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Liver protection from acetaminophen hepatotoxicity using copper(I)-nicotinic acid complex

Ahmed R. Shatat [1,*], Omar B. Ahmed [2,3], Gomaa A.M. Ali [1,*]

[1] *Chemistry Department, Faculty of Science, Al-Azhar University, Assiut 71524, Egypt*

[2] *Institute of Pathology, Charité University Hospital, Berlin, Germany*

[3] *Electron Microscope Unit, Assiut University, Assiut, Egypt*

Abstract

The present study was performed to investigate the hepatotoxicity of acetaminophen and the possible hepatoprotective effect of copper(I)-nicotinate in rats through biochemical light and electron microscopy studies. Two groups of rats (39 in total) were used: group I (15 rats) received acetaminophen by intraperitoneal injection in a dose of 1000 mg/kg. While group II (15 rats) received an oral dose of 800 µg/kg copper(I)-nicotinate dissolved in 0.5 mL of saline three times through 24 h and 1 h after the last dose, and 9 rats were kept as control. Blood and tissue samples for biochemical, light, and electron microscopy were collected after 4, 12, and 24 h. Biochemical results showed a significant elevation in ALT, AST, and nitric oxide levels in rats who received acetaminophen only. In contrast, the corresponding levels of treated rats with the copper complex showed a less significant elevation in ALT, AST, and nitric oxide. Light microscopic examination of liver tissue taken from intoxicated animals showed congestion of the vasculature with hydropic and fatty degeneration. Centrilobular necrosis of the hepatocytes with glycogen depletion was observed in the centrilobular areas. Ultrastructural examination showed fatty degeneration, mitochondrial swelling, severe irregular dilatation of RER and SER, loss of glycogen granules, and pyknosis of the nuclei. Examination of livers of animals that received copper complex before intoxication revealed only cup-shaped mitochondria, a mild degree of fatty degeneration, and glycogen depletion with no evidence of necrosis. This work provides evidence for the antioxidant properties of the copper(I)-nicotinate complex and illustrates the pathological changes induced by acetaminophen in liver tissue.

Keywords: Biochemistry**,** Copper(I)-nicotinic acid, Acetaminophen-induced, Hepatotoxicity

1. Introduction

Accidental or intentional acetaminophen overdose is the most common cause of drug-induced liver injury in developed countries, and it remains a leading cause of acute hepatic failure [1, 2]. Acetaminophen (4-Acetaminophenol) is a common drug administered for the treatment of pain or fever. Although it is considered safe at therapeutic doses, an overdose of acetaminophen produces centrilobular hepatic necrosis in man [3] and experimental animals [4, 5]. N-acetyl-benzo-quinone imine, a highly reactive intermediate of acetaminophen metabolism, normally reacts with sulfhydryl groups in glutathione. However, after large doses of acetaminophen, the metabolite is formed in amounts sufficient to deplete hepatic glutathione. Consequently, reaction with sulfhydryl groups in hepatic proteins increases, and

*Corresponding author e-mail: ahmed.rashad@alborglab.com (A.R. Shatat), gomaasanad@azhar.edu.eg (G.A.M. Ali)
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hepatic necrosis can occur [6]. Despite the considerable research, the mechanism responsible for the hepatotoxicity of acetaminophen is not fully understood.

Copper complexes with amino acids were reported to have potent anti-inflammatory, anti-ulcer effects, and antioxidant properties, beneficial in combating oxidative stress [7]. Copper(I)-nicotinic acid complex has been tried in several disease models such as a peptic ulcer in shay rats [8], fatty liver in chicken and rat [9], aflatoxicosis [10]. The antioxidant properties of the copper(I)-nicotinic acid complex suggest a hepatoprotective effect against acetaminophen that produces oxidative stress in hepatocytes before inducing necrosis [11].

This work aimed to describe the pathological changes in the liver after a toxic dose of acetaminophen and to investigate the possible hepatoprotective effects of copper nicotinic acid complex against this type of insult.

