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The Histological Effects of Diazinon on the Liver and Kidneys of Rats

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ABSTRACT

Introduction: Diazinon is an organophosphate pesticide that has widespread applications in both agriculture and household settings. Diazinon poisoning can have detrimental effects on the cardiovascular, gastrointestinal, and central nervous systems. The aim of this study was to investigate the effects of chronic exposure to diazinon on histological features of the liver and kidney of rats.

Materials and Methods: Twenty adult male Wistar rats, aged 10 - 12 weeks and weighing 150-200 grams, were purchased and divided into two groups of 10. In the treatment group, diazinon was administered at a dose of 20 mg/kg/day to each animal for 3 months, while the control group was maintained on a normal diet and drinking water. After 12 weeks of exposure, the animals were prepared for H & E staining, and the samples were examined under a light microscope.

Findings: The structural integrity of the liver and kidney was preserved in the diazinon-treated group compared to the control group, but mild effects of diazinon were observed in the liver tissue, such as foci of inflammation and hyperplasia of Kupffer cells, and in the kidney, such as the presence of epithelial cells in the distal tubule and cell detachment from the basement membrane.

Conclusion: Based on the results of this study, the rats exposed to diazinon did not show significant changes in histological features in the liver and kidney, and these changes were subtle, possibly indicating a need for higher doses or longer exposure durations to diazinon.

Keywords:

Choronic; diazinon; histological changes; liver; kidney.

INTRODUCTION

Various chemical compounds are used to control pests and insects, every year. Uncontrolled use of these pesticides leads to food contamination in the environment (1, 2). Organophosphate pesticides, such as diazinon, are chemical agents widely used in agricultural and household applications. Owning to their extensive use and easy accessibility, poisoning from these substances poses a global problem for human and animal health, accounting for over 50% of all poisoning cases worldwide (3). Diazinon is an organophosphate insecticide that has significant applications in both agriculture and household. It has many harmful effects on humans and animals (4), including cardiotoxicity (5), neurotoxicity (6), and alterations in blood factors, plasma testosterone levels, and blood glucose levels (7, 8). Compared to other pesticides, these compounds exhibit the highest toxicity among vertebrates, and their use in agriculture has led to severe environmental contamination and acute and chronic poisoning. Therefore, there is growing public concern regarding the accumulation of these insecticides in food products and water reservoirs (9). These pesticides phosphorylate the amino acid serine at the active site of the enzyme acetylcholinesterase, forming a strong bond with the enzyme, which inhibits it and increases acetylcholinesterase levels, leading to cholinergic disruption (10). In addition to their enzymatic effects, many studies have focused on the increased release of free radicals, such as nitric oxide (NO), in the body and oxidative stress caused by these compounds (4, 5, 11). Exposure to diazinon results in increased serum creatinine levels due to kidney damage and liver cirrhosis, minor changes in the

pancreatic tissue, necrosis, and hemorrhage of hepatocytes, along with elevated levels of AST, ALT, and serum glucose (12). Therefore, considering the above, the aim of this study was to investigate the effect of diazinon on histopathological indices of the liver and kidneys of male rats.

MATERIALS AND METHODS

Animal

In this case-control study, 20 male Wistar rats weighing between 150-200 grams were obtained from the Pasteur Institute of Iran. They were then kept in groups of four in standard conditions of 12 hours light and 12 hours dark, with a temperature range of 22 to 25 $^{\circ}C$ and normal humidity, in the animal room of Alborz University of Medical Sciences. All animals had free access to food and water. The study protocol was approved by the ethical committee of Alborz University of Medical Sciences, Iran (ethical code: IR.ABZUMS.REC.1399.275).

Experimental Procedure

The animals were randomly divided into two groups of 10. The treatment group received a dose of 20 mg/Kg/day of diazinon (dissolved in drinking water) for 3 months via gavage (13, 14), which corresponds to one-quarter of the LD50 (lethal dose for 50% of the population) of this toxin. During this period, the control group was provided with a normal diet and drinking water. After the completion of 12 weeks, for histological examination, the liver and right kidney of the animals were removed under deep anesthesia with chloral hydrate at a dose of 80 mg/Kg.

