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Histological Evaluation Of The Stomach Using Standard Dose Of Copper Sulfate In Rats (Feed Supplement Dose)

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ABSTRACT

Background & aim: Therefore, the current study aimed to determine the histological effects of copper sulfate on the stomach, study the long-term effects of copper sulfate, and use different concentrations of copper sulfate to understand the effect on the stomach tissue structures.
Materials and Methods: This study was conducted at the Animal House of the College of Veterinary Medicine, Tikrit University, during the period from September 1 to October 1, 2024. Forty-five rats were used for the current study. They were divided into two main groups: the control group and the copper sulfate groups. The rats were given 8 mg / kg B.W / day of a copper sulfate solution dissolved in distilled water.
Results: Histological examination of the transverse sections of all groups dosed with copper sulfate showed many tissue lesions represented by many of simple columnar cells appear to have sloughed off in the gastric lumen. The gastric glands in the basal lamina have undergone shrinkage from the surrounding basement membrane. There is also atrophy of the cells lining these glands, and debris from sloughed epithelial cells is present in the lumen of the tubular glands. also, the loose connective tissue around the glands and gastric pits contained fibroblasts and scattered leukocytes. Masses of necrotic secretory cells were also found and the sections showed degeneration of several chief cells, and cervical mucosal cells
Conclusions: it is concluded that copper sulfate has very harmful effects on gastric tissues, including the destruction of the epithelial lining and gastric glands, and that the longer the exposure period to copper sulfate, the more severe the tissue effects.

Key words; copper sulfate, stomach, histopathological changes, mucosa.

INTRODUCTION

Copper (Cu) is an essential nutrient that plays a vital role in the biochemistry of living organisms. It acts as a cofactor for numerous proteins and enzymes known as copper synthases. Copper is involved in fundamental mechanisms including oxygen transport, energy production, cell metabolism, cell signaling, and hematopoiesis (1,2). The average daily intake of copper in humans has been estimated to be between 0.5 and 1.5 mg, which can come from various sources such as seeds, nuts, beans, shellfish, and liver (3). Copper is absorbed from the diet, particularly from the duodenum and proximal jejunum, although some can be absorbed in the stomach and distal small intestine. It is excreted either via the gastrointestinal tract as absorbed and unabsorbed ions or via bile (4). Cu+ and

Cu2+ ions present in the lumen of the small intestine are absorbed into enterocytes primarily by copper transporter 1 (CTR1), and to a lesser extent by divalent metal transporter 1 (DMT1). From enterocytes, Cu2+ ions enter the blood via the copper-transporting ATPases ATP7A and ATP7B (5). Ozkul et al., (6) reported a significant decrease in copper content in rats fed a copper-free diet for 28 days compared to animals fed a diet containing 15 mg copper/kg during this period. In animals, the most common form of copper toxicity is the prolonged ingestion of copper compounds of various origins. This means that animals raised far from factories ingest copper from industrial deposits via feed or air throughout their lives. The liver primarily controls copper regulation, as it can be transported into the circulation or excreted via the bile (7,8). In cases of chronic copper poisoning, copper gradually accumulates in the liver without causing any noticeable symptoms. When the liver's copper storage capacity is exceeded, this can lead to hepatocellular necrosis, and the subsequent release of copper from the liver into the bloodstream can lead to hemolysis, jaundice, and renal insufficiency (9,10). Copper is therefore a strong oxidant, as it can bind to cellular molecules during high loads. It can therefore generate highly reactive hydroxyl radicals, thereby affecting certain cellular functions. Consequently, high dietary copper levels in mice led to lipid peroxidation in mitochondrial membranes (11,12,13). Furthermore, a study conducted by Ashish et al., (14) indicated that copper ions administered to mice in the form of copper salts caused a range of histological changes in the gastrointestinal tract. Copper ions caused gastrointestinal toxicity and intestinal damage, leading to changes in the gastrointestinal mucosa and a range of degenerative and necrotic changes in stomach and intestinal cells. The researcher indicated that doses exceeding 0.5 grams caused nausea and toxicity, as copper at high doses is a potent inhibitor of enzymes. Therefore, the current study aimed to determine the histological effects of copper sulfate on the stomach, study the long-term effects of copper sulfate, and use different concentrations of copper sulfate to understand the effect on the stomach tissue structures.

MATERIALS AND METHODS

Laboratory Animals

This study was conducted at the Animal House of the College of Veterinary Medicine, Tikrit University, during the period from September 1 to October 1, 2024. Forty-five male rats were obtained from the Animal House of the College of Veterinary Medicine, Tikrit University. The animals' ages ranged between 8-10 weeks, and their weights ranged between 120-180 grams, with an average weight of 150 grams. The animals were placed in standard plastic cages measuring 46 x 28 x 13 cm and maintained under suitable environmental conditions, with temperatures ranging between 20-25 degrees Celsius.

