Research Article

The Impact of Chronic Exposure to Copper Sulfate on the Liver, and the Role of Ascorbic Acid in Improving Liver Function in Local Rabbits (*Cuniculus lepus*)

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Abstract | The present study was conducted to evaluate the impact of chronic exposure to copper sulfate ($CuSO_4$) on liver function and to investigate the potential protective role of vitamin C (ascorbic acid). Adult male rabbits (*Cuniculus lepus*) were divided into four groups (n = 8 per group), including a control group. Two experimental groups (groups 2 and 3) were exposed to $CuSO_4$ at doses of 100 mg/kg^{-1} and 200 mg/kg^{-1}, respectively. Group 4 received a combined treatment of $CuSO_4$ (200 mg/kg^{-1}) and ascorbic acid (200 mg/kg^{-1}). Biochemical analysis revealed a significant increase in total protein (TP) in group 3 and elevated albumin (Alb) levels in both groups 2 and 3 compared to the control group. Other parameters including glucose, aminotransferases (AST/TGO and ALT/TGP), and alkaline phosphatase (ALP) showed no statistically significant changes ($P > 0.05$). In the group treated with both $CuSO_4$ and ascorbic acid, there was a significant increase in TGP, TP, and Alb compared to the control, and in TGO compared to group 2, along with a significant decrease in ALP relative to the control group ($P < 0.05$). Histopathological examination revealed notable liver alterations in rabbits exposed to $CuSO_4$, whereas co-administration of ascorbic acid ameliorated structural damage and improved hepatic tissue integrity. In conclusion, the findings suggest that ascorbic acid may mitigate the hepatotoxic effects associated with chronic $CuSO_4$ exposure.

Keywords | Copper sulfate, Chronic exposure, Ascorbic acid, Liver function

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INTRODUCTION

Copper (Cu) is the metal that man discovered from the beginning of history. For ten thousand years, it was considered the second most important element in terms of utility for human beings, after iron (Stern *et al.*, 2007). However, this metal is an essential trace element for biological life for humans, plants, animals and microorganisms. The cupric concentration in the human body ranges from 1.4 to 2.1 mg per kg. It is found in the liver, muscles and bones, and is transported through the bloodstream by a protein called ceruleoplasmin (Touri, 2012). according to Hart *et al.* (1928) Cu is essential in animals, where there is a close correlation between the signs of Cu deficiency and the decrease in the activity of various enzymes dependent on this metal. Trocello *et al.*

(2010) find that the Cu deficiency resulted in a decrease in the plasma concentration of ceruloplasmin, which causes a misuse of iron and Cu enzymes, on the other hand Cu is used by many enzymes that participate in the synthesis of neurotransmitters, the formation of connective tissue or in the respiratory chain as cofactors. An excess or shortage of Cu can lead to harmful effects, such as cell damage in various organs (Bahloul, 2018), it is recommended to have a daily consumption of 0.15 mg kg^{-1} body weight (Arena *et al*., 2018). For guinea pigs and rabbits, a dose as low as 2 mg kg^{-1} of $CuSO_4$ is fatal when administered intravenously or by injection into a vein (Hartley and Kidd, 1983).

In humans, Cu toxicity can result from mutations in the genes that control the biological processes related to this metal. High levels can cause liver damage due to viral infection or cholestatic disease. Today, cases of Cu poisoning are increasingly common in a variety of animals (Blood *et al*., 1983), it is suggested that the cellular redox buffer can reduce acute oxidative stress in response to an excess of Cu, but cannot compensate for secondary biochemical effects such as altered lipid metabolism, altered transcriptional activation or mitochondrial fragmentation (Huster *et al*., 2007; Zischka and Lichtmannegger, 2014).

According to Perestrelo *et al*. (2021) as a salt, Cu is toxic and it is used in various fields, especially in agriculture as a pesticide and fungicide, as well as in the production of adhesive glues, dyes and in the photography industry (Gamakaranage *et al*., 2011).

