Tikrit Journal of Veterinary Sciences (2026) 5(1): 27-35
DOI : 10.25130/tjvs.5.1.4

IRAQI Academic Scientific Journals

TJVS Tikrit Journal of Veterinary Sciences

Tikrit Journal of Veterinary Science

Histopathological changes of the small intestine using a standard dose of copper sulfate in rats (feed supplement dose)

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ARTICLE INFO.

Article history:
-Received: 1/9/2025
-Received in Revised Form:10/11/2025
-Accepted: 18/11/2025
-Available online: 30/6/2026

Keywords:
Anatomy, Histology, Spleen, Puppies

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ABSTRACT

Background & aim: Data from human studies indicate that gastrointestinal symptoms may occur due to consuming beverages or drinking water containing high levels of copper. Therefore, the current study aimed to determine the histological effects of copper sulfate on the small intestine tissue structures.

Materials and Methods: Forty-five rats were used for the current study. They were divided into two main groups: the control group and the copper sulfate group. The rats were given 8 mg/kg B.W./day of a copper sulfate solution dissolved in distilled water.

Results: Histological examination of copper sulfate groups shows that the mucosa of the small intestine contains short villi on its surface, degeneration of simple columnar epithelial cells, and their scarcity, with a large number of mucosal cells and droplets. The core of the villi contains massive infiltration of white blood cells extending to the bases of the villi. The basal plate of the small intestine contains intestinal glands with degeneration of main cells and mucus droplets of different sizes, along with white blood cells between the glands. The area between smooth muscle fibers contains degenerated ganglionic nerve cells. Thickening of the surface of the villi includes large numbers of mucus droplets from goblet cells and loss of epithelial cells. The core of the villi contained white blood cells spread to the basal lamina around apparent intestinal glands, with residual cells. There was a disintegration of the interstitial tissue between the glands in the basal lamina.

Conclusions: It is concluded that copper sulfate has very harmful effects on intestinal tissues, including the degeneration of simple columnar epithelial cells, with the presence of mucus droplets of different sizes and the appearance of white blood cells between the glands.

Introduction

Copper (Cu) is an essential minor metal cofactor for the formation of heme and the absorption of iron. After iron and zinc, it is the third most prevalent minor element in human tissue. High electrical and thermal conductivity, alloy ability, minimal corrosion, and malleability are some of its physical characteristics. Intrauterine devices (IUDs) contain copper, and their ability to effectively contracept is dependent on copper release. Adults typically consume between 0.6 and 1.6 mg of copper per day, with dietary consumption being a significant source [1,2]. Copper in the bloodstream is initially linked to albumin and subsequently transferred by the hepatic portal circulation to the liver, where it is integrated into ceruloplasmin, an alpha globulin produced in liver microsomes. There are two types of serum copper: 7% is loosely linked to albumin, and 93% is strongly bound to ceruloplasmin. The portion of serum copper that is toxically active is represented by the copper-albumin complex [3,4]. All tissues contain copper, but the liver, cardiovascular system, kidneys, brain, and muscle have the largest quantities. Metallothionein is the primary binding agent for intracellular copper. Red blood cells contain large amounts of copper in the form of erythrocuprein, along with additional proteins. Eighty percent of excreted copper comes via biliary and fecal excretion. The urine excretes around 4% of it [5,6]. Symptoms of copper sulfate poisoning are partly due to the liver, in addition to hemolysis. On the second or third day following medication consumption, jaundice manifests. Oxidative stress-induced liver mitochondrial dysfunction is the cause of liver injury. The nature of liver damage is characterized by cell necrosis and obstruction. Obstructive jaundice is primarily observed compared to toxic hepatitis. Bilirubin levels are directly proportional to the severity of poisoning. Liver biopsy shows centrilobular necrosis, mononuclear infiltrate, and cholestasis. Intravascular hemolysis plays a major role in the pathogenesis of renal failure [7,8]. At copper concentrations exceeding 3 mg/L, researchers observed a higher frequency of nausea and other gastrointestinal side effects. With an average daily intake of water of 1.6 L, the average daily copper consumption via drinking water was 4.8 mg. This study suggests that 4.8 mg/day is the threshold for acute gastrointestinal symptoms from copper in water. People might, however, be able to adjust to increasing copper levels in the water they drink. Adults who drank water with 8.5 to 8.8 mg/L of copper for over twenty years, beginning in infancy (0 to 5 years), have not had any negative gastrointestinal consequences [9].