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 rat was given 8 mg / kg B.W / day of a copper sulfate solution dissolved in distilled water. The mice were further divided into two subgroups as follows:
The control group: 5 rats were given distilled water only and were dissected at the end of the experiment.
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 every three days over a period of four weeks.

Institutional Animal Care and Use Committee (IACUC)
Before conducting any experiment, 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
Rat stomach pieces were taken, fixed with 10% formalin, paraffin-processed, cut with a rotary microtome to a thickness of six micrometers, and stained with Hematoxylin and Eosin (H&E) histological stains (15). Through the use of an Optica microscope (Italy), sections were inspected.

RESULTS & DISCUSSION

Stomach
Control group
Microscopic examination of prepared sections reveals that the stomach wall is lined with simple columnar cells. Between these cell rows, broad grooves were found within the basal lamina lined with chief cells and parietal cells. Mucus neck cells were also found, and leukocytes were found between these cells in the basal lamina (Figure 1).

Copper sulfate groups
Daily dosing for one week
Histological examination of this group shows the gastric mucosa lined with simple columnar cells, many of these cells appear to have sloughed off in the gastric lumen. The gastric glands in the basal lamina have undergone shrinkage from the surrounding basement membrane. There is also atrophy of the cells lining these glands, and debris from sloughed epithelial cells is present in the lumen of the tubular glands (Figure 2).

Daily dosing for two weeks
Histological examination of this group shows the gastric mucosa lined with columnar epithelial cells, with most of these cells sloughing off into the gastric lumen. The gastric glands in the basal lamina showed atrophy of chief cells and parietal degeneration of partial parietal cells. The loose connective tissue around the glands and gastric pits contained fibroblasts and scattered leukocytes (Figure 4-6).

Daily dosing for three weeks
Under a light microscope, the gastric mucosa was found to contain degeneration and sloughing of the epithelial cells lining the gastric wall facing the gastric lumen. The primary lamina of the stomach contains gastric glands lined with chief cells, some of which appeared sloughed off in the lumen of the glands, with a lack of parietal cells in those glands. Masses of necrotic secretory cells were also found (Figure 3).

Daily dosing for four weeks
Microscopic examination showed that the surface of the gastric mucosa contained degeneration and sloughing of columnar epithelial cells. Gastric units extending from the epithelial surface to the basal lamina were surrounded by gastric glands, which showed degeneration of several chief cells, chief cells, and cervical mucosal cells (Figure 4-13).

Dosing every three days (first week)

Histological examination reveals that the surface of the gastric mucosa has lost its epithelium and the cytoplasm of other cells has become swollen. The basal lamina has shrunken gastric glands, and some of the principal cells have necrotic and detached from the glands. These necrotic and sclerotic cells have extended deep into the basal lamina with thickened nuclei (Figure 5).

Dosing every three days (second week)
Microscopic examination reveals the gastric mucosa containing the basal lamina, a number of tubular gastric glands, chief cells, and some parietal cells. The interstitial tissue contains loose connective tissue with scattered white blood cells extending throughout the basal lamina. The surface of the gastric mucosa is lined with atrophied epithelial cells, continuous with the cervical mucosal cells at the gastric units descending to the basal lamina (Figure 6).

Dosing every three days (third week)
Histological examination revealed degeneration and necrosis of the cells lining the gastric glands, including the chief and parietal cells, with some of these cells sloughing off into the gland cavity and surrounded by macrophages. In addition, there was an infiltration of white blood cells extending into the mucosal muscle. The submucosal layer was characterized by disintegration of connective tissue and dispersion of white blood cells. There was also disintegration of smooth muscle fiber bundles (Figure 7).

Dosing every three days (fourth week)
Microscopic examination revealed that the gastric mucosa contained gastric sloughing of the columnar epithelial cells and the presence of other atrophied epithelial cells at the mucosal surface. Expansion of gastric units lined with atrophied cervical mucosal cells was found, along with degenerated parietal cells showing cytoplasmic vacuolation (Figure 8).

