Modulation effects of quercetin against copper oxide nanoparticles-induced liver toxicity in rats

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Background and objectives
Despite the several benefits of nanotechnology, studies indicated that certain nanoparticles (NPs) may cause adverse effects because of their minute size and unique properties. The aim of this study is to investigate the role of quercetin (que) in attenuating toxicity by copper oxide (CuO) NPs in rat liver.
Materials and methods
The effect of CuO-NPs on the liver was induced by two injections of CuO-NPs (size >20 nm) at the dose 3 mg/kg and 50 mg/kg intraperitoneally in different groups of female rats for 7 days. The effects of NPs were tested by evaluating liver function enzymes: alanine aminotransferase, aspartate aminotransferase, and alkaline phosphatase; antioxidant biomarkers: nitric oxide, catalase activity, reduced glutathione, and total antioxidant capacity; DNA damage as shown by comet assay; and histopathological examination of hepatic tissue. Flavonoids que was administered orally to intoxicated rats at the dose of 200 mg/kg for 30 consecutive days.
Results and conclusion
The present results indicated significant depletion in serum hepatic enzyme activities and improvement in the cellular antioxidant status of CuO-NPs-intoxicated rats after administration of que. Histopathological examination of hepatic tissue treated with que confirmed the previous biochemical results, which showed normal architecture of hepatic tissues. However, treatment of intoxicated rats with que led to a significant reduction in the DNA damage, tail length, and tail moment. Oxidative stress could be considered to play a key role in liver toxicity by CuO-NPs. This research also showed that que is an effective free-radical quencher and could represent a potential valid therapeutic for hepatotoxicity.

Keywords:
comet assay, copper oxide, liver toxicity, nanoparticles, quercetin

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Introduction

Owing to the unique properties of nanoparticles (NPs), they have received increasing attention as a result of their large specific surface areas and minute sizes. The properties of materials at the nanoscale can be completely different from those at a larger scale [1]. Therefore, they have wide-spread applications in all aspects of industries and sciences, and this field is opening new avenues in science and technology [2]. The increasing use of nanomaterials has challenged the world in terms of their effect on biological systems, giving rise to a demand for parallel risk determination. NPs can invade the human body in various ways such as inhalation, ingestion, and injection and may lead to crucial toxicological risks according to the difficulty of assessing the probable toxic effects of such pollutions [3]. The toxicological effects are a major concern as they are highly reactive, cause oxidative stress, and generate free radicals [4].

Metal oxide NPs have been receiving considerable attention for a large variety of applications such as optoelectronics, nanodevices, nanoelectronics, nanosensors, information storage, and catalysis [5]. However, these nanomaterials are potential toxicants and few trials have been conducted to evaluate their ecotoxicity in biological systems [6]. NPs of some metal oxides can pass through physiological barriers resulting in an increase in inflammatory responses, and can cause severe damage in DNA and protein structures, therefore causing mutations [7].

Copper oxide (CuO) NPs, because of their unique physiochemical properties, are being used extensively in catalysis, batteries, gas sensors, heat transfers fluids,

and solar-energy [8], and are being marketed for many applications, including plastic, ceramics, electronics, and propellants [9]. They are also being used in antimicrobial preparations [5], heat-transfer fluent, semiconductors, and intrauterine contraceptive devices [10]. Therefore, there should be a focus on the study of their biological and toxicological effects on human as well as animal organs. However, CuO-NPs were found to be highly toxic compared with other metal oxide NPs under in-vitro conditions [11]. They may cause toxicity once they pass the physiological endurance limits *in vivo* [12].

In addition, CuO-NPs causes severe hepatotoxicity and nephrotoxicity; toxicity is caused by oxidative stress on the liver and kidney tissues [13]. Furthermore, exposures to NPs were found to increase the activities of intracellular enzymes such as transaminases and alkaline phosphatase (ALP), indicating cellular leakage and loss of the functional integrity of cell membranes in the liver [14]. Moreover, CuO-NPs-induced histopathological abnormalities in the liver tissue by triggering reactive oxygen species (ROS) production and DNA damage [4]. Cells exposed to CuO-NPs showed decreased catalase activity (CAT) and glutathione (GSH) reduced enzymes activity while increasing GSH peroxidase activity, thereby implying that CuO-NPs not only generate ROS but also block cellular antioxidant defenses [15].