Cu intoxication (inhalation, ingestion or local exposure) leads to intravascular hemolysis followed by hepatic and renal insufficiency (Perestrelo *et al*., 2021), et clinically, it is characterized by non-specific signs and symptoms such as dysgenesis, abdominal pain, vomiting, muscle cramps or spasms, diarrhea, inky urine, jaundice, anemia and convulsions (Gunay *et al*., 2006; Gamakaranage *et al*., 2011).

Tests on animals that were poisoned with $CuSO_4$ revealed acute toxicity symptoms in the spleen, liver and kidney (Clayton and Clayton, 1981). Damage can also occur to the brain, liver, kidneys and gastrointestinal tract in response to overexposure (Hartley and Kidd, 1983). Blood (1979) showed that poisoning is frequently cumulative, resulting from prolonged exposure to Cu. It is possible that the metal will accumulate gradually in the liver lysosomes until it reaches a specific threshold for each species, where the liver loses its ability to store it (Blood, 1979; Schilsky and Stemlieb, 1993). In some species, an increase in the cupric concentration in the blood can cause an acute hemolysis crisis (Sutter *et al*., 1958; Schilsky and Stemlieb, 1993). It has been shown that a number of nutrients interact with Cu and modify its cellular effects, among these nutrients vitamin C (Vit C). *In vivo* studies on this vitamin show that the compound is beneficial and can reduce oxidative damage caused by Cu (Gaetke and chow, 2003).

Based on existing theoretical data and toxicological research, this study aims to evaluate the effects of chronic exposure to copper sulfate ($CuSO_4$), as a source of copper, on liver function and cellular structure in male rabbits (*Cuniculus lepus*). The investigation involves measuring selected biochemical markers and assessing liver histology to determine the extent of hepatic damage, as well as examining the potential protective role of ascorbic acid in mitigating Cu-induced hepatotoxicity.

MATERIALS AND METHODS

Chemicals products

The Chemical product used in this study « Neela Thotha » or copper sulfate ($CuSO_4$) is a compassing bright-blue or clear crystal in pentahydrate form [$CuSO_4$ $5H_2O$] (Gamakaranage *et al*., 2011), and 100% Pure Vitamin C surfine powder (highly assimilable) (CAS 50-81-7) was purchased from pharmacia group saidal.

Animal

This experiment was conducted on 32 adult male rabbits (*Cuniculus lepus*), aged between 6 and 8 months, with body weights ranging from 1600 to 1700 grams. The animals were obtained from a rabbit breeding center (Landjas Zhori, Centre Hallouya, Soumaa, Blida, Algeria). The rabbits were housed under controlled environmental conditions, with an ambient temperature of approximately 27°C and a spring-like atmosphere. They were provided with free access to water and a diet consisting of pelleted feed formulated with a balanced composition of proteins, carbohydrates, lipids, vitamins, and minerals. In addition to pellets, their diet was supplemented with lettuce and crusty bread. The feed was sourced from the Agricultural Cooperative WChefoun, Boumerdes Province, Algeria.

Experimental design

The rabbits were separated into four groups (n= 8 per group). The experimental protocol is as follows:

- Group 01: control group: Granulated feed + lettuce and crispbread + water.
- Group 02: $CuSO_4$-treated group: Granulated feed mixed with 100 mg of $CuSO_4$ + lettuce and crispbread + water.
- Group 03: $CuSO_4$-treated group: Granulated feed mixed with 200 mg of $CuSO_4$ + lettuce and crispbread + water.
- Group 04: mixture of $CuSO_4$ and ascorbic acid-treated group: Granulated feed mixed with 200 mg $CuSO_4$ and 200 mg of ascorbic acid+lettuce and crispbread + water.

Rabbits received the treatment every day for 5 weeks.

Table 1: The variations of the biochemical parameters in rabbits exposed to different doses of $CusSO_4$ with and without ascorbic acid.

