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Original Article

Ameliorative effect of vitamin E on copper sulfate-induced liver damage in Wistar rats

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Abstract

BACKGROUND AND AIM: Significant human exposure to heavy metals such as copper sulfate is a major health concern due to its adverse consequences to body tissue. Vitamin E, a potent anti-oxidant is being investigated for its potential to protect against heavy metal toxicity. This study aimed to investigate the role of vitamin E on copper sulfate-induced liver damage.

METHOD: Twenty-five (25) adult Wistar rats weighing between 160g and 220g were divided equally into five groups (A to E). They received 1 ml of distilled water, 200 mg/kg of copper sulfate for 30 days, 200 mg/kg of vitamin E for 30 days, 200 mg/kg of copper sulfate for 30 days followed by 200 mg/kg of vitamin E for 30 days, 200 mg/kg of copper sulfate for 30 days followed by 1 ml of distilled water for 30 days. At the end of the study, blood and liver tissues were collected for analysis of serum Alanine aminotransaminase(ALT), Aspartate aminotransferase(AST), Alkaline Phosphatase(ALP), Total protein(TP), Total bilirubin(TB), Conjugated bilirubin(CB), liver Malondialdehyde(MDA), Superoxide dismutase(SOD), Catalase(CAT), Glutathione Peroxidase(GPx) and histology of the liver.

RESULTS: There was significant increase in in ALT, AST, ALP, TB, CB, liver MDA and a significant decrease in TP, liver SOD, CAT, and GPx in copper sulfate treated rats when compared to control which were reversed with vitamin E treatment. Histological findings showed a reversal of vascular stenosis and inflammation induced by copper sulfate following co-treatment with vitamin E.

CONCLUSION: Vitamin E possesses ant-oxidant and anti-inflammatory properties against copper-induced liver damage.

Keywords:
Liver; copper sulfate; Vitamin E; Hepatoprotective; Ameliorative

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INTRODUCTION

Copper, an essential micronutrient, plays a crucial role in various physiological processes, including enzymatic reactions, cellular respiration, and the synthesis of connective tissue (Angelova *et al.,* 2011). Copper is naturally found in various foods, including seafood (oysters, crab), nuts, seeds, legumes, organ meats (liver beef), dark leafy greens, chocolate, avocado, and mushrooms (Shahidi *et al.* 2021). A well-balanced diet incorporating these sources ensures a healthy intake of copper. However, excessive copper accumulation or exposure can disturb cellular homeostasis, leading to liver damage characterized by oxidative stress, inflammation, and impaired antioxidant defense mechanisms (Alhusaini *et al.,* 2018). Copper is absorbed through ingestion, primarily in the upper gastrointestinal tract. It is absorbed particularly in the duodenum but some are also absorbed in the stomach. After ingestion, it dissolves in gastric acid in the stomach, forming copper ions (Espinosa and Stein, 2021). Once absorbed, copper is transported in the blood, bound to proteins, and distributed to various tissues, with the liver playing a key role in regulating copper levels (Roberts and Sarkar, 2008). Copper sulfate plays crucial roles in enzymatic reactions and serves as a cofactor for several metalloenzymes (Shim and Han, 2023).

Copper exists in different forms, may exist in its pure state, as a compound, as a salt, or as an alloy. It is commonly used in industries and agriculture. It may even be employed as household pesticide, but upon inordinate exposure, this compound can be detrimental to the body.

Vitamins are antioxidants and they increase tissue protection from oxidative stress due to their easy, effective, and safe dietary administration in a large range of concentrations (Garewal and Diplock (1995). One of the most recognized vitamins for its protective role is vitamin E. A fat-soluble antioxidant essential for cellular health (Traber and Stevens, 2011). Vitamin E is commonly found in foods like nuts, seeds, and vegetable oils. Its ability

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ability to neutralize lipid peroxidation products and modulate oxidative stress pathways has been well-documented in various experimental models It supports the immune system, contributes to cardiovascular health, exhibits anti-inflammatory effects, promotes skin health, and additionally plays a role in neurological function (Lewis *et al.*, 2019)..

MATERIALS AND METHOD

Experimental design

Twenty-five (25) adult Wistar rats were used for this study. They weighed between 160g and 220g and were purchased from the animal house of the Department of Anatomy, University of Benin, were used for this study. They were allowed to acclimatize for two weeks before administration. The rats had free access to conventional rats feed and water. The Research Ethics Committee's Guidelines for Animal Care and Handling of the College of Medicine, University of Benin were implemented.