Nowadays, the treatment of liver injury with synthetic antioxidants can promote tumor formation as well as result in huge costs because of common hepatic treatments. From this point of view, the application and exploration of natural antioxidants have received more attention [16]. This study represents the improving effect of quercetin (que), which is one of the most abundant flavonoids against CuO-NPs-induced liver toxicity in rats.

Que (3,3′,4′,5,7-pentahydroxyflavone) is a plant pigment that is present in large amounts in vegetables and fruits. It has received considerable attention because of its antioxidant activity against the free radicals in the body, which damage cell membranes, tamper with DNA, and induce cell death [17,18]. Que exerts hepatoprotective effects versus the toxicity induced by environmental contaminations [19,20]. It can also stabilize the cells that discharge histamine in the body and thereby have an anti-inflammatory effect [21]. In addition, it acts as a metal chelator [22–24]. Que shows therapeutic potential against several diseases: ischemic heart disorder, liver fibrosis [25], atherosclerosis, renal injury [26], and chronic biliary obstruction and cancer [27].

Accordingly, the aim of the current study is to investigate the curative efficacy of que as an antioxidant in attenuating toxicity induced by CuO-NPs in rat liver as well as further investigate its effective role as a free-radical quencher.

Materials and methods

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).

Synthesis of copper oxide nanoparticles

CuO-NPs (particle size <20 nm) were synthesized by the precipitation technique using copper chloride and sodium hydroxide [28]. 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.

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.

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 a low dose of

CuO-NPs (3 mg/kg) [29]. The other principal group was administered a high dose of CuO-NPs (50 mg/kg) [29] for 7 consecutive days. At the end of the CuO-NPs 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 [30] and the other subgroup was treated with que at a dose of 200 mg/kg [31]. Both standard silymarin and que were administered orally for 30 consecutive days.

Preparation of serum from blood and liver tissue homogenate

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. 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.

Histopathological investigation of liver tissue

Samples of liver tissue of all experimental animal groups were collected and kept in 10% neutral-buffered formalin for 48 h, and then embedded within paraffin. Solid sections of 4 μm thickness were prepared using a rotary microtome. The sections were stained with hematoxylin and eosin and then observed by light microscopy for histopathological changes [32].

Detection of DNA damage by the comet assay

Liver DNA damage was measured using a single-cell gel electrophoresis technique (comet assay) [33]; 0.5 g of crushed samples were transferred to 1 ml ice-cold PBS. This suspension was stirred for 5 min and filtered. The cell suspension (100 μl) was mixed with 600 μl of low-melting agarose (0.8% in PBS). One hundred microliters of this mixture was spread on precoated slides. The coated slides were immersed in lyses buffer [0.045 mol/l Tris/Borate/EDTA (TBE), pH 8.4, containing 2.5% Sodium dodecyl sulphate (SDS)] for 15 min. The slides were placed in an electrophoresis chamber containing the same TBE buffer, but without SDS. The electrophoresis conditions were 2 V/cm for 2 min and 100 mA. Staining with ethidium bromide 20 μg/ml was performed at 4°C. While the samples are still humid; the DNA fragment migration patterns of 100 cells for each dose level were evaluated using a fluorescence microscope [with excitation filter 420–490 nm (issue 510 nm)]. The comets' tails lengths were measured from the middle of the nucleus to the end of the tail with a 40× increase for the count and the size of the comet was measured. For visualization of DNA damage, observations were made of ethidium bromide-strained DNA using a ×40 objective on a fluorescent microscope. Although any image analysis system may be suitable for the quantification of SCGE data, we used Comet 5 image analysis software developed by Kinetic Imaging Ltd (Liverpool, UK) linked to a CCD camera to assess the degree of quantitative and qualitative DNA damage in the cells by measuring the length of DNA migration and the percentage of migrated DNA. Finally, the program calculated the tail moment. Generally, 50–100 randomly selected cells are analyzed per sample.