The Aims of the study:

- To determine the histological effects of copper sulfate on the stomach and small intestine
- To study the long-term effects of copper sulfate.
- To evaluate the potential toxic effects of copper sulfate on body tissues, including the stomach and small intestine.
- To use different concentration of copper sulfate doses to understand the effect on the histological structures of the stomach and small intestine.

Material and Method:

Laboratory Animals

Between 1/9 -1/10/ 2024, this study was carried out in the animal shelter of Tikrit University's College of Veterinary Medicine. We acquired 45 male rats from the Tikrit University College of Veterinary Medicine's Animal House. The animals weighed between 120-180 gr, with an average of 150 grams, and ranged in age from 8 to 10 weeks. The animals were kept in an appropriate environment with temperatures between 20-25 C°, in ordinary plastic cages that measured 45X28X13 cm.

Experimental Design

Forty-five rats were used for the current study. They were divided into two main groups: the control group and the copper sulfate group. The rats were given 8 mg/kg B.W./day of a copper sulfate solution dissolved in distilled water. The rats were further divided into two subgroups as follows:

1. The control group: 5 rats were given distilled water only and were dissected at the end of the experiment.
2. The copper sulfate group: 40 adult rats were divided into two subgroups as follows:

• The copper sulfate group: 20 rats were given copper sulfate for one month, and five rats were dissected weekly for four weeks.

• The copper sulfate group: 20 rats were given a dose of copper sulfate every three days, and five rats were dissected three days later, at a rate of four weeks.

Institutional Animal Care and Use Committee (IACUC)

Prior to any experiments being conducted, the methodology and protocols used in this research were reviewed and approved in accordance with animal welfare ethics standards by the Scientific Committee of the Department of Physiology, Biochemistry, Pharmacology, and Toxicology,

College of Veterinary Medicine, Tikrit University, Salahaddin, Iraq (TU.VET.30).

Histological study

Sections of rat intestine were removed, paraffin-processed, fixed with 10% formalin, sliced to a six-micrometer thick using a spinning microtome, and labeled with histological stains of Hematoxylin and Eosin (H&E) (10). The slices were examined using an Optica microscope (Italy).

Results & Discussion

Intestine

Control group

Microscopic examination of the prepared sections shows that the mucosa of the small intestine contains villi lined with simple columnar cells and mucous goblet cells. The core of the villi contained white blood cells extending to the bases of the villi. Between the villi were found crypts or grooves continuous with the intestinal glands in the basal lamina. The muscular layer was composed of smooth muscle fibers that surrounded the lumen of the intestine (Fig. 1).

Copper sulfate groups

Daily dosing for one week

Histological examination of this group shows that the mucosa of the small intestine contains short villi on its surface, degeneration of simple columnar epithelial cells, and their scarcity on the surface of the intestine, with a large number of mucosal cells and their droplets. The core of the villi contains a massive infiltration of white blood cells extending to the bases of the villi (Fig. 2).

Daily dosing for two weeks

Histological examination shows that the intestinal villi are mostly devoid of columnar epithelial cells, in addition to most of the goblet cells, and their walls are torn. The medulla of the villi contains a nodular infiltration of white blood cells, especially dilated lymphocytes. The basal lamina, the intestinal glands are short and contain white blood cells. The bases of the villi also contain blood vessels and are hemolyzed (Fig. 3).

Daily dosing for three weeks

Examination under a light microscope shows the basal plate of the small intestine, which contains intestinal glands with degeneration of the main cells, with the presence of mucus droplets of different sizes and the appearance of white blood cells between the glands. The area between the smooth muscle fibers contains degenerated ganglionic nerve cells (Fig. 4).

