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**ORIGINAL ARTICLE**

# Nephro Defensive Efficiency of Cichorium Intybus Against Toxicity Caused by Copper Oxide Nanoparticles

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## ABSTRACT
The penetration of nanotechnology into the medical world is constantly growing, and this raises concerns about its toxicity. This study aimed to illustrate the defense ability of Cichorium intybus (CI) against the nephrotoxicity induced by copper oxide nanoparticles (CuONPs) in a rat model. 32 laboratory rats were evenly distributed into 4 groups, each containing 8 animals. CON rats did not receive any dosage, whereas, CuONPs animals were dosed with copper oxide nanoparticles. As for the CuONPs + CI group, intoxicated rats were dosed with Cichorium intybus extract. Lastly, CI rats were given C.intybus extract only. The parameters of renal function, oxidative stress, and renal histological changes were measured. Animals treated with CuONPs showed negative changes in all examined parameters of kidneys compared to the CON group. However, co-administration of plant extract with NPs showed a clear improvement effect of these toxic changes. We concluded that the high dose of CuONPs causes significant kidney damage, and CI extract has a defensive effect against this toxicity.
**Keywords**: Nephrotoxicity, nanoparticles, oxidative stress, histological changes.

## INTRODUCTION
Nanomedicine has undergone rapid development in the twenty-first century [1,2], due to its great role in many delicate medical branches, especially related to cancers [3,4]. The strong adsorption capacity of nanoparticles has greatly enhanced their performance and applications [5]. Copper oxide nanoparticles (CuONPs) have acquired further attentiveness due to their distinct advantages compared to the rest of the metal oxides [6]. CuONPs have shown unique antioxidant, anticancer and antibacterial actions making them a portentous appliance for medical implementations [7,8]. In line with the increasing uses of nanoparticles, concerns have increased about the potential negative effects of their toxicity [9,10]. Several previous studies mentioned that nanoparticles may aggregate in different organ tissues of animal models, in particular the kidneys, causing adverse effects depending on dose and duration of exposure [11,12]. Even today, many developing countries are tending to consume medicinal herbs for their likability by the body as well as their fewer side effects [13].

Cichorium intybus (CI) is a perennial plant belonging to the family Asteraceae, characterized by being hardy with few green leaves. It grows in the wild within the temperate climate zone, especially in Eurasia and North Africa [14]. All parts of it contain several chemical compounds such as amino acids, vitamins, minerals, polyphenols, tannins, flavonoids and others. Therefore, it is widely used in traditional medicine CI extracts possess several health-promoting benefits including anti-inflammatory, antioxidant, and detoxifying [15]. This experimental study was designed to evaluate the defensive role of CI extract upon copper nanoparticle-induced nephrotoxicity in a rat model.

## MATERIALS AND METHODS
**Nanoparticles and plant extract:** Nano liquid copper oxide dispersion has been used with the following properties: particle size from 30 to 50 nm, purity of nanopowder 99%, brown-black color, dissolved in water, application of catalyst and sensing as well as antibacterial, industrial grade, HWNANO brand, place of origin: Jiangsu, China. Organic Chicory (Cichorium Intybus) roots dried capsules 100 % natural herbal supplement, GMO-free, VEGAN (USA) were used in this experiential study.

**Animals and design of study:** We used thirty-two albino males, aged between 5-6 months, and their weights 190-235 gm, in the current experimental study. They were obtained from animal houses within Iraqi universities. They were placed inside cages designated for them in a room that was ideally prepared in terms of temperature, ventilation and lighting. Rats were conditioned for 7 days before conducting the experiment. They were distributed into four groups with equal numbers as shown in Table 1. Upon termination of the experiment, all animals were anesthetized and dissected. Then, blood was collected from all animals used in the experiment by puncturing the heart, and the serum needed for assess kidney function was obtained. In addition to acquisition kidney tissues to assess oxidative stress, as well as to perform histological microscopic examination.

Table 1: Dosing of experimental groups.

| Groups | Treatment dosages |
|---|---|
| Cont | Rats were not given any treatment. |
| Cuonps | Rats were dosed with copper oxide nanoparticles at 250 mg/kg, orally for 14 consecutive days [16]. |
| Cuonps+ ci | The rats received CUONPs, followed shortly afterwards by an extract of Cichorium intybus at 200 mg/kg also orally [17]. |
| Ci | Animals dosed only with Cichorium intybus extract. |

**Kidney function tests:** To evaluate the efficiency of renal function, the levels of creatinine (Cr), blood urea (BU), and uric acid (Ur) were measured by Rock's kits with an automatic biochemical analyzer.