Biochemical analysis

Determination of liver function enzyme activities

Serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities were determined according to the method described by Reitman and Frankel [34]. ALP was estimated colorimetrically according to the method described by Belfield and Goldberg [35].

Determination of antioxidant biomarkers

Nitric oxide (NO) was determined in the liver homogenate according to the method described by Tousson *et al.* [36]. Initially, nitrate was converted into nitrite by the enzyme nitrate reductase, and then the nitrite released was detected colorimetrically by Griess? reagent. The resulting azo dye has a bright reddish-purple color that can be measured at 540 nm.

Glutathione (GSH) levels depend on the reduction of 5,5′-dithiobis(2-nitrobenzoic acid) with GSH, producing a yellow color whose absorbance is measured at 405 nm according to Beutler *et al.* [37].

CAT reacts with a known quantity of hydrogen peroxide (H_2O_2) and the reaction is stopped after 1 min with the catalase inhibitors. In the presence of peroxidase, the remaining H_2O_2 reacts with 3,5-dichloro-2-hydroxybenzene sulfonic acid and 4-aminophenazone to form a chromophore with a color intensity inversely proportional to the amount of catalase in the sample. The absorbance was measured at 510 nm as described by Aebi [38].

Total antioxidant capacity (TAC) was performed by the reaction of antioxidant in the serum samples with a defined amount of exogenously added H_2O_2. The antioxidants in the samples eliminate a certain amount of hydrogen peroxide added. The residual H_2O_2 is determined colorimetrically by an enzymatic reaction that involves the conversion of 3,5 dichloro-2- hydroxyl benzensulfonate into a colored product. The absorbance was read at 505 nm as described by Koracevic *et al.* [39].

Statistical analysis

The experimental data were analyzed using analysis of variance (ANOVA) combined with the Co-state computer program.

Results and discussion

NPs have the ability to penetrate, translocate, and damage living organisms [40]. This capability results mainly from their small size, which allows them to enter the physiological barriers, and circulate within the circulatory system [41]. However, to date, few studies have directly or indirectly evaluated the toxic effects of these nanomaterials and, presently, there is a lack of clear guidelines to quantify these effects. It is recorded in the scientific literature investigated that liver is one of the target organs of suchlike NPs toxicity after the path to the body through any of the probable ways [42]. The liver is a primary site of detoxification and plays a vital role in metabolism to maintain energy levels and structural balance in the body [43]. It is also an organ where biotransformation occurs and a toxic substance is transformed into a less harmful form to lower toxicity [44].

As shown in Table 1, CuO-NPs-intoxicated groups that received a low dose (3 mg/kg) and a high dose (50 mg/kg) showed a significant increase in AST, ALT, and ALP activities compared to the control group. Low-dose toxicity recorded percentage increase reached up to 16.15, 30.03, and 35.60%, respectively, whereas the high-dose recorded percentage increase reached up to 20.77, 61.42, and 55.05% for AST, ALT, and ALP enzyme activities. The activity of these enzymes is normally used to evaluate the liver function. ALT is an enzyme that helps metabolize protein, whereas AST is the mitochondrial enzyme that plays a role in the metabolism of the amino acid alanine. Under normal circumstances, ALT and AST reside within cells of liver whereas ALP is located in the cell membrane. When the liver is injured, these enzymes enter into the blood stream. The level is increased in cases of liver cell death resulting from shock or drug toxicity [45]. The relevant increase in enzyme activities leads to leakage and loss of functional integrity of cell membranes in the liver [46]. Similar results were reported by Mohammadyari *et al.* [14], who reported a significant increase in liver enzyme activities during an in-vivo toxicity assessment of CuO-NPs in Wistar rats.