The animals were grouped into 5 groups of 5 rats per group. Group A Control, received 1 ml of distilled water. Group B was given 200mg/kg body weight of copper sulfate daily for 30 days (Kumar *et al.*, 2016). Group C was given 200mg/kg body weight of Vitamin E daily for 30 days (Huang *et al.*, 2006). Group D was given 200mg/kg body weight of copper sulfate daily for 30 days followed by 200mg/kg body weight of vitamin E daily for 30 days. Group E was given 200mg/kg of copper sulfate daily for 30 days followed by 1 ml of distilled water daily for 30 days.

All animals had free access to conventional rats feed and water *ad libitum.* Copper and vitamin E were administered using oral gavage

Tissue harvesting, processing and Staining Techniques

At the end of the 30 and 60 days study, the rats were sacrificed using chloroform anaesthesia. Five millilitres (5mls) of blood was collected into sterile sample bottles for analysis of serum Alanine aminotransaminase (ALT), Aspartate aminotransferase (AST), Alkaline Phosphatase (ALP), Total protein (TP), Total bilirubin (TB), Conjugated bilirubin (CB). A section of the liver tissue was harvested and placed in physiological saline in universal bottles and processed for liver oxidative biomarkers of MDA, SOD, CAT, GPx. The Liver tissue were also preserved in 10% formal saline for 72 hours before been processed histologically and stained with Haematoxylin and Eosin stains. The liver sections were examined using research microscope (Leica DM750) and photomicrographs of liver sections were taken at 400x magnification.

Data analysis

Data analysis was done using Statistical Package Graph Pad Prism version 9.0. Data obtained were expressed as Mean ± SEM (standard error of mean). The differences between the means were determined by one-way analysis of variance (ANOVA) and values were considered statistically significant if P value was less than 0.05 ($p < 0.05$).

RESULTS

There was a statistically significant increase ($P<0.05$) in Alanine Transaminase (ALT) levels for rats treated with 200mg/kg of copper sulfate when compared with control and a statistically significant decrease ($P<0.05$) in Alanine Transaminase (ALT) levels for rats treated with 200mg/kg of Copper sulfate + 200mg/kg of Vitamin E and rats treated with 200mg/kg of Copper sulfate + Distilled water when compared with rats treated with 200mg/kg of Copper Sulfate only (Fig 1).

There was a statistically significant increase ($P<0.05$) in Aspartate Transferase (AST) levels for rats treated with 200mg/kg of copper sulfate when compared with control and a statistically significant decrease ($P<0.05$) in Aspartate Transferase (AST) levels for rats treated with 200mg/kg of Copper sulfate + 200mg/kg of Vitamin E and rats treated with 200mg/kg of Copper sulfate + Distilled water when compared with rats treated with 200mg/kg of Copper Sulfate only (Fig 2).

There was a statistically significant increase ($P<0.05$) in Alkaline Phosphatase (ALP) levels for rats treated with 200mg/kg of copper sulfate when compared with control and a statistically significant decrease ($P<0.05$) in Alkaline Phosphatase (ALP) levels for rats treated with 200mg/kg of Copper sulfate + 200mg/kg of Vitamin E and rats treated with 200mg/kg of Copper sulfate + Distilled water when compared with rats treated with 200mg/kg of Copper Sulfate only (Fig 3)

There was a statistically significant decrease ($P<0.05$) in Total Protein (TP) levels for rats treated with 200mg/kg of copper sulfate when compared with control and a statistically significant increase ($P<0.05$) in Total Protein (TP) levels for rats treated with 200mg/kg of Copper sulfate + 200mg/kg of Vitamin E and rats treated with 200mg/kg of Copper sulfate + Distilled water when compared with rats treated with 200mg/kg of Copper Sulfate only (Fig 4).

