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ULTRASTRUCTURAL CHANGES OF KIDNEY INDUCED BY COPPER NANOPARTICLES AND THE THERAPEUTIC ROLE OF VITAMINS E AND C AGAINST CYTOTOXICITY

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SUMMARY

The ability of nanoparticles as anti-bacterial materials has led to their widespread application in many health products. However, there are many concerns about the exposure of individuals to these particles, addressed the cellular effects caused by copper nanoparticles on the kidneys using transmission electron microscopy, and investigating the potential therapeutic role of vitamin E and C against the toxicity of copper nanoparticles and their cellular harmful effects.

*The study used 56 rats (*Rattus norvegicus*) divided into seven groups and dosed over a month with copper nanoparticles and then dosed for another month with both vitamins E and C, either alone or together, the results showed that the animals of the groups that were treated with copper nanoparticles showed micro-structural changes in the kidneys. Changes were found in the renal tissue at the level of the podocytes forming the endothelial cells in the visceral layer of the renal glomeruli. It was also observed that mitochondria were affected in the epithelial cells of the Proximal convoluted tubules. In addition to an increase in the number of lysosomes and cytoplasmic vacuoles. The effective therapeutic role of vitamin C, E has been shown in improving the size and number of organelles and returning them to their almost normal state. The study recommends the need to use vitamins C and E at a dose of 250 mg/kg/day, which showed a positive role in reducing the toxicity of copper nanoparticles.*

Keywords: Copper Nanoparticles; Kidney; Cellular Effects; Vitamins C and E

INTRODUCTION

Industrial and technological progress has accompanied the spread of hundreds of nanocomposites, which have become at the forefront of the most important sciences whose applications are used in various fields that are highly relied upon in daily life which pose a real threat to human health and safety (Dziendzikowska *et al*., 2012; Sun *et al*., 2021), Nanoparticles are produced in the range of 1 - 100 nanometers (Thomas and Sayre, 2005) and may enter the body with water, food or cosmetics, medicine, etc. (Hoet *et al*., 2004; Oberdörster *et al*., 2005) by ingestion, inhalation, or through skin pores, accumulating in vital organs (Li *et al*., 2008).

Copper is an essential element that helps maintain homeostasis function in living organisms (Nawaz *et al*., 2006). In latest years, different types of nanomaterials have been applied in many fields, due to their particular physical and chemical properties (Amin *et al*., 2020). including Copper nanoparticles are widely which used as wood preservatives, additives in lubricants, inks, and in plastics to reduce friction (Sau and Rogach., 2010; Kwon *et al*., 2012; Liu, *et al*., 2004) and to reduce the growth of microorganisms such as fungi (Cioffi *et al.*, 2005)

Copper nanoparticles cause a range of toxic effects such as hemolysis and jaundice, stimulate a range of toxic activities in the liver, such as cirrhosis (Zietz*et al*., 2003). Studies have shown that kidneys, liver, and spleen being the main target organs of copper particles with regard to acute toxicity which may lead to death in mice (Manna *et al*., 2012; Sarkar and Das, 2006; Liao and Liu, 2012; Chen *et al*., 2006)

A study of AL-Bairuty *et al.* (2013), which dealt with the pathological effects of copper nanoparticles and copper sulfate on *Oncorhynchus mykiss* trout, reported hepatitis in addition to lacerations in renal tubes when trout were exposed to copper nanoparticles at a concentration of 100 mg/kg and for multiple time periods. A comparison study was made between titanium oxides, copper, zinc and iron nanoparticles, with regard to toxicity and DNA damage, and the results showed that copper nanoparticles are the most effective in terms of cytotoxicity and DNA damage (Kim *et al*., 2009). The study aimed to know studying cellular effects resulting from the effect of copper nanoparticles on the kidneys using Transmission Electron microscopy.

MATERIALS AND METHODS

This experiment was conducted according to the criteria set by the Ethics Committee of Scientific Research at King Saud University, Ethical Approval No. KSU-SE-19-32.

Fifty six male rats (*Rattus norvegicus*) with weights ranging between 120 ± 10 g, and 5-6 weeks of age were used. They were obtained from the animal house - College of Science - King Saud University in Riyadh, Saudi Arabia. The animals were placed at a room temperature of 25 ± 5 ° C and humidity 45±5%, 12/12 hour light-dark cycle.