2. Materials and Methods

2.1. Animals

This study was performed on 39 albino rats aged six months with body weights ranging from 160 to 205 g. These animals were obtained from Helwan farm for laboratory animals breeding (Helwan, Egypt). Rats were kept at room temperature and housed in the toxicology laboratory in Assiut University's animal house. Rats were housed in metal cages (four per cage) and were kept for two weeks before treatments for acclimation under laboratory conditions. Rats were housed on a 12:12 light/dark h and fed commercial food with water ad libitum. Every possible effort was made to minimize animal suffering.

2.2. Chemicals and Kits

Copper(I)-nicotinate complex ([copper(I)-(nicotinic acid)$_2$]+Cl$^-$) was synthesized as described by Gohar and Dratviscky in 1975 [12]. Acetaminophen (4-Acetaminophenol) was purchased from Fluka Company, France (Cat. No. 00370). Alanin transaminase kits and Aspartate transaminase kits (Spectrum diagnostics, Germany; Cat. No.: 264, 260 respectively); Griess reagent (Promega Corporation, USA; Cat. No.: G2930) and total protein kits (Stanbio Laboratory, USA; Cat. No.: 0251, 0256).

2.3. Study design

All animals were fasted for 18 h before injecting acetaminophen [13]. The acetaminophen (hepato-toxicity) group contained (n = 15) received an intraperitoneal injection of acetaminophen in a dose of 1000 mg/kg dissolved in 20%, Tween-80 in saline to induce acute model according to Gardner *et al.* [13]; 1 h after the copper-nicotinate vehicle (0.5 mL of saline) administration. Five of the induced animals with acute treatment were dissected for each period of 4, 12, and 24 h post-injection. The copper-nicotinate (hepato-protection) treated group contained another 15 rats were treated similarly to the group above, but the dose was preceded with an oral dose of 800 μg/kg copper(I)-nicotinate dissolved in 0.5 mL of saline three times through 24 and 1 h after the last dose, rats received acetaminophen [9]. Five of these rats were dissected after 4, 12, and 24 h post-injection. Nine rats were kept as control (vehicle) and sacrificed at the same time intervals as the treated groups.

2.4. Blood sampling and tissue processing

Blood samples were taken from the eye vein of rats and centrifuged at 3000 rounds per minute (rpm) for 10 minutes; then, the obtained sera were kept at -20 ºC until the time of analysis. About 1 g of liver tissue was taken and washed in cold saline then kept at -20 ºC in labeled tubes for biochemical assays. For electron microscopy, 8-10 blocks from the liver 1x1 mm in size were taken and fixed using 5% glutaraldehyde and kept at 4 ºC until the time of processing. Suitable specimens from the liver of all animals were taken and fixed using 10% neutral buffered formalin for a light microscope.

2.5. Light Microscopy

After good fixation, specimens from all animals were embedded in paraffin, and sections of 4 μ thick were taken as usual. H&E and PAS stains were applied. Also, toluidine blue-stained semithin sections (0.5-1 μ) were examined.

2.6. Transmission Electron Microscopy

Immediately after sacrificing the rats, 10-12 small pieces were taken from the liver and fixed in 5% glutaraldehyde for 2-24 h. The specimens were then washed in cacodylate buffer (0.1 M, pH 7.2) 3-4 times for 20 minutes at every time and then postfixed in 1% osmium tetroxide for 2 h. After repeated washing in cacodylate buffer (4x20 minutes), dehydration was done using ascending grades of ethyl alcohol up to 100% (30, 50, 70, 80, 90, and 100%/2 h) and using gelatine capsule they were embedded in Epon 812. For polymerization, the embedded samples were kept in an incubator at 35 ºC for one day, at 45 ºC for another day, and three days at 60 ºC.

Using LKB ultramicrotome, semithin sections with a thickness of 0.5-1 µ were prepared from prepared blocks. The sections were stained by toluidine blue, examined by light microscope, photographed, and regions for preparation of ultrathin sections were oriented, and by Leica ultramicrotome, the ultrathin sections in thickness of 500-800 Å were made and fixed on copper grids (200 µ meshes). The ultrathin sections were then contrasted in uranyl acetate for 15 minutes and lead citrate for 5 minutes and examined by a transmission electron microscope (Jeol, CX11) in electron microscope unit, Assiut University.