There was a statistically significant increase ($P<0.05$) in Total Bilirubin (TB) levels for rats treated with 200mg/kg of copper sulfate when compared with control and a statistically significant decrease ($P<0.05$) in Total Bilirubin (TB) levels for rats treated with 200mg/kg of Copper sulfate + 200mg/kg of Vitamin E and rats treated with 200mg/kg of Copper sulfate + Distilled water when compared with rats treated with 200mg/kg of Copper Sulfate only (Fig 5)

There was a statistically significant increase ($P<0.05$) in Conjugated Bilirubin (CB) levels for rats treated with 200mg/kg of copper sulfate when compared with control and a statistically significant decrease ($P<0.05$) in Conjugated Bilirunin levels for rats treated with 200mg/kg of Copper sulfate + 200mg/kg of Vitamin E and rats treated with 200mg/kg of Copper sulfate + Distilled water when compared with rats treated with 200mg/kg of Copper Sulfate only (Fig 6)

There was a statistically significant increase (P<0.05) in Liver Malondialdehyde (MDA) levels for rats treated with 200mg/kg of copper sulfate when compared with control and a statistically significant decrease (P<0.05) in Liver Malondialdehyde (MDA) levels for rats treated with 200mg/kg of Copper sulfate + 200mg/kg of Vitamin E and rats treated with 200mg/kg of Copper sulfate + Distilled water when compared with rats treated with 200mg/kg of Copper Sulfate only (Fig 7)

There was a statistically significant decrease (P<0.05) in Liver Superoxide Dismutase (SOD) levels for rats treated with 200mg/kg of copper sulfate when compared with control and a statistically significant increase (P<0.05) in Liver Superoxide Dismutase (SOD) levels for rats treated with 200mg/kg of Copper sulfate + 200mg/kg of Vitamin E when compared with rats treated with 200mg/kg of Copper Sulfate only (Fig 8).

There was a statistically significant decrease (P<0.05) in Liver Catalase (CAT) levels for rats treated with 200mg/kg of copper sulfate when compared with control and a statistically significant increase (P<0.05) in Liver Catalase (CAT) levels for rats treated with 200mg/kg of Copper sulfate + 200mg/kg of Vitamin E when compared with rats treated with 200mg/kg of Copper Sulfate only (Fig 9).

There was a statistically significant decrease (P<0.05) in Liver Glutathione Peroxidase (GPx) levels for rats treated with 200mg/kg of copper sulfate and 200mg/kg of Vitamin E when compared with control and a statistically significant increase (P<0.05) in Liver Glutathione Peroxidase (GPx) levels for rats treated with 200mg/kg of Copper sulfate + 200mg/kg of Vitamin E when compared with rats treated with 200mg/kg of Copper Sulfate only (Fig 10).

Histological findings

Histological findings showed normal liver architecture in the control group (plate 1). There was evidence of vascular dilatation, stenosis and congestion with presence of inflammatory cells in rats liver treated with 200 mg/kg of copper sulfate only (plate 2). Rats liver given 200 mg/kg of vitamin E only showed normal hepatocyte architecture with presence of lymphocytes in the periportal region (plate 3). Rats liver given 200 mg/kg of copper sulfate followed by 200 mg/kg of vitamin E showed normal hepatocyte architecture (plate 4). However, rats liver given 200 mg/kg of copper sulfate followed by distilled water showed evidence of persistent inflammatory cells in the periportal of the liver region (plate 5).

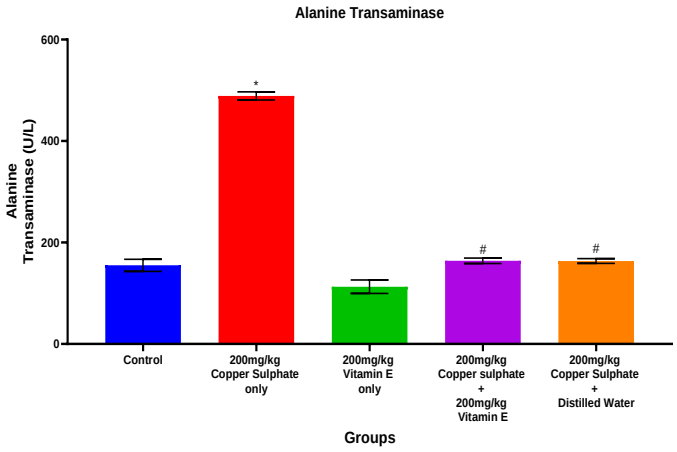

Fig 1. Alanine Transaminase Levels. *Represent comparison with control (P<0.05); #Represent comparison with 200 mg/kg of copper sulfate only group (P<0.05)