Experimental design:

Copper nanoparticles were obtained from Sigma-Aldrich Company with a size of 10 nm and purity of 99.9%, their size was confirmed by transmission electron microscopy and their purity using XRD device, they were given to rats by oral administration at a dose of 100 mg/kg/day for 4 weeks, then rats were given vitamins C and E at a dose of 250 mg/kg/day for another four weeks. The rats were divided into 7 groups, (n=8), as follows:

The first group was given only drinking water without any additives and was considered a control group, the second group was given vitamin E at a dose of 250 mg/kg/day and the third group was given vitamin C at a dose of 250 mg/kg/day, the fourth group was dosed with copper nanoparticles at a dose of 100 mg/kg/day, The fifth group dosed with 100mg/kg/day copper nanoparticles and vitamin E 250mg/kg/day, the sixth group dosed with 100mg/kg/day copper nanoparticles and vitamin C 250mg/kg/day, the seventh group dosed with 100mg/kg/day copper nanoparticles and vitamins E and C 250mg/kg/day, and the autopsy was done in two stages. The first stage included four rats from each group at the end of the first month and the second stage included the rest of the rats which were dissected at the end of the second month, and kidney samples were taken from the dissected animals quickly to reduce the rate of change in the sample, The animals were dissected immediately after anesthetization and the samples were quickly taken to the glutaraldehyde fixative in a Petri dish and they were cut in the fixative so that some of the rapidly degrading organelles such as the mitochondria (which degrade after about five minutes) would not be adversely affected.

The samples were cut into appropriate sizes in the form of cubes of about (1 mm^3) in size to facilitate the penetration of the fixative into the sample, especially the osmium tetroxide fixative, which penetrates only to a distance of one millimeter through the sample. The samples were cut with very sharp blades, and the cellular sectors of the samples were prepared as described by Eisenman and Alfert, (1982).

RESULTS

Size and purity of copper nanoparticles:

The transmission electron microscopy (TEM) image (Figure 1-a) showed the size and distribution of copper nanoparticles, where it was found that the particles had an average size of 10 nm and this was also shown by the Gaussian curve in Figure (1-b).

(a) **(b)**

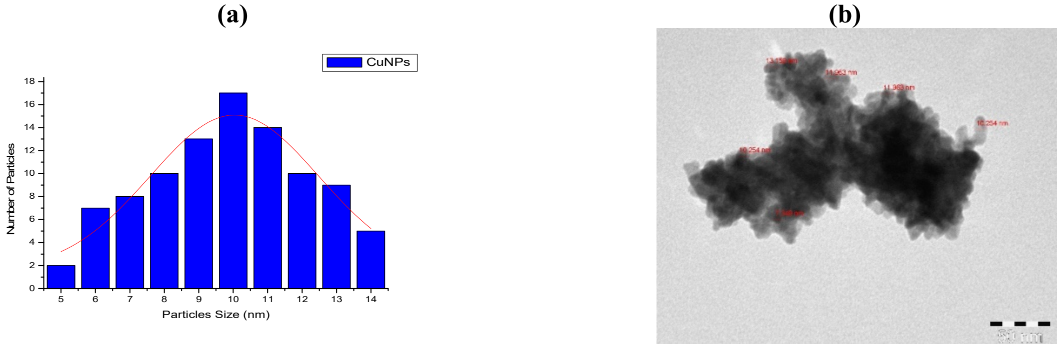

Figure 1. The size of copper nanoparticles by transmission electron microscopy (TEM) and their average size using the Gaussian curve.

Changes in the cellular structures of the renal tissue

The results of the cytological examination of kidney tissue in animals treated with copper nanoparticles and comparing them with the control group and with the groups treated with copper particles and vitamin C, E, each separately or together, showed that there were changes in the fine structure of the kidney tissue that can be illustrated in the following points (Figs. from 2 to 17)

- In animals of the groups treated with copper nanoparticles, it was observed that there was a disturbance in the secondary processes and primary processes extending from the podocytes that make up the endothelial cells in the visceral layer in the renal glomeruli, which are based on the basement membrane, compared to the animals of the control group and the two groups of vitamin C. E, and a significant improvement was found in the groups treated with copper nanoparticles and vitamin C, E, separately or together, compared to the cellular sectors of the groups treated with copper nanoparticles.
- As for the mitochondria, it was observed that they were negatively affected in the endothelial cell of the proximal convoluted tubule in the animals treated with copper nanoparticles, which showed swelling and shortening of their cristae and decomposition in the matrix, the effective role of vitamin C and E has appeared, separately or together, in improving the cristae and matrix of mitochondria without reducing their swelling.
- An increase in the numbers of lysosomes and cytoplasmic vacuoles was observed in the

endothelial cells of the proximal convoluted tubule in animals treated with copper nanoparticles compared to the control group and the two groups of vitamins C and E.