Hematoxylin and Eosin (H and E) Staining

The liver and right kidney were removed and fixed in 10% formaldehyde solution for at least 48 hours. The liver and right kidney were then processed and embedded in paraffin. Next, the tissue preparation steps were performed according to standard protocols. The samples (cubic shape of $1\times1\times1\ cm^3$) were labeled and washed in running tap water for at least 2 hours before processing in serial alcohol, xylene, and melting paraffin using an automatic tissue processor (Autotecknicen). The serial steps were 70 % ethanol, 80% ethanol, 2 steps of 95% ethanol, 2 steps of xylene, and 2 steps of paraffin. The duration used in each step was 1 hour and 30 minutes. The processed tissue was then embedded in a paraffin block. The paraffin-embedded liver and kidney were transversely sectioned at 5 µm by microtome (Didsabz Company). The sectioned tissues were then stained with hematoxylin and eosin (H and E). One section out of each 10 sections was considered (10 sections for each tissue) using light microscope (NiKon Company) equipped with projecting camera (KECAM Company) to assess the staining cells of the liver and right kidney.

Histopathological Indices of Liver and Kidney

Histopathological Indices of the Liver

The liver tissue was evaluated based on the condition of hepatocytes (cellular swelling, fatty changes, and cholestasis), portal tracts (proliferation of bile ducts and cholestasis), and the lobular structure of the liver (presence of lymphocytes, condition of the limiting plate, and preservation of the lobular structure of the liver).

Histopathological Indices of the Kidney

The histopathological indices of the kidney were examined concerning proximal and distal tubules (cellular detachment from the basement membrane, cytoplasmic staining changes, and nuclear changes concerning karyorrhexis), glomeruli (glomerular size, cellularity, and condition of the glomerular basement membrane), interstitial tissue (vascular changes, presence of inflammatory cells, and fibrosis), and acute tubular necrosis (ATN).

Statistical Analysis

The data were analyzed qualitatively based on the observed slides under the microscope.

RESULTS

Qualitative evaluation of diazinon-induced hepatotoxicity

In the histopathological study of the liver in the control and diazinon-treated groups, the hepatocyte condition, portal tracts, and the status of the lobular structure of the liver were examined. The hepatocytes appeared normal, cuboidal, or polygonal with a red cytoplasm and one or two spherical nuclei. The lobular structure of the liver was maintained in both control and diazinon-treated groups. Mild foci of inflammation in liver cells (lobular hepatitis) were observed in tissue sections of the diazinon-treated group. A slight increase in collagen fibers and mild edema were also noted, indicative of regeneration (Figure 1).

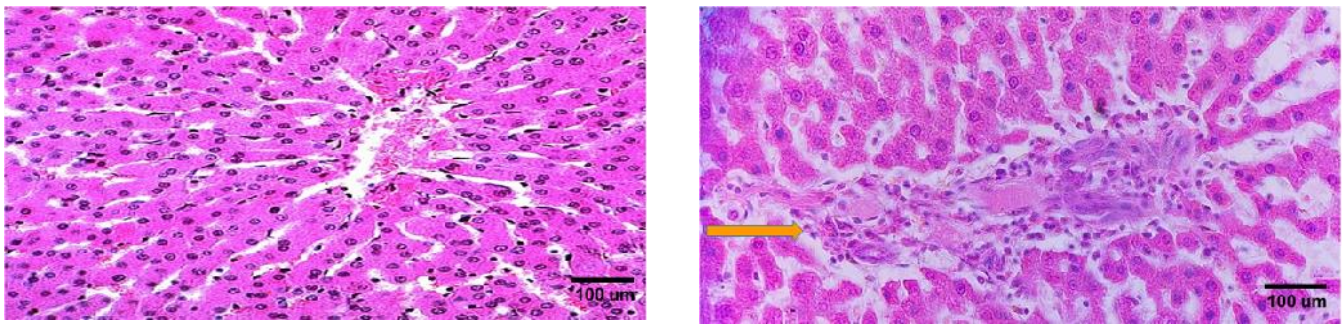

Figure 1: Histological aspects of diazinon in the liver tissue of the control and diazinon-treated groups of rats. In the left image (control group), the liver revealed normal histological features with normal shape and distribution of the hepatocytes with cytoplasm, and one or two spherical nuclei. There was no evidence of lymphocytic infiltration. In the right image (diazinon - treated group), lymphocyte infiltration was observed (orange arrow), indicating lobular hepatitis. H & E, x100, scale bar = 100 um.

