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Original Research Article

Copper Induced Hepatic Toxicity in Male Albino Rats and the Possible Protective Effect of Vitamin D

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ABSTRACT

Background: Copper (Cu), a trace element is safe to consume at low levels. Copper toxicity is often caused by unintentionally ingesting too much Cu from corroded water pipes and from Cu salt-containing creams for burn treatments due to its antimicrobial properties if applied to large areas of burned skin. Unfortunately, Cu-toxicity can extend up to liver cirrhosis. Vitamin D3 (VitD) is known for its efficient antioxidant, anti-inflammatory effects, antiapoptotic and antifibrotic properties.

Aim: The present study is designed to detect the possible protective effect of VitD against the toxic effects of Cu on the liver in rats.

Material and Methods: Thirty adult rats were divided into three groups (10 mice each): Control group, Cu Group B received Cu at a dose of 0.03 mg/kg body weight by gastric gavage and VitD+ Cu Group C received Vitamin D3 at a dose of (600 IU daily) 10 IU/ kg daily together with Cu for 14 days. Blood samples were collected to assess liver functions, liver tissue was examined for oxidative markers, histological and immunohistochemical examinations.

Results: The Cu group B revealed significant increase in AST, ALT and oxidative stress markers. Light microscopic examination showed disturbed hepatic architecture, ballooning of hepatocytes around widened central vein with less acidophilic cytoplasm, pyknotic nuclei and vacuolation. Hepatic sinusoids were dilated and congested between the rays of hepatic cells with dilated portal tracts and inflammatory cellular infiltration around. Liver sections stained by Masson trichrome detected fibrotic changes with increased fibrotic index. Immunohistochemical study showed increase in the proinflammatory cytokine TNF α and decrease in anti-inflammatory IL-10, while TUNEL technique detected increased number of apoptotic cells. Administration of VitD in group C led to a significant reduction in liver dysfunction and oxidative stress, accompanied by marked improvements in histological, immunohistochemical findings and decreased fibrosis and apoptosis.

Conclusion: VitD offers significant protective effects against Cu induced liver damage It has antioxidant, anti-inflammatory, anti-apoptotic and antifibrotic properties on the cellular and functional levels. So, VitD supplementation is recommended either as a therapeutic or a prophylactic measure in liver diseases to reduce OS, alleviate hepatic damage and interrupt the fibrotic process.

KEYWORDS: Copper toxicity, Microscopic anatomy of the liver, Oxidative stress, Pathological anatomy of hepatic fibrosis, Vitamin D.

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INTRODUCTION

Copper (Cu) is widely used all over the world due to its wide and antimicrobial activity. It is one of the heavy metals to which people are exposed in daily life. Copper, a trace element mostly stored in muscles and liver, is safe to consume at low levels. Though it's only needed in tiny amounts, the daily dose of Cu shouldn't exceed 2 mg/day [1, 2].

About 50 percent of Cu is excreted in the bile, while the rest is excreted through gastrointestinal secretions. So Cu homeostasis is mainly regulated by the gastrointestinal tract [3].

Although being an essential element and an important cofactor for many proteins, high level of concentrations of Cu are toxic due to its ability to generate hydroxyl radicals, and can cause damage to cellular components. A delicate balance between the uptake and efflux of Cu ions determines the amount of cellular Cu. Excess Cu induces not only oxidative stress but also DNA damage and cell proliferation [4, 5].

Symptoms of Cu toxicity arise when there is high intake of Cu, increased absorption or reduced excretion due to an underlying pathologic process such as Wilson disease; an inherited metabolic disease caused by a variant in the gene encoding Cu-ATPase enzyme leading to excessive Cu accumulation. For example, Cu-IUD does not cause any harm in normal female unless they already have a condition that affects the liver's ability to process Cu [6, 7].

Water supplies can contain high levels of Cu as by farm operations or industrial waste that drain into public wells. Also, Cu pipes, especially if corroded, may contaminate water through absorption of Cu particles. Less common, acidic foods cooked or stored in uncoated or corroded copper cookware or brass vessels [8-10].

Topical creams or compresses for burn treatments contain Cu salts can cause Cu toxicity if applied to large areas of burned skin. Another iatrogenic cause is dental amalgams, alloys and Cu tubing used in hemodialysis is. Additionally, Cu-sulfate is easily accessible and is even sold over the counter in many countries, therefore its bright blue color is attractive to children and is a frequent reason for accidental poisoning. It is also used in pesticides and leather industry. [11-13]. Copper toxicity can range from mild gastroenteritis limited to nausea, vomiting, and diarrhea to severe toxicity with hemolytic anemia, anuria and Cu liver cirrhosis [14, 15].

Vitamin D (VitD) is a steroid hormone, acquired by exposure to sunlight UV-rays and dietary intake, then becomes physiologically active through activities of several hepatic and renal isoenzymes. It is a known for its effective antioxidant and anti-inflammatory effects [16, 17]. Recent studies suggest that the administration of antioxidants could potentially mitigate the detrimental effects of Cu induced hepatotoxicity. While VitD is traditionally known for its role in bone metabolism, it has been found to have various additional therapeutic applications. Vitamin D exhibits several cytoprotective properties, including anti-fibrotic and anti-inflammatory activities, making it a promising candidate for minimizing the harmful effects of Cu toxicity on the liver [18, 19].