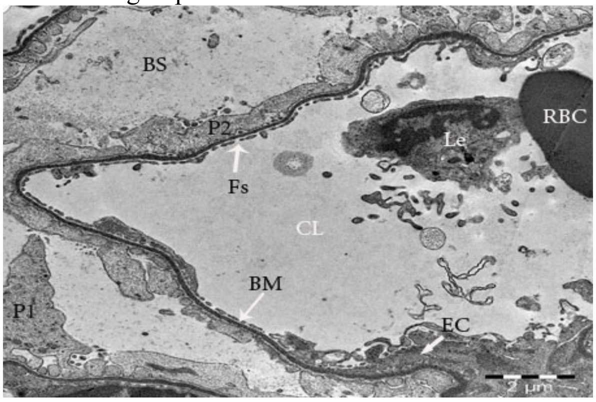

Figure 2. Transmission electron micrograph of the kidney from the control group, showing a part of the glomerulus with a section of the glomerular capillary (GC) where the podocytes appear (P), from which it extends primary processes (P1) and secondary processes (P2). The basement membrane (BM) surrounding the renal glomerulus is also visible, Bowman's space (BS) is also observed, and in the glomerular capillary, the Capillary lumen (CL) is noted, and there is also erythrocyte (RBC) and Leukocyte (Le), endothelial cells (EC), and filtration slits (FS) within the glomerulus (2000x).

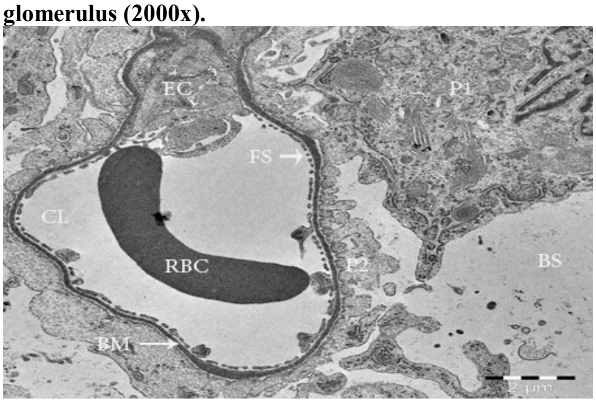

Figure 3. Transmission electron micrograph of the kidney from the group that was treated with vitamin E, showing a part of the glomerulus with a section of the glomerular capillary, where the podocytes appear, from which it extends primary processes (P1) and secondary processes (P2) extend on the basement membrane (BM) that surrounds the renal glomerulus, Bowman's space (BS), the capillary lumen (CL), erythrocyte (RBC), and endothelial cells (EC), filtration slits (FS) inside the glomerulus (2000x).

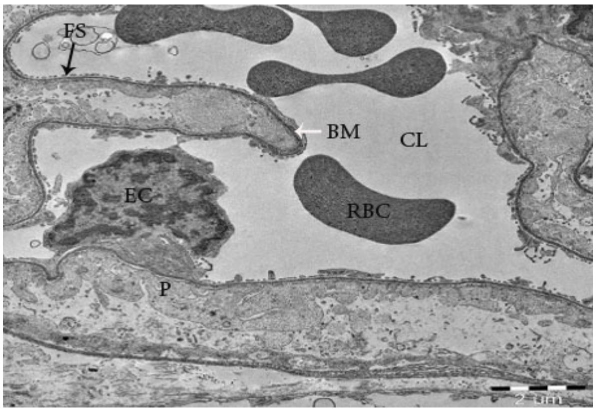

Figure 4. Transmission electron micrograph of the kidney of the vitamin C-treated group, showing a part of the glomerulus with a section of the glomerular capillary where the podocytes (P) and the basement membrane (BM) surrounding the renal glomerulus appear, while in the glomerular, capillaries the capillary lumen (CL), erythrocytes (RBC), endothelial cells (EC), Filtration slits (FS) are also found within the glomerulus (2000x).