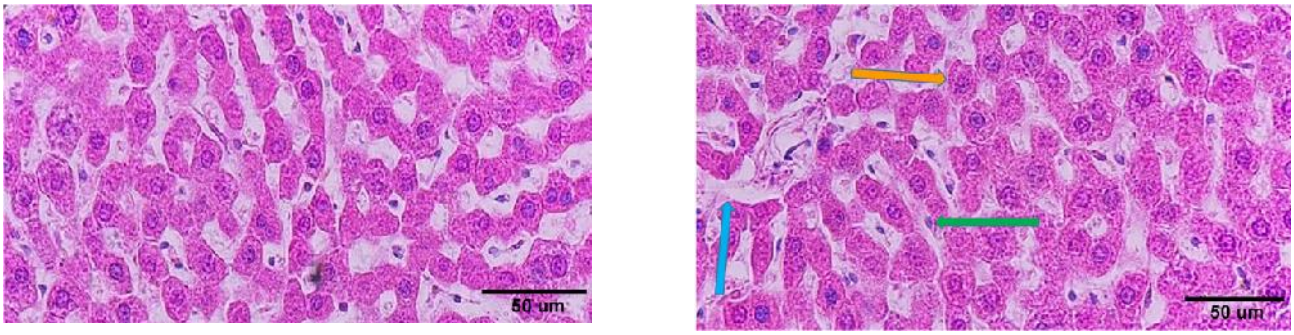

Figure 2: Photomicrographs of the liver tissue exposed to diazinon in the control and diazinon - treated groups of rats. The hepatocytes appeared normal, cuboidal, or polygonal in the left image (control group). The number of Kupffer cells increased in the right image (diazinon-treated group). Blue arrow (sinusoids), green arrow (Kupffer cells), orange arrow (hepatocytes). H & E, x400, scale bar = 50 um.

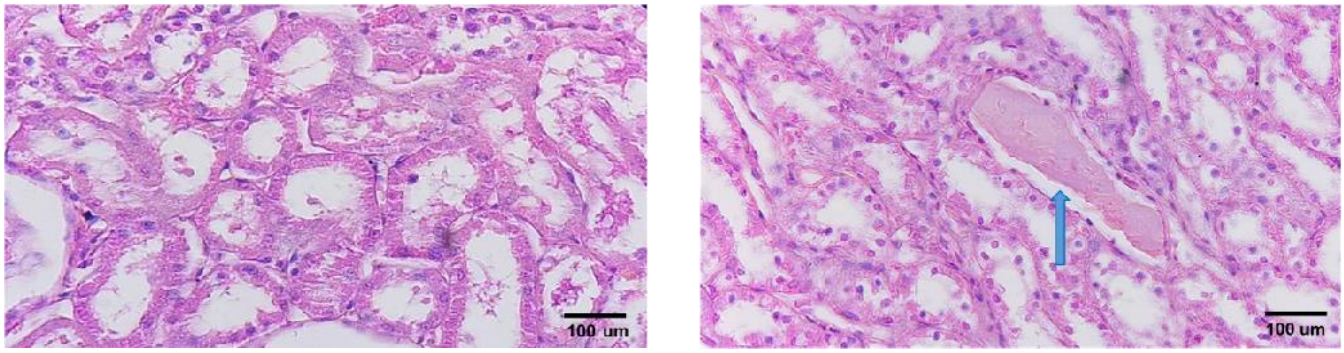

Figure 3: Effect of diazinon on the kidney tissue of the control and diazinon-treated groups of rats. In the left image (control group), the parameters of the proximal and distal tubules, glomeruli, and interstitium were normal. In the right image (diazinon-treated group), hyaline casts were visible in the collecting tubule. The blue arrow indicates hyaline casts. H & E, x400, scale bar = 100 um.

The presence of numerous Kupffer cells - tissue macrophages of the liver - alongside endothelial cells in the diazinon-treated group is particularly noteworthy. Higher-than-normal numbers indicate hyperplasia in this group of cells. However, overall, this is a non-specific finding that can also be observed in other pathologies (Figure 2). In general, histological changes in the diazinon-treated group were subtle.

Qualitative evaluation of diazinon-induced nephrotoxicity

In the histopathological study of the kidneys in the control and diazinon-treated groups, parameters of the proximal and distal tubules, glomeruli, and interstitium were examined.

The blood vessels in both the control and diazinon-treated groups were normal. No evidence of edema or infiltration of inflammatory cells was observed. Upon detailed histological examination, no fibrosis or vascular changes were observed. Overall, there was no evidence of acute tubular necrosis (ATN) in these tissues. Cross-sections of the collecting ducts were observed in the medulla. A small amount of hyaline cast was observed in the collecting tubules of the kidney in the diazinon-treated group (Figure 3).

