

The histological and histochemical changes in the kidney of rabbit induced by diazinon

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Abstract

Diazinon (DZN) is a commonly used Organophosphorus (OP) pesticide to control a variety of insects in agriculture and in the environment. The aim of this project is to study the effects of organophosphorous pesticide (Diazinon) on histological and histochemical of kidney in male rabbit. The present study is based upon observations made on 24 male Baladi rabbits, which was divided into: 3 groups: Eight animals each, the whole body of the rabbit was dipped in 10 liters of the diazinon solution, while sparing the head for 10 seconds. Group one (control group) was dipped in water only. Animals of Group 2; were dipped in diazinon solution dipped every 2 days for 4 weeks, and Group 3 were dipped in diazinon solution every 7days for two months. Decreased in body weight was noticed in group 2 and group 3 may be due to increases in the exposed doses and increased accumulation of DZN in the blood of the exposure animals. Histological changes in group 2 and 3 are similar, renal tubules were severely damaged with cytoplasmic vacuolation and atrophy of some glomeruli. Blood capillaries in between the degenerated tubules were congested; the urine spaces are widening, and fibrosis inters tubules in group 3 only. Exposure of animals to diazinon caused extensive changes in histopathologically as well as histochemically in the kidney of group 2 and group 3. But very marked changes in group 3.

Keywords: Diazinon, kidney

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التغيرات النسيجية والكيمياء النسيجية التي يحدثها عقار الديازينون في كلية الارانب

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فرع التشريح، كلية الطب، جامعة الموصل، الموصل، العراق

الخلاصة

الديازينون: هو المبيد الفوسفوري العضوي الذي يستخدم عادة للسيطرة على مجموعة متنوعة من الحشرات في الزراعة وفي البيئة، هدف هذا المشروع هو دراسة التغيرات النسيجية و الكيمياء النسيجية في كلى ذكور الأرانب المعرضة للمبيد الحشري العضوية الفسفوري (ديازينون). تستند هذه الدراسة على الملاحظات التي أجريت على 24 من ذكور الأرانب البلدي، والتي تم تقسيمها إلى ثلاثة مجموعات: ثمانية حيوانات لكل مجموعة، نغطس الجسم كله في 10 لترا من محلول الديازينون، في حين نرش الرأس لمدة 10 ثواني. و نغطس المجموعة الأولى (المجموعة الضابطة) في الماء فقط. نغطس حيوانات المجموعة 2 كل 48 ساعة و لمدة 28 يوم، ونغطس المجموعة 3 كل 7 أيام و لمدة 56 يوم. التغيرات النسيجية في المجموعة 2 و 3 كانت الأنابيب الكلوية قد تضررت بشدة، وأظهرت بعض خلاياها فارغه من السيتوبلازم وتضخمت بعض الكبيبات. احتقان الشعيرات الدموية الموجودة ما بين النبيبات الكلوية، تليف داخل الأنبوب، الباحة البولية متوسعة. فقدان وزن الجسم في المجموعة 2 و 3 وهذا قد يكون راجعا إلى زياده في عدد الجرعات وبالتالي يؤدي زيادة تراكم السموم في دم الحيوانات المعرضة. تعرض الحيوانات للديازينون تسبب تغيرات واسعة في الأنسجة وكذلك الكيمياء النسيجية في الكلى وكانت التأثيرات ملحوظة جدا في المجموعة 2 و المجموعة 3. ولكن هذه التغيرات تكون اكثر وضوحا في المجموعه الثالثة.

Introduction

Organophosphorous compounds (OP) are part of large group of pesticides that have been developed in the last 50 years. Diazinon is the common name of an organophosphorous insecticide used to control pest insects (1). Diazinon poisonings effects on mammalian cells were studied by researchers (2), Diazinon toxicity induce oxidative stress resulting in hematological changes, neurotoxicity, hepatotoxicity, nephrotoxicity, cardiac toxicity and both male and female reproductive toxicity (3).