Treatment of intoxicated rats with que showed a significant reduction in liver function enzyme activities in comparison with CuO-NPs-induced rats. This

Table 1 Therapeutic effects of quercetin and silymarin on aspartate aminotransferase, alanine aminotransferase, and alkaline phosphatase enzyme activities in copper oxide nanoparticles-induced toxicity in rats

Groups	AST (U/ml)	ALT (U/ml)	ALP (U/l)
Control	160.61±5.90[a]	123.55±11.90[a]	212.40±12.98[a]
LD CuO-NPs	186.55±10.87[b]	160.65±13.80[b]	288.02±13.87[b]
% Change	16.15	30.03	35.60
HD CuO-NPs	193.97±7.98[c]	199.44±12.67[c]	329.32±14.86[c]
% Change	20.77	61.42	55.05
LD CuO-NPs+que	165.82±8.75[a]	125.74±4.89[a]	223.21±9.08[a]
% Change	3.24	1.77	5.09
% Improvement	12.90	28.26	30.51
HD CuO-NPs+que	168.55±9.75[a]	128.13±11.90[a]	224.64±16.81[a]
% Change	5.26	3.71	5.76
% Improvement	15.83	57.72	49.28
LD CuO-NPs+silymarin	161.66±11.21[a]	124.94±10.13[a]	214.63±10.76[a]
% Change	0.65	1.13	1.05
% Improvement	15.50	28.90	34.57
HD CuO-NPs+silymarin	161.80±12.90[a]	128.50±5.75[a]	216.80±11.80[a]
% Change	0.74	4.01	2.07
% Improvement	20.03	57.42	52.98

Data were expressed as mean±SD (n=10). ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; 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 $P \leq 0.05$. Shared means similar. Unshared means different.

reduction indicated that que has antioxidant properties and is known to modulate the activities of various enzymes because of its interaction with different biomolecules. The hepatoprotective potential of que has been reported previously in cadmium-induced hepatotoxicity by Renugadevi and MiltonPrabu [25]. In addition, the preventive role of que against hepatic tissue injury has been documented previously [47].

Table 2 shows that NO levels were significantly elevated in both low and high doses CuO-NPs-intoxicated rats compared with the control group, with percentage changes reaching 5.15 and 8.91%, respectively. The elevation in NO in CuO-NPs-intoxicated rats may have occurred because of the following: NPs can induce oxidant systems, possibly because of the production of ROS [48]. Furthermore, NO can react with ROS or oxygen, yielding reactive nitrogen species, which cause damage of biological molecules such as enzymes, lipids, and DNA by intrastation, oxidation, and nitration [18]. With respect to the percentage of improvement, it was

Table 2 Therapeutic effects of quercetin and silymarin on nitric oxide, glutathione, catalase activity, and total antioxidant capacity copper oxide nanoparticles-induced toxicity in rats

Groups	NO (μmol/l)	GSH (mmol/g tissue)	CAT (U/g tissue)	TAC (mmol/l)
Control	114.92±6.89^{a}	12.26±1.00^{a}	119.83±5.76^{a}	1.53±0.07^{a}
LD CuO-NPs	120.84±5.87^{b}	9.59±0.98^{b}	99.22±4.54^{b}	1.04±0.09^{b}
% Change	5.15	21.78	17.19	32.01
HD CuO-NPs	125.16±4.12^{c}	7.67±0.65^{c}	80.68±4.21^{c}	0.90±0.07^{c}
% Change	8.91	37.44	32.67	41.18
LD CuO-NPs+que	105.59±10.10^{d}	10.16±1.10^{d}	118.49±5.23^{a}	1.54±0.04^{a}
% Change	8.12	17.13	1.12	0.65
% Improvement	17.03	4.95	16.08	32.68
HD CuO-NPs+que	109.25±6.12^{d}	10.49±0.89^{d}	118.66±3.90^{a}	1.50±0.50^{a}
% Change	4.93	14.44	0.98	1.96
% Improvement	13.84	23.00	31.69	39.22
LD CuO-NPs+silymarin	113.25±5.76^{a}	10.35±0.87^{d}	117.13±3.68^{a}	1.50±0.05^{a}
% Change	1.45	15.58	2.25	1.96
% Improvement	6.60	6.19	14.95	30.06
HD CuO-NPs+silymarin	113.89±6.01^{a}	10.29±1.00^{d}	118.21±4.78^{a}	1.49±0.03^{a}
% Change	0.89	16.07	1.35	2.96
% Improvement	9.81	21.37	31.32	38.56