Daily dosing for four weeks

Microscopic examination reveals the sloughing off of large numbers of cells lining the villi in the intestinal lumen and the presence of widespread loss of epithelial cells at the surface of the villi and the medulla of the villi, which are filled with white blood cells and scattered in the basal lamina and around the intestinal glands and accumulation of mucus droplets in the lumen of the intestinal with degeneration of nerve cells in the muscular layer (Fig. 5).

Dosing every three days (first week)

Microscopic examination revealed that the small intestine villi contained large numbers of goblet cells containing an increase in mucus droplets, with sloughing and loss of columnar epithelial cells from the surface of the villi. The medulla of the villi contained infiltration of white blood cells within the connective tissue of those villi (Fig. 6).

Dosing every three days (second week)

Histological examination shows that the intestinal villi are covered with large numbers of mucus-secreting goblet cells and have a limited number of columnar epithelial cells. Some of the goblet cell walls are torn, with several goblet cells sloughing off into the intestinal lumen. The medulla of the villi is filled with white blood cells extending to the bases of the villi and the basal lamina (Fig. 7).

Dosing every three days (third week)

The prepared slides from the intestines of this group show that the wall of the small intestine has mucosal villi and contains many atrophied villi and others that have been sloughed off. The core of the villi contains white blood cells surrounding the intestinal glands and extending into the submucosal layer. The muscular wall of the intestine is composed of smooth muscle fibers that are internally circular in direction and externally longitudinal in direction (Fig. 8).

Dosing every three days (fourth week)

Microscopic examination showed that the thickening of the surface of the villi contained large numbers of mucus droplets of goblet cells with loss of epithelial cells on the surface of the villi. The core of the villi contained white blood cells spread to the rest of the basal lamina around the apparent intestinal glands and in it were relapsed cells. There was also a disintegration of the interstitial tissue between the glands in the basal lamina (Fig. 9)

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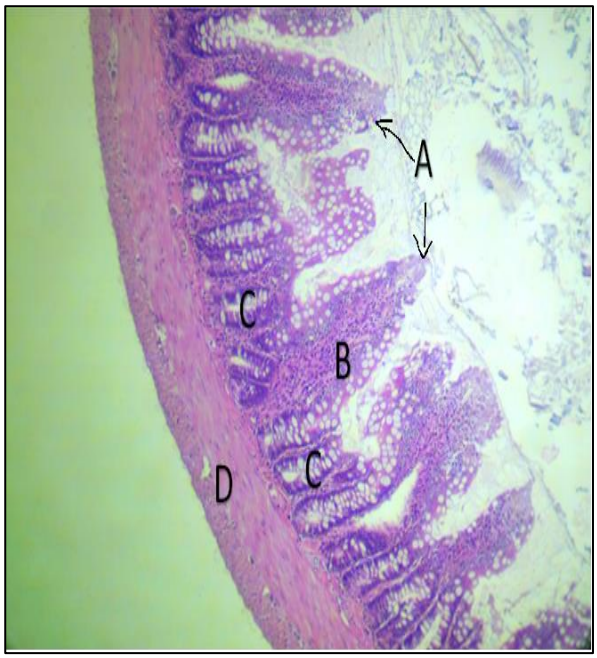

Figure (1): Intestine of the control group showing intestinal villi lined with columnar and mucosal cells (A), medulla of villi containing white blood cells (B), intestinal glands in the basal lamina (C), intervillous tufts (D), smooth muscle layer (E), H&E X10.

