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Histopathological effects of chronic fluorosis on the liver of mice (Swiss albino)

Yusuf ERSAN[1], Evren KOÇ[2], İsmail ARI[3], Başaran KARADEMİR[4]

Aim: Fluoride intake in high doses has toxic effects on various organs. Chronic fluorosis results in tubular degenerations, inflammation, fibrosis, parenchymatous nephritis, cloudy swellings, and dilations of convoluted tubules. In addition to these effects, fluoride causes deteriorative effects on the skeleton, teeth, and soft tissues. The goal of this study was to examine the impacts of chronic fluorosis on the liver tissues of mice.

Materials and methods: One control group and 3 experimental groups, each group consisting of 4 male and 4 female mice, were formed to conduct the experiment. A stock solution including 2000 ppm fluoride was prepared by solving 4.44 g of NaF in 1 L of distilled water. Water with 10, 20, and 40 ppm fluoride was obtained by diluting the stock solution to give to the animals. Animals in the control group had free access to tap water with 0.3 ppm fluoride. Animals in experimental group 1 were orally given distilled water with 10 ppm fluoride, water with 20 ppm fluoride was orally given to the animals in experimental group 2, and the animals in experimental group 3 were orally given distilled water containing 40 ppm fluoride for 90 days. The animals were killed under light ether anesthesia to obtain specimens from the livers. Slides were prepared under the light microscope to examine histopathological anomalies and then photographs were taken.

Results: Histopathological disorders were observed on the slides prepared from the liver specimens of the animals exposed to chronic fluorosis depending on doses of chemicals given to the animals. Hyperemia, local necrosis, hydropic degeneration, vacuolar degenerations, and swelling on hepatocytes around the central vein were detected.

Conclusion: Depending on the doses of fluoride applied to the animals, degenerations in the tissues around the central vein increase.

Key words: Chronic fluorosis, mouse, liver, histopathology

Kronik florozisin fare (Swiss albino) karaciğeri üzerine histopatolojik etkisi

Amaç: Yüksek dozda alınan flor, vücuttaki çeşitli organlarda toksik etki oluşturur. Kronik florozis, tübüler ve parankimatik iltihaplara, bulanık şişkinliklere ve sarmal tübüllerin genişlemesine neden olur. Bu etkilere ilaveten, flor, iskelet, diş ve yumuşak dokular üzerinde kötü etki oluşturur. Bu çalışmanın amacı, kronik florozisin karaciğer üzerine etkisini belirlemektir.

Yöntem ve gereç: Deney yapılmak üzere her bir grubu 4 dişi ve 4 erkek fareden oluşan 1 kontrol ve 3 deney grubu oluşturuldu. Sodyum florün stok çözeltisi hazırlanıp, bu stok çözeltisinden deney grubundaki hayvanlara verilmek üzere 10 ppm, 20 ppm, ve 40 ppm'lik flor içeren çöeltiler deney grubundaki hayvanlara 90 gün verildi. Kontrol grubundaki farelere ise 0,3 ppm flor içeren içme suyu verildi. Hayvanlar hafif eter anestezisi altında karaciğerden örnek almak için öldürüldü. Preparatlar ışık mikroskobunda histopatolojik bozuklukları incelemek için hazırlandı ve daha sonra fotoğrafları çekildia.

Bulgular: Hayvanlara verilen kimyasalın dozuna bağlı olarak kronik florozise tabi tutulan hayvanların karaciğer örneklerinden hazırlanan preparatlarda histopatolojik bozukluklar gözlendai. Hiperemia, local nekrozis, vacuolar dejenerasyon ve merkezi topardamar çevresindeki hepatositlerde şişmeler gözlendi.

Sonuç: Hayvanlara uygulanan florun dozuna bağlı olarak, merkezi toplardamarın çevresindeki dokularda dejenerasyon (hydropic degeneration) artmaktadır.

Anahtar sözcükler: Kronik florozis, fare, karaciğer, histopatoloji

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[1] Department of Biology, Faculty of Arts and Sciences, Kafkas University, 36100 Kars - TURKEY
[2] Department of Biology, Faculty of Arts and Sciences, Kafkas University, 36100 Kars - TURKEY
[3] Department of Biology, Faculty of Arts and Sciences, Kilis 7 Aralık University, 79000 Kilis - TURKEY
[4] Department of Internal Diseases, Faculty of Veterinary Sciences, Kafkas University, 36100 Kars - TURKEY
Correspondence: *İsmail ARI, Department of Biology, Arts and Sciences Faculty, Kilis 7 Aralık University, 79000, Kilis - TURKEY*
E-mail: ismailari@kilis.edu.tr

