MEDICAL RECORDS-International Medical Journal

Research Article

Investigation of the Effects of Pendimethalin on Liver and Kidney Tissue in Mice by Histological Methods

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Abstract

Aim: Pesticides, which provide many benefits in terms of production and yield, can also be toxic substances that can harm human health. In this study, the effects of pendimethalin (PND), a type of pesticide, on the liver and kidney tissue of mice were examined by histopathological methods.

Material and Method: A total of 30 mice were used in our study. They were divided into 5 groups, 6 mice in the control group and 6 mice in the other groups. One of the groups was reserved as the control group. No application was made to the control group. Group I received 0.1 mg/l PND, group II received 0.2 mg/l PND intraperitoneally on the 1st and 3rd days of the study. Group III received 0.1 mg/l PND intraperitoneally and 0.1 mg/l vitamin A and C orally, group IV received 0.2 mg/l PND intraperitoneally and 0.1 mg/l vitamin A and C orally on the 1st and 3rd days of our study. On the 4th day of our study, the experimental animals were sacrificed by cervical dislocation method under general anaesthesia. Liver and kidney tissues of the mice were histopathologically examined under light microscope.

Results: In our study, sinusoidal enlargement and vascular congestion were observed in the liver tissue of the I. experimental group, while tubular dilatation and intertubular vascular congestion were observed in the kidney tissue. In the second experimental group, in addition to the similar findings in the first experimental group, an increase in the number of pyknotic nuclei and Kuppfer cells in the liver tissue and loss of cells and disrupted areas in the tubules in the kidney tissue were observed. In addition, the findings in this group were more pronounced. In the third experimental group, histopathological findings were similar to those in the first group. Similarly, the findings in experimental group IV and experimental group II, which were given vitamin A and C, were similar.

Conclusion: In conclusion, our findings showed that PND negatively affected the histology of liver and kidney of mice. Vitamins A and C did not contribute positively to these histopathological findings.

Keywords: Liver tissue, kidney tissue, pendimethalin, histopathology

INTRODUCTION

As a result of the rapid increase in the world population, the increase in urbanisation activities in agricultural areas requires maximum yield from the products. This has made the use of pesticides almost mandatory. Therefore, pesticides are recognised as essential substances all over the world (1). Chronic toxicity occurs when pesticides are exposed to pesticides for a certain period of time at long-term or low dosages. Carcinogenicity, mutagenicity, teratogenicity, oncogenicity, liver damage, reproductive disorders, neural damage and allergic symptoms can be listed as defects resulting from chronic effects (2).

Pendimethalin (PND), which is included in the herbicide class among the pesticide types classified according to the field of use, is a dinitroaniline group member. In the studies conducted, PND has been identified as a substance that pollutes water resources (3-6). It also adversely affects water resources as well as air and soil (7). The United States Environmental Protection Organisation (EPA) has classified PND as a probable human carcinogen (8). Another study concluded that PND exposure may induce tumour development (9). Increased incidence of cancer has been found to be associated with PND exposure by some agricultural health study committees (10-12). PND compound has the potential to cause problems in the functioning of the endocrine system (13). Due to the toxic effects of pesticides on non-target organisms and the negative effects on natural resources, there is a need for a more comprehensive evaluation of pesticides (14,15).

CITATION
Ersan Y, Iscen NT. Investigation of the Effects of Pendimethalin on Liver and Kidney Tissue in Mice by Histological Methods. Med Records. 2025;7(1):263-7. DOI:1037990/medr.1565351

Received: 11.10.2024 **Accepted:** 17.12.2024 **Published:** 15.01.2025
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Studies on PND exposure on the liver and kidneys of freshwater fish were carried out and histopathological changes were found (16).

When the necessary literature is reviewed, it is seen that the protective effect of vitamin A and C on the liver and kidney tissue of mice against PND toxicity has not been investigated and there is a need for further studies in this field. In this study, it was aimed to make examinations for the need and to provide an original work to the literature. Mouse liver and kidney tissues were histopathologically examined to determine the damage caused by PND and to see the effect of vitamin A and C in treating the damage. Thus, it was aimed to determine whether vitamins A and C would be effective in preventing the damage that PND exposure may develop in living organisms.

MATERIAL AND METHOD

Experimental Animals

In the study, 30 Balb-c female mice were used. The mice used were 84 days old at the beginning of the study and weighed 32-35 g on average. The experimental animals were housed in polycarbonate standard type 3 cages for 12 hours day and 12 hours night, 08.00 in the morning and 08.00 in the evening. Mice were fed ad libitum with tap water and standard feed. The ambient temperature was kept between 22-24°C, humidity was kept between 45-50% and ventilation was provided automatically.