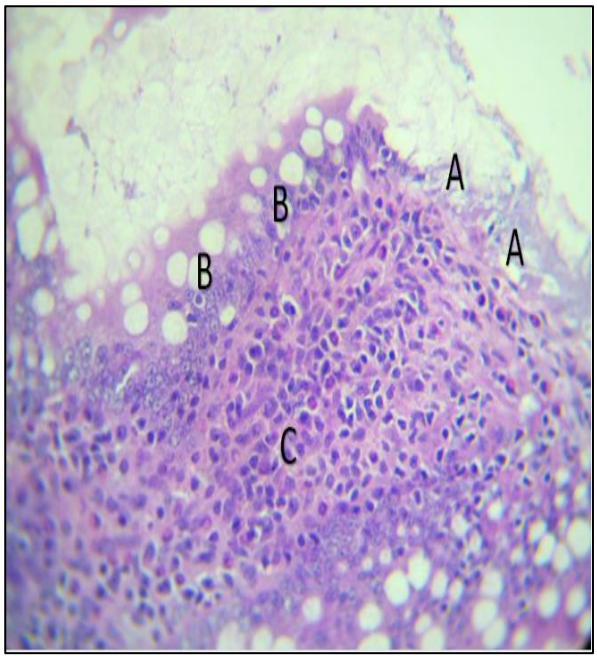

Figure (2): Copper sulfate group (first week) showing degeneration of epithelial cells and their loss from the surface of the villi (A), numerous mucus droplets on the surface of the intestine (B), large infiltration of white blood cells and the core of the villi (C), H&E X40.

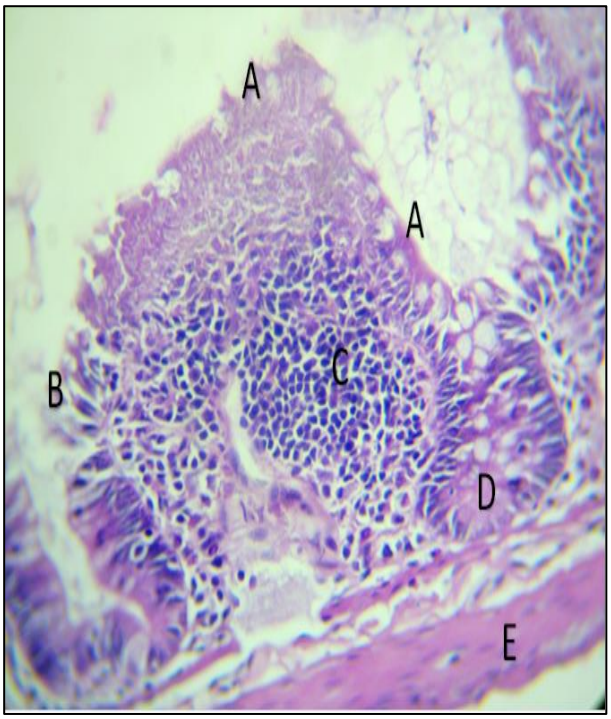

Figure (3): Copper sulfate group (2nd week) showing necrosis of goblet cells (A), loss of epithelial cells from the surface of the villi (B), nodular lymphocytic infiltration in the medulla of the villi (C), capillaries congested with hemolytic blood (D), mucosal muscle (E), H&E X40.

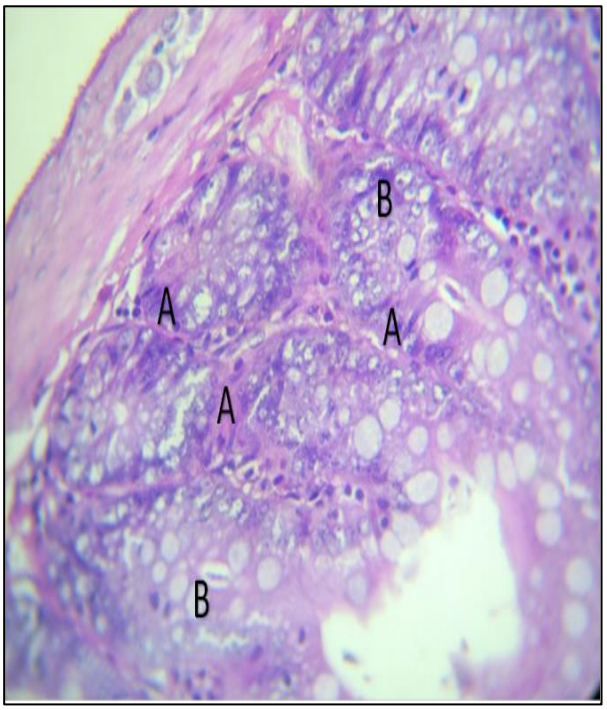

Figure (4): Copper sulfate group (third week) showing the basal plate of the small intestine with degeneration of intestinal cells (A), accumulation of mucus droplets in the lumen of the intestinal glands (B), H&E X40.