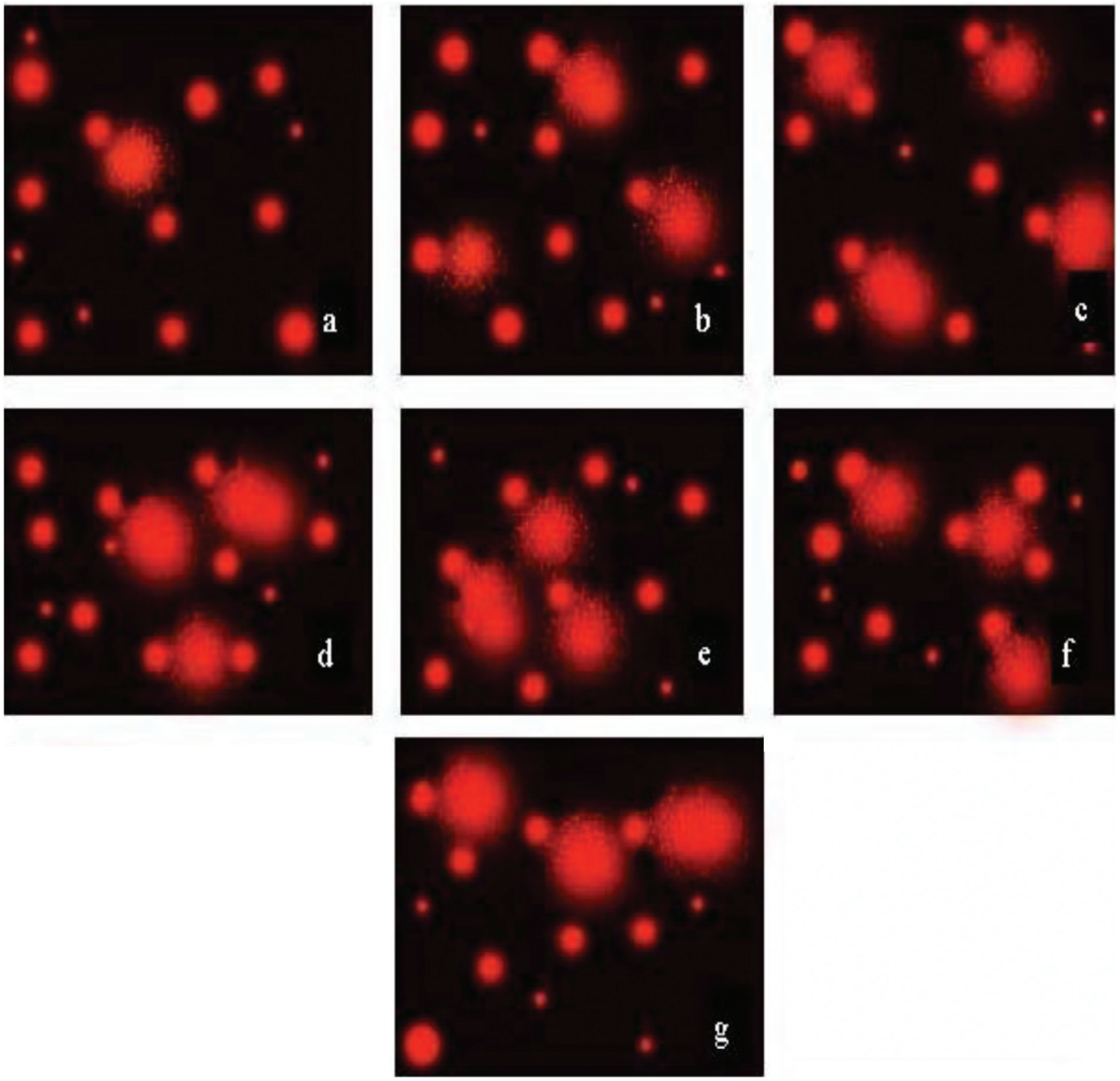
Data were expressed as mean±SD (n=10). CAT, catalase activity; CuO, copper oxide; GSH, glutathione; HD, high dose; LD, low dose; NO, nitric oxide; NP, nanoparticle; TAC, total antioxidant capacity. Shared letters between groups are not significantly different; unshared letters between groups represent the significantly different values at $P \leq 0.05$. Shared means similar. Unshared means different.

