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Therapeutic Potential of Quercetin on Biochemical Deteriorations Induced by Copper Oxide Nanoparticles

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Authors' contributions

This work was carried out in collaboration between all authors. Authors ERY and AFA were designed the study, performed the analytical procedure and the statistical analysis. Author HFA wrote the manuscript, and wrote the first draft of the manuscript. All authors Read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Objective: The current research is designed to evaluate copper oxide nanoparitcles (CuO NPs) toxicity on HCT116 in vitro as well as in vivo on liver paraoxanse 1 (PON1) activity and serum B-cell lymphoma -2 (BCl2) in rats.
Materials and Methods: The toxicological role of CuO-NPs on the liver was indicated through intraperitoneal injection of 3 and 50 mg/kg of CuO-NPs (size >20 nm) in female rats for 7 days. The effects of NPs were examined by demonstrating serum levels of antiapoptotic marker (BCl2) and antioxidant enzyme PON1 .Flavonoids quercetin (que) was administered orally to intoxicated rats at the dose of 200 mg/kg for 30 consecutive days.
Results: *In vitro* study showed 100% death of HCT116 cells up to 12.5 ug/ml. The current results declared obvious PON1 inhibition and BCl2 in CuO-NPs intoxicated rats. Also, the results of this study indicated that the two concentrations of copper nanoparticles induced toxicity, while

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attenuation in antioxidant status and antiapoptotic marker was detected upon treated intoxicated rats with que.

Conclusion: It is evident that these nanoparticles cannot be used for human purposes because of their toxicity caused by oxidative stress implicated in liver toxicity by CuO-NPs. This study also declared that que is an effective free radical scavenger and could consider a potential nutraceutical for liver toxicity.

Keywords: Copper oxide; liver toxicity; nanoparticles; quercetin; PON1; BCl2.

1. INTRODUCTION

Recently, increase the need of nanoparticles in the different human activities has increased. So, study of the biological effectiveness of various nanoparticles and nanocomposite substances, particularly their toxicological effects on organs of human and animal need great attention. The primary consequence is the nanoparticles toxicity in humans and their potential risk as well as their corresponding products on health of human. Copper nano- materials are excessively synthesized and used as metal catalyst in tools of machine, semiconductors, and in medications as antibacterial [1,2]. Copper nano-particles are used as engineered nanoparticles in industrial applications; thus, their being released into the environment and the related of their different effects on human health has increased. It is indicated that nanoparticles of copper are dispersed in tissues of animal, causing architecture changes. High dose of nanoparticles of copper has led to dystrophy or tissue necrosis. Kim et al. [3], detected the effect of inhaled nanoparticles of copper on pulmonary function in mice and noticed that nanoparticles of copper release inflammatory reaction, increase in lungs recruitment and neutrophils total cells, as well as elevation in the activity of LDH in the bronchus in comparison with iron oxide, titanium dioxide, and silver [3]. Cytotoxicity and damage of DNA were also demonstrated in A549 type II epithelial cells of lung for CuO, TiO2, ZnO, Fe_2O_3, and Fe_3O_4 atdose 40 and 80 µg [4].One of the most important mechanisms is their ability to induce oxidativedamage [5,6]. It was recently detected that nanoparticles of, copper are highly toxic in vitro compared to other nanoparticles of metal oxide, [2]. On the other hand, quercetin (que) is natural flavonoid present in various fruits and vegetables. Different studies have found that this molecule has many biological qualities; as antiischemic, hypolipidemic, cytoprotective, anti-angiogenic, antispasmitic, anti-mutagenic, anti-platelet, antihypertensive, antioxidant, anti-inflammatory, anti-thrombotic, anti-cancer, anti-proliferative, and anti-viral [7-9]. In several models of toxicity, que was found to protect against toxicity and damage of tissue induced by anti-cancer drugs [8-10]. So, this study is designed to investigate the hepatoprotective effects of que on liver antioxidant PON1 and serum anti-apoptotic marker BCl2 in CuONPs induced hepatic toxicity in rats. Also, the effects of intraperitoneal injection of two doses (3, 50 mg/kg) of copper nanoparticles with <20 nm diameters will be investigated on aforementioned parameters in rats.

2. MATERIALS AND METHODS

2.1 Chemicals and Reagents

Que hydrate, 95%, was obtained from New Jersey, USA. Silymarin was obtained from Sigma-Aldrich Co. (St Louis, Missouri, USA). Biochemical parameters were determined using Biodiagnostic Kits (Biodiagnostics Co., Upton-Upon-Severn, Worcestershire, UK).

2.2 Synthesis of Copper Oxide Nanoparticles

CuO-NPs (particle size <20 nm) were synthesized by the precipitation technique sing copper chloride and sodium hydroxide [2].In brief, each precursor was dissolved in 100 ml deionized water to form a 0.1 mol/l concentration. Then, sodium hydroxide solution was slowly added under vigorous stirring. Black precipitates were obtained at pH 14 and repeatedly washed by deionized water and absolute ethanol several times. The washed precipitates were dried at 80°C for 16 h to obtain a dry powder of CuO-NPs. Finally, the resulting product was calcined at 500°C for 1 h and investigated by radiography diffractrometry. The particle size and size distribution were tested by a transmission electron microscope. CuO-NPs were suspended in 1% Tween 80 and dispersed by ultrasonic vibration for 15 min.