	Control	SCu1	SCu2	SCu+AC
Glucose (g L^{-1})	1.414±0.446^{ab}	1.95±0.418^{a}	0.8925±0.2631^{b}	1.397±0.465^{ab}
TGO (UI L^{-1})	32.13±5.94^{ab}	20.5±12.44^{b}	31.34±6.91^{ab}	44.6±29.5^{a}
TGP (UI L^{-1})	39.75±4.43^{b}	57.9±23.88^{ab}	41.13±16.53^{ab}	71.6±35.1^{a}
ALP (UI L^{-1})	145±23.12^{a}	121.65±22.59^{ab}	112.12±24.48^{ab}	104.4±34.7^{b}
TP (g L^{-1})	57.52±3.42^{b}	63.23±11.35^{ab}	69.21±6.47^{a}	67.6±3.94^{a}
Alb (g L^{-1})	26.2±1.602^{b}	33.63±5.24^{a}	38.51±4.38^{a}	37.08±4.61^{a}

SCu1: dose 1 of $CuSO_4$ (100mg kg^{-1}), SCu2: dose 2 of $CuSO_4$ (200mg kg^{-1}), SCu+AC: 200 mg kg^{-1} of $CuSO_4$ supplemented with ascorbic acid; TGO: Glutamate-Oxaloacetate-Transaminase; TGP: Glutamate-Pyruvate Transaminase; ALP: alkaline phosphatase; TP : Total protein; Alb : Albumin.

Biochemical evaluation

At the end of the experimental period, the rabbits were fasted for 24 hours. Blood samples were collected at the Aquatic and Terrestrial Ecosystems Research Laboratory, University of Mohammed Cherif Messaadia, Souk-Ahras (Algeria), following an experimental protocol in accordance with national guidelines for the care and ethical use of laboratory animals. Blood was drawn and immediately collected into dry tubes, then centrifuged at 5000 rpm for 10 minutes to separate the serum. The serum was subsequently analyzed to assess hepatic function, including the following biochemical parameters: glycaemia, glutamate-pyruvate transaminase (TGP/ALT), glutamate-oxaloacetate transaminase (TGO/AST), albumin (Alb), total protein (TP), and alkaline phosphatase (ALP).

Histopathological evaluation

A fraction of the liver tissue was fixed in 10% formalin and subjected to routine histopathological procedure. The studied sample was dehydrated with ethanol (70~100%), then embedded in a kerosene bath at 58°C. Using a rotary microtomy and kerosene blocks, 4~6 µM sections were prepared and stained with hematoxylin-eosin. Finally, the slides were studied and photographed under a light microscope (Leica DM 500, Leica Light Microscope).

Statistical analysis

The data were analyzed using analysis of variance (ANOVA), followed by Tukey's post hoc test, to determine statistically significant differences between the untreated control group and the treated groups. All analyses were performed using the statistical software Minitab, and results were considered statistically significant at $P \leq 0.05$.

RESULTS

Biochemical results

The results of the statistical analysis of the parameters studied are shown in Table 1, through which the variations in glucose levels revealed no significant difference between the treated and control groups, while SCu1 showed a highly significant increase (*P= 0.000*) compared to SCu2.

The values of TGO and TGP, are reported in Table 1, their levels remain in the standards in the groups treated with $CuSO_4$ compared to the control group, no significant difference was observed between the groups SCu1 and SCu2 ($P > 0.05$). For the SCu+AC group, the TGO rate shows a significant increase compared to the SCu1 group (*P=0.0.35< 0.05*), as for the TGP, it shows a significant increase compared to the control group (*P=0.045< 0.05*).

The results show a significant decrease in the concentration of ALP between the SCu+AC group and the control group (*P=0.025< 0.05*). However, no significant difference was observed between the three groups 1, 2 and 3 ($P > 0.05$).

The circulating concentrations of Alb recorded in Table 1 show that all three treated groups exhibited an increase compared to the control group. This increase was significant for the SCu1 group (*P=0.007<0.05*) and very highly significant for the SCu2 and SCu+AC groups (*P=0.000*), though no significant difference was observed between the treated groups themselves (*P>0.05*).

Regarding TP levels, a significant increase was recorded in the SCu2 and SCu+AC groups compared to the control in the TP rate (*P<0.05*). However, no significant difference was obtained between the three treated groups (*P> 0.05*).