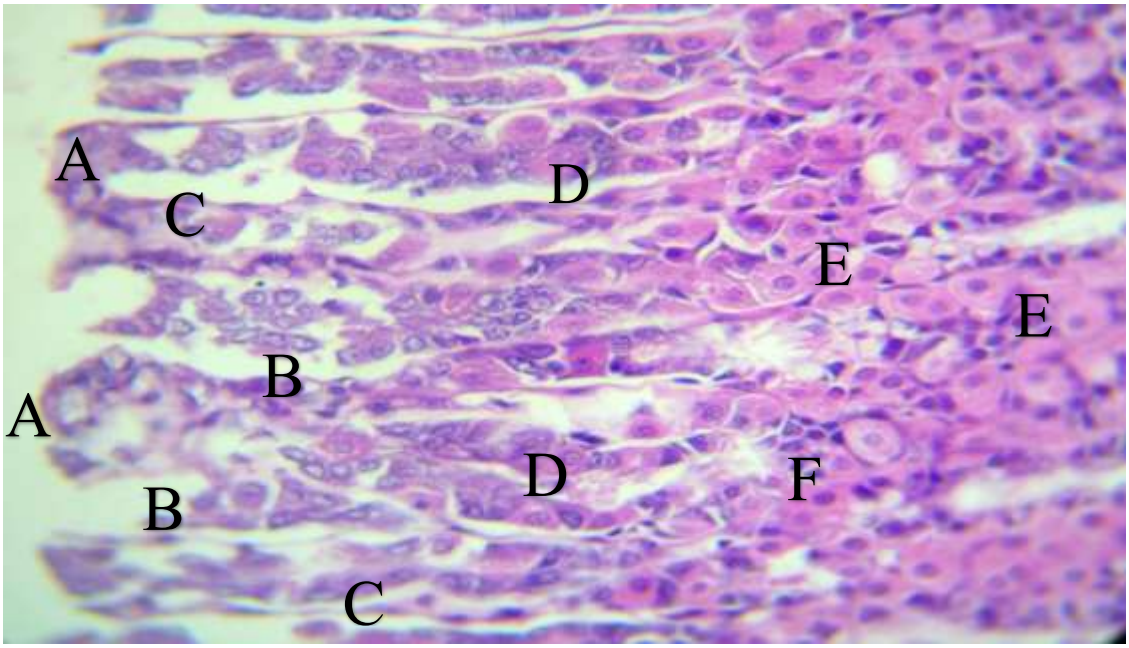

Figure (1): cross section of control stomach, gastric mucosa, simple columnar epithelial cells (A), gastric units (B), mucosal cervical cells (C), chief cells (D), parietal cells (E), leukocytes (F) H&E X400.

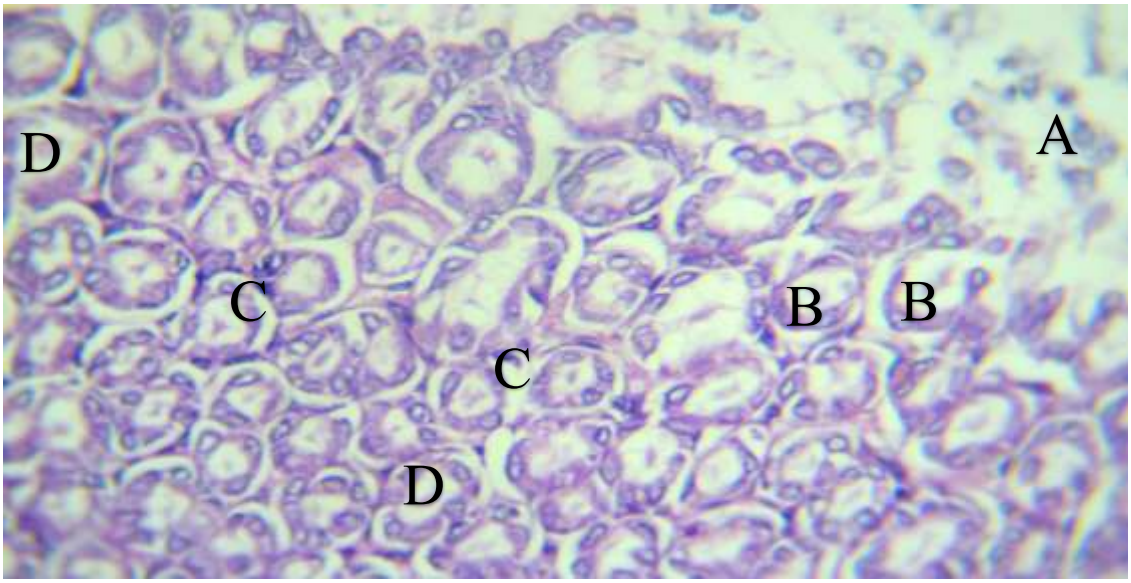

Figure (2): Gastric mucosa, copper sulfate group (first week), sloughing of columnar epithelial cells (A), atrophy of glandular lining cells (B), shrinkage of glands from the basement membrane (C), sloughed cells in the lumen of glands (D), H&E X 40

