# PROPHYLACTIC EFFICACY OF SILYMARIN UPON RENAL DYSFUNCTION INDUCED BY COPPER OXIDE NANOPARTICLE

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(Received 21st June 2023; accepted 22nd September 2023)

**Abstract.** Copper oxide nanoparticles (CuO-NPs) have unique properties encouraged their rapidly growing uses in various fields as biomedical applications and environmental remediation. In this regard, the potential toxicity resulting from repeated exposure increases exponentially, so ensuring the suitability and safety of these nanoparticles for different uses is urgent. This experimental study aims to estimate nephrotoxic effects of CuO-NPs on some renal dysfunction markers along with the possibility attenuated efficacy of silymarin (SLM) in laboratory animals. Twenty four adult male rats were recruited in current study distributed into (4) equal groups. In CON group rats were given distilled water only and considered as control, while rats in CuO-NPs group were poisoned with copper oxide nanoparticles using gastric gavage. In CuO-NPs + SLM group, rats were provided with both CuO-NPs plus silymarin orally. In the SLM group, only silymarin was administered to rats. Studied rats were sacrificed and sera and kidney homogenates were obtained to complete the necessary biochemical and oxidative stress tests. According to the results, rats intoxicated with CuO-NPs recorded deleterious alterations in all studied renal biomarkers levels compared to control rats. However, the data confirmed that the co-administration of SLM with CuO-NPs had positively ameliorated the detrimental changes induced by CuO-NPs. In conclusion, it was suggested that a high dose of copper oxide NPs may cause significant impairment in renal functioning, and silymarin can be considered as a protective agent upon potential nephrotoxicity induced by copper oxide nanoparticle.
**Keywords**: *silymrin, renal dysfunction, nanoparticles, oxidative stress*

## Introduction

Nanomedicine has seen tremendous progress in the twenty-first century, owing to its great contributing role in facilitating the diagnosis and medication of abundant diseases, especially intractable ones (Biswas et al., 2023; Ghareeb, 2022). Nanoparticles with extremely small sizes (1-100 nm) have participated in many biomedical applications for their distinctive physicochemical characteristics, especially the high surface area to volume ratio (Mandhata et al., 2022; Ramadhan and Ghareeb, 2022). Research topics related to NPs, especially metal oxides, have garnered a lot of attention due to their frequent use in modern life (Ghareeb, 2023; Samuel et al., 2022).

Copper oxide nanoparticles (CuO-NPs) are among the notable metal oxide nanoparticles that are frequently consumed in a diversity of technological, industrial, and medical applications, including electronics, catalysts, gas sensors, metallic coatings, herbicides, algaecides, inks, and antimicrobial products (El-Refaey and Salem, 2023; Verma and Kumar, 2019). On the contrary, continued exposure to these particles has potentially harmful effects on human and animal health a like Ghareeb et al. (2021). As these particles have a high ability to enter the circulatory system, penetrate physiological barriers, and reach most vital organs (Zhang et al., 2021). Notably, in vivo

studies suggested that CuO-NPs have toxic effects, including nephrotoxicity (Naz et al., 2023; Ahmed et al., 2022; Pantic et al., 2019). Recently, great emphasis has been placed on the role of herbal plant extracts as protective agents for various diverse toxins (Pandey et al., 2023). In addition, there is great support from scientists around the world for employing medicinal plants in developing original medicines with the least possible side effects (Al-Haidari et al., 2021). Silymarin (SLM), biologically active extract of Silybum marianum (milk thistle) plant, has attracted significant attention for its health benefits against a variety of liver-related toxins (Macit et al., 2023). Previous experimental laboratory studies have concluded that SLM is effective in reducing oxidative stress and mitigating cytotoxicity (Vecera et al., 2022; Yardım et al., 2021). It is a complex blend of plant-derived compounds with flavonolignans, flavonoids, and polyphenolic molecules that are effective antioxidants. Approximately 60% of the SLM complex is silibinin (silybin), which has many pharmacological effects including antioxidant and anti-inflammatory properties (Gillessen and Schmidt, 2020). It also has mitigation susceptibility against the nephrotoxic effects caused by chemicals (Kandemir et al., 2017).This study was done to inspect the potential preventative effect of SLM upon CuO-NPs caused by nephrotoxicity in laboratory rats.