Histological results

The Liver histology in control rabbits showed intact hepatocyte structure and regular morphology. The parenchyma consisted of hexagonal lobules with a centro-lobular vein and peripheral portal spaces. The hepatocytes are regular presenting a normal trabecular disposition. The results of the SCu1 group showed congestion of the central-lobular and portal vein, with distension of the portal spaces by inflammatory fibrosis rich in lymphocytes and plasmocytes. It is also associated with moderate sinusoidal congestion (obstruction of the very small veins

of the liver). In the SCu2 group, microscopic observation of the prepared slide showed an increase in periportal fibro-inflammatory lesions with appearance of minimal lobular necrosis zones, the latter are formed of small hepatocytes with dark reduced cytoplasm and pycnotic nuclei surrounded by inflammatory cells (Figure 1).

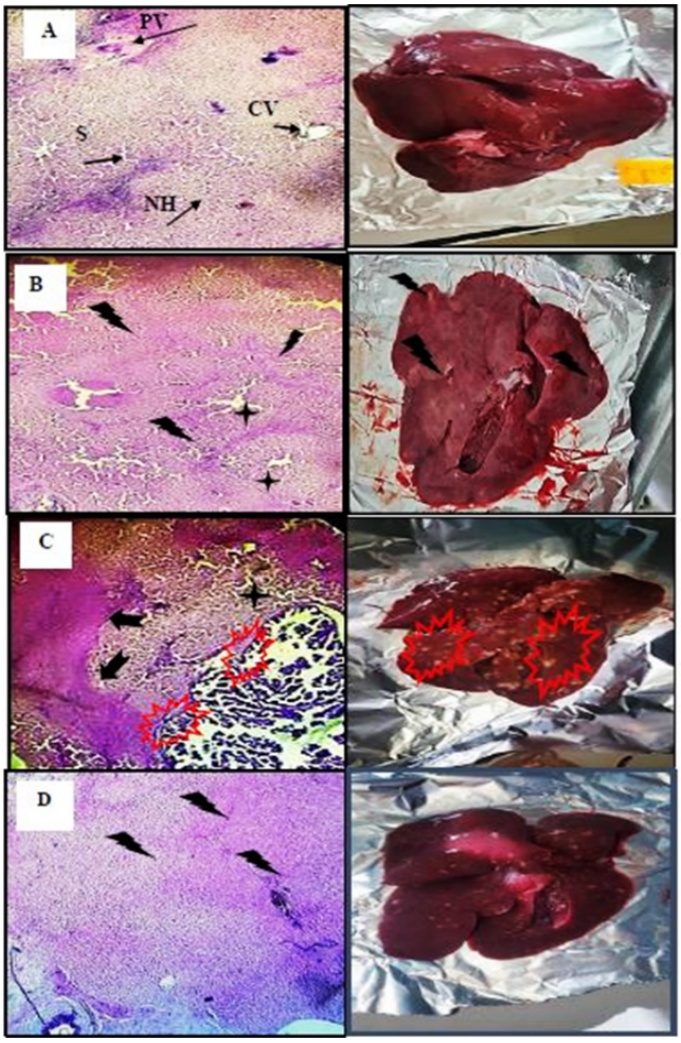

Figure 1: Histological profile of liver tissue from control and treated rabbits (A: Control; B: SCu1 group; C: SCu2 group and D: SCu+AS group).
CV: Central Vein; PV: Portal Vein; S: sinusoid; NH: Normal Hepatocyte; ✦: congestion; ↗: Inflammatory fibrosis; ✹: zone of necrosis; ➡: Fibro-inflammatory lesions.

DISCUSSION

Cu as a salt is toxic and can damage several organs. Poisoning with this element causes intravascular hemolysis, followed by liver and kidney failure, which can be fatal (Perestrelo *et al*., 2021). The chronic Cu toxicity mainly affects the liver, because this is the first site of its deposit and it is usually manifested by the development of liver cirrhosis with episodes of hemolysis and lesions of the renal tubules (Gaetke and Chow, 2003).

The results obtained in our study concerning glucose concentrations show no difference in the $CuSO_4$-treated groups in compare with the control group, whereas the SCu2 group showed a decrease compared to the SCu1 group.