2.7. Statistical Analysis

Results were given as means ± standard error (X± standard error, SE). The difference between groups was tested for significance using student -t- test-taking $p< 0.05$ as significant (*), $p< 0.01$ as very significant (**), and $p< 0.001$ as highly significant (***). Statistical analysis was done using analysis of variance (ANOVA) followed by Tukey ’s multiple comparison test using the Statistical Package for the Social Science (S.P.S.S. 11).

3. Results

3.1. Biochemical analysis

Biochemical results of the toxicity study showed a significant elevation in ALT, AST, and nitric oxide levels in rats who received acetaminophen only. In contrast, the corresponding levels of treated rats with the copper complex showed a less significant elevation in ALT, AST, and nitric oxide as demonstrated in Fig. 1(a-c). Glutathione levels measured in the liver tissue showed a significant decline in animals who received acetaminophen only. While, in animals treated with acetaminophen after treatment with a copper complex, the level of GSH was within the normal level (Fig. 1d).

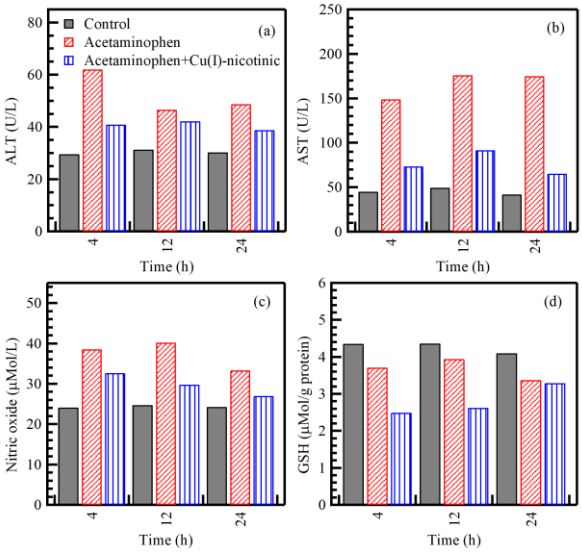

Fig. 1. ALT (a), AST (b), nitric oxide (c), and GSH (d) levels in sera of intoxicated animals.

3.2. Microscopy analysis

By light microscope, animals sacrificed after 4 and 12 h of acetaminophen injection showed congestion of vasculature with degeneration of the hepatocytes as shown in Fig. 2a,b, compared to control (Fig. 3a,b). The degenerative changes which were observed in the hepatic cells were mostly found in centrilobular areas. These changes were in the form of dissociation of the hepatic cords and vacuolation in the cytoplasm of the hepatocytes (Fig. 2a). There was no evidence for necrotic changes. An increase in the size and number of Kupffer cells was also observed (Fig. 2b). Moderate depletion of glycogen was observed around central veins.

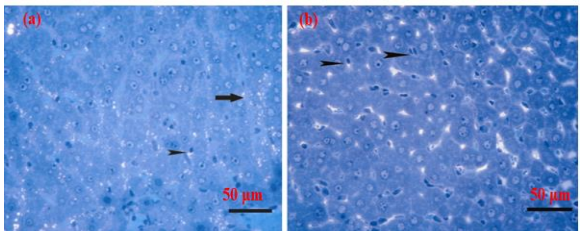

Fig. 2. (a) Rat liver after 12 h of acetaminophen injection (a) showing swelling of the hepatocytes and compression of the sinusoidal lumen (arrowheads) with the presence of variable-sized vacuoles. (Arrows; Semithin section stained with t.b.; Bar = 50 um), and (b) showing numerous swollen Kupffer cells (arrowheads; t.b. stain; Bar = 50 um).

