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# Silica–Containing Redox Nanoparticles Improve the Antioxidant Activity of Curcumin and its Gastrointestinal Biodistribution Evaluation

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**Abstract:** This study aimed to evaluate the antioxidant potential of designed silica-containing redox nanoparticles (siRNP) to improve curcumin's stability and gastrointestinal (GI) distribution via oral administration. Curcumin-loaded silica-containing redox nanoparticles (CUR@siRNP) were prepared by dialysis, resulting in a particle size of approximately 100 nm. Curcumin was degraded by oxidizing agents including 2,2'-azobis (2-amidinopropane) dihydrochloride) and hydrogen peroxide, while siRNP effectively protected the encapsulated curcumin from oxidative degradation. In addition, CUR@siRNP showed significantly higher antioxidant activities in protein carbonyl assay and lipid peroxidation assay than free curcumin. Furthermore, CUR@siRNP remarkably suppressed the toxicity of curcumin against normal fibroblasts L929 cell line by using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay. The GI distribution *in vivo* showed that CUR@siRNP improved the accumulation of curcumin in the small intestine and colon after oral administration. The results of this study suggested that the CUR@siRNP nanoparticles exhibited great potential carriers for the oral delivery of curcumin in the treatment of GI inflammation.

**Keywords:** curcumin; anti-inflammatory; redox nanoparticles; oxidative degradation; oral delivery; gastrointestinal distribution



## 1. Introduction

Inflammation is the result of a complex series of responses triggered by the immune system. Inflammation can also lead to various physiological and pathological diseases [1]. Inflammatory bowel disease (IBD) is caused by aberrant activation of the immune cells from the gut mucosal boundary, life stressors, and reactive oxygen species (ROS) [2, 3]. Abdominal discomfort, diarrhea, bloody stools, weight loss, and increased neutrophils and macrophages that create cytokines, proteolytic enzymes, and free radicals that lead to inflammation and ulceration are all symptoms of IBD. Nuclear factor kappa-B (NF-κB), which is involved in producing cytokines and chemokines that are important for inflammation, is a key target for many IBD

therapies [4]. Therefore, many natural anti-inflammatory compounds have been used to treat inflammation. However, there are a certain number of side effects associated with the use of drugs when patients receive medication for IBD [5].

Turmeric contains hydrophobic polyphenol curcumin, which is an important yellow phytochemical component [6]. Curcumin is a phytochemical with huge biological properties [7], such as anti-inflammatory, antioxidant, anti-tumor [8], and antimicrobial activities [9], according to preclinical and clinical investigations. Some previous studies reported that the tissue levels of curcumin were very limited in important organs after oral administration of curcumin [10] due to low absorption by the small intestine, substantial liver reductive, and gall bladder excretion [11]. Previous reports have shown that after oral administration, curcumin's systemic bioavailability is low and rapidly removed from the body. Hence the activity of oral curcumin may be limited to the gastrointestinal (GI) tract [12] because of its fast degradation [13]. The curcumin molecule has some limitations when used in therapeutic applications, including limited water solubility at physiological and acidic pH environments, fast hydrolysis in alkaline environments, and light instability [14, 15].

Reactive oxygen species (ROS) have been found to play active roles in various pathways, including oxidative stress, which aggravates chronic inflammation [16]. In addition, the overproduction of ROS in diseased tissue affects the quality of the drug when it enters the body via an oxidation reaction [17]. In general, the most harmful by-product of oxidative metabolism is the hydroxyl radical (OH•), which can cause molecular damage in living systems [18]. Therefore, the production of OH radicals can induce oxidative damage to biomolecules such as proteins, lipids, and DNA [19]. The lipid peroxidation products are highly reactive and have marked biological effects, causing selective changes in cell signaling, protein and DNA damage, and cytotoxicity depending on their concentration. Malonaldehyde (MDA) has attracted a lot of attention as one of the degradation products of lipid hydroperoxides, not only in lipid peroxidation studies of muscle foods but also in connection to tissue damage or oxidative stress [20]. ROS also causes curcumin degradation, reducing its anti-inflammatory and anti-cancer abilities [21]. In several cases, the presence of a reducing antioxidant, such as *N*-acetylcysteine, which neutralized curcumin's effects, proves that ROS has involvement [22].