Few research has delt with the cellular changes of Cu on the liver. The present study is designed to detect the possible protective effect of VitD against the toxic effects of copper on the liver in rats.

MATERIAL AND METHODS

Chemicals: Cupper and Vitamin D3 were obtained from iHerb, Inc. Oxidant and antioxidant stress markers; Malondialdehyde (MDA), total antioxidant capacity (TAC) standard, alanine aminotransferase (ALT) and aspartate aminotransferase (AST), were obtained from Biodiagnostics, Giza, Egypt). Tumor necrosis Factor alpha (TNF-α) antibody and Anti Interleukin-10 (IL-10) antibody were obtained from Sigma-Aldrich, Merck, Darmstadt, Germany).

Experimental animals and conditions: Thirty adult rats approximately 10 weeks old (200-300g) were acquired from the Mowassat animal house, Alexandria University. They were housed in standard environmental conditions with free access to water and food. Experiments were performed in accordance with NIH Guide for care and use of laboratory animals.

The protocol of the study was approved by Research Ethics Committee accordance with "Guide for care and use of laboratory animals" at Faculty of Medicine, Alexandria University, Egypt (#0306800). All experiments were performed in accordance with NIH Guide for care and use of laboratory animals [20].

Experimental design: Rats were categorized into three groups (10 mice each) according to an equal mean body weight and receive the following for 14 days:

Neg Group A: Control received distilled water as a vehicle for Cu and Vit D

Cu Group B: Copper at a dose of 0.03 mg/kg body weight daily dissolved in distilled water was given by gastric gavage.

VitD+ Cu Group C: Vitamin D3 at a dose of (600 IU daily) 10 IU/ kg daily together with Cu (the same as group B) were given by gastric gavage [19].

Collection of blood and tissue samples:

On the day of sacrifaction, blood was collected from the inner canthus of the eye of rats by a capillary tube through the retroorbital method, under mild anesthesia by inhalation of ether. Blood samples were collected in test tubes and left to clot for 10 min, then serum was removed to measure oxidant and antioxidant stress markers MDA and TAC respectively and ALT, AST [21].

Upon completion of the experiment, rats were sacrificed under anaesthesia using 50 mg/kg of sodium pentobarbital by cervical dislocation.

Histological study

The liver was removed and prepared for histological study. Liver segments were fixed in 10% formol saline, processed using a standard procedure, and embedded in paraffin wax. Paraffin blocks were sliced into 5µ sections and stained with hematoxylin and eosin (H&E) and Masson trichrome stain for visualization under a light microscope (LM) for assessment of any possible histopathological finding, hepatic architecture and the deposition of collagen type I [22].

Immunohistochemical study:

Liver sections were incubated with the primary antibodies; Anti-TNFa and anti-IL-10 (rabbit polyclonal IgG) Santa Cruz Biotechnology, Inc., Dallas, Texas, USA) according to the manufacturer's protocol). The primary antibody was then detected using avidin-biotin peroxidase detection solution. Negative controls included non-immune serum. The stained sections were analyzed and photographed with a Leica microscope. The quantification for Immuno-staining colour intensity were identified and measured by immunohistochemistry image analysis toolbox in the software followed by immunostaining calculation "Area Percentage" for TNFa and IL-10 using the Leica QWin 500 image analyzer. The data were expressed as mean ± SD for area %. [21,23].

TUNEL assay this was used to measure the extent of DNA in apoptotic cells. Counterstaining was done using DAPI (#P36962; Thermo Fisher Scientific). The sections were examined at high magnification (x400) under fluorescence microscope (EVOS). DNA damage is observed by emission of a green, fluorescent dye. The percentage of necrotic / apoptotic cells was calculated in 15 randomly selected nonoverlapping fields. The apoptotic index was calculated by the help of the cell counter tool supplied by EVOS fluorescent microscope [24].

Statistical analysis of Data

Data were analyzed using the SPSS software (version 25). All data were tested for normality and homogeneity by Kolmogrov and Levene tests respectively. One-way ANOVA was utilized for the comparison between the study groups. To determine the correlations Pearson's test was used. Statistical significance set at the 5% level.

RESULTS

Serum parameters:

Liver functions: were markedly affected with acute Cu toxicity. Serum levels of AST and ALT were significantly higher in comparison to the Neg group but administration of VitD resulted in a significant reduction. **(Table 1, Figure 1)**.

Table 1: Mean ± SD of liver function parameters and serum 25-OH vitamin D.

