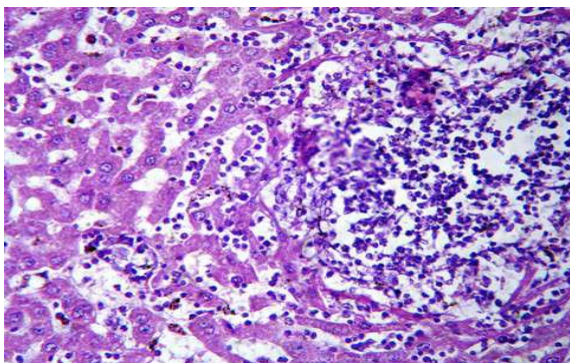


Plate 5. Liver of rat (methomyl 1.66 mg a.i./kg b.wt.) showing intrasinusoidal lymphocytosis and focal replacement by leukocytic aggregations, H and E (X 1200)

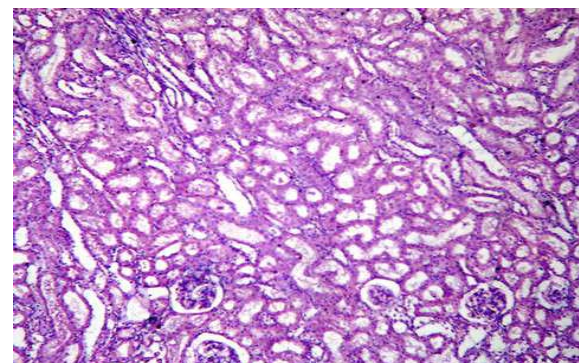


Plate 6. Kidney of rat (control) showing normal renal parenchyma, H and E (X 300)

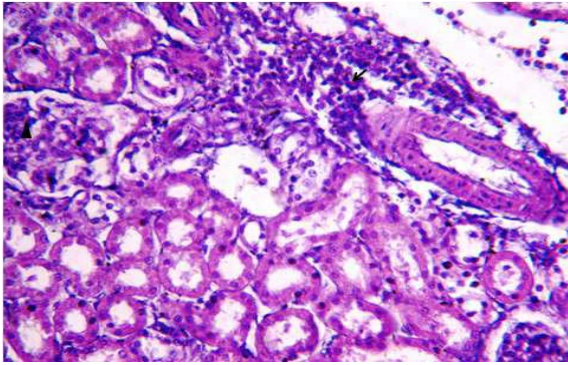


Plate 7. Kidney of rat (methomyl 3.33 mg a.i./ kg b.wt.) showing perivascular lymphocytic aggregations (arrow) and hypercellularity of glomeruli in renal cortex (arrow head), H and E (X 1200)

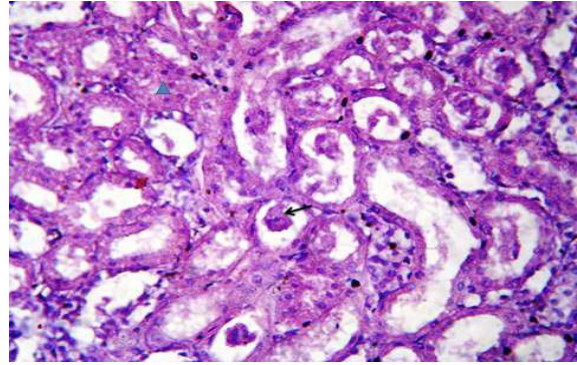


Plate 8. Kidney of rat (methomyl 1.66 mg a.i./ kg b.wt.) showing focal tubular necrosis (arrow head) with cellular casts (arrow), H and E (X 1200)

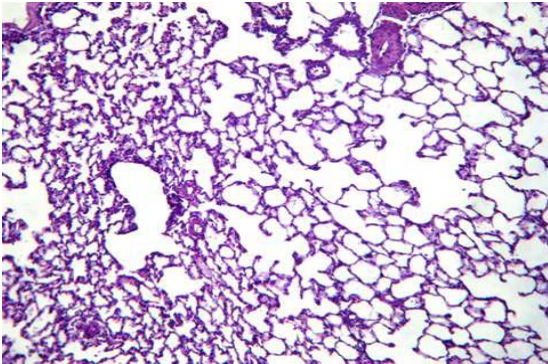


Plate 9. Lung of rat (control) showing normal pulmonary tissue, H and E (X 300)