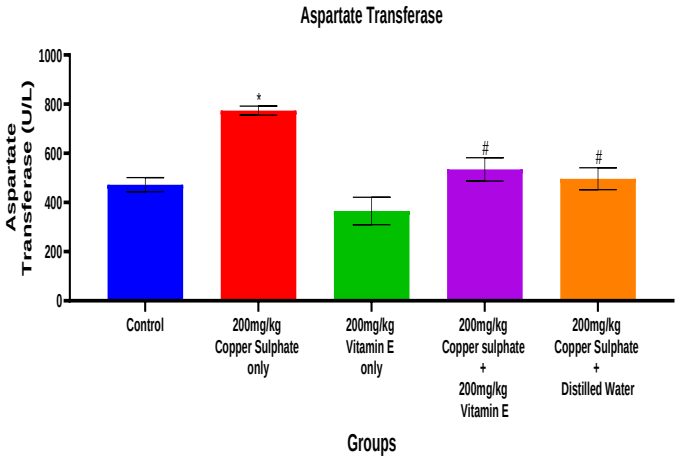

Fig 2. Aspartate Transferase Levels. *Represent comparison with control (P<0.05); #Represent comparison with 200 mg/kg of copper sulfate only group (P<0.05)

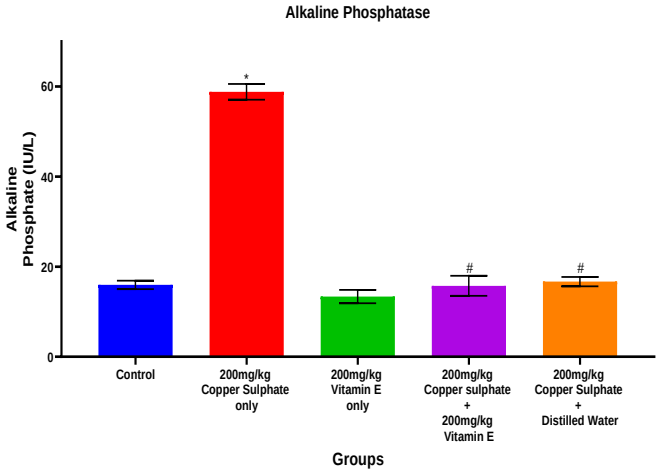

Fig 3. Alkaline Phospahatase Levels. *Represent comparison with control (P<0.05); #Represent comparison with 200 mg/kg of copper sulfate only group (P<0.05)

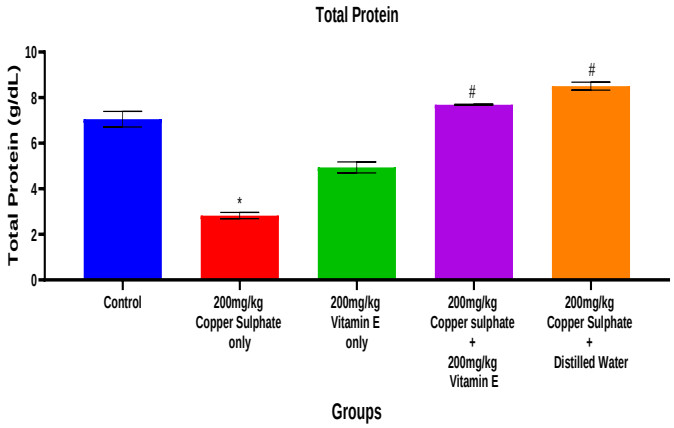

Fig 4. Total Protein Levels. *Represent comparison with control (P<0.05); #Represent comparison with 200 mg/kg of copper sulfate only group (P<0.05)

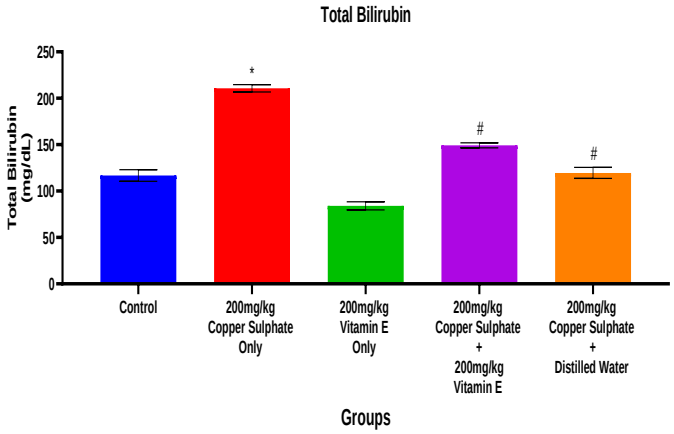

Fig 5. Total Bilirubin Levels. *Represent comparison with control (P<0.05); #Represent comparison with 200 mg/kg of copper sulfate only group (P<0.05)