Liver functions	Neg	Cu	Cu+VitD
AST (IU/L)	102.10 ± 3.35	218.30±5.42 [a]	150.70±5.76 [a,b]
ALT (IU/L)	67.60 ± 3.92	168.20±4.61 [a]	112.20±4.80 [a,b]
Serum 25-OH VD	40.10±2.69	30.09±2.28 [a]	51.38±2.59 [a,b]

a significant at $p<0.05$ compared with Neg group
b significant at $p<0.05$ compared with Cu group

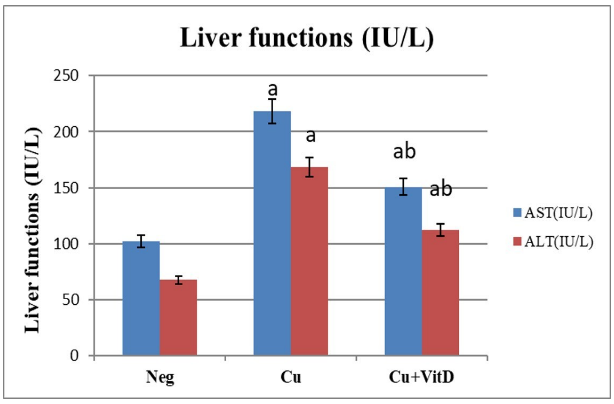

Fig. 1: Liver functions among the studied groups.

a significant at $p<0.05$ compared with Neg group
b significant at $p<0.05$ compared with Cu group

Hepatic tissue concentrations of oxidant and antioxidant markers:

The hepatic tissue homogenates demonstrated a change in the content of oxidative and antioxidant markers. The Cu group showed a significant increase in MDA concentration compared with the Neg group and treatment with VitD resulted in a significant reduction, but it was still above the Neg group. On the other hand, TAC concentration of the hepatic tissue was significantly reduced in the Cu group compared with the Neg group while VitD group showed a significant elevation but still it was less than the Neg group. **(Table 2)**

There was a positive correlation between serum level of vitamin D and the hepatic content of TAC and an inverse correlation between serum vitamin D and hepatic MDA content. **(Figure 2)**

Table 2: Oxidant and antioxidant markers in the studied groups.

Marker	Neg	Cu	Cu+VitD
MDA	0.87±0.02	1.70± 0.08[a]	1.22±0.04 [a,b]
TAC	101.90±3.41	68.30±4.85 [a]	87.40±3.98 [a,b]

a significant at $p<0.05$ compared with Neg group
b significant at $p<0.05$ compared with Cu group

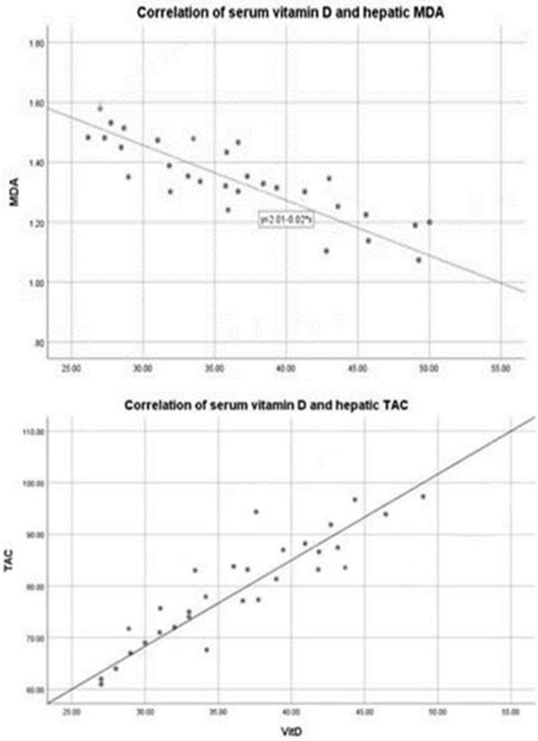

Fig. 2: Correlation of serum vitamin D and hepatic level of MDA and TAC.

Histological results:

H&E stain: The liver tissue of the Neg group showed normal hepatic architecture with normal shaped hepatocytes around the central vein normal sinusoids. The hepatocytes showed acidophilic cytoplasm and vesicular nuclei. The portal tract shows an artery, a vein and bile canaliculus. **(Figure 3A)**

The group treated with Cu demonstrated disturbed hepatic architecture with ballooning of the hepatocytes around the widened central vein. Some hepatocytes showed less acidophilic cytoplasm, others had pyknotic nuclei and a number of cells showed vacuolation. The hepatic sinusoids were dilated and congested between the rays of hepatic cells, also the portal tracts were dilated. There was mild inflammatory cellular infiltration inside the lobules around the dilated central veins and the dilated portal tracts. **(Figure 3B)**

Adding VitD improved the picture where there was marked restoration of the hepatic architecture around the central vein, more acidophilic cytoplasm with less pyknotic nuclei, less vacuolations and less inflammatory cellular infiltration. The hepatic sinusoids were slightly dilated and less congested and the portal tracts were seen more or less normal. **(Figure 3C)**