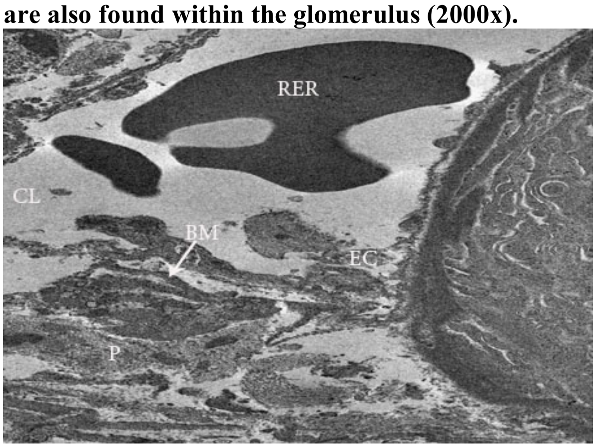

Figure 5. Transmission electron micrograph of the kidney from the group treated with copper nanoparticles, showing a part of the glomerulus with a section of the glomerular capillary where the podocytes (P) appear macerated and the basement membrane (BM) surrounding the renal glomerulus is scattered, either. In the lumen of the capillary (CL) erythrocytes (RBC), the endothelial cells (EC) are macerated and inconspicuous, and there are no filtration slits (FS) within the glomerulus (2000x).

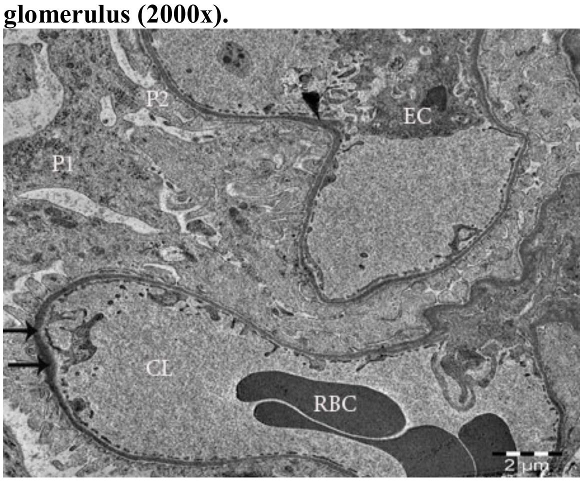

Figure 6. Transmission electron micrograph of the kidney from the group treated with copper nanoparticles and vitamin E, showing a part of the glomerulus with a section of the glomerular capillary where the podocytes processes (P1-P2) appear normal, and the basement membrane (BM) that surrounds the renal glomerulus is regular. and enlarged in some areas (arrows), erythrocytes (RBC) are located inside the capillary lumen (CL), while the endothelial cells are less macerated than the copper nanoparticles group (EC) (2000x).

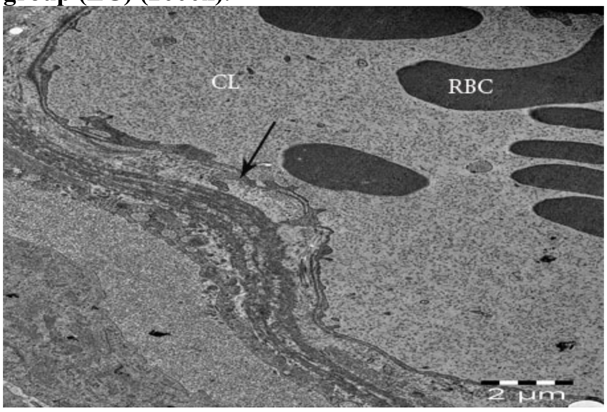

Figure 7. Transmission electron micrograph of the kidney from the group treated with copper nanoparticles and vitamin C, showing a part of the glomerulus with a section of the glomerular capillary, where the podocytes appear macerated and unclear, and the basement membrane (BM) that surrounds the renal glomerulus is irregular (arrow), erythrocytes (RBC) are located within the capillary lumen (CL), and lining endothelial cells are seen to be macerated (EC) (2000x).

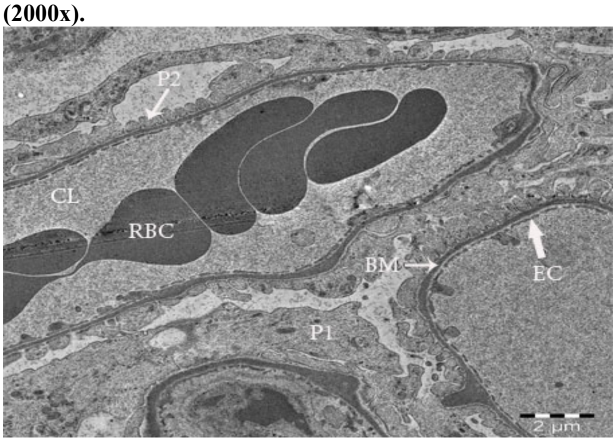

Figure 8. Transmission electron micrograph of the kidney from the group treated with copper nanoparticles and each of vitamin C, E, showing a part of the glomerulus with a section of the glomerular capillary where the podocytes appear, from which the normal primary processes extend (P1) and show the secondary processes (P2), while the basement membrane (BM) surrounding the renal glomerulus appears regular, and erythrocyte (RBC) are found in the lumen of the capillary (CL). (2000x).