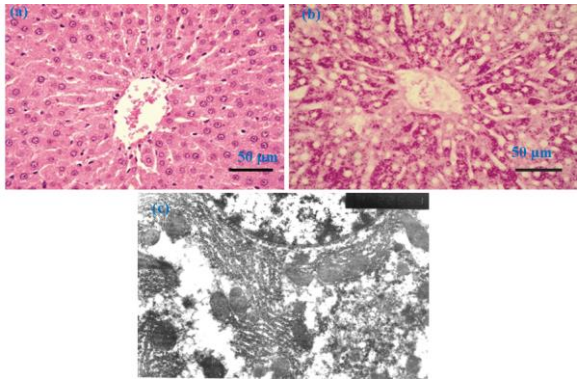

Fig. 3. (a) Liver of control animal dissected 24 h post-AAP vehicle administration. This liver shows the normal structure (H&E stain; Bar = 50 um), (b) light micrograph of a control rat liver showing the presence of glycogen in the hepatic lobule evenly distributed (PAS stain; Bar = 50 um), (c) Transmission electron micrograph showing a normal view of mitochondria, RER, and nucleus in a hepatocyte of a control animal (mag. X 10000).

Transmission electron microscopy of the liver of these groups showed that hepatocytes near the central vein exhibited pathological alterations of the cell organelles compared to control (Fig. 3c). Slight dilatation of rough endoplasmic reticulum and mitochondrial swelling with rupturing of some of them was prominently observed. The swollen mitochondria showed loss of cristae and increased matrix electron density (Fig. 4a, b). Some nuclear changes were seen at this stage, including chromatin clumping and pyknosis of the nucleus. Many hepatocytes showed vacuolation and spherical variable-sized fat globules (Fig. 4c).

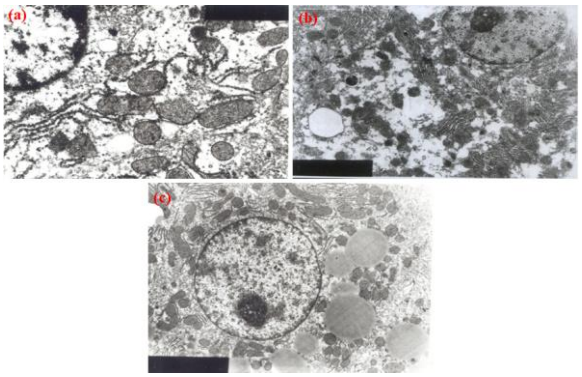

Fig. 4. Transmission electron micrograph (a) showing mitochondrial swelling, rupturing of some mitochondria, irregular dilatation in the rough and smooth endoplasmic reticulum, marked vacuolation with loss of glycogen granules (acetaminophen after 12 h, mag. X 10000), (b) showing a hepatocyte containing numerous mitochondria that lost its cristae and became electron-dense structures, loss of glycogen granules of the hepatocytes, and presence of large membrane-bound vacuole (acetaminophen after 12 h, mag. X 5000), and (c) showing some fat globules appears in the cytoplasm of a hepatocyte (acetaminophen alone after 12 h, mag. X 4000).

After 24 h of acetaminophen injection, the liver showed more severe changes. Dilatation of the central veins and widening of sinusoids were observed in the hepatic lobule. The hepatic cells of this group showed variable cytopathic changes, and these changes were in vacuolation, fatty degeneration, and coagulative necrosis (Fig. 5a). The prominent changes were detected around the central veins and characterized by hepatic cell necrosis. The hepatocytes showed pyknosis, karyorrhexis, or karyolysis of their nuclei, along with an increase in acidophilia of the cytoplasm (Fig. 5a). Peripheral to these necrotic areas, there were greatly swollen hepatocytes with prominent cytoplasmic vacuolation and pyknotic nuclei (Fig. 5b). The peripheral areas of the hepatic lobules showed slight degenerative changes of hydropic type, and some areas showed fatty degeneration. PAS staining of this group revealed profound glycogen depletion in hepatocytes surrounding central veins, while cells away from the central vein showed normal and even distribution of glycogen (Fig. 5c).