The proximal tubules contain a single layer of cuboidal cells adjacent to the glomeruli on the basement membrane. Examination of the glomeruli of both the control and diazinon-treated groups revealed a double-layered Bowman's capsule surrounding them. No significant pathology was observed in the cortical areas or Bowman space. The size of the glomeruli was the same and normal in both control and diazinon-treated groups and the glomeruli in both groups exhibited normal cellularity. In the diazinon-treated group, a slight detachment of cells from the basement membrane was observed in the cortex, indicating signs of cellular ischemic injury. Additionally, slight vascular congestion was noted (Figure 4, right image). Overall, the kidney changes in the diazinon-treated group, such as those in the liver were subtle.

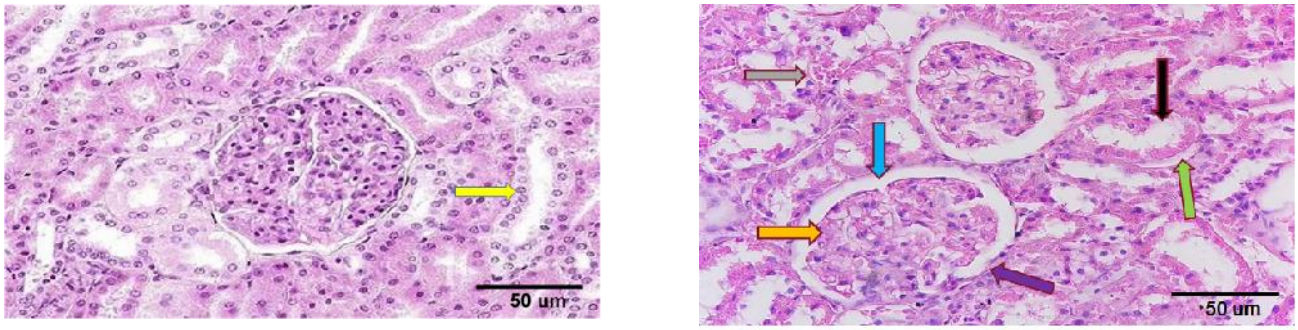

Figure 4: Cross-section of the kidney tissue from the control and diazinon-treated groups of rats. In the left image (control group), all proximal tubule cells had nuclei (yellow arrow). There were no signs of congestion and the cells were locate on the basement membrane. Cell detachment from the basement membrane was observed in the right image (diazinon-treated group), along with slight vascular congestion. Purple arrow (Bowman's capsule), blue arrow (Bowman's space), orange arrow (glomerulus), gray arrow (vascular congestion), green arrow (cell detachment from the basement membrane), and black arrow (distal tubules). H & E, x100, scale bar = 100 um.

DISCUSSION

Organophosphate pesticides are chemical agents that are widely used in agriculture. Owning to their extensive use and easy accessibility, poisoning from these pesticides presents a global problem for human and animal health (3). Several factors, such as the dose used, route of exposure, absorption percentage, physical and chemical properties, and extent of detoxification by the body, play a role in the severity and duration of poisoning (9). The acute toxicity of diazinon, which is characterized by its irreversible inhibitory effect on acetylcholinesterase and the mechanism of chronic toxicity in humans and animals, is also a concern (15). Although the acute toxicity effects of this compound have been assessed, its sub-acute and chronic toxicities have not been thoroughly investigated. Chronic exposure to low doses of diazinon induces neurological diseases, including memory impairment, movement abnormalities, and anxiety-like behaviors (16) . Therefore, this study aimed to evaluate the effects of chronic exposure to diazinon and observe histological changes in the liver and right kidney of rats.

The findings from this study indicate that treatment with diazinon for three months at a dose of 20 mg/kg/day, compared to the control group, did not cause significant pathological changes in hepatocyte status, portal tracts, and lobular structure of the liver. However, changes, such as mild foci of inflammation in liver cells, slight regeneration phenomena, and hyperplasia of Kupffer cells, were observed in the liver tissue of the diazinon-treated group.

In kidney tissue, the status of the proximal and distal tubules, glomeruli, and interstitium in the diazinon-treated group was almost similar to that of the control group. Nonetheless, evidence of cellular damage was noted, including signs of cell detachment from the basement membrane, anucleated proximal tubular cells, and epithelial cells in the distal tubule.

Previous studies have examined the effects of diazinon on various tissues, demonstrating the destructive effects of this pesticide. In a study conducted in 2013, rats were exposed to low (30 mg/kg), moderate (60 mg/kg), and high (120 mg/kg) doses of diazinon for 30 days on a daily basis. In the low dose group, vacuolation of hepatocytes, lymphocyte infiltration in liver parenchyma, and vascular congestion were observed, with overall mild morphological changes (17).