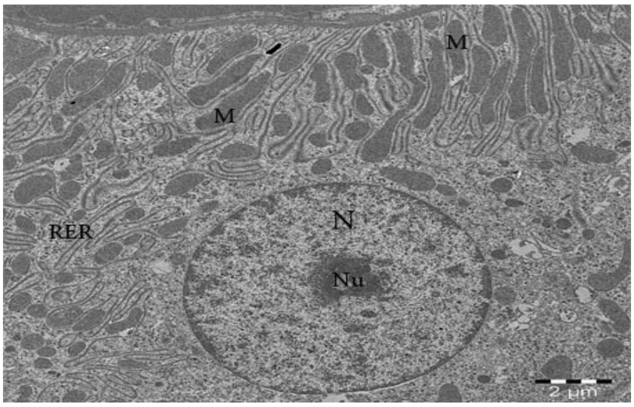

Figure 9. Transmission electron micrograph of a cell from the proximal convoluted tubule of a rat of the control group, showing the nucleus (N), which is circular, regular and devoid of protrusions, as well as the nucleolus (NU), also showing the normal mitochondria (M)and normal rough endoplasmic reticulum (RER) (2000x).

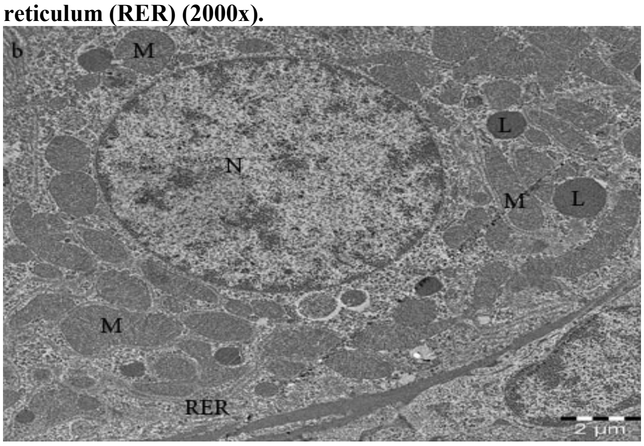

Figure 10. Transmission electron micrograph of a cell from the proximal convoluted tubule of a rat of the group treated with vitamin E, showing the nucleus (N), which is circular, regular, and devoid of protrusions. Lysosomes (Ly), the rough endoplasmic reticulum (RER), part of the normal brush border that distinguishes the proximal convoluted tubule (b) are also shown (2000x).

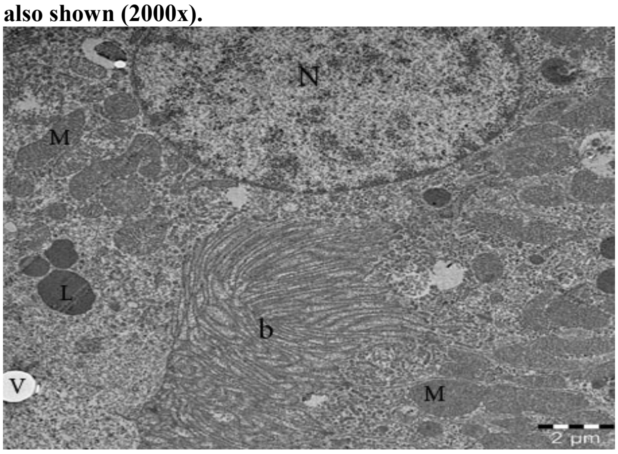

Figure 11. Transmission electron micrograph of a cell from the proximal convoluted tubule of a rat of the group treated with vitamin C, showing the nucleus (N), which is circular in shape, regular, and devoid of protrusions. Lysosomes (Ly), the normal brush border edge that distinguishes the proximal convoluted tubule (b) are also shown (2000x).