Introduction

Excessive intake of fluoride for a prolonged period can induce chronic fluorosis. Because mineral supplements such as calcium phosphate and limestone often contain high levels of fluoride, chronic fluorosis of animals can result in great economic loss in animal production. Fluoride produces deteriorative effects on the skeleton, teeth, and soft tissues. In recent decades, numerous investigations have focused on the relationship between fluoride and free radical reactions. Many studies indicate that excessive fluoride can induce free radical toxicity in human and animals (1). Fluoride has toxic effects on various body organs. Two or 4 weeks' exposure of rabbits to 10 and 50 mg of NaF/kg led to hypoplasia of bone marrow, markedly decreased total nucleated cell count, and anemia. Children living in industrial regions polluted by fluoride had decreased hemoglobin levels and an increase in the number of erythrocytes in peripheral blood (2). In rats intoxicated with 190, 210, 452, 904, and 1356 ppm fluoride in their drinking water, renal injury was reported. Other changes included tubular degeneration, inflammation, fibrosis, parenchymatous nephritis, cloudy swellings, and dilations of convoluted tubules. It was reported that renal degeneration and mineralization occurred in cattle ingesting high fluoride levels (3). The rare occurrence of acute fluoride poisoning is an outcome of exposure to excessive amounts of the chemical (4). In this case, various disorders develop, depending on the results of the binding impact of fluoride to calcium and suppressing effects of the chemical upon enzymes as an outcome of a low amount of calcium, high level of potassium, and decrease of oxygen utilizable in the cells of the stomach, intestine, lung, heart, brain, kidney, and muscle. In chronic fluoride toxicity (which is a result of intake of high levels of fluoride by the body over a long period), disorders in the bones, thyroid gland, pituitary gland, hypothalamus, testes, and teeth are important (5).

Fluoride intake to the body can occur via diets consisting of plant and animal products and breathing air from the atmosphere, and drinking water. Fluoride is rapidly absorbed by the lungs and gastrointestinal system at the rate of 95% (4). It was reported that the concentration of fluoride in drinking water in the provinces in eastern Turkey is varied (6,7). The chemical when absorbed is rapidly distributed into the intracellular and extracellular water of tissues by systemic circulation. More than 90% of the total body burden is retained in the bones and teeth (8).

The major route for the removal of fluoride from the body is the kidneys (8). Removal of a minor amount of the chemical from the body occurs via the skin, perspiration, feces, salvia, and milk (4,9). The aim of this study was to examine the effects of chronic fluoride toxicity on the liver of mice in 3 experimental group and 1 control group by examining histopathological disorders in the liver tissues.

Materials and methods

Animals

The study included 3 experimental groups and 1 control group of mice (Swiss albino) weighing 30-35 g that were obtained from the Veterinary Research Institute of Erzurum, Turkey. Each group contained 8 mice (4 male and 4 female). Animals in the control group had free access to tap water with 0.3 ppm fluoride. Animals in experimental group 1 had free access to distilled water with 10 ppm fluoride, animals in experimental group 2 had free access to distilled water with 20 ppm fluoride, and animals in experimental group 3 had free access to distilled water containing 40 ppm fluoride for 90 days. The animals were housed in a room at a temperature of 18-20 °C and with a 14 h/10 h light/dark cycle. Animals in all groups were fed with chow during the experimental period.

Chemicals

NaF was used to create fluoride intoxication. A stock solution including 2000 ppm fluoride was prepared by solving 4.44 g of NaF in 1 L of distilled water. The stock solution was stored at 4-8 °C for 1 week. The solution was prepared each week. Water with 10, 20, and 40 ppm fluoride was obtained by diluting the stock solution to be used for the experiment animals. To prepare solution with 10 ppm fluoride, 25 mL of stock solution was completed to 5 L by adding distilled water; solution with 20 ppm and 40 ppm fluoride was obtained in the same way. The animals were killed with ether anesthesia between 0900 and 1000 hours on day 91.

Histopathology

Slides for morphological studies were prepared (10). Tissue obtained from animals in the experimental and control groups was fixed in formalin for 24 h and switched to ethanol for storage. The liver sections were processed routinely and embedded in paraffin blocks. Slides were prepared (3-5 μm thick), and stained with hematoxylin and eosin, and then they were analyzed for pathology by light microscopy (Olympus, BX51, Japan), and subsequently photographed.

Results

Hepatocytes of the liver of mice in the control group had a regular morphological structure. The stained structures in the cytoplasm of cells had a regular distribution (Figure 4). However, depending on the dose of the chemical, histological disorders were observed on the slides obtained from the liver of mice treated with NaF. Hyperemia, local necrosis, vacuolar degeneration, and hydropic degeneration, which was characterized by the swollen cytoplasm, in some hepatocytes around the central veins were observed. Light hyperemia on the structure of central veins, local necrotic areas (marked with stars) and swellings (indicated with arrows) in some hepatocytes around the central veins were determined by examining slides obtained from the liver of mice treated with 10 ppm NaF (Figure 1).