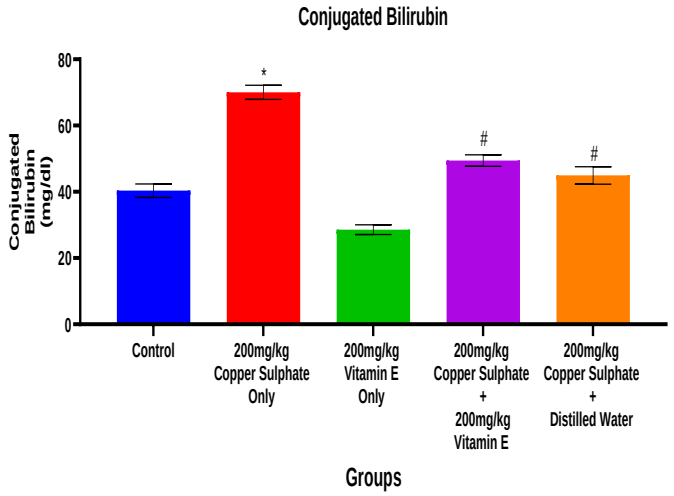

Fig 6. Conjugated Bilirubin Levels. *Represent comparison with control (P<0.05); #Represent comparison with 200 mg/kg of copper sulfate only group (P<0.05)

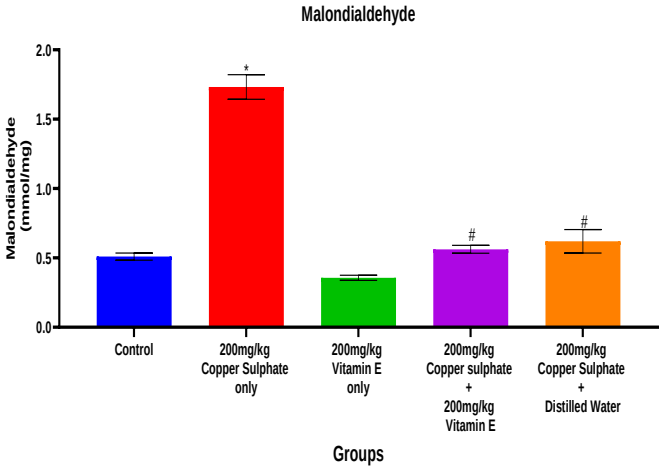

Fig 7. Liver Malondialdehyde Levels. *Represent comparison with control (P<0.05); #Represent comparison with 200 mg/kg of copper sulfate only group (P<0.05)

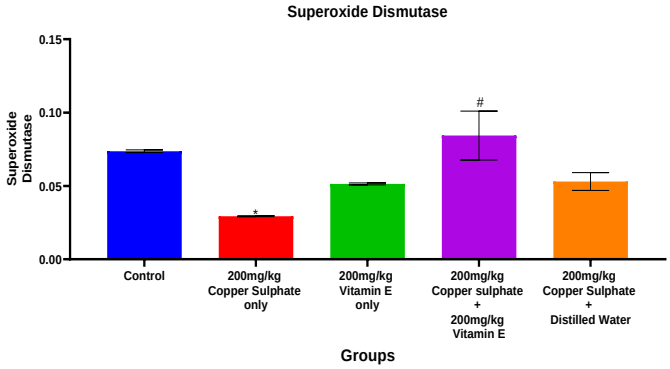

Fig 8. Liver Superoxide Dismutase Levels. *Represent comparison with control (P<0.05); #Represent comparison with 200 mg/kg of copper sulfate only group (P<0.05)