Work Plan and Groups

A preliminary study was conducted before the study. Firstly, it was observed that mice given 0.5 mg/l PND could not survive at this dose for a long time. Likewise, mice were lost at 0.4 mg/l PND dose. At the last dose of 0.2 mg/l, although the mice were restless, they survived. Thus, 0.2 mg/l high dose was determined as 0.1 mg/l low dose in the study.

In the study, the experimental animals were divided into 5 groups consisting of 6 mice, one of which was the control group. One group was divided as the control group. In group I, 0.1 mg/l PND was administered intraperitoneally (ip) on the 1st and 3rd days of the experiment. In group II, 0.2 mg/l PND was administered ip on days 1 and 3 of the experiment. The animals in group III were firstly given 0.1 mg/l PND by ip route and then vitamin A and C were given orally on the 1st and 3rd days of the experiment. In group IV, 0.2 mg/l PND was administered ip, then vitamin A and C were administered orally on the 1st and 3rd days of the experiment.

Tissue Collection and Histological Examination

At the end of the 3rd day, the experimental animals were sacrificed by cervical dislocation method under anaesthesia. Then, the liver and both kidneys were dissected by making a vertical incision in the periumblical region. The tissues were placed in 10% formaldehyde solution and fixed in formaldehyde for 14 days. After fixation was completed, the tissues were kept in tap water overnight to remove the fixative. Complete removal of the water permeating the tissue was achieved by passing through alcohol series. The tissues, which were rendered transparent in xylol to make them transparent to light, were embedded in paraffin blocks. From the obtained blocks, 5 µm thick sections were taken using a microtome (DIAPATH-Galileo Auto). The sections were floated in 38°C water to open the folds and placed on slides. After the sections were opened, they were left to dry in an oven at 37°C on the slides.

After the sections were taken, the tissue samples placed on the slides were stained with haematoxylin-eosin (H&E) double stain. After staining, the slides were covered with coverslips using entellan. Photographs were taken from the obtained preparations in a Light Microscope (LEICA DM 2500) with a digital camera (LEICA DMC 4500) attachment and transferred to digital media for evaluation.

Differences in the H&E method used in dyeing are due to the waiting time in the alcohol used after dyeing. Such phenomena are natural in new and old solutions. You mentioned that bars should be used, the magnification criteria are specifically stated for each image. In addition,the use of bars is not deemed appropriate since the objective lens gives the magnification result more clearly. Here the phrase “increasing the number of image and better explaining the lesions at large and small magnification” is normally true. The cursors here are thought to be more meaningful since they were selected for each texture. I would like to thank you especially for your useful criticism. This work only possible within our means.

RESULTS

In this study, when the data obtained from the control group were examined under light microscope, it was observed that the liver lobule had a normal structure. Hepatocytes were observed to be arranged in a radial pattern. The vena centralis, portal area, sinusoids and sinusoidal Kupffer cells and endothelial cells in the liver tissue were observed in normal structures. Sinusoids were normal in terms of their width and arrangement (Figure 1A, 1B). In the kidney tissue of the control group, normal renal tubule and glomerular structure in the cortex and medulla and regular interstitial space were present (Figure 1C, 1D).

In our study, irregularities in hepatocyte arrangement and necrotic areas were present in group I in which low dose PND was administered. Sinusoidal areas were enlarged and mononuclear cell infiltration was observed in the vena centralis. Vascular congestion in the portal area was also among the findings we observed (Figure 1E, 1F). Tubular dilatation and intertubular vascular congestion were observed in group I kidney tissue. There were also necrotic areas in places (Figure 1G, 1H).

In group II in which high dose PND was administered, histopathological changes were quite remarkable. There was vascular congestion covering almost the entire vena centralis. Necrotic areas and pyknotic nuclei were observed. Sinusoidal areas were more dilated compared to group I. Kuppfer cells were observed more prominently. The presence of erythrocytes in the sinusoids was also observed (Figure 1I, 1J). Haemorrhage foci in the cortex and Bowman's capsule, loss of cells in the proximal tubules and distal tubules were observed. These findings were more prominent compared to the findings in group I.

Degeneration was observed in hepatocytes in group III in which we applied low dose PND, which was one of the groups in which we administered vitamin A and vitamin C. There was also some enlargement in the sinusoids. A slight change was observed in mononuclear cell infiltration in the vena centralis compared to group I in which we administered low dose PND. Tubular dilatation and vascular congestion were observed in group III kidney tissue. Necrotic areas were found to be almost similar to group I. According to these results, the healing effect of vitamins A and C was not significantly observed in our study.

Histopathological findings were found in all groups. These findings included mononuclear cell infiltration, sinusoidal dilatation, vascular congestion, pyknotic nucleus, hepatocyte degeneration and necrotic area in the liver. In the kidney tissue, tubular dilatation and degeneration, intertubular vascular congestion, degeneration in Bowman's capsule were observed as haemorrhage foci and necrotic areas. Group II was found to be the most affected group. It was concluded that this was related to the dose increase. In the groups receiving vitamin A and C, histopathological findings were almost similar to those in the groups receiving the same dose of PND.