**Oxidative stress in kidney tissue:** The levels of both Malondialdehyde (MDA) and reduced glutathione (GSH) in kidney tissues were determined enzymatically by spectrophotometric method previously described [18].

**Kidney histology:** After fixing the kidney tissues with formaldehyde, they are dried using ethanol and then embedded in paraffin. Tissue samples were sectioned and stained with histologically specific hematoxylin and eosin (H&E). Using light microscope (OPTIKA B-380 Series, Italy) histological measurements were made after being captured by a high-speed color camera (OptikaView TCB5.0 Camera, Italy).

**Statistical data analysis:** Graph Pad Prism version 9 was used to analyze the data statistically. Data were displayed as mean ± standard deviation. Using one-way ANOVA followed by Tukey's multiple post hoc test, analyzes of differences between groups were performed. The level of statistical significance was considered at $p$ value $< 0.05$.

## RESULTS
Serological biochemical results related to renal functional markers as presented in figure (1) demonstrated a significant increase in serum levels of Cr (0.71±0.06), BU (30.05± 1.08) and Ur levels (1.61±0.07) in rats exposed to CUONPs compared to control (0.44± 0.04; 19.15± 0.80; and 1.13± 0.07 respectively). Whereas rats dosed with CUONPs and CI assured a considerable decrease in the levels of these kidney function indicators (0.51±0.03; 24.87±0.91; and 1.33±0.06) compared to the CUONPs group. Also, there was no important variance between control and CI groups.

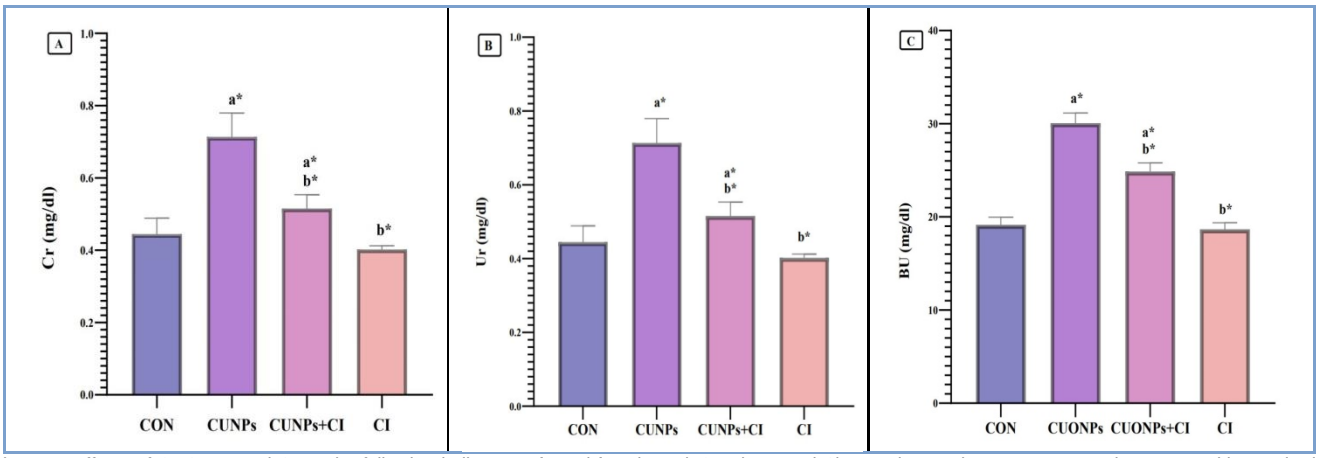


Figure 1: Effects of CUONPs and CI on the following indicators of renal function: A) Cr, B) Ur, and C) BU. The results are represented as mean with standard deviation (N= 8(. a* represents the significant variance compared to CON group, whereas b* represents the significant variance compared to CUONPs group.