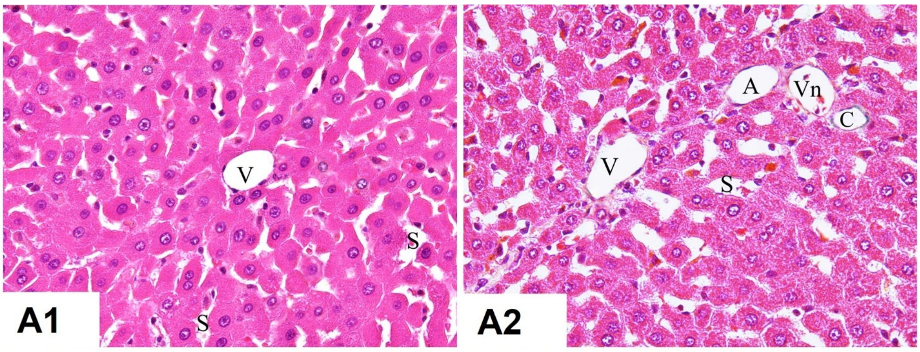

Fig. 3A: A photomicrograph of a section of the liver of the Neg group showing normal shaped acidophilic hepatocytes (A1) with vesicular nuclei around the central vein (V), normal sinusoids (S) and intact portal tract with an artery (A), a vein (Vn) and bile canaliculus (C) (A2). (H&E X400).

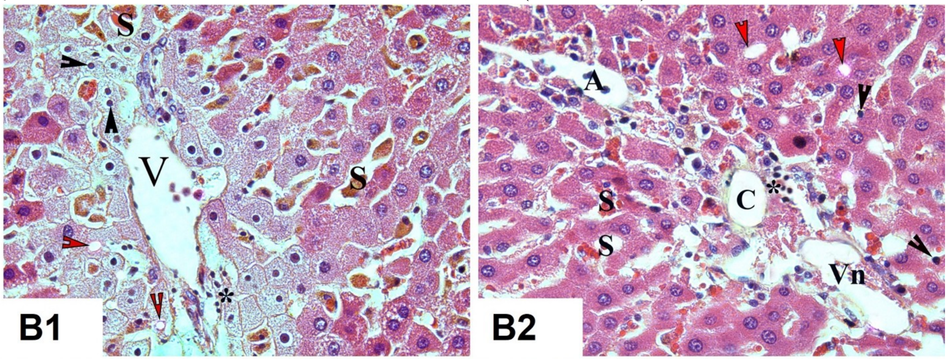

Fig. 3B: A photomicrograph of a section of the liver of the Cu group showing disturbed hepatic architecture with less acidophilic hepatocytes around the dilated central vein (V), dilated and congested sinusoids (S). Some cells show pyknotic nuclei (black arrow), others show vacuolation (red arrow) (B1). Mild inflammatory cellular infiltration (*) is seen around the dilated central vein and dilated portal tract. (A) artery, (Vn) vein and (C) bile canaliculus (B2). (H&E X400)

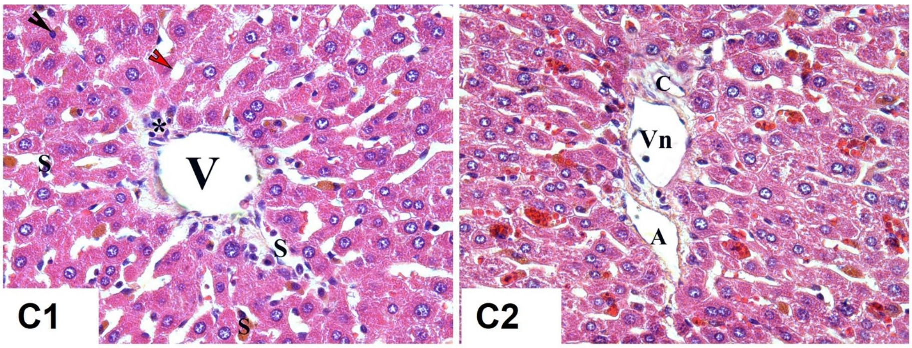

Fig. 3C: A photomicrograph of a section of the liver of the Cu+VitD group showing restoration of the hepatic architecture with more acidophilic hepatocytes around the central vein (V), less dilated and less congested sinusoids (S). Few cells show pyknotic nuclei (black arrow) and few cells show vacuolation (red arrow) (C1). Less inflammatory cellular infiltration (*) is seen and the portal tract is seen normal. (A) artery, (Vn) vein and (C) bile canaliculus (C2). (H&E X400)

