RESEARCH PAPER

Study the Effect of CuO Nanoparticle on the Immunohisto-Chemical Expression of Cadherin in the Renal Parenchyma of Male Mice

Nabaa Yahya khaleel [1], Mazin Kamil Hamid [1], Haider Abdulrasool Jaafar [2]*

[1] *Department of Physiology and Medical Physics, College of Medicine, Al Nahrain University, Baghdad, Iraq*
[2] *Department of Anatomy, College of Medicine, Al Nahrain university, Baghdad, Iraq*

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ABSTRACT

The kidney cortex, containing renal corpuscles and convoluted tubules, is particularly vulnerable. CuO NPs are widely used in industrial and consumer products, raising concerns about their impact on vital organs. Cadherins, transmembrane proteins essential for cell adhesion and tissue integrity, can reflect cellular damage when their expression changes. In this study, sixty Swiss rats were divided into four groups. Group D served as control, while Groups A, B, and C received intraperitoneal CuO NP doses (0.6, 0.45, and 0.3 mg/kg/day) for 45 days. Histopathological and immunohistochemical analyses revealed immune cell infiltration and congested blood vessels in treated groups, especially at higher doses. E-cadherin expression increased with CuO NP dosage, visible as dark brown staining in renal tissue using anti-cadherin IHC and image analysis software. This suggests CuO NPs have an affinity for E-cadherin in nephron epithelium. Their 45–50 nm size may disrupt the renal filtration barrier, typically 30–40 nm wide, impairing glomerular function and inducing apoptosis.

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INTRODUCTION

Nanotechnology refers to the utilization of materials at the atomic, molecular, and supra molecular levels for industrial applications. The term "nanotechnology" originally referred to a specific technological purpose. Molecular nanotechnology involves precise manipulation of atoms and molecules to create macroscale objects. The National Nanotechnology Initiative defines nanotechnology as the manipulation of matter with dimensions ranging from 1 to 100 nanometers [1-5]. Nanotechnology is employed in a variety of medical applications, including the development of innovative medications. Most recently produced pharmacological medicines are insoluble, posing a hurdle in practical use. Nanoparticle are widely used to enhance the solubility of these drugs and reduce their exposure to healthy organs through targeted delivery mechanisms [6]. Research and technology focus on the unique features of matter below certain size thresholds. NPs are small materials with sizes ranging from 1 to 100 nm. They may be divided into many classes based on their qualities, forms, and sizes. Fullerenes, metal NPs, ceramic NPs, and polymeric NPs are among the several classifications. NPs have distinct physical and chemical characteristics due to their large surface area and nanoscale size. Their optical

* *Corresponding Author Email: mazinkamil4@gmail.com*

characteristics are said to be size-dependent, resulting in varied hues due to absorption in the visible area. Their reactivity, hardness, and other attributes are also determined by their particular size, shape, and structure. These properties make them useful for a variety of commercial and home uses. These include catalysis, imaging, medicinal applications, energy-based research, and environmental applications**.** Living organisms are built of cells that are typically 10 µm across. However, the cell parts are much smaller and are in the sub-micron size domain. Even smaller are the proteins with a typical size of just 5 nm, which is comparable with the dimensions of smallest manmade nanoparticles. This simple size comparison gives an idea of using nanoparticles as very small probes that would allow us to spy at the cellular machinery without introducing too much interference [7].Understanding biological processes at the nanoscale is a major driving force for the development of nanotechnology[8]. Among the several size-dependent physical characteristics accessible to those interested in the practical side of nanomaterials, optical and magnetic effects are the most commonly exploited for biological applications [9].