Diazinon inhibits the enzyme acetyl cholinesterase (AChE), which hydrolyzes the neurotransmitter acetylcholine (ACh) in cholinergic synapses and neuromuscular junctions. This results in abnormal accumulation of ACh in the nervous system (4).

Diazinon exposure, whether from ingestion, skin contact, or inhalation can result in nervous system health effects, these effects may include watery eyes, running nose, and loss of appetite, coughing, urination, diarrhea, stomach pain, and vomiting. Typically, these symptoms are followed by head and body tremors, muscle tetany, stiffness, weakness with paresis and paralysis. Tachycardia and mydriasis are also possible with massive oral exposures. Central nervous system signs and symptoms may also be present such as restlessness and/or hyperactivity, depressed respiration, anxiety, depression, clonic-tonic seizures, and coma (5).

The effect of diazinon on body weight was controversial, some studies showed significant decreased in body weight. (6-8) Significant weight loss was appeared after 20 days of dermal contacts with the toxicant (9), with toxic effect of different doses of diazinon on body weight (10).

Histopathological examination showed lesions in kidney tissues produced by Diazinon, and we observed shrinkage of glomerulus, tubular dilation, glomerular hypercellularity, hypertrophy of tubular epithelium, degeneration of renal tubules, interstitial edema, necrotic cells in the glomerulus and renal tubules (11-12).

Materials and methods

Experimental rabbits

Twenty-four male local rabbits weighing 1.2-1.4Kg were housed in the Experimental research unit, College of Medicine, University of Mosul, Mosul, Iraq and used in the present study, at controlled light and temperature and provided with chow and fresh tap water. The whole body of the rabbit was dipped in 10 liters of the diazinon solution, with sparing the head for 10 second. While the control group were dipped in water only. used diazinon-60 EC, made in Jordon (VAPCO), was used as a dip or spray after

dilution with water.1ml/ 1liter, the LD50 of diazinon in males is 250 mg/kg body weight.

Experimental design

The experimental rabbits were randomly divided into three symmetric groups, eight animals for each. Group 1: The first group was dipped into tap water and served as a control group. Group 2: Animals were dipped in diazinon solution every 48 hrs. For 28 days. Group 3: Animals were dipped in diazinon solution every 7days, for 56 days.

Histological procedures

Specimens of kidney were dissected and washed with normal saline, and then cut into slices with an average thickness of 4-5mm, the slices were fixed for more than 24 hours in 10% neutral buffered formalin (13), dehydration of the slices were made in ascending through ethanol alcohol series, using 70% ethanol for 1 hour, followed by two changes of 90% ethanol with a period of 1 hour each, and then two changes of absolute ethanol with the second one left over night, after dehydration the slices cleared twice with xylene for 1 hour each, and then embedded in three changes of paraffin with a melting point of 56-58c, 1 hour each, the section mounted on the glass slides, and kept for 30 minutes in the vacuum-drying oven at 60 deparaffinization in two changes of xylene for 5 minutes each was carried out, then the section transferred to graded ethanol, absolute, 70% and 95% ethanol, two minutes each, and finally, the sections were hydrated in the distilled water for 2 minutes to be ready for staining.

Results

Physical Observations

The animals of the control group stayed alive till the end of the experiment. They were active, responded very quickly to stimuli, and they had good appetite, whereas the animals of the group 2 and 3 suffered from a decrease in activity, and their response to stimuli and food intake was less than normal. Itching, diarrhea.

Histological Findings (Group 2 and group 3)

In comparison to control group (Fig. 1), the sections of kidney showed glomeruli were atrophied, and their cells showed cytoplasmic vacuolation, there is inter renal tubules fibrosis in group 2 only, the urine spaces are widened, diffuse prominent congested blood vessels in some glomerulus, infiltrations of lymphocyte in glomerulus (Fig. 2 and 3).