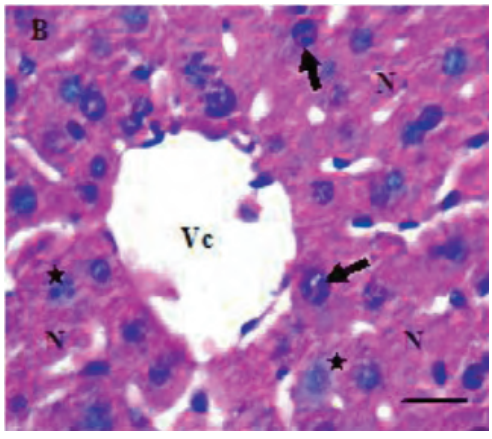

Figure 1. Picture obtained from the liver of a mouse in the first experimental group. Vc. Central vein, N. Local necrotic areas, stars indicate hydropic degeneration H-E., Bar: 20 μm.

On the slides obtained from the liver of mice exposed to 20 ppm NaF, vacuolar degenerations with sharp edges (pointed with arrows) were observed (Figure 2). Furthermore, on the slides prepared from the liver of mice treated with 40 ppm NaF, hyperemia around the central vein and hydropic degenerations (indicated with stars) in some hepatocytes, and necrotic areas were observed (Figure 3).

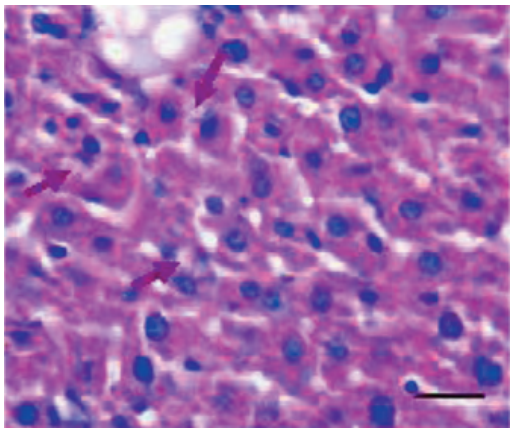

Figure 2. Picture obtained from the liver of a mouse in the second experimental group. Arrows show vacuolar degenerations H-E., Bar: 20 μm.

Discussion

In the hepatic fluoride studies, there are very limited findings about the histological structure of the liver. During the present investigation, the liver of mice treated with 10, 20, and 40 ppm NaF showed swellings, necrosis, and vacuolization of hepatocytes. It was observed that the intensity of swellings, vacuolization, and necrosis increased with rising doses of fluoride. The most intense staining was seen in hepatocytes of the permanent response zone around the central vein (2). In this study, it was found that histological disorders in the liver were observed around the central vein. Hepatocytes with vacuoles with sharp edges, with swellings and necrotic areas were prevalent. Hyperemia in the central vein was observed as well. It was indicated that histological disorders in the liver of mice treated with fluoride existed (5).

The intensity of histopathology increased with rising doses of fluoride. The impact of fluoride on mice was examined and it was reported that an

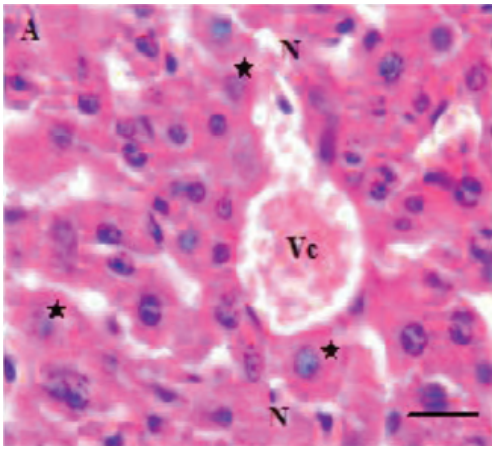

Figure 3. Picture obtained from the liver of an animal in experimental group 3. Vc. Central vein, N. Local necrotic areas, stars indicate hydropic degeneration. H-E., Bar: 20 μm.

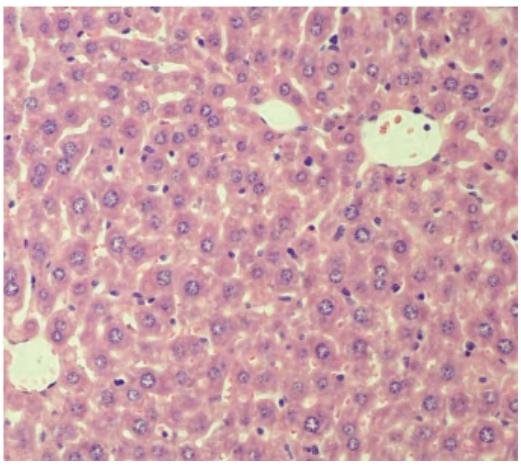

Figure 4. Picture obtained from the liver of an animal in the control group. H-E, 40×.

increasing dose of the chemical retarded the growth of mice (11). The renal changes in rabbits varied with the concentration of fluoride (3). Excessive fluoride can enhance lipid peroxidation and inhibits the oxidative enzymes in the liver, kidney, heart, ovary, brain, and gastrocnemius muscle of animals (1).

Studies with NaF on mice liver are so limited that there is no chance to comprehensively evaluate the effects of the chemical. Data in this study confirm the findings obtained by other researchers. It can be inferred that NaF has effects on the liver not only histopathologically but also morphologically.

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