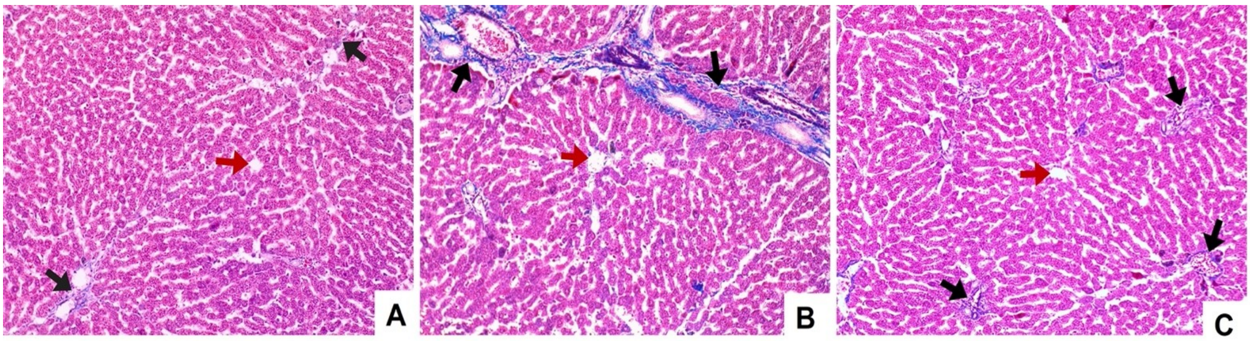

Fig. 4: A photomicrograph of liver sections stained with Masson trichrome showing minimal fibrotic changes (blue colour) in the Neg group (A). The Cu group (B) shows marked fibrotic changes especially around the portal tracts (black arrow). The fibrotic changes were markedly reduced in the Cu+VitD group (C). (D) is the fibrotic index . Central vein (red arrow), portal tract (black arrow). (Masson Trichrome X200)

Masson trichrome stain: liver sections were stained by Masson trichrome stain to detect fibrotic changes. The Neg group showed minimal fibrotic changes especially around the portal tracts, while the Cu group showed significantly increased fibrotic changes especially around the portal tracts. Treatment with VitD significantly reduced the amount of fibrous tissue around the portal tracts. **(Figure 4 A, B, C, D)**

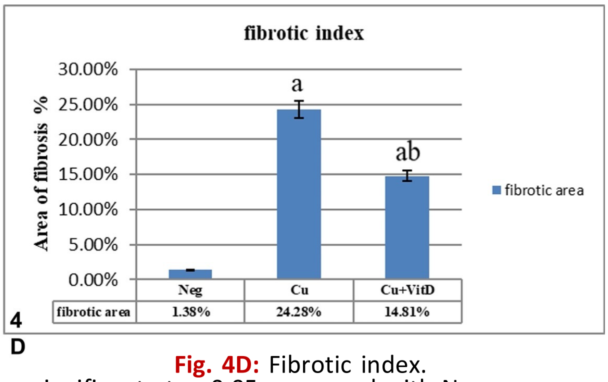

Fig. 4D: Fibrotic index.
a significant at $p<0.05$ compared with Neg group
b significant at $p<0.05$ compared with Cu group

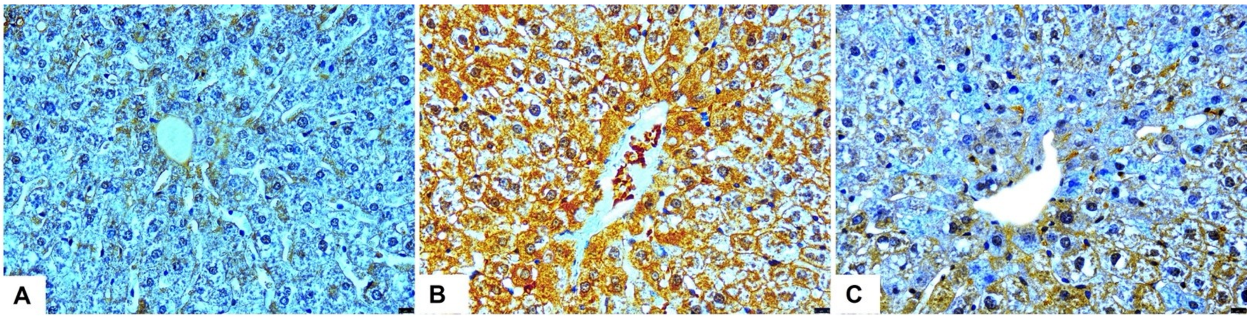

Fig. 5: A photomicrograph of a section of the liver showing immune expression (brown colour) of TNF α in the liver tissues and hepatocytes. The Neg group (A) shows minimal expression, the Cu group (B) has the highest reaction while the Cu+VitD group (C) shows a significant reduction than the Cu group (D). X400

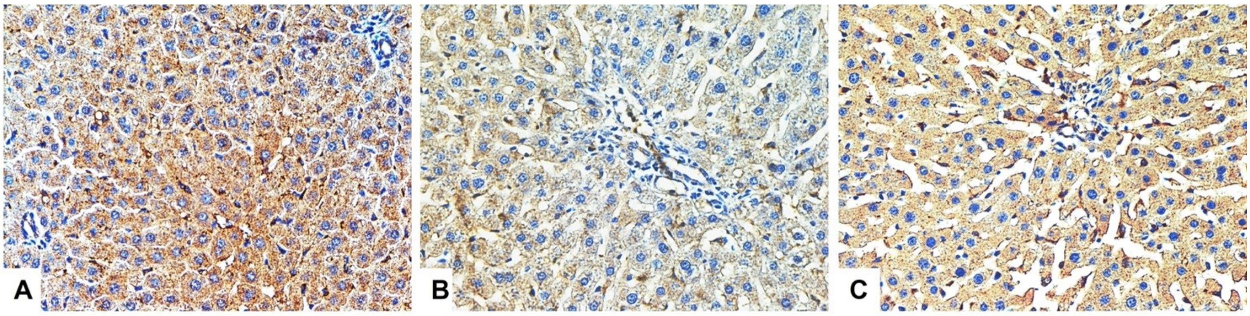

Fig. 6: A photomicrograph of a section of the liver showing immune expression (brown colour) of IL-10 in the liver tissues and hepatocytes. The expression was highest in the Neg group (A), minimal in the Cu group (B) while the Cu+VitD group (C) shows a marked elevation than the Cu group (D). X400

