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The protective role of Vitamin E against copper-induced histopathological changes in rat rectum: An experimental study

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ABSTRACT

Objectives: This study aims to evaluate the impact of Vitamin E supplementation on mitigating copper-induced histopathological changes in rat rectum tissues.

Material and Methods: Twenty-five Wistar rats were divided into five groups and subjected to different dosages of copper sulfate, Vitamin E, or a combination. Tissue samples were collected, processed, and stained with Hematoxylin and Eosin for histopathological examination.

Results: Histopathological analysis revealed significant alterations in the rat rectum following exposure to copper sulfate alone, including decreased cryptgoblet cells and mucosal lymphocyte mobilization. Vitamin E supplementation showed mitigated effects, with signs of active mucosal congestion and lymphocyte mobilization. However, when rats were administered copper sulfate followed by Vitamin E, histopathological changes were normalized, indicating a protective role for Vitamin E. Conversely, rats exposed to copper sulfate without subsequent Vitamin E supplementation exhibited severe inflammatory responses and submucosal vascular ulceration.

Conclusion: Vitamin E demonstrates a protective effect against copper-induced histopathological changes in rat rectum tissues, potentially mediated through its antioxidant properties. Further molecular studies are warranted to elucidate the underlying mechanisms. Hematoxylin and Eosin staining proved instrumental in identifying and characterizing these histopathological alterations.

Keywords: Antioxidant protection, Copper sulfate, Histopathological changes, Rat rectum, Vitamin E

INTRODUCTION

The importance of Vitamin E in maintaining various aspects of health cannot be overstated. It acts as a potent antioxidant, safeguarding cell membranes from oxidative damage caused by free radicals.[1] This protective function extends to immune support, heart health, skin maintenance, and eye health.[2] Vitamin E is primarily obtained through a diet rich in fruits, vegetables, nuts, and seeds.[3]

Research indicates that Vitamin E has a multifaceted role within the body, particularly as a fat-soluble antioxidant.[4] It neutralizes free radicals by donating hydrogen atoms, thus preventing oxidative damage to cells.[5] Moreover, it influences gene expression and enzyme activity regulation,[6] notably in the modulation of protein kinase C activity.[7]

Vitamin E's impact on gene expression involves its antioxidant properties, which can affect signaling pathways and transcription factors.[7] This modulation of gene expression extends to hepatic functions, cholesterol homeostasis,[8] and age-related changes.[9] Long-term deficiency may lead to alterations in hepatic gene expression, affecting various metabolic processes.[10]

Vitamin E plays a vital role in maintaining overall health by acting as a powerful antioxidant and modulating gene expression and enzyme activity. Its deficiency is rare and is usually associated with fat digestion issues rather than dietary insufficiency.[11]

Copper (II) sulfate, also known as cupric sulfate, is a versatile compound widely used across various industries.[12] Its production involves different methods tailored to scale and purity requirements, including direct synthesis and bioleaching.[13] The compound is commonly employed in laboratories due to its high purity and convenience.[13] However, its environmental impact and toxicity, dose-dependent and primarily linked to its copper content, raise concerns.[14] High concentrations can harm aquatic life and pose health risks to humans through various exposure routes.[15] Acute exposure may result in severe symptoms, while chronic exposure, though rare, can lead to long-term health effects.[16] In ecosystems, copper sulfate can disrupt aquatic fauna and ecosystem balance, necessitating careful management in pesticide use to avoid ecological disturbances.[17,18]

The rectum is an integral part of the gastrointestinal tract, serving as the terminal section of the large intestine.[19] It plays a crucial role in the process of digestion by being the final stop before fecal matter is expelled from the body.[20]

The rectum's primary role is to act as a temporary storage facility for feces, playing a key part in the defecation process.[21]

The rectum, being the final section of the large intestine, is susceptible to various forms of toxicity, which can manifest as inflammation, pain, bleeding, or more severe health conditions.[22]

MATERIAL AND METHODS

Experimental rats

Twenty-five Wistar rats of weight between 120 g and 170 g were used. The rats were given a 2-week acclimatization period before the administration method began. They were given free access to conventional rat feed and water. The research ethic committee's guidelines for animal treatment at the University of Benin's College of Medicine were espoused and fully implemented.

Experimental protocol

All administrations were done by gavage and lasted for 60 days [Table 1].[23]

Copper sulfate and Vitamin E were administered using orogastric tube by following a modified method by Alflen *et al.*[24]

Tissue collection, processing and staining, histopathology

The rats were sacrificed and the rectum was taken at the end of the 30-day and 60-day study. The tissue was immediately sent to the University of Benin Teaching Hospital's Chemical Pathology department. The rectum tissue was preserved for 24 h in 10% buffered formalin before being histologically processed and stained with Hematoxylin and Eosin (H&E) using standard procedures.[25] The sections obtained were examined and photomicrographs were taken using a Leica DM750 research microscope with an attached digital camera (Leica CC50). The tissues were photographed digitally at magnifications of ×100.