Copper oxide nanoparticles are cost-effective, photocatalytic, and exhibit stable chemical and physical characteristics. CuO-NPs have a large surface area and attractive crystal morphologies, making them more effective anti-infective agents [10]. Copper is a structural component of several enzymes. Cu^{++}'s harmful effects on microbiological pathogens require a high concentration. CuO nanoparticles can cause oxidative stress in cells by producing excessive reactive oxygen species (ROS), resulting in DNA and organelle damage and intercalate nucleic acid strands. Cu^{++} release may affect amino acid synthesis in several bacteria [11]. Copper oxide nanoparticles (CuO NPs) have several biological applications, including excellent bacteria killing, reaction acceleration, cancer cell inhibition [12] inorganic, CuO NPs have a longer shelf life than their organic counterparts. CuO NPs offer potential in biomedicine, but their toxic effects on vertebrates and invertebrates raise questions regarding their use for diagnosis and therapy. CuO NPs toxicity is mostly due to size, surface charge, and dissolution, both in vitro and in vivo [13], CuO NPs in vitro and in vivo toxicity subjected to species (bacterial, algae, fish, rats, human cell lines) used for toxicological hazard assessment. The factors that influence the toxicity of CuO NPs such as particle shape, size particle, surface functionalization, time–dose interaction and animal and cell models. CuO NPs if increase human exposure this causes the risk related to their short- and long-term toxicity. when CuO NPs exposure to the living systems results in reactive oxygen species generation, oxidative stress, inflammation, cytotoxicity, genotoxicity and immunotoxicity [14].

NPs may be get released to the environment during production stage, usage, recycling, or disposal and persist in air, soil, water, or biological system NPs can enter the human or animal body though the skin, orally or via respiratory tract the exposure to NPs causes inflammatory cells, allergic, neoplastic disease [15]. moreover, the exposure to ultrafine particles can causes pulmonary, cardiac, and central nervous system disease [16-18].

Metal oxide nanoparticles have been linked to genotoxicity, cytotoxicity, and immunotoxicity in several studies. Metal oxide nanoparticles' mechanism remains unclear despite intensive research. Surface features have a significant impact on their toxicity and interactions with biological systems. To assess nanomaterial toxicity (genotoxicity, cytotoxicity, and immunotoxicity), factors such as shape, size, crystallographic appearance, chemical composition, surface properties (chemistry and charge), and aggregative behavior should be characterized [19]. Metal ions released by NPs contribute significantly to their hazardous potential. The breakdown of metal ions from metal oxide NPs and their exposure to the environment are key factors in causing toxicity [20]. Among many NPs, that had been evaluate the toxicity of CuO. Misunderstanding the toxicity of CuO NPs stems from their binding, interaction with live cells, and resulting surface chemical changes. the toxicity of CuO NPs, it's important to examine their surface modification, exposure routes, and underlying mechanisms [21].CuO nanoparticles are characterized by their size, shape, and charge. Smaller NPs result in higher surface-to-volume ratios, and vice versa. The size of a particle determines its penetration and reactivity. Certain authors suggested that NPs larger than 100 nm may breach cell membranes and enter cells, whereas those smaller than 40 nm can enter blood and reach cell nuclei. The size of NPs influences cellular uptake and interaction processes and intercellular stability [22] had

shown that the toxicity rate increases as a result of increased exposure to copper oxide nanoparticle While the average person is exposed to it in proportions that the body needs in some foods, including cocoate CuO NPs are mostly absorbed by inhalation, hence several hazard evaluations of inhalation exposure to CuO NPs are underway [23] recently, inhalation exposure to CuO NPs was found to produce respiratory toxicity by creating excessive reactive oxygen species (ROS), leading to oxidative stress [24]. Exposure to these substance produces extensive inflammatory cell infiltration of the lung tissue, leading to oxidative stress and inflammation, which can ultimately end in lung failure [25]. Copper (Cu) plays an important part in the proper functioning of the human body by maintaining homeostasis, whereas excessive Cu consumption causes jaundice, hemolysis, and eventually death. When specific thresholds are exceeded, it can cause toxicity in the respiratory system, gastro intestinal tract (GIT), and skin problems. Due to the high impact use of CuO NPs, there is an urgent need to rigorously analyze their toxicity. However, there is currently inadequate understanding about the possible negative effects of human exposure to CuO NPs [25].

The bean-shaped kidney that are situated on either side of the back, directly below the rib cage. The crucial functions of the kidney are excretion of waste materials and toxins including urea, creatinine, and uric acid, as well as the control of extracellular fluid volume [26]. The kidney has the cortex and medulla. The cortex is composed of renal corpuscles, loop of Henle, collecting tubules, collecting ducts, and vasculature [27].