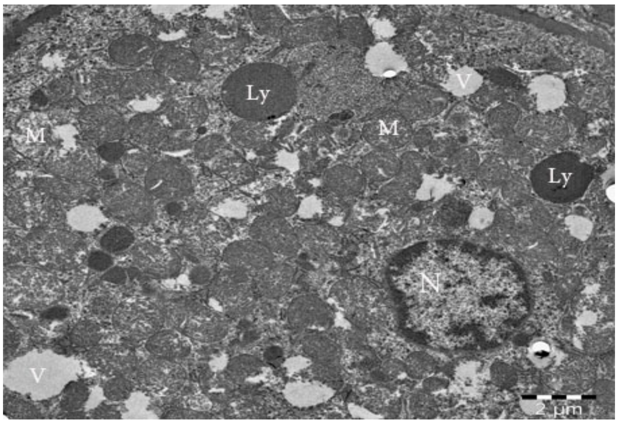

Figure 12. Transmission electron micrograph of a cell in the proximal convoluted tubule of a rat treated with copper nanoparticles. The nucleus (N) shows irregular shape, in addition to swelling and lacerations in the mitochondrial cristae, and the presence of many cytoplasmic vacuoles (V), and lysosomes (Ly) (2500x).

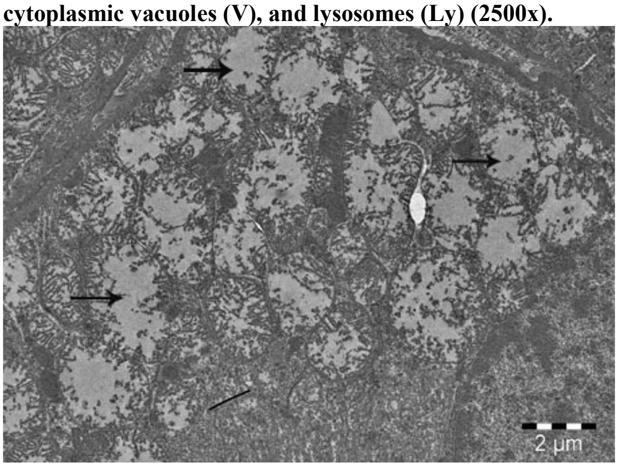

Figure 13. Transmission electron micrograph of a cell in the proximal convoluted tubule of a rat treated with copper nanoparticles, showing swelling and laceration in the cristae and matrix of mitochondria (M) (arrows) (2000x).

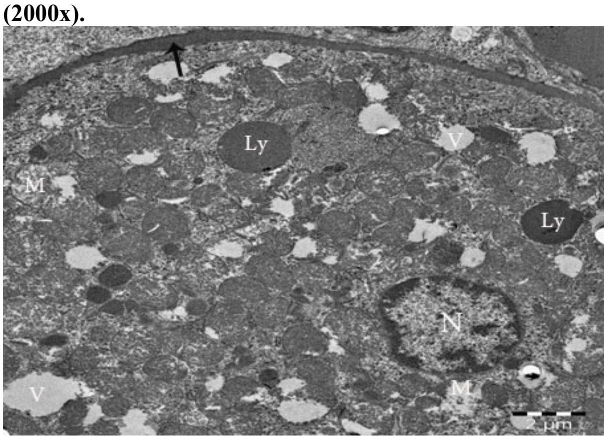

Figure 14. Transmission electron micrograph of a cell in the proximal convoluted tubule of a rat treated with copper nanoparticles, showing the small size of the nucleus (N) and its irregularity, and the laceration of some of the mitochondria (M), the presence of many cytoplasmic vacuoles (V), and many of lysosomes (Ly) in addition to the thickening of the basement membrane (BM) that surrounds the cell (arrow) (2000x).

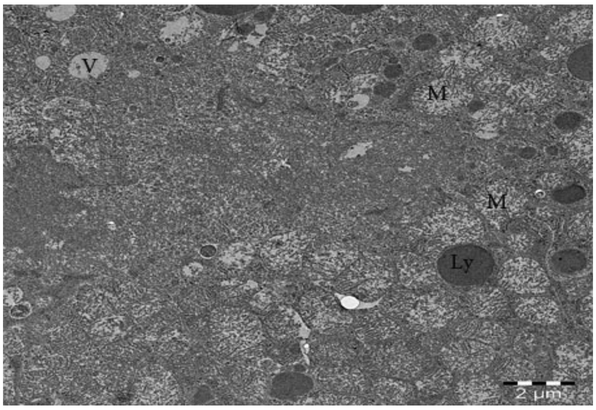

Figure 15. Transmission electron micrograph of a cell in the proximal convoluted tubule of a rat treated with copper nanoparticles and vitamin E, showing an improvement in the cristae of mitochondria (M) with swelling in some of them (arrows), with the presence of some vacuoles (V), and many lysosomes (Ly) (2000x).