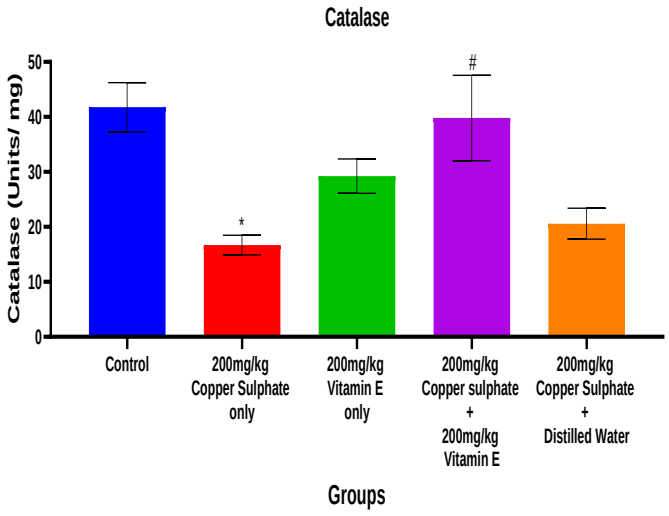

Fig 9. Liver Catalase Levels. *Represent comparison with control (P<0.05); #Represent comparison with 200 mg/kg of copper sulfate only group (P<0.05)

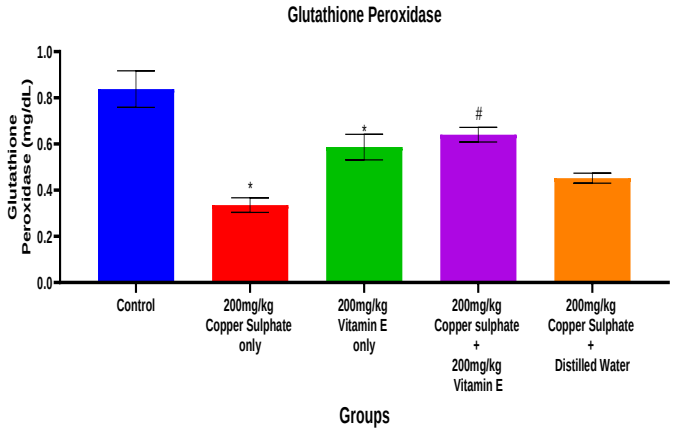

Fig 10. Liver Glutathione Peroxidase Levels. *Represent comparison with control (P<0.05); #Represent comparison with 200 mg/kg of copper sulfate only group (P<0.05)

PHOTOMICROGRAPH OF LIVER

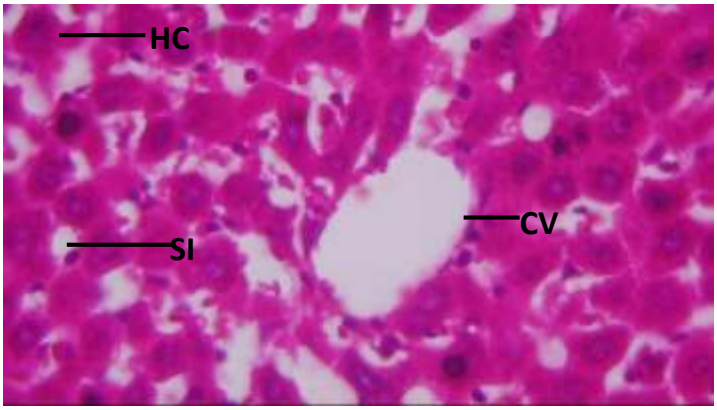

Plate 1. Rat liver. Control. Showing normal architecture composed of: hepatocytes (HC), sinusoids (SI) and central vein (CV): H & E 400x

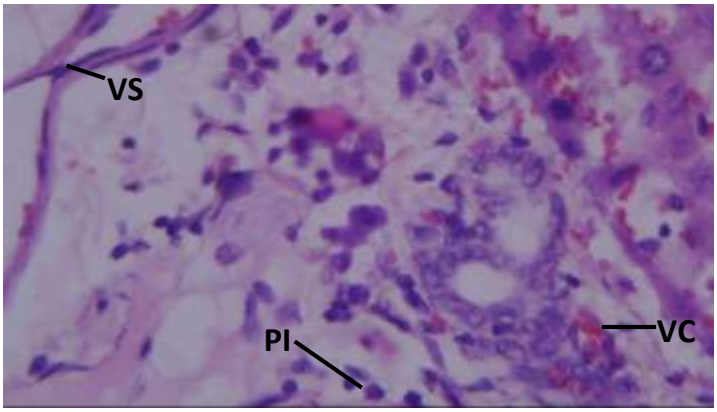

Plate 2. Rat liver given Copper only showing: vascular stenosis (VS), vascular congestion (VC), periportal infiltrates of inflammatory cells (PI): H&E 400x