2.3 Cytotoxic Effect on Human Colon Tumor Cell Line (HCT116)

CuO NPs was tested against the human colon tumor cell line (HCT116). The sample

concentrations range between (100 to 0.78 ug/ml) using MTT assay. Cell viability was assessed by the mitochondrial dependent reduction of yellow MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) to purple formazan [11]. Procedure: The procedures were done in a sterile area using a Laminar flow cabinet biosafety class II level(Baker, SG403INT, Sanford, ME, USA). Cells were suspended in RPMI 1640 medium for HCT116) , 1% antibiotic-antimycotic mixture (10,000 U/ml Potassium Penicillin, 10,000 µg/ml Streptomycin Sulfate and 25 µg/ml Amphotericin B) and 1% L-glutamine at 37ºC under 5% CO2.

3. ANIMALS

Seventy adult female albino rats with an average weight of 120±5 g were obtained from the animal house of the National Research Centre laboratory, Egypt. Animals were acclimated in a controlled environment (22±5°C, 12 h light/dark cycle) with free access to water and pelleted standard rat chow diet during the study. The present study was approved by the Ethical Committee of National Research Centre, Egypt, provided that the animals will not suffer at any stage of the experiment.

3.1 Experimental Design

After 1 week of acclimatization, 70 rats were divided randomly into a control group of 10 rats and two principal equally tested groups. The initial principal group was injected intraperitoneally with low dose of CuO-NPs (3 mg/kg) [2]. The other principal group was administered a high dose of CuO-NPs (50 mg/kg) [2], for 7 consecutive days. At the end of the CuONPs injection, 10 rats from each principal group were left untreated (intoxicated groups). The remaining rats from each principal group were subdivided equally into two subgroups: the first subgroup from each principal group was treated with standard Silymarin drug at a dose of 50 mg/kg [12] and the other subgroup was treated with que at a dose of 200 mg/kg [13]. Both standard Silymarin and que were administered orally for 30 consecutive days.

3.2 Preparation of Serum

After 24 h of the last dose administration, rats were fasted overnight, anesthetized by diethyl ether, and their blood collected by puncture of the sublingual vein in the clean and a dry test tube. Serum was separated by centrifugation at 3000 rpm at 4°C for 10 min and kept at −20°C for different biochemical analyses of BCl2. However, liver tissues were carefully separated, washed in ice-cold saline, and blotted with a filter paper. The homogenate was prepared in phosphate buffer, pH 7.4, using a Potter Elvehjem homogenizer (Report Fraud and Corruption, Jiangning, Nanjing, Jiangsu Province, China) with a Teflon pestle (20% w/v). The resulting homogenate was centrifuged at 5000 rpm at 4°C for 15 min. The resulting supernatant was used for the biochemical analysis of PON1.

4. BIOCHEMICAL ANALYSIS

4.1 Determination of PON1

We measured the rate of hydrolysis of paraoxon by monitoring the increase of absorbance at 405 nm and at 25°C. The basal assay mixture included 1.0 mM paraoxon and 1.0 mM CaCl2 in 0.05 M glycine buffer, pH 10.5. One unit (IU) of paraoxonase activity is defined as 1 µmol of p-nitrophenol formed per min, and activity was expressed as U/l of serum (22) [14].

4.2 Determination of BCl2

The level of serum B-cell leukemia/lymphoma 2 (Bcl-2) was determined by double-antibody sandwich enzyme-linked immunosorbent assay kit according to the manufacturer's instructions (Biosystems, Egypt).

Percentage change:

$$\frac{\text{Mean Control- Mean of Test}}{\text{Mean of control}} \text{X100}$$

Percentages of improvement:

$$\frac{\text{Mean disease – Mean of treated}}{\text{Mean of control}} \text{X100}$$

4.3 Statistical Analysis

Statistical analysis is carried out using SPSS computer program (version 8) combined with costate computer program, where unshared letters are significant at P≤0.05.

5. RESULTS AND DISCUSSION

In vitro study showed 100% death of HCT116 cells up to 12.5 ug/ml (Table 1).

Table 2 demonstrated significant reduction in bothPON1 and BCl2 levels in CuO NPs reached to 48.80 and 26.62 %, respectively in low dose of Cuo NPs intoxicated rats, while severe reduction was detected in PON1 and BCl2 upon treated rats with high dose of CuONPs (76.10 and 31.54%, respectively). On the other hand, treatment of que to low dose of CuONPs recorded amelioration percentages in PON1 and BCl2 21.73 and 20.83%, respectively compared to standard Silymarin drug (24.86 and 19.29%, respectively). While the percentages of improvement of que in PON1 and BCl2 reached to 23.57 and 15.91%, in high dose CuO NPs compared to 44.01 and 20.77%, respectively for Silymarin.