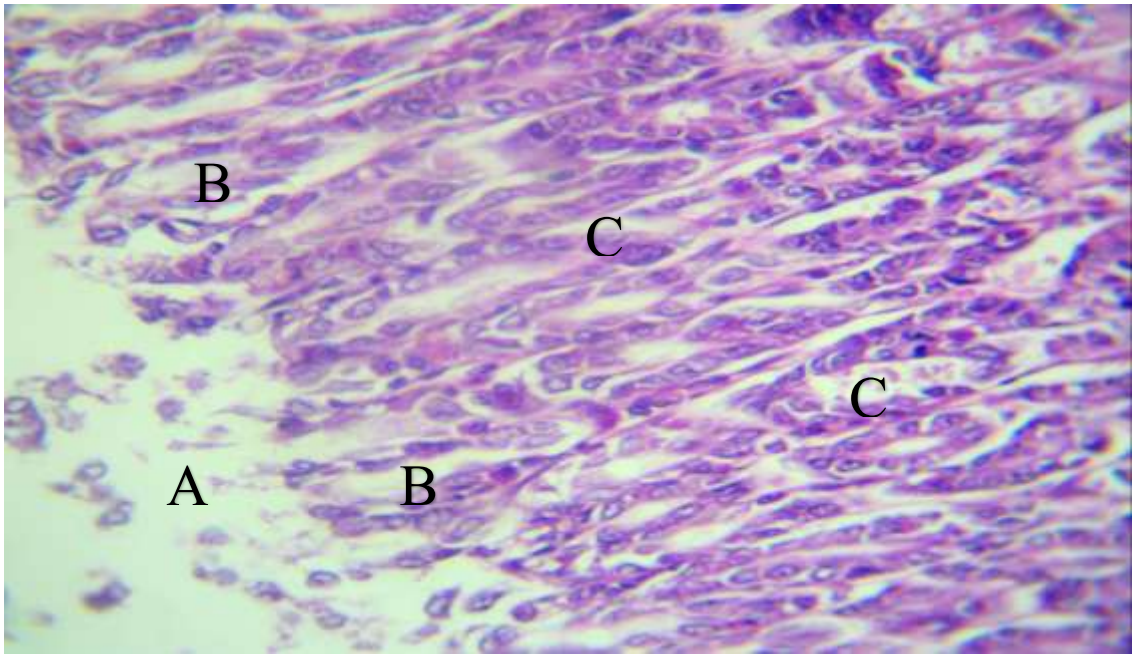

Figure (3): Stomach of copper sulfate group (third week), gastric mucosa, epithelial cell sloughing (A), atrophy of mucosal cervical cells in gastric units (B), relapsed chief cells in the lumen of gastric glands (C), H&E X 40

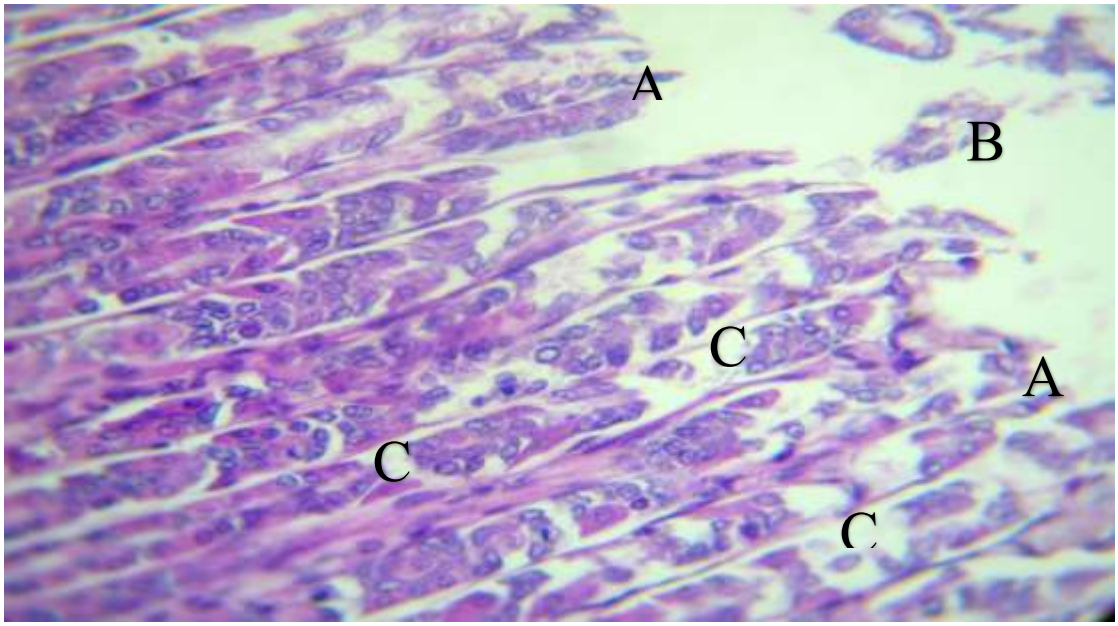

Figure (4): Stomach of copper sulfate group (4th week), gastric mucosa with epithelial cell degeneration (A), sloughing of other cells (B), degeneration of chief cells (C), and gastric units (D). H&E x40.