Table 3 Therapeutic effects of quercetin and silymarin on DNA degradation indices in copper oxide nanoparticles-intoxicated rat

Groups	Tailed (%)	Untailed (%)	Tail length (μm)	Tail intensity DNA (%)	Tail moment (units)
Control	3.00±0.01^{a}	97.00±0.02^{f}	2.47±0.02^{e}	1.82±0.01^{k}	4.50±0.02^{p}
LD CuO-NPs	8.00±0.02^{b}	92.00±0.01^{g}	3.46±0.01^{a}	3.05±0.01^{l}	10.55±0.03^{q}
% Change	166.76	5.15	40.08	67.58	134.44
HD CuO-NPs	14.00±0.03^{c}	86.00±0.02^{h}	3.82±0.02^{a}	4.78±0.03^{m}	18.26±0.02^{r}
% Change	366.67	11.34	54.66	162.64	305.78
LD CuO-NPs+que	5.00±0.02^{d}	95.00±0.05^{j}	3.11±0.01^{c}	2.91±0.01^{0}	9.05±0.01^{s}
% Change	66.67	2.06	25.91	59.89	101.11
% Improvement	100	3.09	14.17	7.69	33.33
HD CuO-NPs+que	11.00±0.05^{e}	89.00±0.03^{i}	3.31±0.07^{c}	4.16±0.04^{n}	13.77±0.06^{t}
% Change	266.67	8.25	34.01	128.57	206
% Improvement	100	3.09	20.65	34.07	99.78
LD CuO-NPS+silymarin	5.00±0.02^{d}	95.00±0.03^{j}	3.15±0.01^{c}	3.01±0.02^{l}	9.48±0.05^{s}
% Change	66.67	2.06	27.53	65.38	110.67
% Improvement	100	3.09	12.55	2.20	23.78
HD CuO-NPS+silymarin	10.00±0.04^{e}	90.00±0.02^{i}	3.51±0.01^{c}	4.02±0.03^{n}	14.11±0.04^{t}
% Change	233.33	7.22	42.11	120.88	213.56
% Improvement	133.33	4.12	12.55	41.76	92.22

Data were expressed as means±SD (n=10). Tail moment (U)=tail length×DNA (%). 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 $P \leq 0.05$. Shared means similar. Unshared means different.