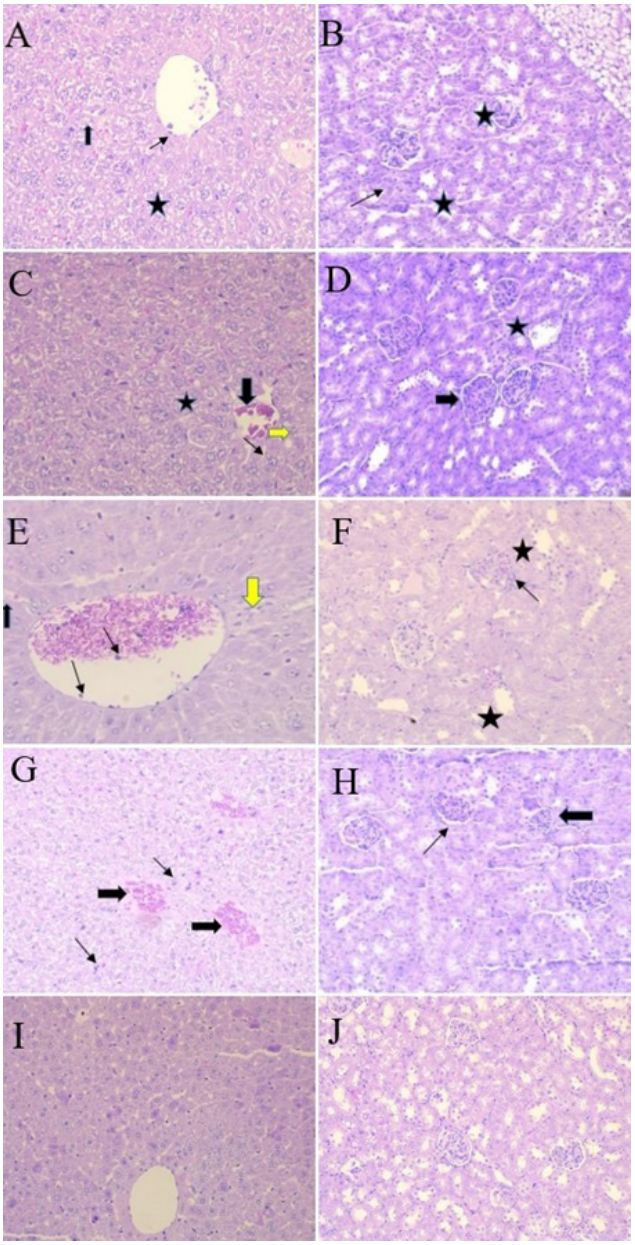

Figure 1. Histopathologic findings liver tissue of group I (**A**). Hepatic mononuclear cell infiltration (thin arrow), necrotic areas (asterisk) and dilatation of sinusoidal areas (thick arrow) seen in the vena centralis H&E X20. Kidney tissue of group I (**B**). Tubular dilatation (asterisk) and intertubular vascular congestion (thin arrow). H&E X20. Mouse liver tissue from group II (**C**). Vascular congestion in the vena centralis; (thick arrow) sinusoidal dilatation, (thin arrow) necrotic area (asterisk) and pyknotic nucleus (yellow arrow). H&E X20. Kidney tissue from group II (**D**). Foci of hemorrhage (thick arrow) and tubular dilatation (asterisk) are seen in Bowman's capsule and cortex. H&E X20. Liver tissue from group III (**E**). Vena centralis shows mononuclear cell infiltration (thin arrow) sinusoidal dilatation (thick arrow) and hepatocyte degeneration (yellow arrow). H&E X40. Kidney tissue from group III (**F**). Tubular dilatation (asterisk) and vascular congestion (thin arrow) are seen H&E X20. Mouse liver tissue from group IV (**G**). Vascular congestion (thick arrow) and Kuppfer cells (thin arrow) in the vena centralis H&E X20. Kidney tissue from group IV (**H**). Vascular congestion in Bowman's capsule (thin arrow) and Bowman's capsule with narrowed borders (thick arrow) are seen. H&E X20. Images of liver (**I**) and kidney (**J**) tissue in the control group.

DISCUSSION

Chemical pesticides have been an agent that has helped nations in their endeavours to eradicate insect-based problems, ensure adequate food supplies, and protect agricultural lands and forests. However, overuse of more toxic and cheaper pesticides, especially in developing countries, leads to acute health problems and causes environmental and global pollution (17). The damages of pesticides that cause changes in antioxidants and cause ROS formation have been clearly demonstrated (18,19). Vitamins A, C and E are vitamins with antioxidant properties (20). In our study, it was wondered how vitamin A and vitamin C, which are antioxidants, would affect the damage caused by pesticides, and vitamin A and C were included in the study.