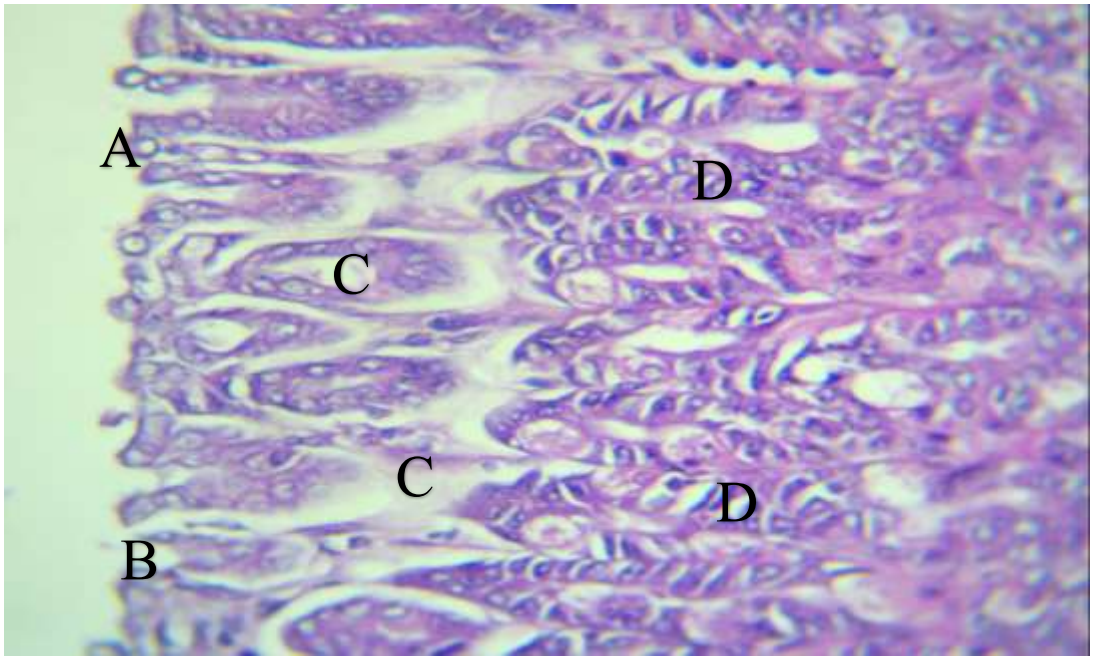

Figure (5): Stomach of copper sulfate group every three days (first week), gastric mucosa, atrophy of epithelial columnar cells (A), loss of epithelial cells from the mucosal surface (B), shrinkage of gastric glands (C), necrosis of chief cells (D) H&E X40.

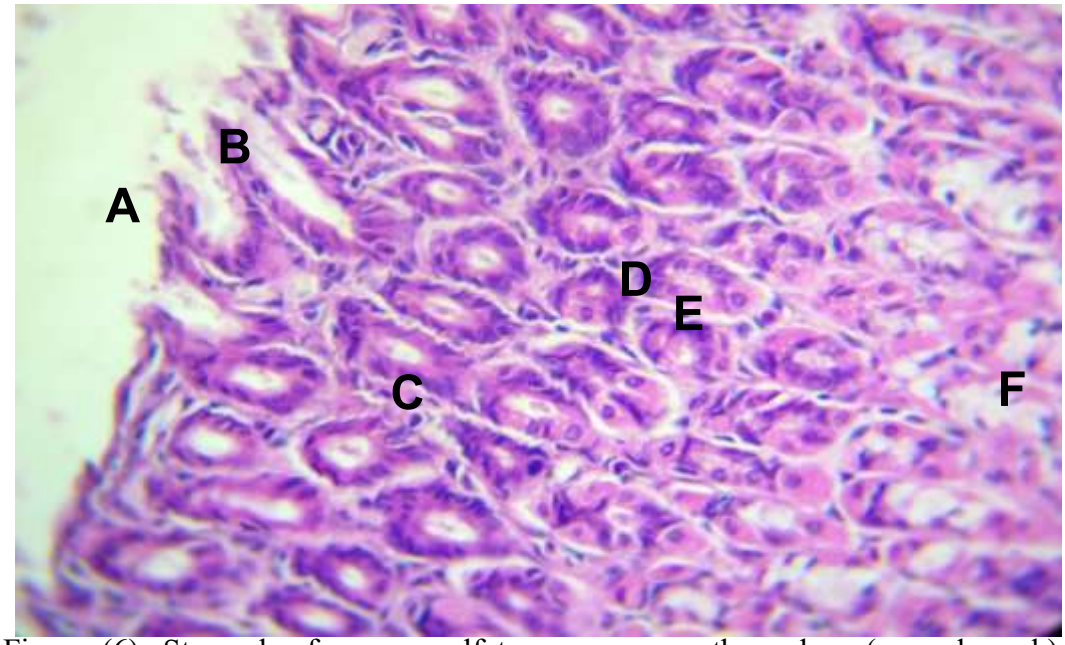

Figure (6): Stomach of copper sulfate group every three days (second week), gastric mucosa, atrophy of columnar epithelial cells (A), atrophy of mucosal cervical cells in gastric units (B), chief cells (C), parietal cells (D), infiltration of leukocytes and macrophages (E), degeneration of gastric glandular cells (F) H&E X40.