Figure 1

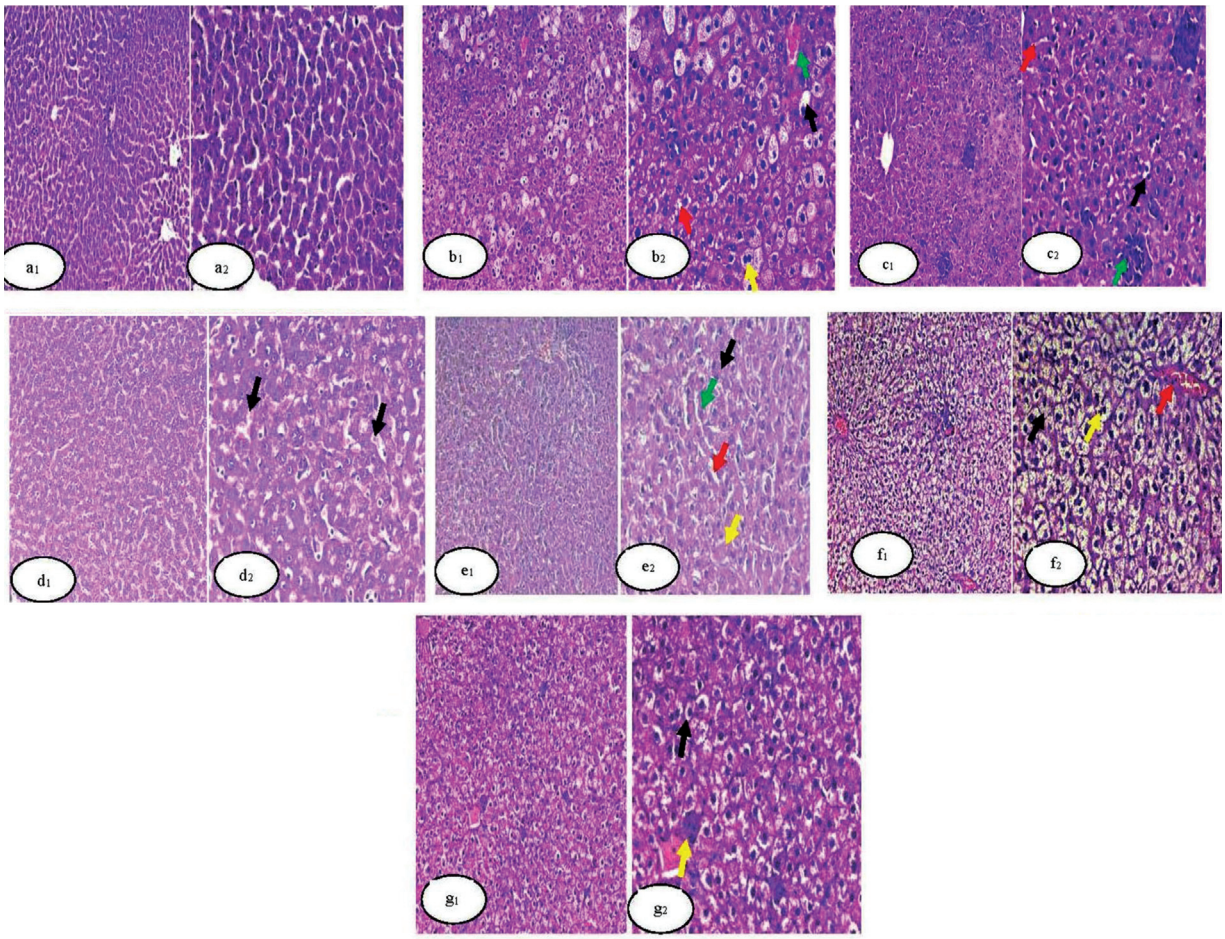


DNA degradation pattern in liver cells (comet technique): (a) control group, (b) low-dose (LD) copper oxide (CuO) nanoparticles (NPs) group, (c) high-dose (HD) CuO-NPs group, (d) LD CuO-NPs+silymarin group, (e) HD CuO-NPs+silymarin, (f) LD CuO-NPs+quercetin (que), and (g) HD CuO-NPs+que

noteworthy that que exerted an ameliorative effect on NO levels. Other studies have reported that que has a potent inhibitory activity against the production of NO and tumor necrosis factor in lipopolysaccharide-stimulated Kupffer cells [49]. Que was also shown to reduce the serum levels of NO in streptozotocin-treated rats [50].

However, CuO-NPs-intoxicated rats showed a significant decrease in GSH level, CAT activity, and TAC compared with normal controls, with a decrease in percentage of 21.78, 17.19, and 32.01% at a low dose, whereas the percentage reached 37.44, 32.67, and 41.18% for GSH, CAT, and TAC at a high dose of CuO-NPs (Table 2). Our results are in agreement with the earlier findings of Sandhu *et al.* [51], who observed a reduction in GSH and CAT, along with increased concentrations of ROS, suggesting that oxidative stress might be the earliest mechanism for toxicity of CuO-NPs in rats following their exposure. Furthermore, CuO-NPs lower the concentrations of CAT activity, which may lead to the aggregation of O_2^-, H_2O_2, or their products of disintegration. Loss of CAT activity results in oxygen intolerance and causes a number of adverse reactions such as protein and DNA oxidation and cell death [52]. These data strongly suggested that CuO-NPs could induce the generation of free radicals directly or indirectly through reduction of antioxidant defense mechanisms depending on the dose that caused lowering the activity of antioxidant systems [53].