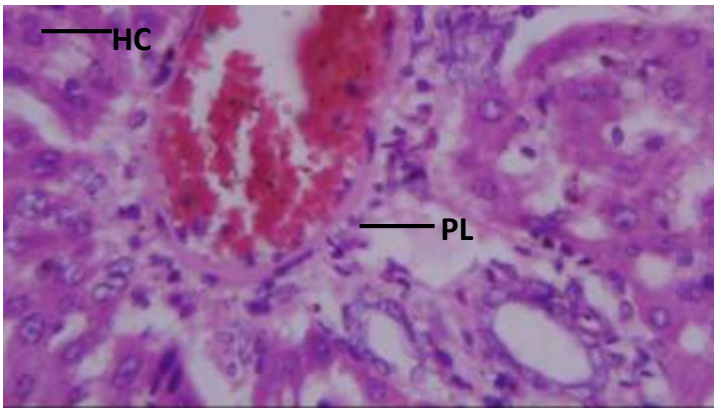

Plate 3. Rat liver given Vit. E only showing: normal hepatocytes (HC), periportal mobilization of lymphocytes (PL) : H&E 400x

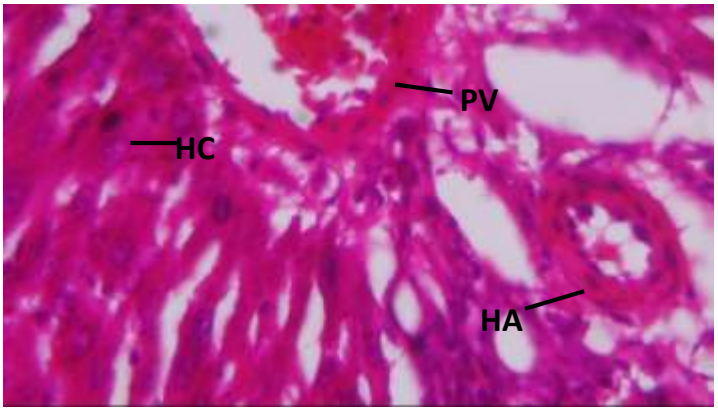

Plate 4. Rat liver given Copper for 30 days, then Vit. E for 30 days showing: normal architecture: hepatocytes (HC), portal vein (PV) and hepatic artery (HA): H&E 400x

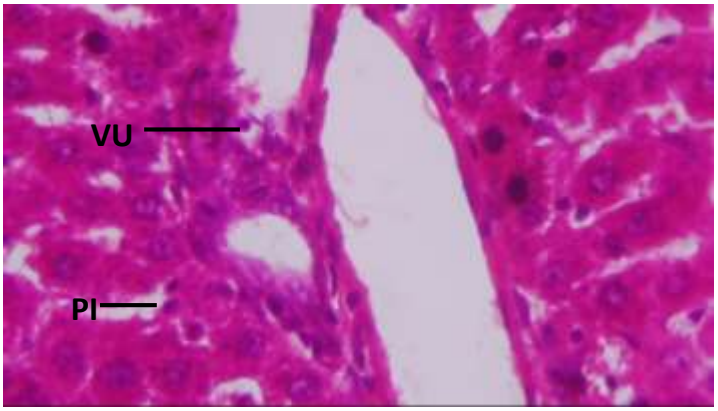

Plate 5. Rat liver given Copper for 20 days, then distilled water for 30 days showing: vascular ulceration (VU), periportal infiltrates of inflammatory cells (PI) : H&E 400x

DISCUSSION

Copper sulfate ($CuSO_4$), an inorganic compound plays vital role in various metabolic pathway in the body. It participates in electron transfer reactions in the respiratory chain and serves as a cofactor

for enzymes such as cytochrome C oxidase (Yoshikawa and Shimada, 2015). Copper-containing enzymes are essential for processes like iron metabolism, antioxidant defense, and connective tissue formation (Krupanidhi *et al*., 2008).