The viability of plasticity epithelium cells and are formed by a family of proteins known as desmosomal cadherins, and due to disruption of intercellular adhesion that is pivotal role in the cancer activation. Desmosomal-Cadherins are proteins with Ca dependent trans-membrane that are related to actin-microfilament of certain proteins in the cytoplasm. E-cadherin plays functional roles in tubule integrity, programmed cell death, and renal inflammation. They underscore the potential of E-cadherin restoration as a novel therapeutic strategy for AKI [28]. E-cadherin as a newfound indicator for diabetic nephropathy, which might be employed to ascertain the progression of DN. Consequently, evaluating the levels of E-cadherin in diabetic individuals could potentially have a significant effect on decelerating or possibly preventing end-stage renal disease [29]. In the rat kidney, E-cadherin was expressed mainly in the basolateral domain of the collecting duct and papillary surface epithelial cells

Is to evaluate effects of CuO NPs on the histological, histomorphometric and immunohistochemical expression of cadherin of kidney in male mice.

MATERIALS AND METHOD

Supplements (nanoparticles)

Providing CuO NPs (copper oxide nanoparticles) (nanopowder with an average size about 45- 50nm) Well-scattered in distilled water were utilized in the current investigation. NP was characterized with spherical shape

Mice and housing

The current research had been conducted on sixty apparently healthy mature male Swiss albino mice weighing 27.8-35g was bought and with average 3 months age. Rats were housed in a controlled environment with ideal conditions such as a constant temperature of 20 to 23 °C and Under hygienic conditions, the rats were kept in transparent polypropylene cages, 15 rats/cage, with free access to water and dry rat pellet feeds. The rats were allowed to acclimatize for a week before starting the experiment for accommodation.

Experimental protocol

The general histological preparation of paraffin blocks

The specimens were histologically prepared for paraffin section as follows:

Fixation, dehydration, clearing, impregnation, embedding, and sectioning de-waxing, staining and mounting. these steps were followed during tissue preparation for kidney paraffin blocking [30].

Immunohistochemical Reaction

Immunohistochemical detection kit E-cadherin of the kidney

Anti E-cadherin polyclonal (IgM) antibody was used, it is specific for the E-cadherin protein in mouse, that acts as junctional complexes protein. The procedure was done according to that was received from the manufactured company Sigma Aldrich-Germany and the staining kit received from Abcam United Kingdom.

The IHC detection kit components

Hydrogen peroxide block 5ml, Blocking reagent (protein block) 5ml, Antibody Amplifier 5ml, Goat anti rabbit HRP conjugate (HRP polymer) 5ml, DAB chromogen 1ml, DAB substrate reagent 5ml

Examination and photography

Photography: The prepared histological tissue slide, were stained with hematoxylin & eosin and IHC by DAB staining for E-cadherin, were examined for histological evaluation and estimation by using light microscope (LEICA DM750, Germany). The observed fields were snapshot using Digital Microscope Camera (Model MC500) with 5 mega pixel resolution and the pictures were saved in a JPEG format. All the examination procedure took place in the anatomy laboratories at the department of Anatomy, College of medicine/AL-Nahrain University.

Morphometry

Image J analysis was done using the software Image J (Java –based image processing program developed at the National Institutes of Health, USA) version 1.47p which already installing in a personal computer. Image J software is the keystone in the morphometric study by which different processing and procedures can be performed that can read certain image formats including TIFF, GIF, JPEG, and BMP [31]. It can calculate area and pixel value statistical of user defined selection. Also, it can measure distance and angles [32].

Statistical analysis of the data

The analysis of the present study was performed by statistical package of social sciences (SPSS) program V22 with the use of T-Test to determine the significance and to show the difference in the mean ± stander deviation between control group and other groups was used to measure the significance of differences in the mean diameter of bowman capsule, glomeruli, PCT.(T-Test). The positive pixels of immunohistochemical reactivity of E-cadherin protein marker expression in Nephron cells of the different dose of CuO NPs groups. A p value less than 0.05 was considered statistically significant.

Characterization of copper oxide nanoparticles

Characterizing CuO nanoparticles (NPs) is necessary to establish their characteristics for specific applications. X-ray diffraction (XRD) is one of the most extensively used procedures for determining the crystalline structure and phase