According to the work of Tomaszewska *et al*. (2017) the concentration of glucose in the groups of rats studied did not mark differences, this was explained by the proper functioning of the enzymatic activity that is responsible for the metabolic conversion of glycogen in the body. A 2018 study revealed that excess Cu can disrupt glucose metabolism by binding to the sulfhydryl groups of the enzyme glucose-6-phosphatase (Anant *et al*., 2018). In another study, low blood glucose levels were reported in rats treated with Cu nanoparticles, which could be due to intestinal damage and malabsorption (Tang *et al*., 2018). Similarly, performed on a group of workers at an electrical cable manufacturing plant showed that levels of glucose were significantly lower than those in the control, this is justified by the massive oxidation of glucose to provide more energy to stressed cells (Boubsil *et al*., 2017).

In the hepatotoxicity studies, the enzymes most commonly tested are TGP and TGP, but others such as ALP can also provide valuable information. The serum increase in these parameters may indicate liver damage as a result of cell death of hepatocytes due to cell toxicity (Pantic *et al*., 2019).

Our results show no significant difference in TGO and TGP levels between the three groups: Control, SCu1 and SCu2. This observation is also reported by Galhardi *et al*. (2004) and Boubsil *et al*. (2017).

In addition, a 66-year-old man chronically poisoned with $CuSO_4$ souffers from hepatic cytolysis, which is explained by the increase in transaminase levels: the TGO:162 UI L^{-1}, the TGP: 143 UI L^{-1} (Perestrelo *et al*., 2021). Research have shown that subchronic exposure to nano-Cu leads to an increase in serum TGO and ALP levels, which can be attributed to liver damage (Bugata *et al*., 2019; Tang *et al*., 2019).

Regarding the ALP level, no significant difference was recorded between the two groups treated with $CuSO_4$ compared with the control group. In contrast to our results, Kumar and Sharma (1987) report that exposure of rats to Cu leads to a significant increase in ALP activity, which is probably due to the leakage of these enzymes from damaged tissues into the blood. In a later study, an increase in ALP was described in Cu-treated subjects (Powell and Kumaratilake, 1985; Al-Naimi *et al*., 2014; Tang *et al*., 2019), which is probably due to the leakage of these enzymes from damaged tissues into the blood. In a

later study, an increase in ALP was described in Cu-treated subjects (Powell and Kumaratilake, 1985; Al- Naimi *et al*., 2014; Tang *et al*., 2019). On the other hand, Mohammed *et al*. (2014) expressed the increase in ALP levels in rats treated with $CuSO_4$ by the accumulation of Cu in the liver, kidneys and heart, which consequently releases their intracellular enzyme into circulating blood.

In contrast to our results of the level of TPs in the three groups: Control, SCu1 and SCu2, Tomaszewska *et al*. (2017) found that protein levels were the same in Cu-treated and control rats. However, the Cu nanoparticles (NPS-Cu) cause a decrease in the rate of TP and this could be explained by the presence of severe liver damage by decreasing protein synthesis (Tang *et al*., 2019).

Our animals have significantly high levels of albuminism in the two groups treated with $CuSO_4$ compared to the control. It was reported that the increase of TGP and TGO levels indicated significant liver damage, leading to disturbances in protein synthesis and a decrease in Alb levels (Tang *et al*., 2018). In addition, the Alb is a Cu transport protein (Linder and Azam, 1996). Hepatocytes use a transport mechanism similar to the Cu-histidine complex to transfer Cu from the Alb. In acute intoxication, Alb binds excess Cu instead of ceruloplasmin (Barceloux and Barceloux, 1999). Conversely, this parameter was significantly unchanged in acute and subacute studies of toxicity by NPs-OCu (Bugata *et al*., 2019).

Ascorbic acid in high levels is considered us an antagonist of metabolism and function of the trace element 'Cu' in some mammals including rabbits and rats. The effect of ascorbic acid on the metabolism of Cu appears to be mainly due to its absorption through the gastrointestinal tract (Finley and Cerklewski, 1983).

Several studies have, showed that ascorbic acid reduces mercury-induced liver toxicity in rats (Hounkpatin *et al*., 2012; Moumen *et al*., 2022). In 2005, a study has demonstrated the protective effect of ascorbic acid against lead toxicity, either by preventing its intestinal absorption, inhibiting lipid peroxidation enhanced by this metal or by protecting cells against the mechanism of apoptosis (Shalan *et al*., 2005).