## Materials and Methods

### *Nanoparticles and silymarin*

A dispersion of CUO nanoparticles (3 wt%) was obtained from Nanoshel (UK) Limited, with the following specifications: color: black, APS: 3-6 nm, solvent: isopropyl alcohol, purity: 99.9% , and PH=3. Besides using Pure Silymarin Envelopes, a dietary supplement available in the markets.

### *Animals and study design*

Twenty-four adult male albino rats, weighing between (190-225) g, were obtained from animal houses designated for scientific research within Iraqi universities. The rats were housed in custom cages of appropriate dimensions under controlled laboratory hygienic conditions (temperature, lighting, and ventilation), along with easy access to designated food and water. They were acclimated to the laboratory environment for seven days, in preparation for study starting. Equally, all animals were set into four groups of six in each. The study protocol was established according to the administered doses of CuO-NPs and SLM to rats groups as shown in *Table 1*. Dosing continued for 14 consecutive days, and one day after completion, all studied rats were generally anesthetized and dissected. All procedures of this experiment were done in full accordance with the directives of the Ministry of Education and Scientific Research regarding the ethics of dealing with laboratory animals.

***Table 1.****Demographic characteristics of the Sample of respondents (N=1067).*

| Animals groups | Treatment and doses for 14 uninterrupted days |
|---|---|
| CON | Animals received distilled water, served as control. |
| CUO-NPs | Animals were poisoned with CuO-NPs of 100mg/kg by gastruc gavage (Abdelazeim et al., 2020). |
| CUO-NPs + SLM | Intoxicated animals with CuO-NPs were provided with SLM of 100mg/kg orally (Taher and Ghareeb, 2022). |
| SLM | Animals were dosed with SLM of 100mg/kg only. |

### *Kidney function analysis*

Blood samples were collected by cardiac puncture from all studied rats, and the sera were separated by centrifugation for several minutes to check the levels of renal function markers including blood urea nitrogen, creatinine, and uric acid (BU, CR, and UR respectively) measured in mg/dl units, using privet diagnostic kits.

### *Oxidative stress analysis*

Kidney tissues were also acquired from autopsied animals to evaluate oxidative stress in studied rats. Kidney homogenization was performed in a homogenization solution containing appropriate molar amounts of potassium buffer, potassium chloride, and EDTA for one and a half minutes, to obtain kidney tissue supernatants for analysis of levels of glutathione as well as Malondialdehyde (MDA) by measurement of the reactants of thiobarbituric acid reactive substances (Anreddy, 2018).

### *Statistical analysis*

Results were statistically processed by SPSS (version 26),with presented all data as mean and standard deviation (M±SD).To determine the difference between the experimental groups, Tukey test was interpreted post hoc one-way analysis of variance (ANOVA). A P-value of $\leq 0.05$ was adopted as a significant variation.

## Results and Discussion

The results related to the levels of serum indicators of kidney function recorded a significant increase in levels of BU (30.76±0.93), CR (1.04±0.01), and UR levels (1.74±0.09) in rats poisoned with CUO-NPs compared to control (20.61±0.74; 0.69±0.03; 1.12±0.05 respectively). On other hand, a clear decline in serological levels of these renal markers (25.14±0.54 ; 0.81±0.02; and 1.34±0.04) was observed in CUO-NPs + SLM group compared to CUO-NPs rats. Also, there was no significant difference between the control and the SLM groups, as displayed in *Figure 1*. As for the evaluation of SLM effect on the oxidative stress caused by nanoparticles in the kidney homogenates, it was noted that CUO-NPs induced a higher concentration of MDA metabolites in exposed rats (0.24±0.012) with an apparent reduction in GSH (38.06±4.17) compared to control normal ones (0.09±0.007 and 63.53±2.28). However, rats treated with CUO-NPs plus SLM manifested respectable amelioration in both oxidative stress indices (0.18±0.006 and 50.04±3.58) compared to rats intoxicated with CUO-NPs as shown in *Figure 2*.

