HISTOPATHOLOGICAL CHANGES THAT INDUCED IN THE INTERNAL ORGANS OF WHITE RAT AFTER EXPOSURE TO DIAZINON

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ABSTRACT

A pesticide diazinon is a widely used and easily available in the world so, the general health risks of this compound for environment, human and animals were investigated with emphasis on histopathological effects by oral gavage. Sixteen adults laboratory rats from tow sex with diazinon solution for about six weeks. The animals were divided into two groups with (8)animal in each group. The control group was given orally distill water, while the experimental group was given high dose of diazinon 10mg/ml for the first three weeks and up to 20mg/ml for the last three weeks.

The most important histopathological changes observed in the internal organs (liver, kidney, heart muscle, lung, stomach and nervous tissue) of the experimental rat include congestion, degeneration, fibrosis, necrosis, vaculation and edema in compared with the control animals.

INTRODUCTION

Diazinon (DZ) is a contact organophosphorus pesticidewith broad spectrum insecticide activity (1). It has been widely used throughout the world to control flies, lice and other insect pests of ornamental plants and food crops (2) as well as a veterinary ectoparasiticide(3). The extensive use of this compound has caused great concern due to
the hazardous side effects on human beings as well as wild and domestic animals (4). This compound is a colorless fluid, with the boiling temperature of 84°C, 12 hours half life (in animals body) and 300 mg/kg lethal dose in rats (5, 6).

The toxic effects of diazinon on animals were studied by some investigators (7,8). Diazinon was also found to lead to alterations in blood factors, plasma testosterone and glucose levels in male rats (9,10). However, it may induce imbalance in the free radicals production/elimination processes with consequent induction of cellular damage (11,12,13,14). Additionally, several studies showed that DZN was capable of inducing histopathological, biochemical and physiological alterations (15,16,17,18,19, 20). Diazinon exposure caused organ pathologies in mouse, such as necrotic degeneration of spleen and thymus, hyperplasia of thymus, spleen and lymph nodes, and sometimes haemorrhage from all tissues (21,22). The prolonged exposure to the most commonly used agricultural pesticides increase the risk of the lung lesions, as well as cancer, in the farmers and commercial pesticide users (23). Also, the histological changes in the alimentary canal of experimental animals due to pesticides exposure have been documented (24,25).

Diazinon affects mainly the nervous system regardless of the route of exposure (26).(27) reported that diazinon induced histopathological changes in liver and pancreas of rats. However, several studies showed that diazinon exposure led to an increase in lipid peroxidation with tissue specific alterations in the liver, kidney, heart, testis and brain (28,29,30).

The aim of this study is to examine the histopathological effects of the diazinon pesticide on some organs (liver, kidney, heart muscle, lung, stomach and sciatic nerve) of adults laboratory rats.
MATERIALS AND METHODS:-

Determination of diazinon:-
The test procedure was done as in (31) from 60% standard of diazinon which contain 100 ml take 1 ml added 5 ml of distilled water which is act 10 mg/kg and 2 ml added 10 ml of D.W. which act 20 mg/kg which give to animals.

Experimental Animals:-
The study was done on 16 rats divided 2 groups, the first one as control group of 8 rats include 4 males and 4 females gave orally distill water, the other group of 8 rats include 4 males and 4 females which is act as high toxicity group treated with 10 mg/ml of diazinon for the first three weeks and up to 20 mg/ml for the last three weeks of the experimental period. The study was run for six weeks by oral gavage.

Histopathological parameters

Procedure of Tissue Processing
In brief the routine sequence of events according to (32) as follows:-
After obtained the tissue from necropsy of animals after 6 weeks from the experimental design. Fix it for 24 hours or more in an appropriate fixative buffered formalin 10%. Dehydrate through ascending alcohol (increasingly higher concentration) alcohols Overnight. And then Replace alcohol (clear) with xylene. Then infiltrate with paraffin. Embed in a block of paraffin. Cut thin sections on the microtome (5μm- thick). Mount the section on glass slides. And remove (dissolve) the embedding medium by putting the slides on hot plate overnight. Then rehydrate the sections in descending alcohols. Stain the section with an appropriate staining sequence (H&E).

Staining Procedure
In the staining procedures used haematoxylin and eosin stains according to (33) for paraffin sections

RESULT
In the last 3 weeks of study of diazinon were given orally, the rats had severe damage in the stomach. The most important results of histopathological changes of
experimental rats of the internal organs (liver, kidney, heart muscle, lung, stomach and nervous tissue) as follows:-

**Liver:** The liver of experimental rats showed congested central vein with degeneration of hepatic cells (figure 1). Increase amount of fibrosis in septal region with proliferation of bile duct cells lining (figure 2).

**Kidney:** The epithelial cells of renal tubules are swelling and tubular necrosis in some other cells (figure 3).

**Heart:** The heart showed marked vacuolation of cardiac muscle cells with area of infiltration adipose tissue between myocardium cells (figure 4).