RESULTS

The histopathological analysis showed notable differences in the rat rectum among the treatment groups. The control group had a normal mucosal lining, crypts, submucosa, and muscularis propria. Rats exposed to copper sulfate alone had a significant decrease in crypt goblet cells and increased mucosal lymphocyte activity, indicating damage to the mucosal barrier. In contrast, those given only Vitamin E showed active mucosal congestion and lymphocyte mobilization, suggesting an immune response. Rats that received copper sulfate followed by Vitamin E had normal crypt goblet cells, mural vasodilatation, and active congestion, demonstrating Vitamin E's protective and restorative effects. However, those given copper sulfate followed by distilled water developed severe mucosal inflammatory infiltrates and submucosal vascular ulceration, indicating that the tissue damage persisted and worsened without Vitamin E intervention.

Rat rectum. Control. Showing: Mucosa Epithelium (ME), crypts (Cr), submucosa (SM), and muscularis propria (MP): Hematoxylin and Eosin (H&E) ×100 [Figure 1].

Table 1: Experimental design.

Groups	Dosage
Group A	Served as control and were fed with animal feed and water ad libitum.
Group B	Received 200 mg/kg of copper sulfate daily for 30 days.
Group C	Received 200 mg/kg of Vitamin E daily for 30 days.[23]
Group D	Received 200 mg/kg of copper sulfate daily for 30 days followed by 200 mg/kg of Vitamin E daily for 30 days[23]
Group E	Received 200 mg/kg of copper sulfate daily for 30 days followed by distilled water daily for 30 days.

Rat rectum given copper only showing: Decreased Crypts goblet cells (GC) and mobilization of mucosal lymphocytes (ML): H&E ×100 [Figure 2].

Rat rectum given Vitamin E only showing: Active mucosal congestion (MC) and mobilization of Mucosa lymphocytes: H&E ×100 [Figure 3].

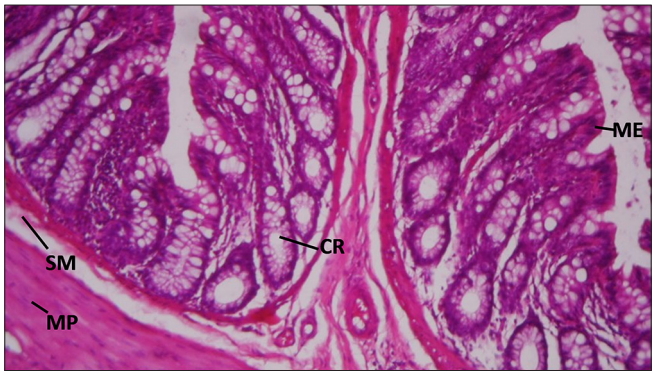

Figure 1: Section of Rat's rectum in control group. ME: Mucosa epithelium, CR: Crypts, SM: Submucosa, MP: Muscularis propria. Hematoxylin and Eosin ×100.

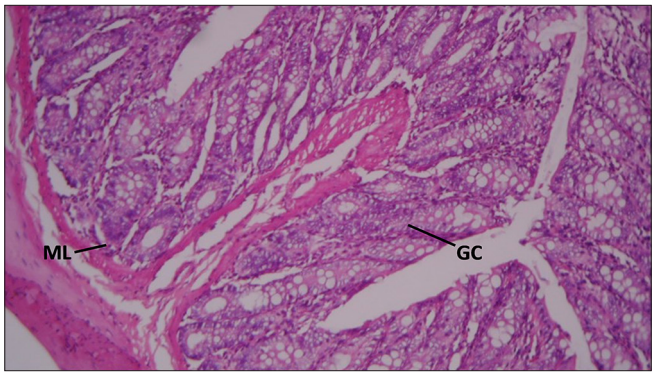

Figure 2: Section of Rat's rectum administered 200mg/kg of copper sulfate only. GC: Goblet cells, ML: Mucosal lymphocytes. Hematoxylin and Eosin ×100.

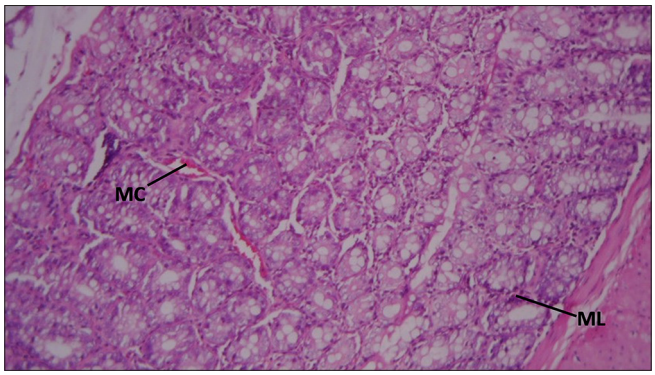

Figure 3: Section of Rat's rectum administered with 200 mg/kg of Vitamin E only. MC: Mucosal congestion, ML: Mucosa lymphocytes. Hematoxylin and Eosin ×100.