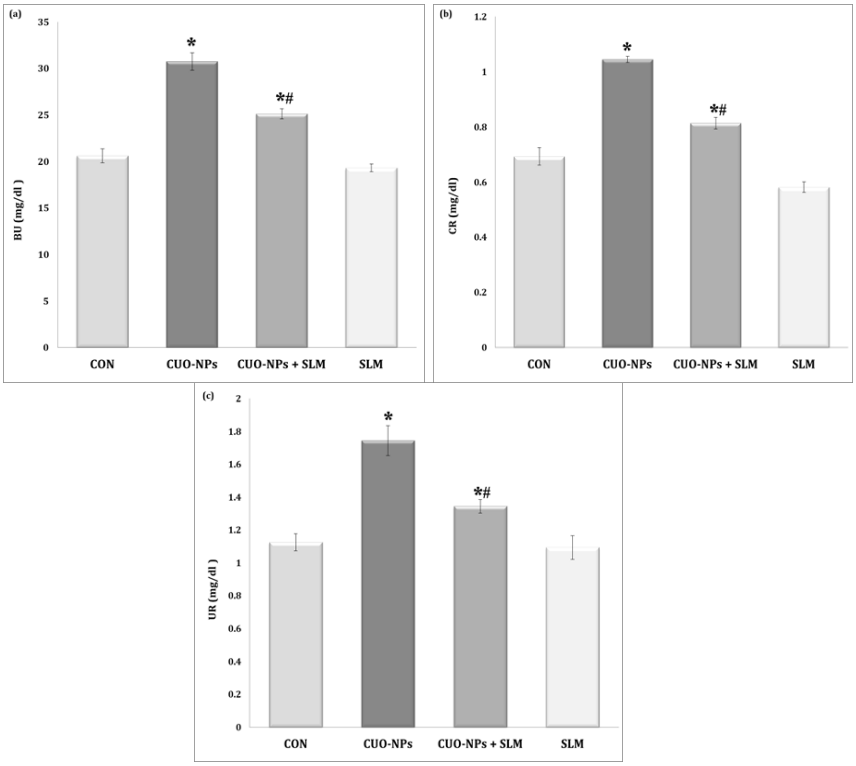


***Figure 1.*** *Serum renal markers levels: a) BU, b) CR, and c) UR, for studied rats. The symbol (*) mark a significant variance with CON group, and (#) mark a significant variance with the intoxicated CUO-NPs rats.*

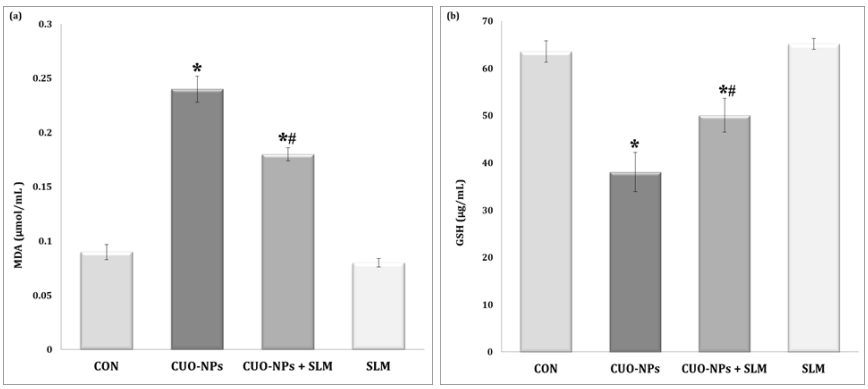


***Figure 2.*** *The values of oxidative markers: a) MDA, and b) GSH, for studied rats. The symbol (*) mark a significant variance with CON group, and (#) mark a significant variance with the intoxicated CUO-NPs animals.*