In measurements made in various soil types, it was observed that heterotrophic activity was adversely affected where PND was intensively present (21). The consumption of agricultural products grown in soils contaminated with PND causes people to be exposed to this pesticide in some way. Therefore, PND was considered to be a pesticide type that should be investigated and used in our study.

El-Sharkawv et al. evaluated the toxic effect of PND on commercially important fish by measuring growth performance, biochemical parameters, histopathological findings and genotoxic effect. The results showed that there were significant decreases in body weight and weight gain in fish exposed to PND depending on the doses given, while serum glucose, aspartate amino transaferase (AST), alkaline phosphatase, total protein and cholesterol were significantly increased. When liver and kidney tissues were examined histologically, necrotic areas and degenerative changes were observed similar to our study (22).

In a study using low dose PND, oxidative stress, DNA damage and mitochondrial dysfunction triggering apaptosis were evaluated in human lymphocytes and rat bone marrow cells. It was found that PND stimulated micronucleus formation showing clastogenic potential. The results showed that DNA damage was 35.6 times higher in PND-treated human lymphocytes. In addition, imbalance in antioxidant enzymes was observed. In this study, histopathological findings were found to be consistent with our results. In conclusion, PND has been shown to have genotoxic and apoptotic potentials in human and animal test models (23).

In the study conducted by Ahmad et al. male rats were given PND at various doses of 62.5, 125 and 250 mg/kg. Toxic effects were evaluated in terms of oxidative stress, DNA damage, histopathological changes, stimulation of anti-inflammatory and apoptotic responses. Significant changes were recorded in oxidative stress indicators and antioxidant defence mechanisms in liver and kidney tissues. Significant DNA damage was also detected in this study. PND-induced cellular stress induced anti-inflammatory and apoptotic changes. When histopathological changes were examined, leukocyte infiltration, pyknotic nuclei, necrosis, large Bowman's capsule and narrowed renal cortex were observed in liver and kidney tissues. The results of this study showed that PND causes cellular toxicity and genetic disorders affecting normal physiological functions in rats (24). Similar histopathological findings were found in our study. Unlike this study, Bowman's capsule was narrowed in our study.

In a study conducted on Nile tilapia fish, four groups were formed, one of the groups was separated as a control group, the other two were given two different doses of PND, and the fourth group was given Moringa plant, which may show antioxidant effect in addition to PND, similar to our study. At the end of this period, haematological and biochemical changes and oxidative stress biomarkers were analysed. PND treatment caused significant decreases in haemoglobin concentration in white and red blood cells, while PND treatment caused significant decreases in hemoglobin concentration in white and red blood cells, significant increases were detected in serum total protein, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), creatinine, uric acid, glucose, cortisol, cholesterol and lactate dehydrogenase (LDH) levels. On the other hand, serum total protein, albumin, globulin and acetylcholinesterase (AChE) decreased. Hepatic superoxide dismutase (SOD), catalase (CAT), total antioxidant capacity (TAC) and glutathione peroxidase (GSH-Px) levels were significantly increased compared to the control group. Addition of Moringa oleifera leaf extract to water overcame the negative effects of pendimethalin and the parameters examined were almost normalised compared to the control group (25). In our study, similar to this study, we investigated the protective effects of antioxidant vitamins A and C on the toxic effects of PND given together with PND. Unlike this study, no significant normalisation was observed in our study. This was attributed to the fact that only two doses of vitamin A and C were given.

In this study, histopathological findings were detected in all groups. These findings were observed as mononuclear cell infiltration, sinusoidal dilatation, vasculer congestion, pyknotic nükleus, hepatocyte degenerasyon and necrotic area in the liver. Tubuler dilatation and, degeneration, intetubuler vasculer congestion, degeneration in Bowman's capsule were observed as bleeding foci and necrosis areas in the kidney tissue. The most affected group is II. İdentified as a group. It was concluded that situation was due to dose increase. This histopathological findings in the groups receiving the same dose of PND.

CONCLUSION

According to the results of the study, it was observed that PND adversely affected liver and kidney tissue. Longer-term studies with different doses of PND will be useful to evaluate the damages of this pesticide in more detail. In addition, we think that the antioxidant effects can be seen more clearly when the doses of vitamin A and C and the number of days on which the mice are given are increased.

Financial disclosures: *The study was conducted with project code 2069 at Karabük University Supported by the Scientific Research Projects (BAP) Coordination Unit. Karabük University supported the study with project code no. 2069.*

Conflict of interest: *The authors have no conflicts of interest to declare.*

Ethical approval: *For this study, Saki Yenilli Experimental Animal Production and Application Laboratory Permission for the study was obtained with the decision of the Local Ethics Committee for Animal Experiments (Decision No: 07-24.04.2019).*

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