Figure 2



Hematoxylin and eosin (H&E)-stained liver sections (×200, ×400). (a_1, a_2) Control group with a normal structure and architecture; (b_1 and b_2) low-dose (LD) copper oxide nanoparticles (CuO-NPs)-intoxicated rats showing preserved lobular hepatic architecture, hepatocyte with ballooning (black arrow), binucleated hepatocytes (yellow arrow), microsteatotic changes (red arrow), and central vein congestion (green arrow); (c_1 and c_2) high-dose (HD) CuO-NPs-intoxicated rats showing preserved lobular hepatic architecture, hepatocyte with ballooning (black arrow), binucleated hepatocytes (yellow arrow), sinusoidal dilatation (red arrow), and lymphocytes aggregates (green arrow); (d_1 and d_2) LD CuO-NPs+quercetin (que) rats showing hepatic tissue with normal architecture, congestion, and dilatation of the sinusoids (black arrow); (e_1 and e_2) HD CuO-NPs +que group showing hepatic tissue with mild hydropic degeneration (black arrow), intracytoplasmic vacuoles (green arrow), congestion, and dilatation of the sinusoids (red arrow) and intralobular lymphocytic collection (yellow arrow); (f_1 and f_2) LD CuO-NPs+silymarin rats showing hepatic tissue with moderate ballooning of the hepatocytes and intracytoplasmic vacuoles (black arrow), congestion, and dilatation of the central vein (red arrow) and binucleated hepatocytes (yellow arrow); (g_1 and g_2) CuO-NPs+silymarin rats showing hepatic tissue with mild hydropic degeneration (black arrow) and intralobular lymphocytic collection (yellow arrow)

As shown in Table 2, animals treated with que showed a significant improvement in the activity of GSH, CAT, and TAC as antioxidant biomarkers compared with CuO-NPs-intoxicated rats. The current results were supported by Shayesteh *et al.* [54], who found that the intake of que significantly attenuated CuO-NPs-induced oxidative stress by an increase in the TAC levels and return to control values. The protective effect of que on oxidative damage was a result of its free-radical scavenging action and antioxidant nature [54].

With respect to tail length in Table 3 and Fig. 1, the two toxicities rat groups, CuO-NPs revealed an increase in DNA damage by 40.08 and 54.66% in low and high doses, respectively. These data are supported by Faddah *et al.* [18], who reported that NPs might damage DNA directly or indirectly by oxidative stress and/or inflammatory responses. A previous study [55] showed that CuO-NPs could induce ROS cytotoxicity and genotoxicity in A549 cells. Upon treatment with que, it was found that the percentage of DNA damage decreased to 25.91 and 34.01%. Also, improvements of 14.17 and 20.65% were observed at both low and high doses, respectively. However, que is a flavonoid known for its its ability to donate hydrogen ions to the free radicals, causing their reduction, thus decreasing the production of hydroxyl radical [56]. Other studies using the comet assay have shown the protective role of que as a scavenger of ROS by decreasing DNA strand breaks and oxidized bases [57].

Liver injury was also confirmed histopathologically by the appearance of several abnormalities in the liver of CuO-NPs-intoxicated groups compared with the control rat group (Fig. 2). CuO-NPs groups showed extensive liver injuries characterized by lobular hepatic architecture, hepatocytes with ballooning and binucleated cellular infiltration, microsteatotic changes, sinusoidal dilatation, and central vein congestion. In the que-treated group, only slight congestion and dilatation of the sinusoids were found. This may be because of the antioxidant activity of que. Our result supported the earlier finding [58], showing that que significantly reduced the histopathological changes in cadmium-treated rats.

Conclusion

Oxidative stress has been shown to play an essential role in the toxicity mechanisms of NPs. The mechanisms have been attributed to their small size and specific surface area, which are generally believed to generate ROS. From the present study, it can be concluded that que treatment leads to therapeutic improvements against CuO-NPs-induced liver toxicity because of its antioxidant, anti-inflammatory activities, capable of inhibiting DNA damage, and is considered a good metal chelator. Hence, que could represent a potentially promising ameliorating agent for liver injury, which may be used as candidate nutraceuticals such as food supplements.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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