Histochemical findings

A strong positive reaction to Periodic acid Schiff's (PAS) and Bests carmine (B.C) was seen in the cytoplasm of proximal convoluted tubular epithelium, the tubular

luminal brush border, basement membrane of tubules, basement membrane of glomerular capsule and the glomeruli, (Fig. 4 - 9).

Body's weight results

Clinical signs indicated that diazinon induced toxicity was shown by a marked decrease in body weight in group 2 and group 3.

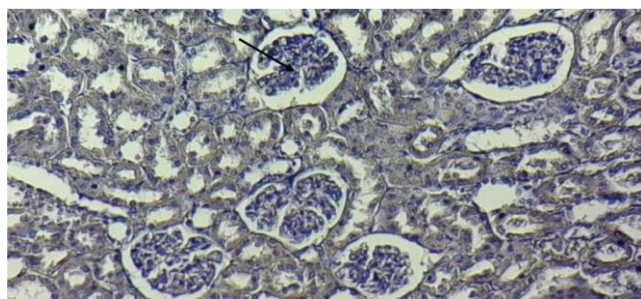


Fig. 1: Photomicrograph from rabbit kidney of a control rabbit showing normal renal tubules (blue arrows) and glomerulus (black arrows), (X 200).

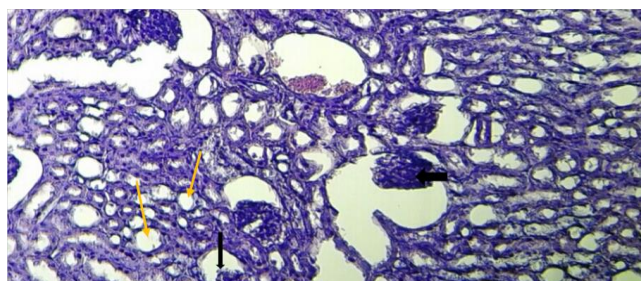


Fig. 2: Photomicrograph from rabbit kidney, of group 2, treated with diazinon for 4 weeks showing atrophy of a glomerulus (black arrows) with degeneration in the lining epithelial cells of renal tubules (yellow arrows), (X 200).

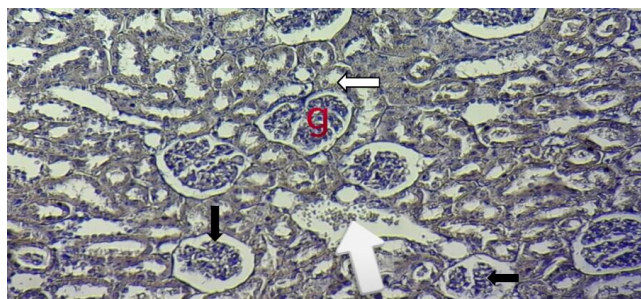


Fig. 3: Photomicrograph from rabbit kidney, of group 3, treated with diazinon for 8 weeks showing atrophy of a glomerulus (black arrows) with degeneration in the lining epithelial cells of renal tubules (d), and congestion between renal tubules (white arrow) (X 200).

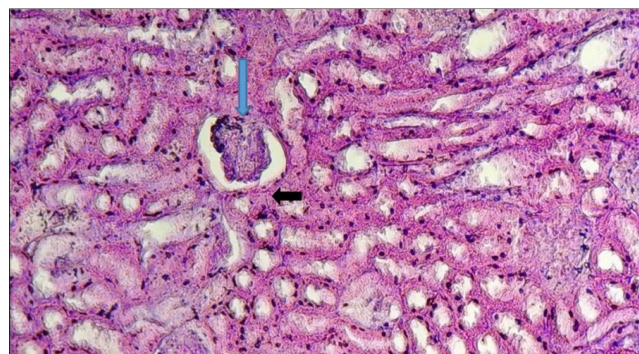


Fig. 4: Photomicrograph from rabbit's kidney of group 1 showing strong positive reaction to PAS stain, demonstrates the prominent BB, glomerulus's (blue arrow) given red reactions in the basal lamina of the capillary loops.