In the moderate dose group, significant

hepatocyte vacuolization, increased diameter of central veins, increased number of Kupffer cells, and notable lymphocyte infiltration were observed. Significant lymphocyte infiltration and severe congestion were observed in the high dose group. In all treated mice exposed to diazinon at all dosages, the area of hepatocytes decreased compared to that in the control group. Additionally, the nuclear area of all mice in the diazinon group showed a reduction in volume, but none of these changes were statistically significant.

Histopathological changes in the kidneys of diazinon-treated mice exposed to low doses included degeneration of glomeruli, cortical hemorrhage, hypertrophy of Bowman's capsule cells, and glomerular atrophy. In the diazinon group exposed to moderate doses, significant lymphocyte infiltration, clear vascular congestion in the cortex, hypertrophy of Bowman's capsule cells leading to glomerular absorption, congestion of capillaries within the glomeruli, and lymphocyte infiltration in the medullary area were observed.

Histopathological changes in the kidneys of diazinon-treated mice exposed to high doses were more pronounced than those in other exposure groups. In this group, many glomeruli disappeared, and extensive vascular congestion in the cortex was visible. This study found that glomerular infiltration was more prominent in mice from groups exposed to moderate doses. The results of this study in all mouse groups exposed to different doses of diazinon showed a significant decrease in the number of glomeruli and renal corpuscles in the diazinon group compared to the control group. The findings in the liver tissue were similar to those in our study, but in the kidney tissue, even at low doses, they contradicted the results of our study. In our study, the numbers of glomeruli and renal corpuscles were normal. In a study by El-Shenawy et al. on the effects of diazinon on kidney and liver changes in mice after 14 days of exposure, contrary to the results of previous study, exposure to low doses of diazinon (30 mg/kg) did not cause changes in the histopathology of the mouse liver. However, exposure to higher doses results in sinusoidal dilation, severe lymphocyte infiltration in all portal spaces, and hepatocyte vacuolization. In the kidney tissue, this study also indicated that exposure to low doses of diazinon had little effect on histological changes, with observed changes limited to slight dilation of the Bowman's capsule and glomerular atrophy. However, at high doses, lymphocyte infiltration in the cortex area and extensive vascular congestion were observed (18).

Diazinon directly inhibits acetylcholinesterase (AChE) and its oxidation product, diazoxon is an even more potent inhibitor of the enzyme (19). In addition, oxidative stress has been suggested as an additional mechanism of action for organophosphorus pesticides, such as diazinon, particularly relating to chronic effects on the central nervous system (20). Diazinon increased intracellular levels of reactive oxygen species and lipid peroxidation in the myocardial cells of rats administered diazinon in a single 235 mg/kg oral dose (21). Diazinon increased cellular levels of oxidized glutathione without varying the levels of reduced glutathione. Although diazinon-induced cytotoxicity was not changed by cholinergic antagonists, it was reduced by the calcium chelator, BAPTA-AM. Together, these findings indicate that diazinon cytotoxicity includes glutathione-modulated production of ROS and may include intracellular calcium homeostasis (22).

Evidences concerning the potential of diazinon-induced endocrine effects are restricted. One study reported testicular atrophy and arrested spermatogenesis in three male dogs administered encapsulated diazinon at a dose of 20 mg/kg/day for 8 months (15).

Micro-RNA (miRNA) expression is highly vulnerable to environmental toxicants, stress, or medications (23). Li et al. in 2024 reported the underlying molecular mechanisms associated with diazinon-induced hippocampal toxicity were mediated by integrated miRNA and mRNA profiling. They stated that aberrations in ECM-receptor interaction, AMPK signaling pathway, and MAPK signaling pathway may be involved in hippocampal neurotoxicity activated by diazinon (24).

According to the above cellular and molecular mechanisms, diazinon is capable of genomic and non-genomic damage, although in the present study, this damage was subtle because of the low dose of the toxin.

CONCLUSION

In this study, the histological changes in the

liver and kidney tissues of rats exposed to diazinon, were mild and minor compared with those in the control group. Additionally, the results indicated that the destructive effects of diazinon were more pronounced in kidney tissues than in liver tissues, as evidenced by the broader pathological changes in the kidneys. By comparing the dose used in this study (20 mg/kg/day) with other doses in different studies, it can be noted that diazinon is capable of causing serious damage to the liver and kidney tissues at higher concentrations. Therefore, it can be concluded that the effects of diazinon were highly dose-dependent.

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CONFLICT OF INTEREST

All authors claim that there are no conflicts of interest.

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