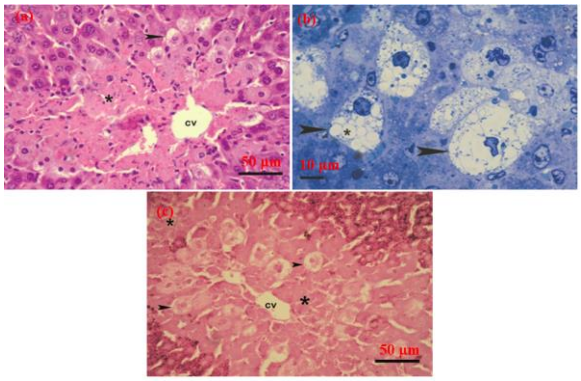

Fig. 5. Liver of rats injected with acetaminophen at 24 h showing (a) extensive coagulative necrosis and cytoplasmolysis of the hepatocytes at the periphery (H&E; Bar = 50 um), (b) prominent vacuolation (star) with pyknosis of the nuclei of hepatocytes (Arrowheads; Semithin section stained with toluidine blue; Bar = 10 um), and (c) extensive coagulative necrosis with cytoplasmolysis of some necrotic cells (stars) and cell swelling (arrowheads) in a glycogen depleted zone around the central vein (stars; PAS stain; Bar = 50 um).

Ultrathin sections of the liver taken from this group showed a more advanced stage of liver damage. The hepatocytes around the central vein showed features of necrosis. Such necrosis is expressed ultrastructurally as the disintegration of cytoplasm with extensive mitochondrial swelling accompanied by cristae disruption and breakdown of membranes of the rough endoplasmic reticulum. Pyknosis,

karyorrhexis, karyolysis of nuclear chromatin, or complete absence of the nucleus with difficulty distinguishing cell boundaries were observed. Vacuolated hepatocytes observed by light microscopy peripheral to the necrotic areas ultrastructurally appeared in the form of many variable-sized clear vacuoles. These vacuoles do not contain fat and appear to be formed by coalescence of largely dilated endoplasmic reticulum-forming vesicles. The nuclei of these cells were pyknotic with condensed or clumped nuclear chromatin (Fig. 6a). The Kupffer cells were increased in size and rich in cell organelles filling most of the siuosidal lumen. The other cells that showed fatty degeneration were characterized by an extraordinary number of fat globules of medium electron density and variable size with no membrane bounding (Fig. 6b).

Mitochondria of some hepatocytes showed an increase in matrix density, size, and several dense granules. These granules represented calcium precipitates. In extreme cases, mitochondrion may be filled with calcium deposits, and fusion of these precipitates may result in irregular electron-dense masses. Some mitochondria lost their cristae, and others showed damage to the outer mitochondrial membrane. Numerous mitochondria showed C-shaped or O-shaped profiles. The hole within such forms may be placed centrally or eccentrically and usually contains a cytoplasmic matrix and various structures such as ribosome or glycogen. These mitochondria are called ringed-shaped, C-shaped, or cup-shaped mitochondria (Fig. 6c).

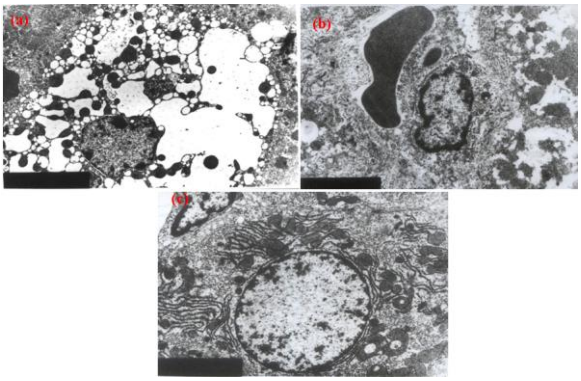

Fig. 6. Transmission electron micrograph showing (a) pyknotic nucleus condensation of chromatin in swollen hepatocytes (acetaminophen after 24 h, mag. X2700), (b) activated Kupffer cell (acetaminophen after 24 h, mag. X5000), and (a) Cup-shaped mitochondria, mitochondrial swelling, and disintegration of the cristae in a hepatocyte (Acetaminophen after 24 h, mag. X 5000).