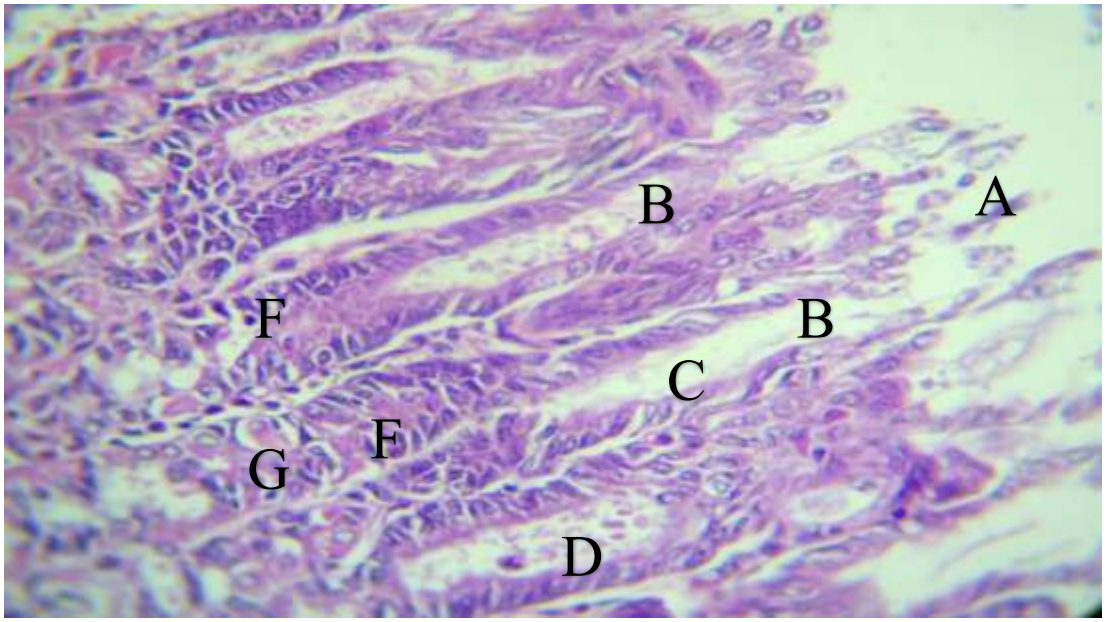

Figure (7): Stomach of copper sulfate group every three days (third week), sloughing of gastric mucosal epithelial cells (A), atrophy of mucosal cervical cells (B), expansion of gastric units (C), sloughing of epithelial cells in the lumen of the glands (D), degeneration of chief cells (F), degenerated parietal cells (G) H&E X40.

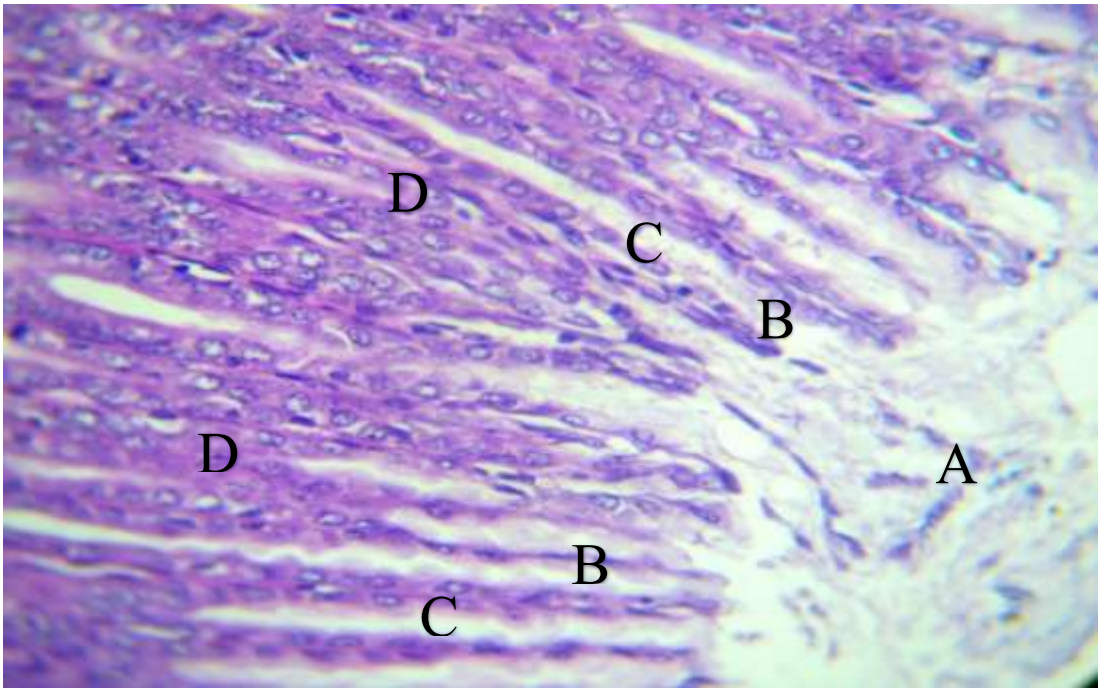

Figure (8): Stomach of copper sulfate group every three days (fourth week), gastric mucosa with epithelial cell sloughing (A), atrophy of mucosal cervical cells (B), expansion of gastric units (C), cytoplasm of chief cells (D) H&E X40.