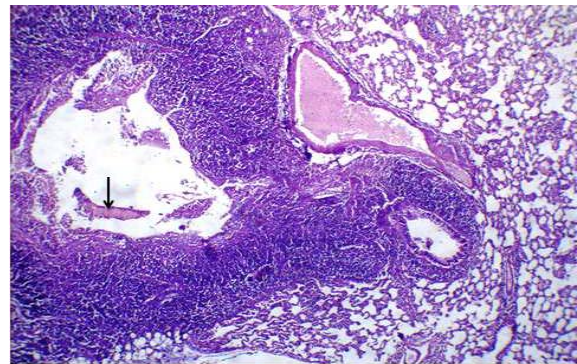


Plate 10. Lung of rat (methomyl 3.33 mg a.i./ kg b.wt.) showing thickened bronchiolar wall by leukocytic infiltration, peribronchial lymphoid hyperplasia and desquamated epithelium (arrow), H and E (X 120)

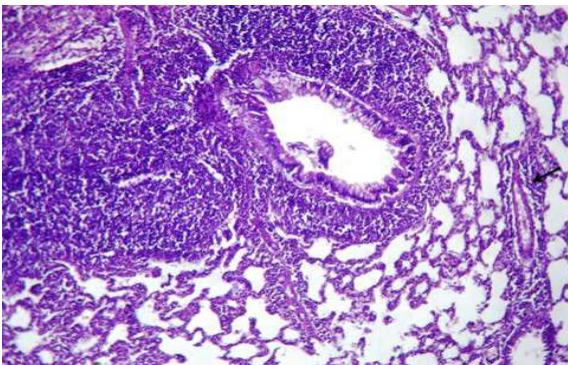


Plate 11. Lung of rat (methomyl 3.33 mg a.i./ kg b.wt.) showing focal lymphocytic aggregations replacing the pulmonary tissue with mild perivascular lymphocytic infiltrations (arrow), H and E (X 300)

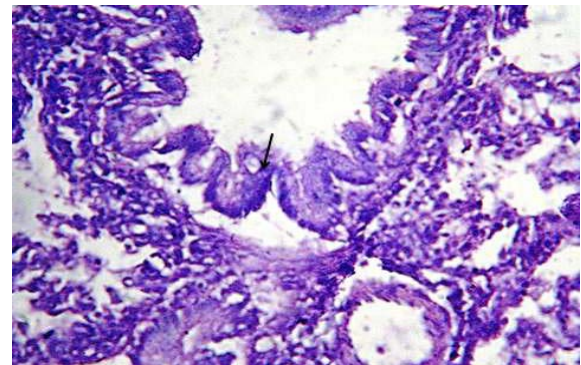


Plate 12. Lung of rat (methomyl 3.33 mg a.i./ kg b.wt.) showing hyperplastic bronchiolar epithelium (arrow) and thickened interalveolar septae, H and E (X 1200)

aggregations beside focal alveolar emphysema (Plate 13).

In lungs of rats treated with methomyl at the 1/20 LD₅₀ level, mild hyperplasia of peribronchial lymphoid tissue, with mild thickening of interalveolar septae accompanied with minute perivascular lymphocytic aggregations were the common pulmonary lesions (Plates 14 and 15).

Kidd and James (1991) and El-Khawaga (2005) attributed the pulmonary lesions to the extensive storage of methomyl in the lungs and its excretion in expired air. These effects are the same as those of Bretveld *et al.* (2007) who reported that exposure to pesticides was a potential risk factor for subfertility. Radad *et al.* (2009) confirmed that lesions in the lungs consisted of necrosis and sloughing of bronchiolar epithelial lining and lymphocytic perivascular accumulations. There were also alveolar emphysema, congestion of alveolar capillaries and thickening of alveolar septa as well as haemosiderin laden macrophages in the alveolar walls and lumens.

Pathological Findings in Heart

Apparently untreated normal cardiac muscles are presented in Plate 16. In rats exposed to methomyl at the 1/10 LD₅₀ value (Plates 17 and

18), severe dilatation and widening of intramuscular blood vessels with partial hyalinization of some myocardial muscle fibers beside intramuscular edema were found. While, normal cardiac muscle and intramuscular blood vessels were obtained with methomyl treatment at the 1/20 LD₅₀ value.