**Lung:** The lung of experimental rats suffered areas of congested blood vessels, edematous fluids in dilated alveoli and emphysema (figure 5).

**Stomach:** The stomach of experimental rats showed degeneration of superficial mucous membrane of gastric glandular region (figure 6) and present ectopic non-glandular region in glandular stomach area which act as proliferative non glandular epithelium appear as preneoplastic lesion (figure 7).

**Sciatic nerve:** The sciatic nerve of experimental rats revealed vacuolated, degenerated of the nerve fiber with areas of interstitial edema (figure 8).

**Brain:** After experimental period end microscopic examination of brain tissue shows pericellular edema (figure 9).
(Fig. 1) Section of rat liver treated with diazinon. Show congested central vein (c) & degeneration of hepatic cells (D). X40. H&E

(Fig. 2) Section of rat liver treated with diazinon show congested central vein (c) & septal fibrosis (f). X10. H&E

(Fig. 3) Section of rat kidney treated with diazinon. Show degeneration of epithelial cells (d) and tubular necrosis of other cells (n). X40. H&E

(Fig. 4) Section of rat myocardial muscle treated with diazinon. Show vaculated of cells (v). X10. H&E.
(Fig. 5) Section of rat lung treated with diazinon. show dilated alveolar (a) and emphysema (e), congestion B.V. (c) X10. H&E.

(Fig. 6) Section of rat stomach treated with diazinon. show degeneration of glandular region of superficial mucosal membrane (d) X10. H&E.

(Fig. 7) Section of rat stomach treated with diazinon. show Ectopic non glandular region (Ec). X10. H&E.

(Fig. 8) Section of rat sciatic nerve treated with diazinon. show vacuolated nerve fiber (v), interstitial edema (e) X10. H&E.
DISCUSSION

The toxic chemicals have been found in a variety of samples including water, air, soil and house dust and their presence has been noted in the tissues of non-occupationally people particularly in the adipose tissue, blood and urine(34). Result of the present study revealed that diazinon insecticide caused different histopathological changes were manifested in myocardial muscle cells, brain and sciatic nerve like vacuolated in cells, desquamation of epithelial cells, hemorrhage because of the tissue protein and polysaccharides were generally inhibited in various animals to which organophosphorus was applied. This result supports the findings of (35,36,17,37) who reported the levels of blood total protein were decreased in experimental animals exposed to DZN and other pesticide. Diazinon is a synthetic organophosphorous compound with a broad-spectrum insecticidal activity (38). The main mechanism of action of diazinon is acetyl-cholinesterase enzyme inhibition (39), however, it may induce imbalance in the free radicals production/elimination processes with consequent induction of cellular damage (39,40,38,41). Several experimental and clinical studies have reported diazinon induced toxicities on several organs(38,41,42,43).

The main histopathological alterations in the stomach of experimental rats supports the finding of(44) who reported desquamation, hemorrhage and necrosis of the epithelial cells of the stomach and intestine were noticed post α-cypermethrin insecticide
oral administration in rats. Also, the histological changes in the alimentary canal of experimental animals due to pesticides exposure have been documented(24),(45) discussed that such these histopathological changes in the epithelial cells of the mucosal membrane and digestive gland is known to be a protective mechanism that an animal usually shows in response to chemical or toxic stresses. Moreover, These layers were hemorrhagic as revealed by the presence of excess blood cells. Besides, the stomach tissue showed clumping of blood cell in the lamina propria just beneath the mucosal epithelium which exposed to pesticides. The toxic effects of diazinon on animals were studied by some investigators (7, 8).

The major histopathological investigation of the internal organs (heart, lung liver, and kidney) of the experimental rats revealed that the diazinon pesticides are pathogenic to rats. The histopathological changes induced by diazinon toxicity which caused multi-organ injuries(20). This result supports the findings of (46,47,1,4) who reported that the diazinon can be highly toxic for animals and human kind. Additionally, several studies showed that DZN was capable of inducing histopathological, biochemical and physiological alterations (15,16,17,18,14,19).

Organophosphorus insecticideides are generally short-lived and tend not to accumulate in plant or animal tissues to any great extent. They are considered as anticholin esterase insecticides and the mechanism by which they elicit their toxicity is identified and is associated with the inhibition of nervous tissue (48,49,50) and other neurophysiological abnormalities(51). (2) reported that s diazinon induced histopathological changes in liver and pancreas of rats. Diazinon acts on the nervous system through the inhibition of the acetylcholine esterase activity at the synapses and neuromuscular junctions and is manifested by overstimulation of acetylcholine receptors and impeded neurotransmission(52). The ubiquitous distribution of both nicotinic and muscarinic cholinergic receptors together with induction of oxidative stress in various tissues(53) may have genotoxic, immunotoxic, nephrotoxic, hepatotoxic, and cardiotoxic effects (54).