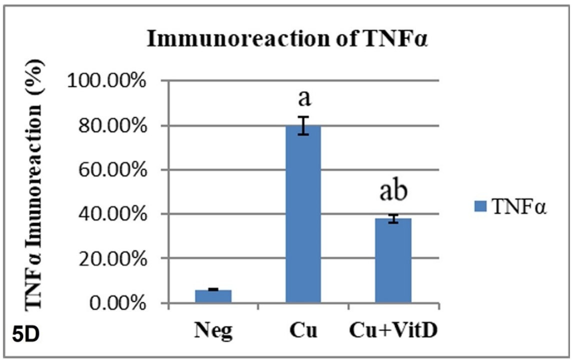

Fig. 5D: Immunoreaction of TNF α in hepatic tissue.
a significant at $p<0.05$ compared with Neg group
b significant at $p<0.05$ compared with Cu group

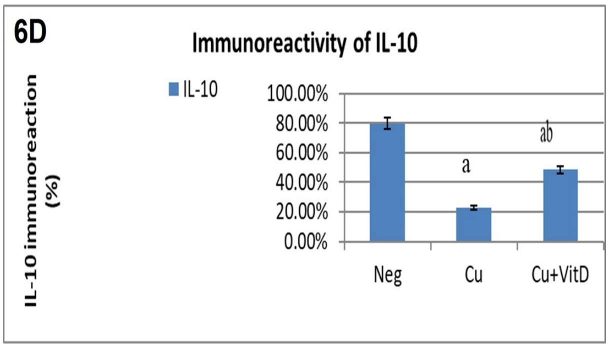

Fig. 6D: Immunoreaction of IL-10 in hepatic tissue.
a significant at $p<0.05$ compared with Neg group
b significant at $p<0.05$ compared with Cu group

Immunohistochemical results:

TNF : The liver tissue of the Neg group showed minimal immunoreaction of Proinflammatory cytokine TNFα that showed a significant increase in the Cu group compared with the Neg group. Treatment with VitD showed a significant reduction of the immune expression but the reaction was still higher than the Neg group. **(Figure 5 A, B, C, D)**

IL-10: The liver tissue of the Neg group showed the highest immunoexpression of the anti- inflammatory IL-10 that showed a significant decrease in the Cu group compared with the Neg. Treatment with VitD showed a significant increase of the immunoreaction but still lower than the Neg group. **(Figure 6 A, B, C, D)**.

Cu-induced liver cell damage (TUNEL technique): The liver tissue of the Neg group showed a minority of hepatic cells that were positive for apoptosis with a significant increase in the number of apoptotic cells in the Cu group. On the other hand, Cu+VitD group showed a marked reduction of the number of apoptotic cells. **(Figure 7 A, B, C, D)**

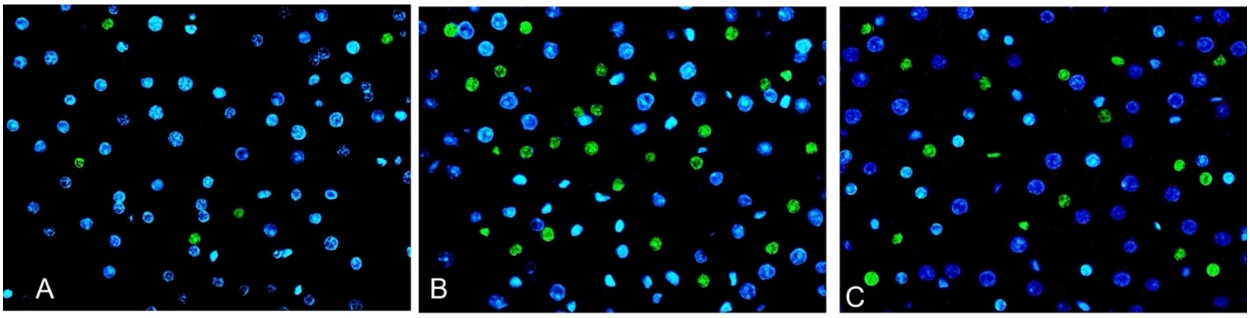

Fig. 7: A photomicrograph of a section of the liver revealing TUNEL reaction and apoptotic cells (green colour). The apoptotic cells are minimal in the Neg group (A) and show a significant increase in the Cu group (B), while the Cu+VitD group (C) shows a significant reduction. (D) is the (%) of apoptotic cells. X400