In the same context, researchers demonstrated that vitamins E and C (Vit E/ C) possess antioxidant properties capable of counteracting the effects of nanoparticles by neutralizing free radicals and has proven effective in restoring liver and kidney enzymes to levels close to normal (Shotop and Al-Suwiti, 2021). This aligns with earlier research indicating that Vit C can mitigate the detrimental effects of Cu nanoparticles (NPs-Cu) on Nile tilapia (*Oreochromis niloticus*) (El-Keredy *et al*., 2017). In addition, it was found that ascorbic acid reduced the plasma activity of ceruloplasmin and decreased the concentration of Cu in plasma and tissues, in particular the liver, when it was introduced into the diet with the recommended Cu content. This is consistent with studies using diets containing concentrations of 10 to 50 g kg-1 (Van Campen and Gross, 1968; Smith and Bidlack, 1980; Johnson and Murphy, 1988). As well Van Den Berg and Beynen (1992) proves that a diet with ascorbic acid can stimulate hepatic absorption of Cu. The intake of this compound clearly affects tissue Cu concentrations as low as 0.1 g kg^{-1} body weight. In our study, the Vit C did not alter the glucose concentration. A decrease in glucose levels showed in mice treated with Vit C and/or E compared to the nickel (Ni)-treated group, and suggest that these vitamins cause increased membrane permeability to modify insulin receptors in muscles and adipose tissue, which improves glucose absorption (Daddouh, 2016). Similar results were shared in other study in 2013 (Layachi, 2013).

Concerning enzyme activities, TGO showed an increase in the SCu+AC group compared to SCu1, while TGP showed a significant increase only compared to the control. It has previously been found a decrease in TGO and TGP in groups treated with antioxidants compared to those contaminated with metals (Layachi, 2013; Daddouh, 2016).

For ALP values, the SCu+AC group recorded a decrease compared only to the control group, Similar results were observed in other studies as well as Layachi (2013) and Daddouh (2016). According to Gupta and Tikoo (2012), the antioxidants can protect hepatocyte membranes and reduce enzyme flux in plasma.

Significant increases were recorded in TPs and Alb levels. The same results reported in both parameters in mice and rats treated with Ni and Cd with vit C and/or E (Layachi, 2013; Daddouh, 2016), notably these vitamins improve the proper functioning of the liver protein biosynthesis (Hattiwale *et al*., 2013).

A histological study of the liver of rabbits was conducted to better understand the effect of Cu at high doses and even their structural alteration in the level of liver cells.

Similar to our results, hepatocyte necrosis and central vein congestion were reported in mice after chronic Ni exposure (Daddouh, 2016). The same result was reported in 2013, where deleterious alterations in hepatic architecture were mentioned, with centrilobular vein dilatation, cytological vacuolization, irregular ballooned hepatocytes, inflammation and necrosis (Layachi, 2013). These alterations are at the origin of an oxidative stress according to a previous research which results the activation of cellular

inflammations and a degradation of lipid products (Prabu *et al*., 2011). Our result is explained by that of Ludwig *et al*. (1980) who showed in their study of Bedlington terriers suffering from a hereditary Cu storage disease, that Cu first accumulates in hepatocyte cells near the central veins, when their hepatic concentration increased above 500 and 1500 µg g^{-1} dry weight, the accumulation extended to the interlobular and periportal regions respectively.

A histo-hepatic study conducted on Wistar rats showed that exposure to different doses of CuSO4 causes vascularization of the central veins and vessels of the triad porte and loss of hexagonal lobules in the liver (Doudi and Setorki, 2014). In the same context Wang *et al*. (2014) histological changes in the liver including vacuolar degeneration and necrosis which may be due to oxidation of the membrane component of liver cells resulting from free radicals in mice, Mohan *et al*. (2003) reported that oxidation is associated with programmed cell death (apoptosis).