Animals received copper complex before acetaminophen administration and sacrificed after 4, and 12 h of acetaminophen showed obvious activation of Kupffer cells with no evidence of fatty degeneration or hepatocellular necrosis. The hepatocytes showed the normal arrangement of the hepatic lobule with normal nuclei and prominent nucleoli (Fig. 7a). However, after 24 h of acetaminophen administration liver revealed a mild degree of fatty degeneration in hepatocytes manifested by the presence of circumscribed empty spaces in the cytoplasm (Fig. 7b). Glycogen distribution in the hepatocytes in the hepatic lobule was uniform in distribution resembling control animals (Fig. 7c).

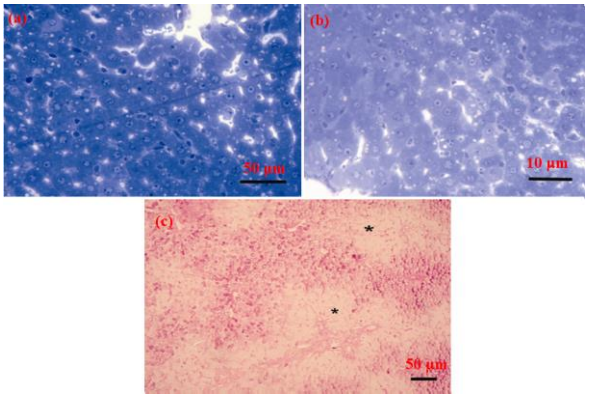

Fig. 7. Rat liver received acetaminophen and copper complex at 12 h showing (a) numerous kupffer cells, (A semithin section stained with t.b. Bar = 50 um), (b) showing the presence of small empty spaces representing fat globules with the prominent hepatic cell nucleus and active Kupffer cells. (t.b. stain; Bar = 50 um), and (c) a slight degree of glycogen depletion around the central vein (PAS stain; Bar = 100 um).

Examination of those livers by transmission electron microscope revealed that hepatocytes appeared ultrastructurally more or less similar to control one (Fig. 8a). No evidence for hydropic degeneration or fatty degeneration was seen. Peculiar to this group, numerous c-shaped mitochondria were observed after 12 and 24 h of acetaminophen administration. These mitochondria assumed C-shaped or O-shaped profiles with a hole in the center containing cytoplasm. The number of these altered organelles was more than that found in hepatocytes of animals that received acetaminophen only and were examined after 24 h (Fig. 8b).

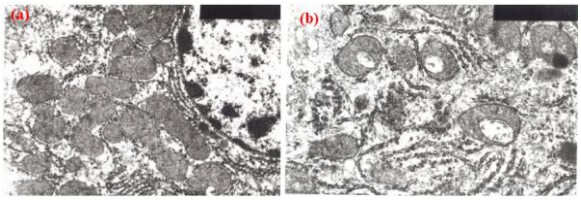

Fig. 8. Transmission electron micrograph showing (a) more or less normal hepatocyte (acetaminophen and complex after 4 h, mag X 10000), (b) cup-shaped mitochondria (acetaminophen and complex after 12 h, mag. X 14000).

4. Discussion

After therapeutic doses of acetaminophen, the reactive metabolite was efficiently detoxified by conjugation with GSH. However, after toxic doses, GSH was depleted by the conjugation reaction and the metabolite covalently bound to protein. Covalent binding may lead to the development of toxicity [14]. Biochemical analysis in the present study was consistent with the morphological changes. Necrotic changes in the hepatocytes of intoxicated animals were associated with a proportional rise in transaminases level in the same animals and considered a pointer to necrotic changes [15]. Copper nicotinate complex had proven to have some hepatoprotective properties against acetaminophen intoxication. This was clear in decreasing the levels of transaminases in intoxicated animals that received copper complex before paracetamol. This effect is due to its ability to help cells retain their normal biochemical mechanisms depending on copper-containing enzymes [7, 9, 10].