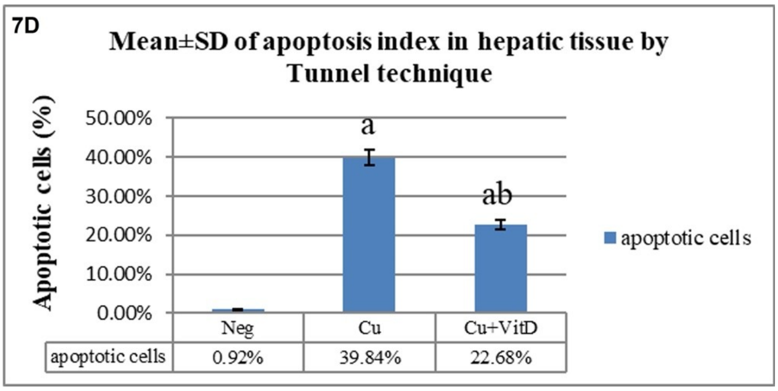

Fig. 7D: Apoptosis index.
a significant at p<0.05 compared with Neg group
b significant at p<0.05 compared with Cu group

DISCUSSION

Copper, a heavy metal and essential trace element, is detected in many human enzymes and transcription factors. Copper plays a major role in different processes, including tissue repair, antioxidant, and regulation of enzyme activity. Acute cupper toxicity is usually the result of ingestion of excessive amounts, often as a suicide attempt or even accidental ingestion of coins by children. Acute hepatotoxicity impairs liver functions after 24-72 hours with elevations of liver enzymes, prothrombin time and ensuing jaundice; whereas chronic toxicity may give a picture similar to Wilson's disease ending in liver failure [7, 25-27].

The present study demonstrated obvious liver damage in the copper group confirmed on the cellular level by disturbed hepatic architecture with early signs of necrosis as pyknotic nuclei and vacuolations of the hepatocytes around dilated central veins together with dilated sinusoids and portal tracts as well as mild inflammatory cellular infiltration accompanied by elevated levels of liver enzymes AST and ALT. The inflammatory effects of Cu were demonstrated as TNF-α, a proinflammatory protein was elevated while the anti-inflammatory marker; IL-10, was reduced. Similar findings were obtained by Ahmed et al.,[28] Ghonimi et al.,[29] El Bialy et al.,[30] Al-Bairuty et al.,[31] Ostaszweska et al.,[32] Jian et al.,[33] and Cho et al [34].

An inflammatory mechanism for Cu toxicity was studied by many authors. Jian et al., [33] found that copper resulted in inflammation and fibrosis of the lung tissues via increasing myeloperoxidase activities and stimulating the nuclear factor kappa b (NF-kB) signaling pathway that stimulates the transcription of proinflammatory cytokines which are responsible for the influx of inflammatory cells and attenuating the mRNA and protein expression of anti-inflammatory cytokines. Moreover, Cu-induced oxidative stress (OS) and excessive reactive oxygen species (ROS) production in the lung tissue might contribute to lesions in the lung up to its malfunction.

Also, a study made by Cho et al.,[34] attributed Cu-induced cytotoxicity to the increased expression of the pro-inflammatory cytokine TNF-á that may lead to inflammatory cellular infiltration and aggravate OS and mitochondrial dysfunction initiating apoptosis.

The hepatotoxic effects of Cu may be accentuated by its water-soluble properties inducing structural changes, cytotoxic and apoptotic effects. It was reported that Cu decreases cell viability by binding to DNA resulting in DNA damage and apoptotic changes [35]. This was proved in the present study that showed increased number of apoptotic cells together with elevation of the oxidant marker MDA, and depression of the antioxidant marker TAC. Multiple studies mentioned early changes of Cu toxicity that tend to occur in the mitochondria, nuclei, and peroxisomes with cell senescence and impaired function [35-40].

The accumulation of intracellular copper is associated with generation of ROS and OS which is always significantly associated with DNA damage, perturbing cellular function. Abudayyak et al., [35] and Chen et al.,[41] reported organ damage mainly in the liver and brain due to Cu toxicity through generation of ROS and oxidation of thiol groups, lipid and protein peroxidation, inhibition of pyruvate dehydrogenase activity leading to mitochondrial dysfunction, with loss of mitochondrial membrane potential rendering the membrane permeable to ROS with DNA damage resulting in cell death. Moreover, Cu-induced OS impairs the proliferation of hepatic cells that also ends in apoptotic changes and subsequent liver dysfunction [38-41]. Also, Cu may induce stress in the endoplasmic reticulum that can promote hepatic apoptosis in mice by activating caspase signaling pathway [42].

The present study proved the permanent residual and destructive effect of Cu toxicity through inducing fibrotic changes in the hepatic tissues. Sinkovic et al.,[27] also reported that Cu toxicity affected several organs including the gastrointestinal tract, especially the liver ending in fibrosis. On the other hand, Zhu et al.,[36] found Cu-induced cell senility and renal fibrosis, while Janssen et al.,[43] showed that Cu over-accumulation is associated with lung fibrosis.