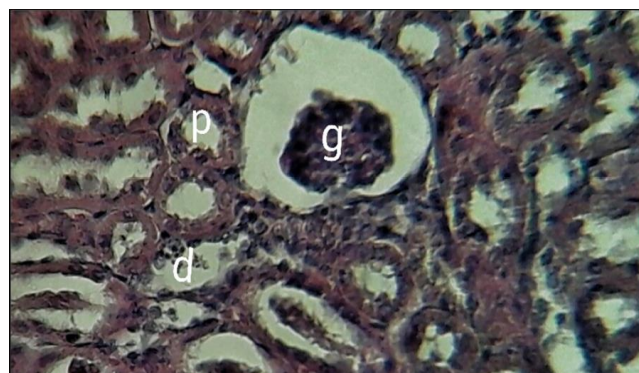


Fig. 5: Photomicrograph from rabbit's kidney of group 1 showing strong positive reaction to Best's carmine stain, demonstrates the prominent BB, glomerulus given magenta reactions in the basal lamina of the capillary loops.



Fig. 6: Photomicrograph from rabbit's kidney of group 2 showing positive reaction to PAS stain, demonstrates the prominent BB, glomerulus's (blue arrow) given red reactions in the basal lamina of the capillary loops.

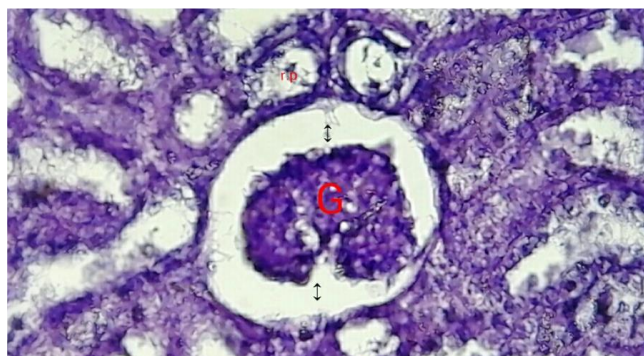


Fig. 7: Photomicrograph from rabbit's kidney of group 2 showing positive reaction to Best's carmine stain, demonstrates the prominent BB, glomerulus given maganed reactions in the basal lamina of the capillary loops, widened of urinary space.

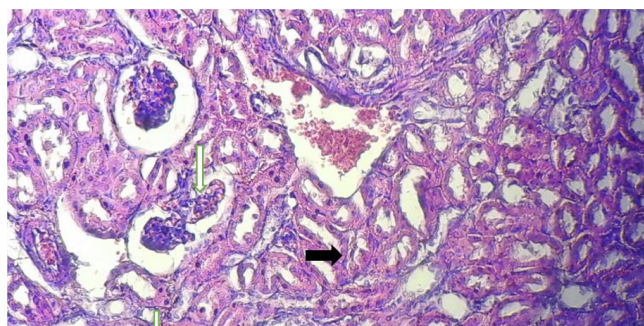


Fig. 8: Photomicrograph from rabbit's kidney of group3 showing strong positive reaction to PAS stain, demonstrates the prominent BB, glomerulus's given red reactions in the basal lamina of the capillary loops, atrophy of glomeruli (white arrows), blood and congestion (black arrow).

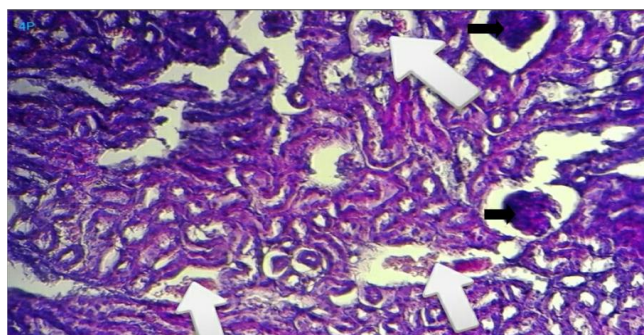


Fig. 9: Photomicrograph from rabbit kidney of group 3 showing strong positive reaction to Best's carmine stain, demonstrates the prominent BB, glomerulus given maganed reactions in the basal lamina of the capillary loops, blood and congestion (Wight arrow), atrophy of glomeruli (black arrows).