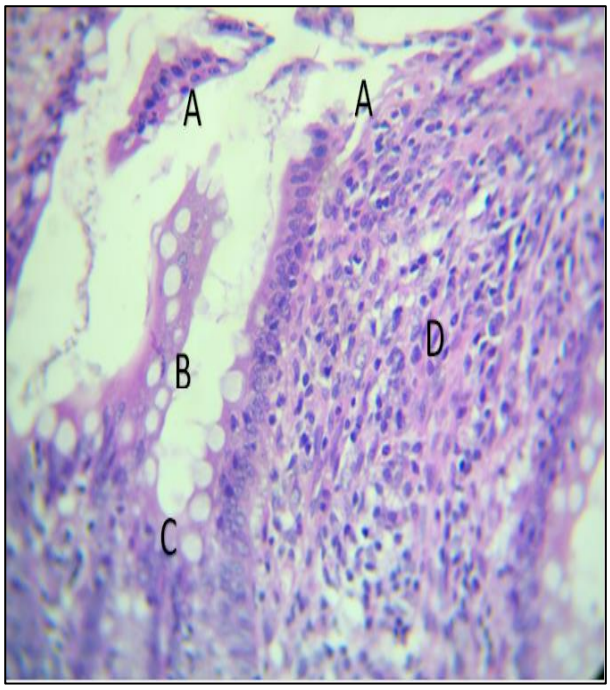

Figure (5): Copper sulfate group (fourth week) showing the basal plate of the small intestine with degeneration of intestinal cells (A), accumulation of mucus droplets in the lumen of the intestinal glands (B), degeneration of nerve cells in the muscular layer (C), H&E X40.

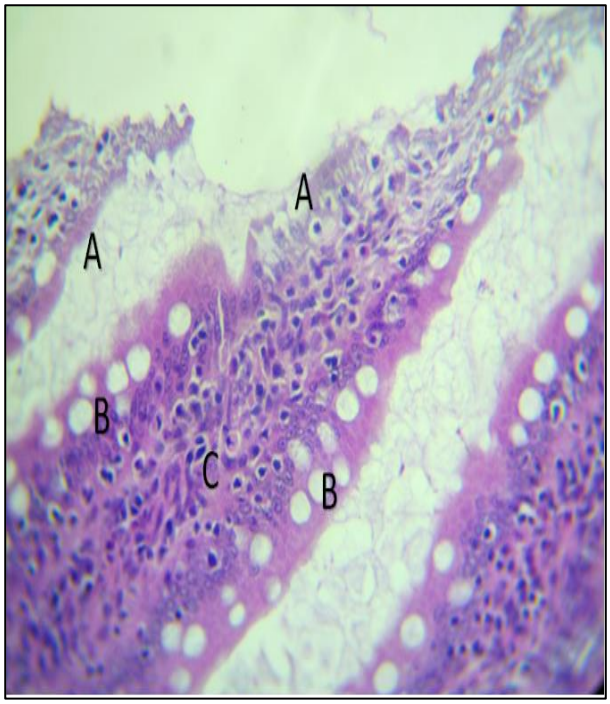

Figure (6): Copper sulfate collection every three days (first week) showing intestinal villi with sloughing and loss of columnar epithelial cells (A), accumulation of mucus droplets (B), villi core with white blood cells (C), H&E X40.