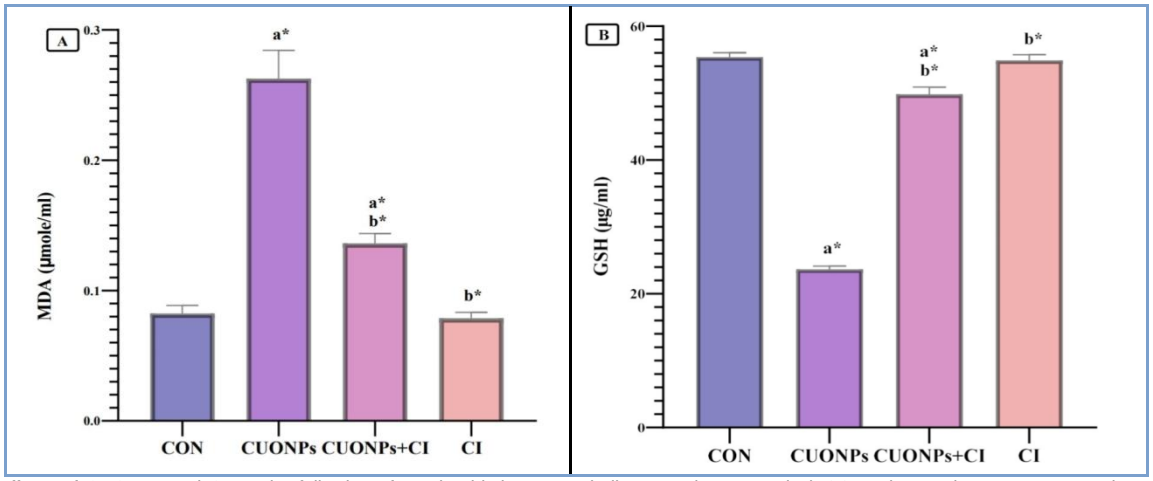


Figure 2: Effects of CUONPs and CI on the following of renal oxidative stress indicators: A) MDA and B) GSH. The results are represented as mean with standard deviation (N= 8). a* represents the significant variance compared to CON group, whereas b* represents the significant variance compared to CUONPs group.

As for the activity of oxidative stress biomarkers of the kidneys, rats treated with CUONPs assured a remarkable increase in MDA (0.26 ± 0.02) with a clear decrease in GSH (23.65 ± 0.46), when compared with the control groups (0.08 ± 0.01 and 55.37 ± 0.65). But rats treated with CUONPs and CI showed significant improvement in both oxidative indices (0.13 ± 0.01 and 49.80± 1.09) compared to those dosed with CUONPs as shown in figure (2).

While figures (3-8) show the histological changes that are additional indicators of the nephrotoxic effect of copper oxide NPs as well as the defensive effect of Cichorium intybus extract.

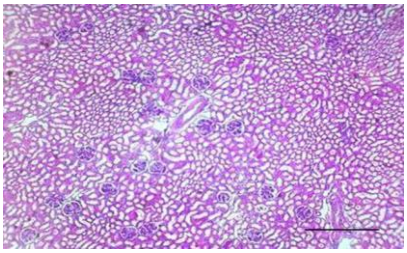

Figure 3: Micrographs of the kidney tissue of CON shows the normal histological structure of glomeruli and renal tubules (H&E X10, Scale Bar= 100µm).

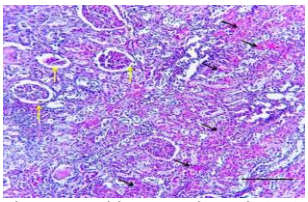

Figure 4: Kidney section of CUONPs displays renal congestion at the cortecomedullary junction of the kidney (black arrows) with the glomerulus detached from Bowman's capsule (yellow arrows) (H&E X20, Scale Bar= 50µm).

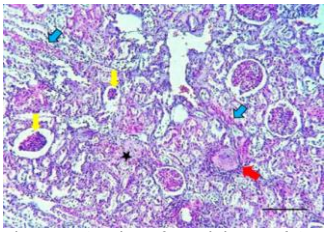

Figure 5: Section of renal tissue of CUONPs looks over coagulative necrosis (black star) with shrinkage of the glomerulus (yellow arrows), complete glomerular sclerosis(red arrow)and intertubular hemorrhage (blue arrows) (H&E X20, Scale Bar= 40µm).