The extensive use of pesticides is usually accompanied with serious problems of pollution and health hazards. It is now well established that many pesticides in common of use can produce some toxic adverse effect on liver, kidney, lung and heart when tested in variety of experimental animals. Therefore, studying the effect of this widely used pesticide (methomyl) on these aspects has become major health concern. Methomyl exerts its toxic effect *via* peroxidative damage to the hepatic and renal and induces DNA damage in these organs (El-Khawaga, 2005).

In conclusion, methomyl was found to be potentially toxic to liver, kidneys, lungs, and heart. The observed hepatic, renal, and heart damages could predispose to hepatic insufficiency and renal failure in exposed individuals.

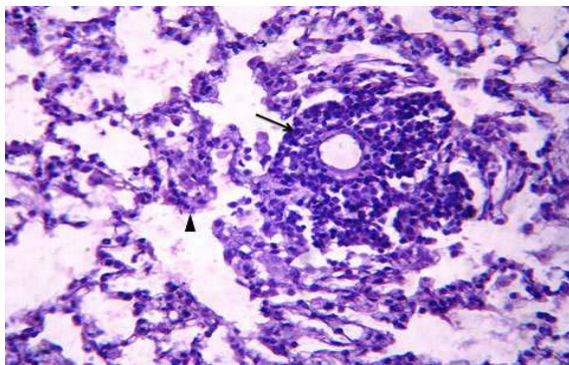


Plate 13. Lung of rat (methomyl 3.33 mg a.i./ kg b.wt.) showing thickened interalveolar tissue by proliferative pneumocytes and lymphocytic infiltrations (arrow head) together with vasculitis and perivascular leukocytic aggregations (arrow) beside focal alveolar emphysema, H and E (X 1200)

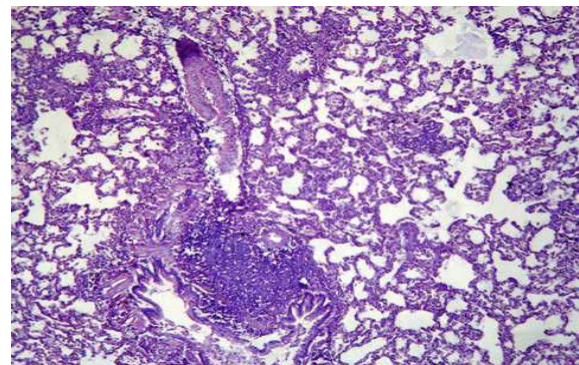


Plate 14. Lung of rat (methomyl 1.66 mg a.i./ kg b.wt.) showing minute perivascular leukocytic aggregations and mild thickening of interalveolar septae, H and E (X 300)

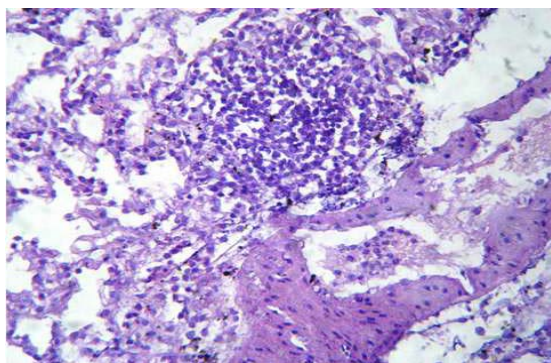


Plate 15. High power of the previous picture to show perivascular lymphohistiocytic aggregations and thickened septae, H and E (X 1200)



Plate 16. Heart of rat (control) showing normal myocardial tissue, H and E (X 300)

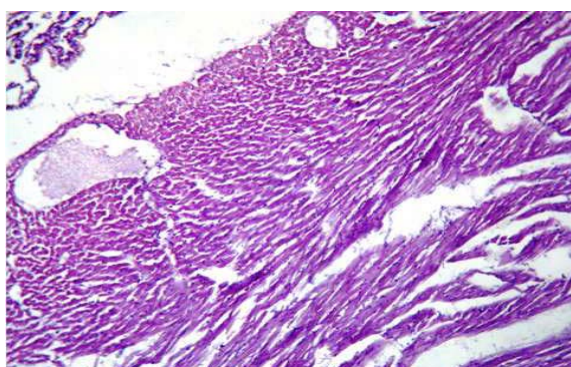


Plate 17. Heart of rat (methomyl 3.33 mg a.i./ kg b.wt.) showing dilated intramuscular blood vessels and intramuscular edema, H and E (X 300)