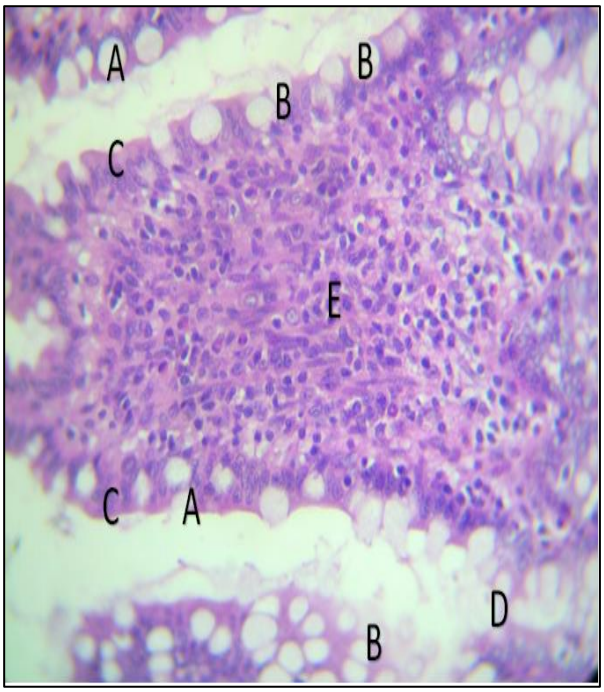

Figure (7): Copper sulfate collection every three days (second week) showing intestinal villi with hyperplasia of goblet cells (A), rupture of the walls of some goblet cells (B), scarcity of columnar epithelial cells (C), continuous villus grooves with intestinal glands (D), massive infiltration of white blood cells in the basal plate (E), H&E X40.

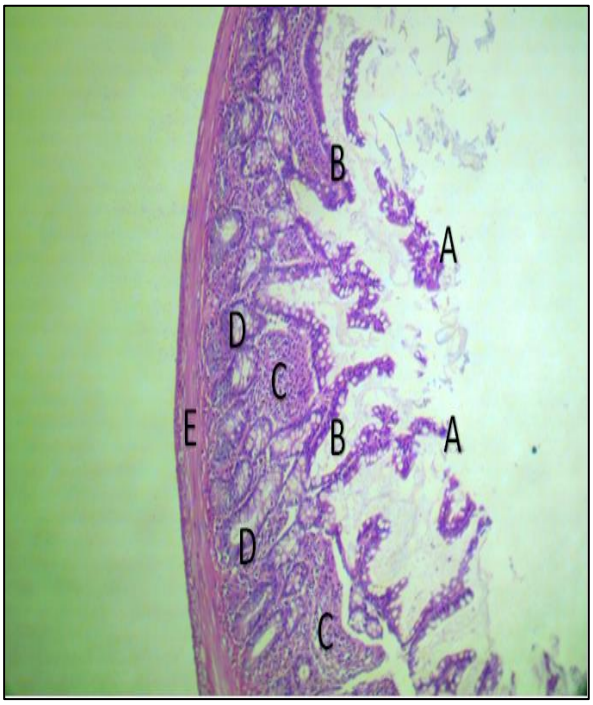

Figure (8): Copper sulfate group every three days (third week) showing the wall of the small intestine, sloughed villi (A), atrophic villi (B), the core of the villi containing white blood cells (C), intestinal glands containing mucus droplets (D), the muscular layer (E), H&E X10.