In rats and mice, the chronic Cu intoxication induces cupric overload in hepatocyte lysosomes, which is released into the cytoplasm, leading to inflammation and necrosis (Hebert, 1993). A number of histological studies showed that centrilobular coagulating necrosis was one of the most important changes recorded in the liver of rats intoxicated by CuSO4 with a beginning in the center and a peripheral progression in the lobule, and affirm that these changes are the result of Cu accumulation in hepatocyte mitochondria and lysosomes (Hojo *et al*., 2000; Al-Naimi *et al*., 2014). However, Yaqub *et al*. (2018) found that Cu was significantly toxic to liver tissue, which showed signs of degeneration. The Sublethal doses of the latter caused a rupture of hepatocyte cells, dilation of sinusoidal space, hemorrhage and congestion of the central vein. In the SCu+AC group, the histopathological study revealed an improvement in the functional structure of the liver, with disappearance of sinusoidal congestion and persistence of slight dilation of the centro-lobular vein and slight peri-portal inflammatory fibrosis.

Other research described that ascorbic acid played a protective role, and that it was able to correct the toxic effects of mercury (Hg) in the liver of Wistar rats, marking a slight vacuolar change in the peris portal hepatocytes and moderate congestion of the hepatic veins (Hounkpatin *et al*., 2017).

These results are also in agreement with Recent studies that indicated that Vit E and through its antioxidant characteristic ensures the regulation of the hepatic structure altered by Cd in rats (Bahri *et al*., 2019). As well as, the Vit C reduces the histopathological impact of Cd chloride on rats, it reduces portal inflammation and hydropic degeneration of hepatocytes, in addition to reducing the infiltration of some leukocyte cells around the central vein (Elgharib *et al*., 2023).

However, other studies in mice have shown that treatment with Vit C and/or Vit E combined with Ni improves the structure of liver tissues, with slight necrosis occurring on the Ni-treated group (Daddouh, 2016).

According to other author, treatment with Vit E and/or C in Cd-exposed rats illustrates vascular congestion with dilated centrilobular veins, but the structure of the liver appears to be intact.

This explains why Vit C and Vit E or/and their mixture have the capacity to attenuate the majority of the harmful effects caused by Cd, inhibiting the threat of oxygen radicals leading to reduced pathological change (Layachi, 2013).

CONCLUSIONS

Our results demonstrate that chronic exposure to $CuSO_4$ disrupts the structural integrity of the liver and alters certain biochemical parameters, such as total protein (TP) and albumin (Alb). However, supplementation with ascorbic acid significantly mitigates the toxic effects of copper and contributes to improved liver function. These findings support the role of ascorbic acid as an effective antioxidant in protecting the rabbit liver against Cu-induced hepatotoxicity.

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NOVELTY STATEMENT

This research, having studied and confirmed the hepatic toxicity of copper sulfate, highlights the reparative effect of ascorbic acid on liver function. For the first time, we demonstrate its effectiveness in local rabbits (*Cuniculus lepus*) in rectifying biochemical indicators and liver structure following prolonged intoxication, thus proposing a new treatment approach.

AUTHOR'S CONTRIBUTION

Nor el yakine Bouaroudj: The corresponding author participates in each step with all authors: Conceptualization (Research idea, objectives, Methodology (Design of experiments), The realization of the practical part, Writing - original version (First draft of the manuscript), Supervision.
Soumaya Boubsil: Formal analysis (Statistical processing of data), Writing - revision and editing,Validation: Verification of results.
Amina Merghad: Investigation, Experimentation/data collection, Validation (Verification of results).
Hadjer Dejmil: Visualization (Creation of tables and figures).
Kouachi Nouha: Resources (materials, equipment, samples, software).
Abdennour Cherif: Data management, Database organization.

Generative AI and AI-assisted technology statement

In preparing this work, the authors used DeepL Translator for translation. The authors critically reviewed, edited, and refined the output of these tools. The authors assume full responsibility for the content of the publication.

Ethical approval

Every procedure used in this investigation involving experimental animals was authorized by Batna 2 University in Batna, Algeria, and complies with the Algerian Ethics Law (Ordinance No. 06-05 of July 15, 2006). The study complied with the national and international ethical guidelines for the use of laboratory animals.

Conflict of interest

The authors declare that they have no competing financial interests or personal relationships that could influence this work.

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