In mammals, copper sulfate is absorbed in the gastrointestinal tract and absorption process is tightly regulated to maintain copper homeostasis (Gaetke *et al*., 2014). Copper ions are transported across the intestinal epithelium into the bloodstream, primarily through the action of specific transporters (Gupta, and Lutsenko, 2009). They are transported in the bloodstream, mainly bound to proteins like ceruloplasmin (Tapiero *et al*., 2003).

In this study, there was significant increase (P <0.05) in serum Alanine transaminase (ALT), (Fig 1), Aspartate transferase (AST) (Fig 2) and Alkaline Phosphatase (ALP) (Fig 3) levels in rats treated with copper sulfate when compared to control. Aspartate transferase is a mitochondrial enzyme, predominantly found in the liver, skeletal muscles and kidneys while ALT is a cytosolic enzyme which is more specific for the liver (.Tiwari *et al,* 2020). This elevated liver enzymes is an indication of liver injury induced by the administration of copper sulfate. However, these effects were reversed with withdrawal of copper sulfate and treatment with vitamin E. The implication of this is that, discontinuation of toxicant like copper sulfate may allow organ like the liver to heal itself.

This study also showed significant decrease (P <0.05) in Total Protein level (Fig 4) in rats treated with copper sulfate when compared with control. Albumin makes up the major component of the the total protein. This decrease in total protein possibly resulted from the severe hepatic injury induced by copper sulfate treatment. The Total bilirubin (Fig 5) and Conjugated bilirubin (Fig 6) levels were significantly increased in rats treated with copper sulfate. Lin *et al*, (2020) found an association between plasma copper and serum bilirubin and an association between dietary copper and serum AST. Bilirubin is metabolized in the liver and an increase in bilirubin level may indicate severe liver and/or bile duct injury or an increase destruction of red blood cells. However, these elevated liver parameters were reversed with withdrawal of copper sulfate and treatment with vitamin E.

There was significant increase (P<0.05) in liver MDA level (Fig 7). Malondialdehyde is a product of lipid peroxidation and a bio-marker of oxidative stress (Tsikas, 2017). Its elevation indicates oxidative damage to cell membrane within the liver tissues (Jelic *et al*., 2021). However, this effect was reversed with withdrawal of copper sulfate and treatment with vitamin E. This study also showed a significant decrease (P<0.05) in liver SOD (Fig 8), CAT (Fig 9) and GPx (Fig) levels in copper sulfate treated rats when compared to the untreated control. Superoxide dismutase, Catalase and Glutathione peroxidase activities constitutes first line antioxidant defense mechanisms which are indispensible in the entire antioxidant defense strategy especially in relation to super oxide anion radicals that are generated continuously (Ighodaro and Akinloye, 2018). It was observed in this study that withdrawal of copper sulfate did not reverse the oxidative stress in the liver as depicted by the reduced liver SOD, CAT and GPx levels (Figs 8, 9, 10 respectively) but treatment with Vitamin E caused a significant increase in liver SOD, CAT and GPx levels when compared to the copper sulfate treated rats. This antioxidant effect of vitamin E corroborated the myriads of researchers view and documentation of the antioxidant potential of vitamin E.

Histologically, the liver of rats treated with copper sulfate showed evidence of vascular dilatation, stenosis and congestion. There was also presence of inflammatory cells at the periportal region (plate 2). These features are consistent with hepatic injury. Oguz and Yuksel, (2009) reported granular degeneration and necrosis of hepatocytes with evidence of vascular congestion in chick liver treated with copper sulfate. In vitro studies indicates that treating cells with copper results in alteration of tight junction permeability (Gotteland *et al*, 2001), which tend towards toxic injury in the case of excessive copper sulfate accumulation or overdose.

Vitamin E treated rats (plate 3) showed normal hepatocyte architecture. The presence of lymphocytes in the liver histology of vitamin E treated rats is an indication of possible immunologic potential with vitamin E. Rats treated with vitamin E after initial copper sulfate treatment in this study, showed normal hepatocytes architecture which is a reversal of hepatic injury induced by copper sulfate. However, rats administered distilled water after withdrawal of copper sulfate showed presence of inflammatory cells at the periportal region of the liver. This may indicate that hepatitis induced by copper sulfate administration may persist if left untreated.

Conclusion: Increase consumption of copper sulfate is a potential toxicant that induces oxidative stress and liver injury. Vitamin E possesses antioxidant and anti-inflammatory properties capable of reversing the hepatic damage induced by copper sulfate.

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