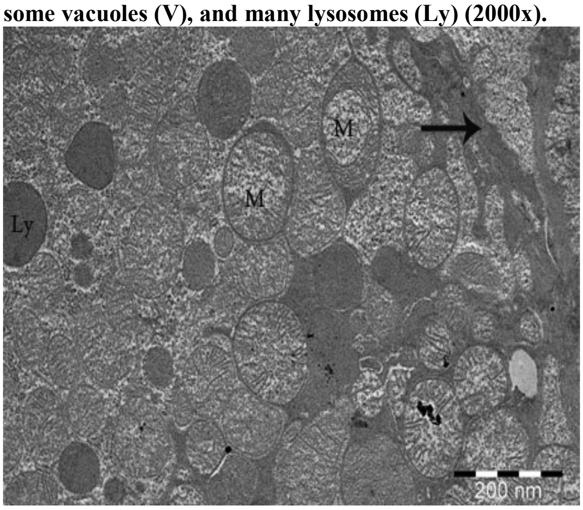

Figure 16. Transmission electron micrograph of a cell in the proximal convoluted tubule of a rat treated with copper nanoparticles and vitamin C, showing an improvement in the shape of the mitochondria (M), but there is a shortening of its cristae and a laceration in its matrix with the presence of some cytoplasmic gaps (V), and lysosomes (Ly) and dispersal in the cell basement membrane (BM) (arrow) (2500x).

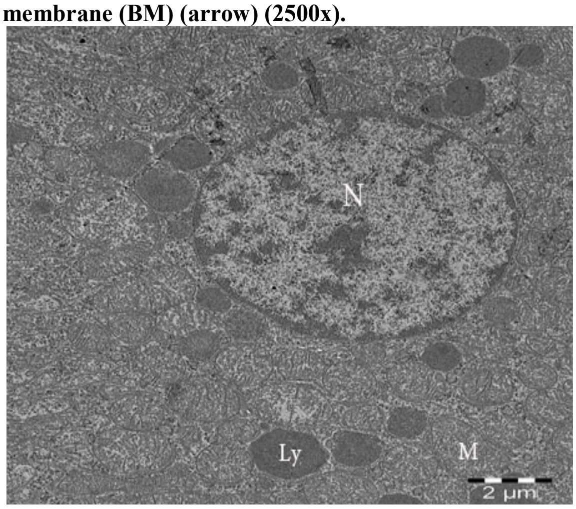

Figure 17. Transmission electron micrograph of a cell in the proximal convoluted tubule of a rat treated with copper nanoparticles and each of vitamin C, E, where the nucleus (N) appears circular, regular, and closer to the control group. with a number of lysosomes (Ly) (2000x).