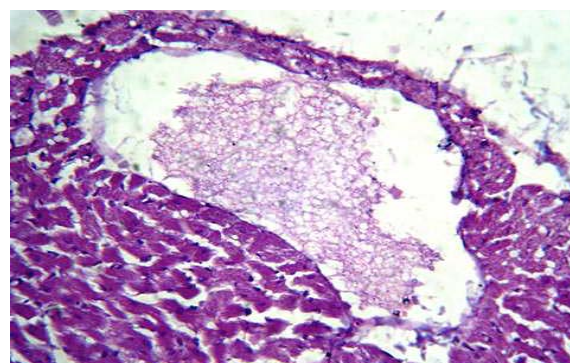


Plate 18. Heart of rat (methomyl 3.33 mg a.i./kg b.wt.) showing dilated intramuscular blood vessels, partial hyalinization and intramuscular edema, H and E (X 1200)

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دراسة التغيرات الهستوباثولوجية على الفئران البيضاء المعرضة لمبيد الميثوميل

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قسم وقاية النبات – كلية الزراعة – جامعة الزقازيق – الزقازيق – مصر

نظراً لما تسببه المبيدات من مشاكل للإنسان والبيئة المحيطة به والتي قد تنتج اثناء عمليات الرش أو التعرض لفترات طويلة لهذه المبيدات، ولما كان الهدف المباشر لعملية مكافحة هو القضاء على الآفات وحماية المحاصيل فمن الضروري أن نستخدم وسائل مكافحة ذات أمان نسبي على الإنسان والبيئة، لذلك فإن المنظمات والهيئات الدولية تشترط قبل استخدام المبيدات أن يتم دراسة التأثيرات الضارة لها وبخاصة التأثيرات التشوهية، ومن هذا المنطلق كان هدف الدراسة هو معرفة التأثيرات الضارة لمبيد الميثوميل على الإناث الحوامل للفأر الأبيض الألبينو أثناء فترة تكشف الأعضاء الجنينية (أى في الفترة من اليوم السابع إلى اليوم الثانى عشر من الحمل) باستخدام جرعات مختلفة من مبيد الميثوميل وهي، ١٠/١ من الجرعة نصف المميته (٣.٣٣ ملليجرام/ كيلوجرام من وزن جسم الفأر) و ٢٠/١ من الجرعة نصف المميته (١.٦٦ ملليجرام/ كيلوجرام من وزن جسم الفأر) بتكرار نفس المعاملة لمدة خمسة أيام متتالية من اليوم السابع إلى الثاني عشر من الحمل عن طريق الفم، وكانت التغيرات النسيجية في بعض الأعضاء الداخلية للأمهات المعاملة كالتالى: أوضحت نتائج فحص أنسجة الكبد للأمهات المعاملة بالميثوميل وجود التالى: مناطق واضحة لموت الخلايا، خلايا رغوية، نشاط خلايا kuffer والخلايا الملتزمة وفجوات تحطمية، وبفحص كلية الأمهات المعاملة فقد لوحظ وجود تغيرات فى الخلايا المبطننة للأنايب الكلوية، واحتقان ونزيف فى الأوعية الدموية للنسيج البيني مع تآكل بعض الخلايا فى الأنايب المجمعمة، وكذلك وجود تغيرات نسيجية أخرى تدل على السمية الكلوية، وبالنسبة للرئة فقد لوحظت تضخمات وتقرحات فى القصيبات الهوائية مع زيادة عدد الخلايا الطلائية المبطننة وذلك عند المعاملة بالجرعة ١٠/١ من الجرعة نصف المميته وكذلك ظهور بعض الفراغات نتيجة اضمحلال بعض القصيبات الهوائية، وفى حالة القلب ظهرت بعض الاستطالات فى العضلات القلبية مع تصلب فى بعض اللبفات العضلية ووجود ارتشاح فى العضلات القلبية، ومن الممكن أن نستخلص من هذا البحث أن مبيد الميثوميل له تأثيرات سامة سيئة على حيوانات التجارب وبالتالي يستوجب الحذر الشديد عند استخدامه فى برامج مكافحة الآفات الزراعية، ولا يوصى باستخدامه فى حقول محاصيل الخضر والفاكهة حتى لا تصل متبقيات المبيد إلى المستهلك.

المحكمون :

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