The significant reduction in antioxidant PON1 as well as antiapoptotic markers BCl2 indicated hepatotoxicity. In this concerns, Doudi1 and Setorki, [2] found hepatic vascular degeneration in periportal regions. They also demonstrated thatat the dosage 6 mg/kg copper nanoparticle, hepatotoxicity and nephrotoxicity are appeared. However, apoptosis could be noticed in periportal area of hepatic tissue and epithelium of kidney tubule post three days and three hours, respectively by three injections of copper nanoparticles [15]. In a good agreement with the present findings, Chen et al.[16] indicated that copper nanoparticles (23.5 nm) induced toxicological effects and acute injuries on the renal , hepatic , and spleen of experimental mice Additionally Li et al [17] recorded severe hepatotoxicity , nephrotoxicity and necrosis in hepatic and renal tissues. by copper nanoparticles at 200 mg/kg/d for 5 days.

Table 1. Cytotoxic activity of CuONPs against HCT116

Sample	LC_{50}(ug/ml)	Remarks
100% up to 12.5 ug/ml		
DMSO	……	1% at 100 ppm
Negative control	……	0%

LC_{50}: Lethal concentration of the sample which causes the death of 50% of cells in 48 hrs

It has been found that, the DNA damage by oxidative stress is the main cause of toxicity of nanoparticles [18].However, various mechanisms must also be implicated. For instance, cell membranes and organelles damage would encourage toxic factors transfer. The mechanisms of genotoxic and allergenic are also possible [19]. Studies of Fahmy et al. [20], indicated that in comparison with normal cells, in cells subjected to nanoparticles of copper, the activity of catalase and glutathione reductase inhibited and elevation of glutathione peroxidase *activity was recorded, suggested that nanoparticles of copper not only produce free* radical, but also they block cellular antioxidant defense. Hence the current results strongly

Table 2. Effect of quercetin on PON1 and BCl2 levels in copper oxide nanoparticles-induced hepatotoxicity in rats

Groups / Biomarkers	PON1 (kU/l)	BCl2 (U/ml)
Control	271.50± 11.78^{a}	264.36± 15.00^{f}
LD CuO-NPs	139.00± 12.00^{b}	194.00± 10.00^{g}
%change	-48.80	-26.62
HD CuO-NPs	65.00± 5.00^{c}	180.98± 9.00^{h}
%change	-76.10	-31.54
LD CuO-NPs+que	198.00± 7.00^{d}	249.06± 15.00^{f}
%change	-27.10	-5.81
%improvement	21.73	20.83
HD CuO-NPs+que	129.00± 6.00^{e}	223.01± 8.00^{k}
%change	-63.17	15.64
%improvement	23.57	15.91
LD CuONPs+silymarin	206.5± 13.00^{d}	245.00± 14.00^{f}
%change	23.94	-7.32
%improvement	24.86	19.29
HD CuO-NPs+silymarin	184.50± 8.00^{m}	205.89± 11.00^{l}
%change	32.04	-22.12
%improvement	44.01	20.77

Data were expressed as Mean±SD (n=10). CuO, copper oxide; HD, high dose; LD, low dose; NP, nanoparticle. Shared letters between groups are not significantly different; unshared letters between groups represent significantly different values at P≤0.05. Shared means similar. Unshared means different

suggested that CuO-NPs could stimulate free radicals generation which decrease PON1 activity in a dose depending manner.

The ameliorative signs of que on BCl2 and PON1 levels post CuONPs intoxication may be related to the beneficial role of que in inhibiting the inflammatory response by down-regulating proinflammatory cytokine protein expression levels [21]. Also, Mostafavi –Pour et al. [10] explained that que plays a preventive role against the imbalance elicited between the production of free radicals and antioxidant defense systems, where cellular destruction is a consequence of reactive oxygen species. Additionally, que is known to attenuate several inflammatory cytokines action that are of particular concern to transplant recipients, including IL-1β, IL-2, IL-6, IL-15 and TNF-α [22,23]. Hushmendy et al. [24] found that que significantly inhibited cytokine levels and T-cell proliferation, suggesting that it may be effective in reducing transplant rejection. The upreglation of antiapoptotic BCl2 marker post que treatment may be attributed to que was associated with lower inflammatory cytokine levels [25].Also, the modulation of PON1 level in que treated rats may be attributed to the antioxidant properties of it. The Qur antioxidant effect is based on its ability to quench hydrogen peroxide [26].

6. CONCLUSION

The findings of the present study showed that the two concentrations of copper nanoparticles were able to induce liver toxicity of rats. Therefore, they cannot be handled by humans due to their toxicity. Nano-copper exposure elevated reactive oxygen species production; one of the most frequently founded nanoparticles-linked toxicity. Nano copper can induce apoptotic intrinsic and extrinsic pathways in oxidative stress .Flavonoid que is proved in the current research to have antioxidant effect ,ameliorating PON1 level and upregulated BCl2 which may be related to its ability to scavenge free radical and eliminating oxidative stress.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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