DISCUSSION

Nanoparticles have the ability to cause damage and generate free radicals in biological devices, which has become one of the important means to explain and interpret the toxicity of many chemical compounds, as they interact with different tissues causing a number of functional and structural imbalances, but antioxidant enzymes may reduce these effects through their activity (Mansour and Mossa, 2009). In the Cellular structures of the renal tissue, the endothelial cell lining the nearby convoluted tubules contain a circular nucleus, while the mitochondria have regular cristae and their matrix is homogeneous, and there is a regular rough endoplasmic reticulum, and some lysosomal bodies of different sizes are scattered within the cytoplasm, This is in agreement with the study of Ansari *et al*., (2016) using mice which found that the renal cells of the control group (in part of the proximal tubules) contained a large spherical nucleus with normal chromatin content, and many well-distributed and organized mitochondria, lysosomes and a small number of cytoplasmic vacuoles, and are similar to the exact structure of the cells lining the distal convoluted tubules, except for the absence of apical appendages and the relative shortness of these cells in length. As for the renal glomeruli, the visceral layer of Bowman's capsule is mutated epithelial cells called podocytes and they have nuclei almost oval in shape, and there are many capillaries in the vascular part of the glomerulus. There were a number of changes in the renal tissue ultrastructure after nanoparticle dosing, where the endothelial cell lining the nearby convoluted tubules contained large lipid droplets, and cytoplasmic vacuoles were found in other cells, and this is consistent with the study of Sarhan and Hussein, (2014) which found damage to kidney cells, including the presence of cytoplasmic vacuoles due to the effect of silver nanoparticles, Also it was found an enlarged nucleus which may be due to the effect of CuNps by damaging the nuclear membrane and interacting with DNA (Wang *et al*., 2012) as well as many lipid droplets, were found within the cells, and the rough endoplasmic reticulum appeared fragmented, with bulging mitochondria, it may be due to ROS generation and oxidative stress toxic mechanism of nanoparticle-induced cell damage (Yang *et al*., 2009). Mitochondria are a major target of oxidative injury and excessive CuNps accumulation in renal cells inevitably which leads to mitochondrial failure and cell death (Lei *et al*., 2008). As for the renal glomeruli, they showed a clear dilation in their capillaries, and with regard to the damage to the mitochondria in the kidneys, they appeared to be of various shapes and sizes, in addition to the lacerations in their cristae and matrix, and some of them were swollen, and this is consistent with the study of Sarhan and Hussein., (2014); Chen *et al*., (2006) that the change in the mitochondria was specially evident in the proximal convoluted tubules, in addition to a significant decrease in the length of the mitochondria, where the mitochondria appeared to be of various shapes and sizes, some of them swollen, lost their crests and contain vacuoles, which indicates a disorder of their functions. This may be because exposure to Nano-copper increased the production of reactive oxygen species (ROS) and reactive nitrogen species (RNS) and altered levels of oxidative stress biomarkers in kidney tissues (Sarkar *et al*., 2011; De Berardis *et al*., 2010).As for the ultrastructural changes of the renal tissues in the groups treated with vitamins, they were less severe, as the secondary and primary processes extending from the bodies of the podocytes that make up the endothelial cells in the visceral layer appeared in the renal glomeruli in their normal size without laceration, in addition to the improvement of the cristae and matrix of the mitochondria without reducing their swelling, As for lysosomes and cytoplasmic vacuoles in the endothelial cells of the proximal convoluted tubules, their numbers were significantly reduced. This may be attributed to the effective effect of vitamin E and C as they are ideal antioxidants to protect tissues and cells from the toxic effects of nanoparticles (Shotop and Al-Suwiti, 2021).

CONCLUSION

In this study, the role of vitamins E and C were addressed in reducing the cytotoxic of copper nanoparticles in the kidneys, and the results showed an effective role of vitamins, individually or together, in improving the cellular structures of the kidneys and returning them to almost normal, and this study recommends the need to use vitamins C, E at a dose of 250 mg/kg/day because of their effective therapeutic role against the nephrotoxicity copper nanoparticles.

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التغيرات التركيبية الدقيقة للكلى الناجمة عن سمية جسيمات النحاس النانوية والدور العلاجي لفيتامين C, E ضد السمية الخلوية

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لقد أدت القدرة التي تتمتع بها الجسيمات النانوية كمضادات للبكتيريا الى تطبيقها وبشكل واسع في الكثير من المنتجات الصحية، ومع ذلك فهناك العديد من المخاوف بشأن تعرض الافراد لهذه الجسيمات، وقد تطرقت هذه الدراسة الى التأثيرات الخلوية الناجمة عن تأثير جسيمات النحاس النانوية على الكلى باستخدام المجهر الالكتروني النفاذ بالإضافة الى معرفة الدور العلاجي المحتمل لفيتامين E وC ضد سمية جسيمات النحاس النانوية وتأثيراتها الخلوية، واستخدمت الدراسة ٥٦ من الجرذان (*Rattus norvegicus*) قسمت الى سبع مجموعات وجرعت على مدى شهر بجسيمات النحاس النانوية ثم جرعت لمدة شهر اخر بكل من فيتامين E وC كلاً على حده او مع بعضهما، وقد أظهرت النتائج ان حيوانات المجموعات التي عوملت بجسيمات النحاس النانوية قد أبدت تغيرات في التركيب الدقيق للكلى حيث وجدت تغيرات في النسيج الكلوي على مستوى الخلايا القدمية المكونة للخلايا الطلائية في الطبقة الحشوية في الكبيبات الكلوية، كما لوحظ تأثر المايتوكندريا في الخلايا الطلائية للنبيبات الملتفة القريبة بالإضافة الى زيادة في اعداد الليسوسومات والفجوات السيتوبلازمية، وقد ظهر الدور العلاجي الفعال لفيتامين C,E في تحسن حجم وعدد العضيات وعودتها الى حالتها الطبيعية تقريبا، وتوصي الدراسة بضرورة استعمال فيتامين C,E بالجرعة ٢٥٠mg/kg/day والتي أظهرت دورا إيجابيا في تقليل سمية جسيمات النحاس النانوية.