Nitric oxide levels in blood samples taken from intoxicated animals were high compared to control values. Paracetamol (toxic doses) was associated with generating oxygen free radicals [16, 17]. Oxygen radicals bind nitric oxide producing nitrogen radicals. Both oxygen and nitrogen free radicals have severed destructive effects on the cell's structural and functional components ranging from a mild effect on the cell membrane until affecting nuclei acids in the nucleus [18-20]. A direct correlation between levels of nitric oxide and histopathological findings could be observed. The generation of reactive oxygen species caused damage to macromolecules in the cell, leading to cell death [21]. The hepatoprotective effect of copper complex against acetaminophen intoxication can be a free radical scavenger and a potent antioxidant. These antioxidant properties are due to its activation of many antioxidant mechanisms in the cell, like some copper-containing enzymes. For example, superoxide dismutase is considered one of the major antioxidant enzymes that shield the cell against attacks of free radicals [8, 9]. On the other hand, copper ion, along with iron, zinc, and manganese, has an important role in raising the reducing capacity of the cell and extracellular spaces.

Reduced glutathione (GSH) is considered the first line of defense against free oxygen and nitrogen radicals and any other destructive compounds that exaggerates oxidative stress in the cell [22, 23]. GSH measured in the liver tissue of intoxicated animals was lower by half or more of its normal values, and this finding ensures the role of oxidative stress on acetaminophen-induced hepatotoxicity. We can speculate that GSH, the main antioxidant molecule, was depleted by its reaction to the increased free radicals. After GSH was depleted, free radicals started to react with cellular macromolecules causing their destruction. These findings agreed with previous reports [24]. Interestingly, treatment of animals with copper complex before paracetamol had elevated GSH levels. This was expected from previous copper complex work that proved its capacity to renew antioxidant agents in the cell and extracellular spaces [9].

A constant feature of Paracetamol-induced hepatotoxicity was Kupffer cell activation [25-27]. In the present study, mild to extensive kupffer cell activation was observed in animals who received acetaminophen only. This activation was ultrastructurally evident and expressed by an increase in phagocytosed materials and vacuoles. One prominent characteristic of activated macrophages is their capacity to release pro-inflammatory cytokines and cytotoxic mediators, which aids in antigen destruction [28]. However, these mediators are non-specific, and thus, when released in excess quantities, they can also destroy normal tissue. In the present study, activation of kupffer cell is indicative of toxicity.

Fatty degeneration is mostly indicative of a sub-lethal injury. In the present study, the fat appeared by TEM examination as medium density, variable-sized non-membrane-bound vacuoles in the cytoplasm of hepatocytes. It seems that injury of the rough endoplasmic reticulum of the hepatocytes resulted in decreased protein synthesis. This deficiency led to low lipoproteins required to export lipids, resulting in

increased fatty acid content in the cell, leading to hepatic lipidosis [29].

A distinctive type of degenerative changes was seen in midzonal cells represented by another type of vacuoles. These vacuolated cells appeared large and had pale cytoplasm by light microscopic examination, and were always seen bordering the necrotic areas around the central veins. Electron microscopic examination of these cells after 12 h of acetaminophen administration showed slight dilatation of the rough endoplasmic reticulum and uniform cellular matrix swelling. After 24 h of acetaminophen administration, severe dilatation of the cisternae of Golgi complex and RER and mitochondrial swelling were observed. This result may represent the ultrastructural counterpart of hydropic vacuoles seen in light microscopy, and the condition is known as acute cellular swelling, which is considered a manifestation of reversible sub-lethal injury.

It is widely accepted that hydropic degeneration occurs after failure to control the cell's osmotic gradient [29]. This means failure of the sodium pump, which is an energy-dependent mechanism for controlling the osmotic gradient. The mitochondrial damage by the reactive metabolite of paracetamol is responsible for insufficient energy production with subsequent failure of the sodium pump and hydropic degeneration of the cell. These results are consistent with those observed [30, 31]. Acute cell swelling can result in necrosis or can be reversible. Earlier work consistently identified two features of irreversible cell injury: First, an inability to restore mitochondrial function, and second, evidence of cellular membrane damage [32].