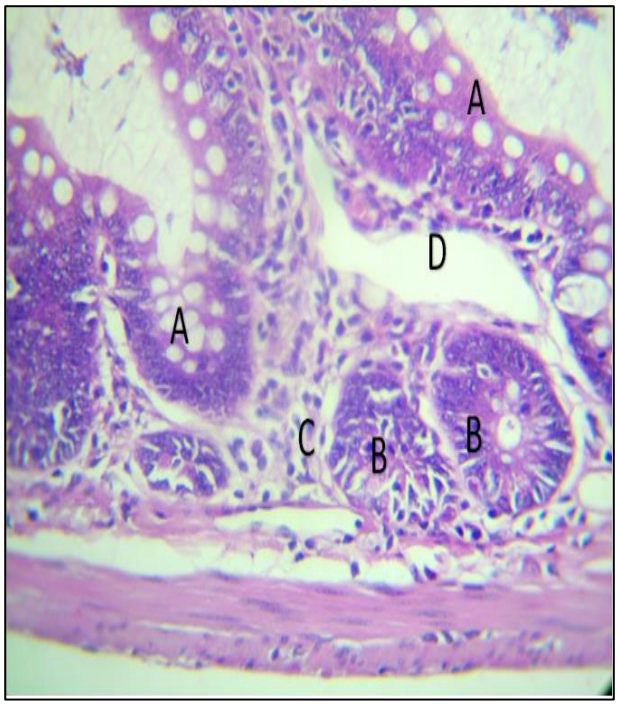

Figure (9): Copper sulfate group every three days (fourth week) shows thickening of the villi surface and large numbers of mucus droplets (A), loss of epithelial cells, atrophy of the intestinal glands with degeneration of their cells (B), infiltration of white blood cells in the basal lamina (C), cavitation of the basal lamina (D), H&E X40.

In the current study, copper sulfate administration resulted in histological lesions in the small intestine, including degeneration of simple columnar epithelial cells and their scarcity on the intestinal surface, with an abundance of mucous cells and their droplets. The medulla of the villi contains a massive infiltration of leukocytes extending to the bases of the villi. The lamina basalis of the small intestine contains intestinal glands with degeneration of chief cells, with the presence of mucus droplets of varying sizes and the appearance of leukocytes between the glands. The area between the smooth muscle fibers contains degenerated ganglion cells. Histopathological studies have shown that the target organs of copper toxicity in mice are the liver and the gastrointestinal tract, as copper in the body's tissues is closely linked to its distribution, accumulation, and release characteristics. Due to its strong stimulating and corrosive effects, its entry into the gastrointestinal tract causes acute reactions and serious pathological damage, manifested by thickening of the serous layer muscles, cracks, and even necrosis of mucosal and submucosal cells, and rupture and destruction of intestinal villi. This is a direct effect of copper salts on the intestinal mucosa, leading to impaired digestive function and the absorption of copper into the body after its primary accumulation in the liver, with a significant increase in copper content [11].

This is consistent with the results of the current study regarding the ability of copper acetate to cause numerous tissue lesions in the small intestine. The current results also showed numerous tissue lesions in the small intestine as a result of copper acetate administration. A possible explanation for these changes induced by copper over such a short period may be the involvement of copper ions in the formation of reactive oxygen species (ROS). Copper is a strong oxidant and can bind to cell molecules during high loads [12]. Copper ions produced by the corrosion of metallic copper can be reduced to copper ions in the presence of biological reducing agents [13]. Copper ions can catalyze the formation of reactive oxygen species (ROS) by decomposing hydrogen peroxide (H2O2) via the Fenton/Haber-Weiss reaction [14,15]. Furthermore, metal ions such as copper exhibit a high affinity for thiol groups and can therefore severely disrupt many cellular metabolic functions [16]. Therefore, oxidative stress, a state of redox imbalance where ROS production overwhelms the cell's antioxidant defense capacity, can lead to adverse biological consequences such as damage to lipids, DNA, or proteins, leading to excessive cell proliferation (overgrowth), apoptosis, or mutation [17]. On the other hand, another explanation for this widespread cellular damage in the small intestine may be the activation of apoptosis and oxidative damage to cellular DNA [18]. Apoptosis is associated with specific morphological changes characterized by chromatin condensation, DNA fragmentation, cell shrinkage, and the formation of membrane-bound compartments known as apoptic bodies [19]. Several researchers have reported on copper-induced cell toxicity in a variety of in vitro and in vivo systems. [20], observed that decreased cell proliferation and increased frequency of nonviable cells depended on copper concentration. Copper compounds have also been shown to delay cell cycle progression and increase cell death in various in vitro cell cultures [21,22]. Previous research provides evidence that copper ions can interact directly with nuclear proteins and DNA, causing site-specific damage [23].