Copper overload in the body usually results in Cu accumulation in the liver with hepatic dysfunction and impaired hepatic secretion of Cu in the bile due to hepatic toxicity. This may lead to hepatic fibrosis ending with hepatic cirrhosis [44]. Also, Cu causes accumulation of cell proteins that bind to thiol residues causing cross-linkage of membrane proteins that elicits fibrotic reactions in addition to the mitochondrial membrane permeability changes and dysfunction with cell senility and fibrosis [36, 45, 46]. Moreover, the release of profibrotic mediators and inflammatory cytokines cause the infiltration of macrophages into the interstitium and induction of fibrosis [47, 48].

To the best of our knowledge, the protective effects of VitD against cellular damage caused by Cu-induced hepatotoxicity have yet not been studied, although the potent antioxidant effects of VitD have been mentioned in previous studies [49].

Liver diseases are acclaimed to be along with disturbed VitD homeostasis, decreased 25-OH VD levels as well as deficient antioxidant capacity that disrupts the function of active VitD. Vitamin D is known to have potent antioxidant, anti-inflammatory, antiproli-ferative, anti-apoptotic together with antifibrotic and fibrinolytic properties [50, 51-53].

In the current study, addition of Vit D affected the oxidant and antioxidant status through reduction of the level of MDA and elevation of the level of TAC. Similar findings were obtained by Elbassuoni et al. [54] and Abdelghany et al [55]. The antioxidant capacity of VitD is well documented as it reduces liposomal lipid peroxidation, inhibits mitochondrial dysfunction, increases antioxidant cellular function, reduces generation of ROS and reduces OS. Thus, ROS scavengers could shield the hepatic cells from Cu-induced damage. [54 ,55, 56]

In the present study, addition of VitD improved the hepatic tissue on the cellular and functional levels. There was restoration of the hepatic tissue architecture, reduction of the hepatic tissue inflammatory cells with significant reduction in the fibrotic changes as well as reduction in the quantity of apoptotic cells. Also, there was a reduction of the expression of the proinflammatory cytokine; TNF-a together upregulation of the anti-inflammatory marker IL-10, with a concomitant improvement of the liver functions evidenced by improvement of ALT and AST levels. Similar results were obtained by ElBoshy et al. [57], Almasmoum et al [58] and Abdelghany et al. [59].

Previous studies reported the anti-inflammatory effect of VitD as it could inhibit the inflammatory process through its receptor (VDR) that is responsible for regulating the inflammatory reactions during tissue injury and activation of VDR signaling that suppresses NF-kB activity so inhibits the stimulation of transcription of proinflammatory cytokines thus the influx of inflammatory cells. Also, the antioxidant effect of VitD may add to its anti- inflammatory properties through preservation of mitochondrial respiratory function and reduction of ROS that may help in the inflammatory cellular influx in the hepatic tissue [60, 61].

Moreover, VitD plays a major role in regulation of the immune system so it has an impact on cells and cytokines involved in the inflammatory process [50, 51, 62, 63].

Collected information show that vitamin D is capable to disrupt liver fibrosis at various levels, namely inhibition of inflammatory and oxidative injury that initiate the fibrotic process, prevention of activation and proliferation of hepatic stellate cells which play a crucial role in hepatic fibrosis, prevention of accumulation of extracellular matrix, and even degradation of collagen by activation of matrix metalloproteinase and inhibition of tissue inhibitor of matrix metalloproteinase [58, 59, 64]. Moreover, VitD through its immunomodulatory and anti-inflammatory properties activates the release of immunoglobulins that reduce the tissue inflammatory response which enhances the fibrotic process [57, 58, 59]. Consequently, the ability of VitD to reduce the cellular inflammatory and fibrotic response in addition to its antioxidant capacity and prevention of mitochondrial dysfunction may add to the anti-apoptotic effects of VitD. Moreover, VitD affects calcium homeostasis as it increases calcium absorption from the gastrointestinal tract and inhibits calcium release from the endoplasmic reticulum and so it inhibits cell apoptosis [65, 66, 67].

CONCLUSIONS

In conclusion, this study proves the antioxidant, anti-inflammatory, anti-apoptotic and antifibrotic properties of VitD against Cu- induced hepatic toxicity on the cellular and functional levels. So, VitD supplementation is recommended either as a therapeutic or a prophylactic measure in liver diseases to inhibit ROS generation, reduce OS, mitigate hepatic damage and interrupt the fibrotic process.

ABBREVIATIONS

Copper - Cu
Vitamin D3 - VitD
Malondialdehyde - MDA
Total antioxidant capacity - TAC
Nuclear factor kappa b - NF-kB
Tumor Necrosis Factor alpha - TNF-α
Interleukin 10 - IL-10
Oxidative stress - OS
Reactive oxygen species - ROS

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AUTHORS’ CONTRIBUTIONS

All authors contributed to the manuscript and approved the final version

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Competing interests: No potential conflict of interest was reported by authors.

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