Rat rectum given copper for 30 days, then Vitamin E for 30 days showing: Normal Crypts goblet cells and mural vasodilatation and active congestion (OC): H&E ×100 [Figure 4].

Rat rectum given copper for 30 days, then distilled water for 30 days, then distilled water for 30 days, showing mucosal infiltrates of inflammatory cells (MI) and submucosal Vascular ulceration (VU): H&E ×100 [Figure 5].

DISCUSSION

The control sample shows a normal histological structure with a mucosal lining (ME), Crs, submucosa (SM), and MP. This serves as a baseline for comparison with treated samples.

Exposure to copper resulted in a decrease in Cr GCs and mobilization of MLs. This suggests that copper may have a detrimental effect on the mucosal barrier, as GCs are responsible for mucus production which protects the intestinal lining.[26] The presence of lymphocytes indicates an immune response, possibly due to irritation or injury to the mucosa.[26]

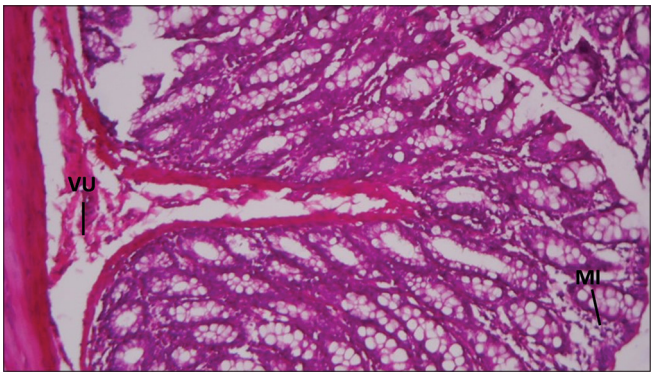

Figure 4: Section of Rat's rectum administered with 200mg/kg of copper sulfate for 30 days then 200mg/kg of Vitamin E for 30 days. MI: Mucosal infiltrates, VU: Vascular ulceration. Hematoxylin and Eosin ×100.

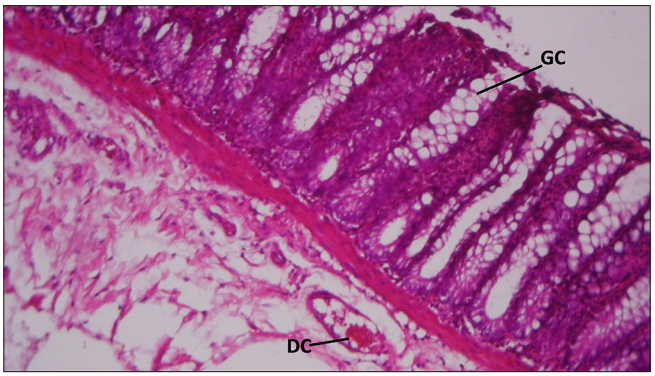

Figure 5: Section of Rat's rectum administered with 200mg/kg of copper sulfate for 30 days then distilled water for 30 days. GC: Goblet Cells, DC: Mural vasodilatation and active congestion.

Vitamin E treatment showed active MC and mobilization of MLs. Congestion could be due to increased blood flow or inflammation, while the presence of lymphocytes again suggests an immune response. Vitamin E is known for its antioxidant properties, which may explain the inflammatory response as the body's attempt to repair tissue damage.[27]

When rats were given copper for 30 days followed by Vitamin E for another 30 days, the samples showed normal Cr GCs and mural vasodilatation with active congestion (OC). This indicates that Vitamin E may have a protective or restorative effect on the damage induced by copper, as evidenced by the normalization of GCs. Vasodilatation and congestion might reflect ongoing reparative processes.[28]

Rats exposed to copper for 30 days and then switched to distilled water for the same duration exhibited mucosal infiltrates of inflammatory cells (MI) and submucosal VU. This severe reaction suggests that without the intervention of Vitamin E, the damage caused by copper persists and may even worsen, leading to ulceration and significant inflammation.[29]

CONCLUSION

The results suggest that copper has a potentially harmful effect on the rat rectum, impairing the mucosal barrier and inducing an inflammatory response. Vitamin E seems to mitigate these effects, possibly due to its antioxidant properties that help in reducing oxidative stress and promoting healing. However, the exact mechanisms of these interactions would require further molecular studies to explain. The use of H&E staining has been crucial in identifying these histopathological changes, as it allows for the clear visualization of tissue structure and cellular components.

Ethical approval: The research ethic committee's guidelines for animal treatment at the University of Benin's College of Medicine were espoused and fully implemented. No ethical number was obtained.
Declaration of patient consent: Patient's consent not required as there are no patients in this study.
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Conflicts of interest: There are no conflicts of interest.
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