Conclusions

It is concluded that copper sulfate has very harmful effects on intestinal tissues, including the degeneration of simple columnar epithelial cells, with the presence of mucus droplets of different

sizes and the appearance of white blood cells between the glands.

Recommendations:

- Conduct studies aimed at revealing the effect of copper sulfate on the histological structure of the remaining body organs, including the heart, kidney, and liver.
- Conduct physiological studies to reveal the levels of some hormones and function of the endocrine glands in the body and the extent to which they are affected by copper sulfate.

Acknowledgement

-The authors are thankful to the Tikrit University / Collage of Veterinary Medicine for all the facilities to achieve this study.

Declaration of interests

The authors declare no comping interests.

Funding Sources

This research received no financial support or specific grant from any funding agency in the commercial, public, or not-for-profit sectors.

Publication consent

The authors consent to the publication of this manuscript.

Data and material availability:

All data analyzed and generated in this study are included in this published research.

Author contribution:

All authors participated in the study design and conception, data analysis, data collection, performance of the results and assent to the final version.

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Tikrit Journal of Veterinary Sciences (2026) 5(1): 27-35
DOI : 10.25130/tjvs.5.1.4

التغيرات النسيجية المرضية في الأمعاء الدقيقة باستخدام جرعة قياسية من كبريتات النحاس في الجرذان (جرعة إضافة علفية)

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الملخص

الخلفية والهدف: تشير البيانات المستمدة من الدراسات التي أجريت على البشر الى ان تناول المشروبات او مياه الشرب التي تحتوي على مستويات عالية من النحاس قد يؤدي الى ظهور اعراض معدية معوية. لذلك، تهدف الدراسة الحالية الى تحديد الاثار النسيجية لكبريتات النحاس على انسجة الأمعاء الدقيقة.

المواد وطرق العمل: استخدمت 45 جرذاً في الدراسة الحالية. تم تقسيمها الى مجموعتين رئيسيتين: المجموعة السيطرة ومجموعة كبريتات النحاس. أعطيت الجرذان 8 مجم/ كجم من وزن الجسم/اليوم من محلول كبريتات النحاس المذاب في الماء المقطر.

النتائج: اظهر الفحص النسيجي لمجموعات كبريتات النحاس ان الغشاء المخاطي للأمعاء الدقيقة يحتوي على زغابات قصيرة على سطحه، وتدهور الخلايا الظهارية العمودية البسيطة، وندرة وجودها، مع وجود عدد كبير من الخلايا المخاطية والقطيرات. يحتوي لب الزغابات على تسلل هائل للخلايا البيضاء يمتد الى قواعد الزغابات. تحتوي الصفيحة القاعدية للأمعاء الدقيقة على غدد معوية مع تنكس الخلايا الرئيسية وقطيرات مخاطية بأحجام مختلفة، الى جانب خلايا بيضاء بين الغدد. تحتوي المنطقة الواقعة بين الياف العضلات الملساء على خلايا عصبية عقدية متدهورة. يتضمن سماكة سطح الزغابات اعداداً كبيرة من قطرات المخاط من الخلايا الكأسية وفقدان الخلايا الظهارية. احتوى لب الزغابات على خلايا دم بيضاء منتشرة الى الطبقة القاعدية حول الغدد المعوية الظاهرة، مع وجود خلايا متبقية. كان هناك تفكك في النسيج الخلالي بين الغدد في الطبقة القاعدية.

الاستنتاجات: يستنتج ان كبريتات النحاس لها اثار ضارة جداً على انسجة الأمعاء، بما في ذلك تنكس الخلايا الظهارية العمودية البسيطة، مع وجود قطرات مخاطية بأحجام مختلفة وظهور خلايا الدم بين الغدد.

الكلمات المفتاحية: كبريتات النحاس، الأمعاء، النسيج المرضي، الغشاء المخاطي.