Necrotic changes in the hepatocytes were seen as early as 12 h following acetaminophen injection. These changes were represented by increased acidophilia of the cytoplasm along with dissociation of the hepatic cords. This was exaggerated in the twenty-four h' group in which frank necrosis was observed in the centrilobular hepatocytes and manifested by increased acidophilia, loss of cell boundaries, and nuclear changes (pyknosis, karyorrhexis and karyolysis of nuclear chromatin or complete absence of the nucleus [32].

In our experiment, there was no evidence for apoptosis as shown by histopathological and ultrastructural examinations, which confirmed the theory that necrosis, not apoptosis, was the major way of cell death after acetaminophen overdose. The presence of necrosis was supported by glycogen depletion, as reflected by the intensity of the PAS staining, in the same centrilobular cells showing features of necrosis. Glycogen depletion can be explained by increasing intracellular calcium accumulation in hepatocytes following acetaminophen overdose [33].

Ultrastructural changes of these necrotic areas were observed in the present study as early as 12 h following acetaminophen administration represented by pyknosis and chromatin clumping with loss of glycogen granules and damaged outer mitochondrial membrane. By 24 h, the disintegration of the cytoplasm with a breakdown of plasma membranes had occurred along with pyknosis, karyorrhexis, and karyolysis of chromatin. These results are similar to those reported [34].

Mitochondrial damage represented by mitochondrial swelling with disruption of cristae and mitochondrial membranes was found in this experiment's hepatocytes of intoxicated animals. These features of mitochondrial dysfunction could be indicative of the involvement of mitochondria in acetaminophen-induced cell death. This was supported by previous reports that directly connected mitochondrial dysfunction and cell death after treatment with paracetamol [24, 35-37].

The cup-shaped mitochondria (Also called C shaped, O shaped, and ringed shaped mitochondria) were observed in the present study after 24 h of injecting paracetamol only and 12 h post-exposure with copper complex paracetamol may have an adaptive physiological response rather than morphological explanation. Cup-shaped mitochondria have been seen in the liver after administration of various toxic agents like carbon tetrachloride, and hence it may indicate a degenerative change [38]. Vickers *et al.* [39] correlated the appearance of cup-shaped mitochondria with a deficiency in GSH. Rosenbaum *et al.* [40] stated that the cup-shaped mitochondria represented an adaptive change of the lung to various oxygen levels, and he proved that it is reversible. From a functional point of view, the shape of the mitochondrion has a greatly increased surface area, and the enclosed cytoplasm is in a more confined relationship with the surface mitochondrial membrane. This condition may facilitate the exchange of metabolic intermediates between the enclosed cytoplasm and the interior of the mitochondria [38]. Regarding to the previous suggestions, the present study could be proved that

cup-shaped mitochondria may be considered as evidence of increased metabolic activity of the cell and consequently more capabilities of facing the harmful effect of active metabolites of paracetamol and subsequent oxidative stress.

5. Conclusions

Treatment with copper nicotinic acid complex in this study proved to minimize or prevent toxicity with acetaminophen, as shown by light and transmission electron microscope. The liver of animals, which received copper complex before acetaminophen administration, showed only slight congestion with Kupffer cell activation. In contrast, pale cells around central veins that represented swollen cells started to appear after 12 and 24 h with no evidence of necrotic changes in hepatocytes. Conclusively, the hepatoprotective action of copper complex against paracetamol intoxication prevents the accumulation of oxygen and nitrogen free radicals caused by the reactive metabolite of paracetamol, thus decreasing the catastrophic actions of these radicals on macromolecules of the cell. This was supported by histological findings of the liver taken from animals treated with acetaminophen alone and with both copper complex and acetaminophen.

6. Conflicts of interest

"There are no conflicts to declare".

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