Discussion

Body weight

In the present study, body weight marked decrease, reduced food consumption and diarrhea was the most important signs of toxicity in the exposed animals.

In group 2, and 3 marked body weight reduced were noticed this is may be due to increases in the exposed doses and increased accumulation of DZN in the blood of the exposure animals.

The present results are generally similar to those reported by Tawab et al. (8), Arab (9) and Maryam et al. (10).

Histological findings

The kidney was the first tissue for toxicant accumulation in the animals exposed to diazinon. The high concentration of diazinon in the renal tissue samples was predictable because of the increased renal blood flow, and it filters large amounts of toxins which can concentration in the kidney tubules (14).

Group 1 (control group)

Kidney tissue of the control group revealed normal histological structures, similar to those observed by other authors (15-17).

Group 2 and group 3

kidney section revealed histological changes which include that of the glomeruli were atrophied with destruction in the lining epithelial cells of renal tubules, cytoplasmic vacuolation and congested blood capillaries in between the degenerated tubules, the urine spaces is widen, diffuse prominent congested blood vessels in some glomerulus, infiltrations of lymphocyte in glomerulus and interstitial and no any changes in endothelial of renal tubules, in group 3 only showed fibrosis inter renal tubules.

This is agreement with the reported mentioned that the glomeruli were hypertrophied. After 4 weeks, the renal tubules were severely damaged and their cells showed cytoplasmic vacuolation and atrophy of some glomeruli. Blood capillaries in between the degenerated tubules were congested. These histopathological alterations in kidney were severer in animals examined in group 2 and group 3 which agreement with result of (18-22).

The histological changes in kidney we can see mild histological changes in group 2 because of Increased time of exposure (28 days), the stepwise increases in the exposure doses (14 doses) through four continuous weeks.

But in group 3 marked histological changes because of the time of exposure of diazinon are long time (for 56 days). Due to increased accumulation (every 7 days) of DZN in the blood of the exposed animals shows the readily

absorption of diazinon through dermis and its potential to access into different tissues. The stepwise increases in the exposure doses through eight continuous weeks (8 dose) lead to high absorption and high concentration of the toxicant in the blood.

Histochemical findings (control group)

The kidney cortex revealed strong PAS-positive in the Basement membrane (BM) and on the brush borders (BB) of the proximal convoluted tubules (23).

In the present study, PAS and Best's carmine was moderately positive in the cells of proximal convoluted tubules and weak positive in the epithelial cells of distal convoluted tubules in control group.

Group 2

In this groups positive reaction to PAS was seen in cytoplasm of proximal convoluted cells, and on the brush borders. Weak positive reaction in the cytoplasm of proximal convoluted cells, and on the brush borders.

Group3

In this groups strong positive reaction to PAS and Best's carmine were seen in proximal convoluted cells, and on the brush borders.

The glycogen content of proximal convoluted tubular cells was more than in the distal tubular cells which is may be related to the high metabolic activity of these cells which would make them having more glycogen compared to that in the cells of the distal convoluted tubules as well as the later are less metabolically active (24).

Decreased glycogen in the proximal tubules was observed after DHC exposure. It may be related to the diazinon treatment induced destruction in the tubular brush border. This makes the tubules lose their physical and chemical protection (25) and in agreement with the decreased glycogen content in brush borders and disruption of Bowman's capsules (7).

Conclusion

Diazinon induced marked decreased in body weight in group 2 and 3. Varies degrees of harmful changes in the histological structure of kidney were observed according to the duration of action of the used diazinon, in the exposed rabbits. It is concluded that diazinon causes depletion in the total carbohydrates in the kidney tissues and this effect were linked with the concentration of the organophosphate. The histochemical finding in groups 2 (positive reaction) are mild effect as compared with group 3 (strong positive reaction).

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