The results of our study proved that copper oxide nanoparticles dispersion induced clearly renal damage by impairing renal function and stimulating oxidative stress in renal tissue. CUO-NPs increased BU, CR, and UR serum levels notably, and the tissue level of GSH reduced, while MDA elevated markedly. This is in agreement with several previous studies documented that CuO-NPs may cause oxidative stress, cytotoxicity, and inflammatory processes in vitro as well as in vivo (Sajjad et al., 2023).It is known that the kidney is primary excretory organ in the body for toxins and chemicals, including nanoparticles, after their penetration and distribution in the circulatory system (TTruskewycz et al., 2022; Zhang et al., 2020). Upon entry of CuO-NPs through any route of exposure including ingestion into the body, these nanoparticles react either with

the acidity of lysosomes or mitochondria (oxidizing organelles), prompting reactive oxygen species) ROS (generation, which represents as a compelling approach to linked toxicity with copper oxide nanoparticles. In doing so, it acts as a pro-oxidant, that is, it promotes oxidative stress by stimulating ROS or inactivating antioxidants. Thus damage to cellular structures as mitochondria and proteins occurs leading to cell death (Liu et al., 2021; Naz et al., 2020; Anreddy, 2018). Several studies have dealt with the toxic effects of CUO nanoparticles on vital organs, including nephrotoxicity. The decrease in glomerular filtration rate in rats intoxicated with CUO-NPs resulted in an increased level of the serum indices BU and CR, indicating impairment of renal function. Otherwise, oxidative stress is a strong potential explanatory cause of renal impairment. As the lipid peroxidase has a deleterious impact on the glomerular basement membrane and thus the renal system (Khalid et al., 2018; Thit et al., 2015). In previous experimental study by Bugata et al. (2019) on the oral toxicity of CuO nanoparticles in female rats, they suggested that acute and sub-acute high-dose treatments induced significant changes in serological and oxidative stress markers. Establishing that the toxicity observed in in liver, kidney tissues may be due to stimulation of excess ROS producing by CuO-NPs (Bugata et al., 2019).

Another study by Elkhateeb et al. (2020), demonstrated adverse effects in rats poisoned with copper oxide NPs orally for 3 months by evaluating indicators of inflammation in the kidneys as well as oxidative stress in laboratory rat (Elkhateeb et al., 2020). Similarity, Ghonimi et al. (2022) concluded in their investigative study that dosing with CuO NPs intraperitoneally for 9 consecutive days has a potential toxicity on liver and kidney tissues of male mature rats that may affect their functions (Ghonimi et al., 2022). On other hand, results of this study found an improvement in kidney function indicators and oxidative stress levels in the CUO-NPs + SLM group. This agreement with a previous study conducted by Abd Eldaim et al. (2021) on male rats, they found that SLM reduced the toxic effects of lead acetate by ameliorative functions of the liver and kidneys and their structures by lipid oxidation reduction, attenuating pathological events in tissues structures, and improving antioxidants (Abd Eldaim et al., 2021). In another study conducted by Guzel et al. (2020) on laboratory rats, they concluded that supplementing vancomycin with silymarin (200 mg) for 8 consecutive days mitigated vancomycin-induced nephrotoxicity by improving oxidative stress perturbations, serum blood nitrogen urea and creatinine levels, and histopathological features (Guzel et al., 2020). Also, Dumludag et al. (2022) found that dosing of silymarin (100 mg/kg) for seven continues days with colistin are able to produce some amelioration in renal tubular necrosis and significantly augmentation antioxidant capability. The prophylactic ability of SLM increased with higher doses and longer treatment duration to prevent renal toxicity (Dumludag et al., 2022). In previous experimental study on 32 laboratory rats, Nouri and Heidarian (2019), found that silymarin had a protective effect against renal damage and oxidative stress caused by diclofenac, by decreasing the levels of serum MDA, urea nitrogen, Cr and TNF-α, and relieving histological injuries in the kidneys (Nouri and Heidarian, 2019).

## Conclusion

Results of this study approved the deleterious effect of copper oxide nanoparticles on of renal function of the studied laboratory rats. However, combining those nanoparticles with silymarin reduced this toxicity by restoring levels of both serum and oxidative

stress renal indices. Thus, silymarin can be considered as a prophylactic agent upon potential nephrotoxicity agents as metal nanoparticles.

## Acknowledgement

This research study is self-funded.

## Conflict of interest

The authors declare no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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