In the current study, copper acetate dosing caused histological lesions in the stomach, including gastric glands lined with parietal cells with vacuolated cytoplasm and some degenerated cells. Chief cell hyperplasia was present deep within the basal lamina, with thickening of some of their nuclei. The gastric mucosa was thickened and infiltrated with leukocytes. The submucosa was composed of loose connective tissue with infiltrated inflammatory leukocytes and devoid of blood capillaries. Some fat cells were also surrounded by a broad smooth muscle layer. Furthermore, gastric glands in the basal lamina showed atrophy of chief cells and partial degeneration of parietal cells. Copper dosing caused methemoglobinemia, which causes a brown discoloration of the mucosa at the onset of hemolysis. Jaundice of the gastrointestinal mucosa worsened if the animals were kept alive for more than two days. A rise in body temperature may have occurred. Death from a large dose of copper is rapid, preceded by diarrhea due to the involvement of the digestive system, including the stomach and intestines (16,17). On the other hand, Ortolani et al., (18) indicated that copper toxicity causes many tissue lesions in the digestive system of sheep. These lesions are represented by degenerative and necrotic changes in the cells and also include infiltration of inflammatory cells. They attributed the reason to the fact that copper toxicity directly affects the metabolic processes of cells and also causes the destruction of cell membranes, which is consistent with the results of the current study. In another study conducted by Kumar et al., (19), they found that exposure to copper nanoparticles led to increased levels of dimalonaldehyde in the blood serum of rats, which indicates rancidity of lipids in cell membranes, leading to cell degeneration and necrosis, which causes lymphocyte infiltration and possible fibrosis at the site of cell degeneration. This was found in the different layers of the stomach in the current study, including cell degeneration, necrosis, and infiltration of inflammatory cells as a result of copper acetate administration. Arefian et al., (20) also indicated that copper sulfate administration causes oxidative stress in the body's cells. They found elevated levels of free radicals (ROS) in the serum of rats that were administered during

the experimental period, which caused increased cell degeneration, necrosis, membrane destruction, and changes in cell metabolism. This is consistent with the results of the current study. The explanation for the many histological lesions diagnosed in gastric cells and layers in the current study as a result of copper acetate dosing is that copper binds to DNA with a higher affinity than other cations, thus promoting DNA oxidation (21), catalyzing the active formation of 8-aminodeoxyguanosine and 8-oxidG (22), and causing apoptosis in cells (23,24). The results of the current study may differ from those of some studies regarding the levels of histological changes that may occur as a result of copper acetate dosing. Most studies indicate that copper exposure depends on the nature of the work, the duration of exposure, the concentration of the element, and its chemical form. The organic form is more toxic than the inorganic form due to its ability to penetrate and accumulate within cells (25). Vascular congestion can be attributed to the animal's inflammatory response to the harmful effects of heavy metals, characterized by increased blood flow to the affected area. It may also be caused by damage and cell breakdown.

Conclusions

Based on the results of the current study, it is concluded that copper sulfate has very harmful effects on gastric tissues, including the destruction of the epithelial lining and gastric glands, and that the longer the exposure period to copper sulfate, the more severe the tissue effects.