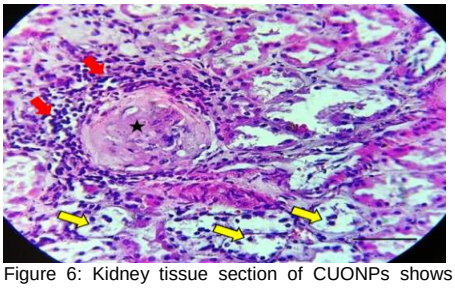

Figure 6: Kidney tissue section of CUONPs shows complete glomerular sclerosis (global sclerosis) with infiltration of mononuclear inflammatory cells mainly lymphocytes (red arrow) with detached the epithelium of proximal convoluted tubules (yellow arrows) and increase in renal interstitial spaces (H&E X40, Scale Bar= 20µm).

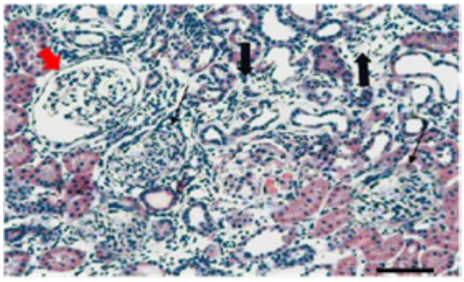

Figure 7: Kidney section of CUONPs+ CI outlines normal appearance of renal corpuscle (red arrow) with normal epithelium of proximal and distal convoluted tubules. There is some global sclerosis (thin black arrows) and thickening of the interstitial connective tissues (thick black arrows) and disappears of renal congestion (H&E X40, Scale Bar= 20µm).

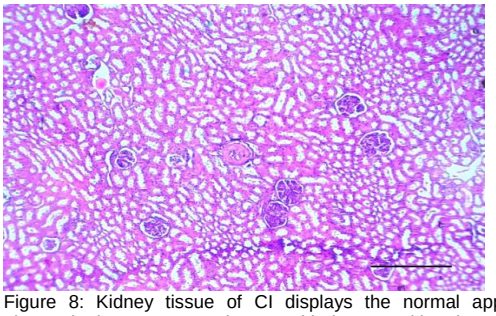

Figure 8: Kidney tissue of CI displays the normal appearance of the glomerular basement membranes with the normal brush margins of the renal tubules (H&E X10, Scale Bar= 100µm).

## DISCUSSION

The kidney is an essential organ for maintaining homeostasis and regulating the extracellular environment, such as detoxification and excretion of toxic metabolites and drugs. Therefore, the kidneys can be considered as the main target organ for exogenous toxic substances [19, 20]. In general, NPs are absorbed into the blood and then cleared by the kidneys. The results of this experiment confirmed the nephrotoxic effects of CUONPs. Where the levels of Cr, Ur and BU in the blood were remarkably high, which indicated that kidney function was damaged [21,22]. Besides, the level of GSH in kidney tissues was observed significantly lower, versus markedly increased MDA. This may explain that induction of reactive oxygen species production is one of the mechanisms followed by nanoparticles, which in turn may impair kidney function [23]. A similar previous study demonstrated changes in these parameters of renal function after one week's high-dose exposure to NPs [24]. We also saw in histopathology of some kidney tissues necrosis, inflammatory cell infiltration and various other histological changes indicating the possibility of tissue damage. Because the induction of oxidative stress and biochemical changes lead to a reduced defense system [25]. Our founding was in agreement with a previous study by Lei and colleagues who found that repeated oral administration of CuNPs for five days caused nephrotoxicity in rats through significant adverse changes in biochemical and oxidative parameters, with widespread necrosis of proximal renal tubules [26]. Bugata et al. conducted a study on the oral toxicity of CuO-NPs for 28 days in female rats. They observed that higher doses induced clear changes in biochemical and antioxidant biomarkers in kidney tissues. Histopathological evaluation also showed marked abnormalities in histological structure when compared to control rats [27]. Co-dosing with CI extract significantly reduced the adverse changes compared to rats dosed with CuONPs. CI extract's oxidative stress-reducing properties have been confirmed by increasing antioxidant enzyme activity and decreasing malondialdehyde levels in vivo as well as in vitro [28,29].Our result in line with the findings of El-Masry and colleagues, who demonstrated that subsequent administrations of hydroxyapatite NPs with CI extract in male rats improved the kidneys functionally and histologically. Whereas, chicory extract provided defensive advantages against the toxicity of these NPs [30].

## CONCLUSIONS

Through this experimental study, we concluded that exposure to copper oxide nanoparticles in high concentrations caused renal toxicity, but with combined use of Cichorium intybus extract, adverse biochemical and histological changes were modified.

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