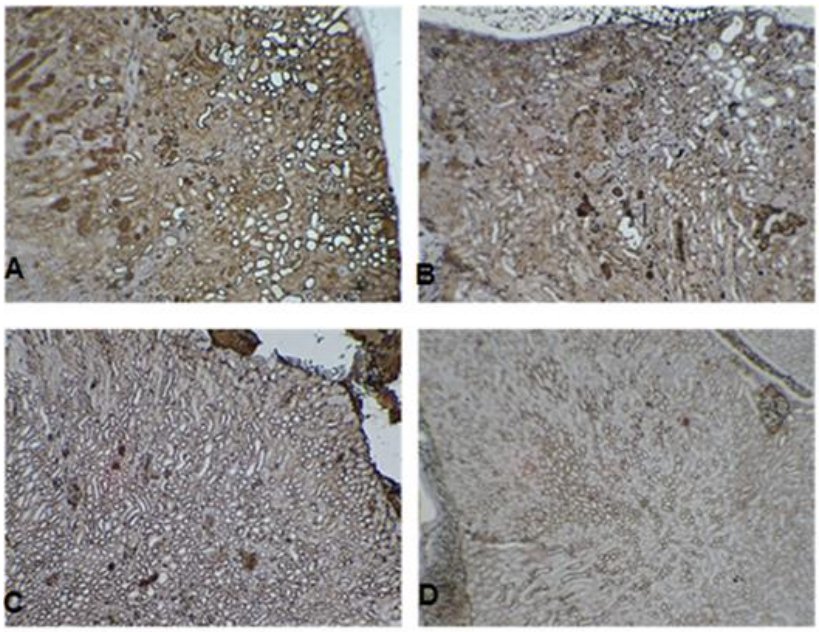

Fig. 1. Images of the histological features of renal cortex for animal treated with different doses of CuO NPs (Magnification of (4X)).

purity.

RESULTS AND DISCUSSION

The renal parenchyma had infiltrations of immune cells as lymphocytic macrophages were seen in treated animals with high doses of CUO NPs than those of low doses or control group as due to immune reaction of immune cells to these CuO NPs.

The histological features of renal cortex for animal treated with different doses of CuO NPs they reveal increment in E cadherin proteins expression with increment in treated dose of CuO NPs as in groups A, B &C in comparison to control group (D) that had been appear as dark brown coloration staining with anti-cadherin IHC at magnification of (4X) as shown in Fig. 1.

The renal cortex of animal treated with different doses of CuO NPs that had been evaluated by image scope Aprio software program at magnification 4X. The E cadherin proteins expression that appears as red color for strong positive while orange for positive and yellow for weak positive especially in groups A, B &C in comparison to control group (D) (Fig. 2, Table 1 and Fig. 3) show the relationship between E-cadherin expression of renal tissue with doses of CuO NPs.

The present study reveals that there was significant increment in the amount of food intake and the weight of the treated mice especially of the high dose animal group and this agree with other researchers who found that Copper oxide nanoparticles (CuO NPs) are consciously used to control the growth of bacteria, fungi, and algae. Several studies documented the beneficial and hazardous effects of CuO NPs on human cells. they found that an increment in the amount of feed intake and gain in the weight of experimental animals were recorded every week [33], while other authors found that there was no significant statistical difference in the weight between the treated groups of the rabbits that treated Copper oxide nanoparticles (CuO-NPs) [34].

The histological features of renal cortex for animal treated with different doses of CuO NPs they reveal increment in E cadherin proteins expression with increment in treated dose of CuO NPs as in groups A, B &C in comparison to control group (D) and this disagree with other researchers who found that the exposure of cells to Nano-CuO for 48 h resulted in decreased E-cadherin. They showed that exposure to Nano-CuO will cause increased ROS generation that lead to cell diseases and later on cell death. This indicating that mitochondria may be the main source of Nano-CuO-induced ROS generation. the