REFERENCES

1. Chen L, Min J, Wang F. Copper homeostasis and cuproptosis in health and disease. Signal Transduct Targeted Ther (2022) 7(1):378.
2. Gao L., Anqi Z. (2023). Copper-instigated modulatory cell mortality mechanisms and progress in oncological treatment investigations. Front Immunol. 14:1236063.
3. Erfanizadeh M., Ali N., Maryam N., Saied K. (2021). The effects of copper sulfate on the structure and function of the rat cerebellum: A stereological and behavioral study. IBRO Neuroscience Reports. 11: 119-127
4. Taylor A.A., Tsuji J.S., Garry M.R., McArdle M.E., Goodfellow W.L., Adams W.J., Menzie C.A. (2020). Critical review of exposure and effects: implications for setting regulatory health criteria for ingested copper. Environ. Manag., 65: 131-159
5. Ognik K., Stępniowska A., Cholewińska E., Kozłowski K., 2016. The effect of administration of copper nanoparticles to chickens in drinking water on estimated intestinal absorption of iron, zinc, and calcium. Poult. Sci. 95, 2045–2051
6. Ozkul H., Kirkpinar F., Mert S., Unlu B., 2011. Effects of high levels of dietary copper sulfate and copper proteinate on growth performance, retention for copper and zinc of rats. J. Anim. Vet. Adv. 10, 1373–1377
7. Tsvetkov P, Coy S, Petrova B, Dreishpoon M, Verma A, Abdusamad M, Rossen J, Joesch-Cohen L, Humeidi R, Spangler RD, Eaton JK, Frenkel E, Kocak M, Corsello SM, Lutsenko S, Kanarek N, Santagata S. Golub T.R. Copper induces cell death by targeting lipoylated TCA cycle proteins. Sci (New York). 2022;375(6586):1254–61.
8. Thi T. T., Tzu-Yu P., Thi H. N., Trang N. B., Ching-Shuen W., Wei-Ju L., Yuh-Lien C., Yang-Che W., I-Ta L. (2024). The crosstalk between copper-induced oxidative stress and cuproptosis: a novel potential anticancer paradigm. Cell Communication and Signaling. 22(353): 1-9.
9. Martins K. P.F., Vitor H.T., Tércio K. D., Marcos A. S., Emanoelly M.S., Marlon R., Asheley H.B., Edson M. (2020). Chronic copper poisoning in beef cattle in the state of Mato Grosso. Pesq. Vet. Bras. 40(9):651-661.
10. Festa R.A., Thiele D.J. (2021). Copper: an essential metal in biology. Curr. Biol. 21(21):R877-R883.
11. Jian Z, Guo H, Liu H, Cui H, Fang J, Zuo Z. et al. Oxidative stress, apoptosis and inflammatory responses involved in copper-induced pulmonary toxicity in mice. Aging (Albany NY).

2020;12:16867-86
12. Chen X, Cai Q, Liang R, Zhang D, Liu X, Zhang M. et al. Copper homeostasis and copper-induced cell death in the pathogenesis of cardiovascular disease and therapeutic strategies. Cell Death Dis. 2023;14:105
13. Tsui KH, Hsiao JH, Lin LT, Tsang YL, Shao AN, Kuo CH, Chang R, Wen ZH, Li CJ. (2024). The Cross-Communication of Cuproptosis and Regulated Cell Death in Human Pathophysiology. Int J Biol Sci., 20(1):218-230.
14. Ashish B., Neeti K., Himanshu R. (2013). Copper Toxicity:A Comprehensive Study.Research Journal Of Recent Sciences. 2 :58-67.
15. Kristensen D. M., Christèle D. L., Abigail L. M., Bernard J. (2018). Ibuprofen alters human testicular physiology to produce a state of compensated hypogonadism. Biological Sciences. 115 (4) E715-E724.
16. Christodoulopoulos G., Roubies N.(2017). Diagnosis and treatment of copper poisoning caused by accidental feeding on poultry litter in a sheep flock. Aust. Vet. J. 85(11):451-3.
17. Bozynski, C.C., Evans, T.J., Kim, D.Y., Johnson, G.C., Hughes-Hanks, J.M., Mitchell, W.J., Rottinghaus, G.E., Perry, J., Middleton, J.R .(2019). Copper Toxicosis with Hemolysis and Hemoglobinuric Nephrosis in Three Adult Boer Goats. J Vet Diagn Invest. 21(3):395- 400
18. Ortolani, L.E., Machado, C.H., Sucupira, M.C.A (2013). Assessment of some clinical and laboratory variables for early diagnosis of cumulative copper poisoning in sheep. Vet Hum Toxicol. 45:289–293.
19. Kumar V., Kalita J., Bora H. K., and Misra U. K., Relationship of antioxidant and oxidative stress markers in different organs following copper toxicity in a rat model, Toxicology and Applied Pharmacology. (2016) 293, 37–43,
20. Arefian Z., Pishbin F., Negahdary M., and Ajdary M., Potential toxic effects of Zirconia Oxide nanoparticles on liver and kidney factors, Biomedical Research. (2015) 26, 89–97.
21. Theophanides T, Anastassopoulou J. Copper and carcinogenesis. Crit Rev Oncol Hematol 2012; 42: 57-64.
22. Sakano K, Oikawa S, Murata M, Hiraku Y, Kojima N, Kawanishi S. Mechanism of metal-mediated DNA damage induced by metabolites of carcinogenic 2-nitropropane. Mutat Res 2001; 479: 101-11.
23. Narayanan VS, Fitch CA, Levenson CW. Tumor suppressor protein p53 mRNA and subcellular localization are altered by changes in cellular copper in human Hep G2 cells. J Nutr 2011;131:1427-32.
24. Tassabehji NM, VanLandingham JW, Levenson CW. Copper alters the conformation and transcriptional activity of the tumor suppressor protein p53 in human Hep G2 cells. Exp Biol Med (Maywood) 2015;230:699-708
25. Sayed Almissari, Ahmed M Athab, Rashid Buthaina Ateyia, Abd, Anas Qays, Aldelaimy and Raed Ahmed (2012) The Effects of Lead exposure to workers in electric generator and car mending, 8(4): 1-8.