Diazinon affects mitochondrial membrane transportation and cytochrome P450 system in hepatocytes (55,56) Administration of diazinon to rats resulted in depletion of
glycogen from the brain and peripheral tissues (57). It has also been shown that diazinon caused an increase in lipid peroxidation in rat erythrocytes (58). Diazinon treatment in rats decreased renal antioxidants and enhanced lipid peroxidation with concomitant renal damage, which are involved in the diazinon-induced renal oxidative stress and toxicity (59).

This study indicates that the diazinon induced histopathological alterations such as congestion, cellular proliferation, vacuolar degeneration, tubular necrosis and edema; this result in agreement with the submission of(10), who reported that, diazinon induced histopathological alterations in the liver of rabbit. The liver showed congestion of veins, leucocytes infiltrations, cytoplasmic vacuolation of the hepatocytes and fatty degeneration. The liver is the primary organ involved in xenobiotics metabolism and is a major target organ for chemicals and drugs. Hepatotoxicity is therefore an important end point in the evaluation of the effect of particular xenobiotics. Clinical chemistry and histopathological evaluations are commonly used methods for detecting organ-specific effects related to chemical exposure (60,61,62). The necrotic conditions observed in the liver of PARA and DIA-treated animals are in corroboration with the observed biochemical changes, wherein an increased level of lipid peroxidation was noticed. Previous studies showed that PARA cause mild focal hepatitis in the lobules and portal areas (63,64).

The present results showed that diazinon treatment led to degeneration of renal tubules, hypertrophy of glomeruli and leucocytic infiltrations. These results indicated that diazinon metabolites caused toxicity in renal system; and the immune system makes a good role for defending against foreign particles. The effect of diazinon on kidney was studied in different animals. Oral administration of diazinon for 2 months to male albino rats showed degeneration of the renal tubules (65). (66) reported that exposing mice to diazinon caused degeneration of renal tubules, atrophy of glomeruli and interstitial inflammatory cells infiltrations. The results of (20,67,16,68,69,17) showed that rats treated with DZN display a pronounced impairment in renal function which is confirmed by the increase of serum creatinine, BUN and uric acid levels, and histopathological alterations. However, the present high activity of
serum CK and LDH demonstrated that the cellular membranes integrity of myocardial tissues may be disturbed. Furthermore, several investigations showed that the exposure to DZN led to cardiotoxicity accompanied with an increase of serum CK and LDH levels in rats and mice (15,16,17,19). Moreover, (4), also reported that oral sub lethal dose of DZ induces time-dependent histopathological lesions in the lung of rats included, edema, congestion, hemorrhage and infiltration of mononuclear cells showed similarity with those recorded in the several previous investigations (70,71,72). It has been shown that these histopathological changes could be associated with the chemical-induced decreases in the antioxidant status of the animal body (73). The congestion of the lung vasculature might impose excessive pressure on the neighboring structures leading to malnutrition, deficient oxygenation and the accumulation of the excretory products(72). (25) results showed that the exposure to the sub lethal doses of diazinon insecticide induced dose-dependent histopathological lesions in the lung of guinea pig. These lesions were mostly represented in the infiltration of the macrophages and mononuclear cells, hemorrhage, congested blood vessels, edema, pyknosis and necrosis.

In conclusion, this study demonstrated a direct correlation between diazinon pesticides exposure and histopathological changes observed in the internal organs (heart, lung, liver and kidney, stomach, brain and sciatic nerve) depend on concentration and period of exposure. Finally, it is hoped that the results of this study will be useful for educating people on health risks that induced by diazinon pesticides exposure and planning of health care. Also, more researches should be done on the genotoxic and cytotoxic effects of the diazinon pesticides.
التأثيرات المرمية النسيجية التي تحدث في الأعضاء الداخلية للجرذان البيضاء بعد التعرض للديازينون
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الخلاصة
المبيد ديازينون واسع الاستخدام ومتوفر بسهولة في العالم لذلك، تم التحقيق في المخاطر الصحية العامة لهذا المركب للبيئة والبشر والحيوانات مع التركيز على التأثيرات المرمية النسيجية عن طريق إعطاء (16) حيوان مختبري من الجرذان البالغة من كلا الجنسين بمحلول الديازينون عن طريق الفم لمدة سنة أساسية. قُمَّت الحيوانات إلى مجموعتين مع (8) حيوانات في كل مجموعة. أعطت مجموعتي السيطرة ماء مقطر عن طريق الفم، في حين أعطت مجموعة التجربة جرعة عالية من الديازينون 10 ملي/مل للأسابيع الثلاثة الأولى وتصل إلى 20 ملي/مل في الأسابيع الثلاثة الأخيرة.

أهم التغييرات النسيجية التي لوحظت في الأعضاء الداخلية (الكبد والكلى و عضلة القلب والرئة والمعدة والأنسجة العصبية) من حيوانات التجربة وتشمل الاحتباس، انحفاظ، نخر، التليف، نزف، وذمة بالمقارنة مع حيوانات السيطرة.

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