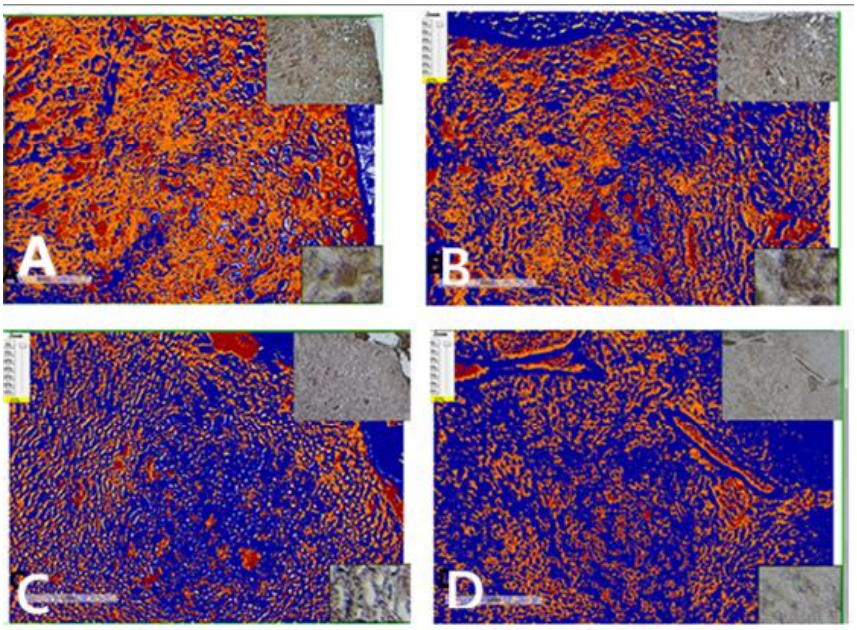

Fig. 2. The E cadherin proteins expression evaluated by image scope Aprio software program at magnification 4X.

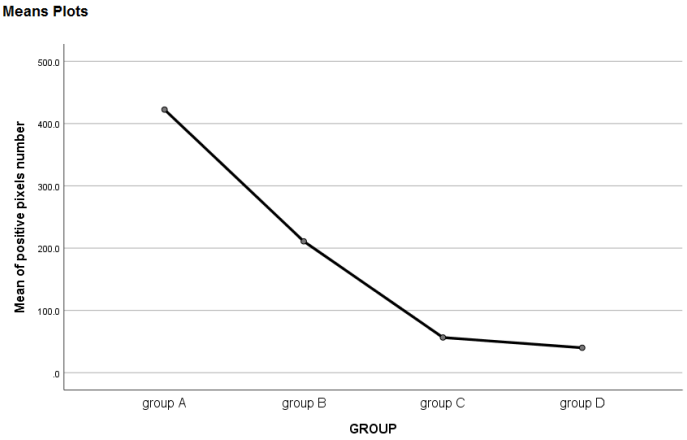

Fig. 3. show the relationship between E-cadherin expression of renal tissue with doses of CuO NPs.

Table 1. reveals the relationship between E-cadherin expression of renal tissue in pixels per micron with the doses of CuO NPs treated mice.

	Positive pixel number				
	Subset for alpha = 0.05				
	Group	N	1	2	3
Turkey HSD[a]	Group D	10	40.013		
	Group C	10	56.491		
	Group B	10		210.907	
	Group A	10			422.467
	Sig.		0.914	1.000	1.000

Means for groups in homogeneous subsetsare displayed.

a. Users Harmonic mean Sample Size= 10.000.

exposure of cells to Nano-CuO for 48 h resulted in decreased E-cadherin expression and increased protein expression of vimentin and fibronectin that lead to Nano-CuO-induced cell toxicity. They demonstrated that Nano-CuO exposure caused mitochondrial ROS generation. They found that these metal Nanoparticles will make cells to undergo Nano-CuO-induced dysregulation of ROS pathway. there is a potential mechanism involved in metal nanoparticle-induced various toxic effects including epithelial-mesenchymal transition EMT and lung pulmonary fibrosis. Expression to (Nano-CuO) are wildly used in medical and industrial fields and our daily necessities. the adverse effects of Nano-CuO on normal human bronchial epithelial cells caused dysregulation of protein MMP-3, an important mediator in pulmonary fibrosis, and its potential role in (EMT) [35].

CONCLUSION

The present investigation can conclude that the CuO NPs have a potential toxicological effect on the hepatic and renal tissues and with increasing the dose, they induced severe histopathological damages that may affect their functions as:

1. The renal parenchyma and the interstitial spaces had congested blood vessels that due to CuO NPs which induce lymphocytic infiltration of immune cells (lymphocytic and monocytes as macrophages) in the renal tissue.
2. The CuO NPs has toxic effects on cells of the renal tissue that lead programmed cell death (apoptosis).
3. High different doses of CuO NPs reveal increment in E cadherin proteins expression. It indicated that copper has affinity to E cadherin proteins in between cells CuO NPs Toxicity on the renal tissue in mice was due to metals with size of 38-50 nm in diameter that may impeded and impacted in the filtration barrier diaphragm of renal corpuscle causing defect in glomerular filtration though the filtration slit diaphragm in

renal corpuscles is typically 38-50 nm wide.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this manuscript.

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