Nanoparticle delivery is known as a potential therapy to improve the bioavailability and bioactivity of the drug [23]. Therefore, the nanoparticles have been applied to protect the drug from the oxidation of ROS. Recently, several previous studies have reported different nanoparticles conjugating with drugs to treat anti-inflammatory [24] and ROS [25]; among them, polymeric redox nanoparticles (RNP) have shown great potential in the treatment of many ROS related-diseases [26]. Orally administered RNP has been claimed to have the ability to treat diseases such as inflammation in the colon [27], colitis-associated colon cancer [28], and fibrosis associated with nonalcoholic steatohepatitis [29]. RNP exhibits a potent therapeutic effect, high biological compatibility through long-term blood circulation, and low toxicity [30, 31]. However, due to the low drug loading capacity character of RNP, it showed low stability via oral administration. Silica-containing redox nanoparticles (siRNP) were designed and developed to enhance the nanoparticle stability and drug loading capacity via possessing silica moieties in the hydrophobic core. Some of the benefits of encapsulating silica nanoparticles in redox nanoparticles

are the enhancement of drug loading capacity, absorption properties, and stability of nanoparticles in acidic conditions [26, 32].

The goal of attaching silica moiety base to redox nanoparticles is to help increase the ability of nanoparticles to stabilize in acidic conditions when the drug enters the body by oral use [33]. In addition, ROS-scavenging nitroxide radical moiety can inhibit curcumin degradation by an oxidative environment to improve the antioxidant activity of curcumin. Previously, curcumin was encapsulated by siRNP (CUR@siRNP) to improve curcumin bioavailability and anti-inflammatory in colitis mice [16]. This study aimed to investigate CUR@siRNP bioactivities against oxidative stress and its distribution in the GI tract after oral administration.

## 2. Materials and Methods

### *2.1. Synthesis of CUR@siRNP.*

The preparation of amphiphilic redox polymer (PEG-siPMNT) was discussed previously [33], through dialysis of PEG-siPMNT polymer against pure water, siRNP, and CUR@siRNP were synthesized. Briefly, a mixture containing 30 mg poly(ethylene glycol)-*b*-poly[4-(2,2,6,6-tetramethylpiperidine-*N*-oxyl)aminomethylstyrene] (PEG-*b*-PMNT), 0.25 µL tetraethyl orthosilicate (TEOS) (0.936 g/mL), 0.25 µL $NH_3$ and 0.5 mL dimethylformamide (DMF) was fully dissolved with pure curcumin. After being loaded into the dialysis membrane, proceed for 24 hours with the occasional change of water every 4 hours.

### *2.2. Characteristic of CUR@siRNP.*

The nanoparticle size and polydispersity index (PDI) were determined by dynamic light scattering (DLS). siRNP, CUR@siRNP was diluted in water or phosphate buffer saline (PBS) and measured at an angle of 173° using Zetasizer - ZS (Malvern, UK).

The encapsulated efficiency (EE) and loading capacity (LC) was evaluated by evaluating the concentration of curcumin in siRNP by measuring the absorption wavelength of 425 nm. The encapsulated efficiency and loading capacity were calculated by the following formula:

$$\text{EE}\ (\%) = \frac{\text{encapsulated drug (mg)}}{\text{total drug added (mg)}} \times 100$$

$$\text{LC}\ (\%) = \frac{\text{encapsulated drug (mg)}}{\text{total polymer added (mg)}} \times 100$$

The release of CUR@siRNP was investigated in various pH conditions (2.5, 5.5, and 7.4) using the dialysis membrane. CUR@siRNP solution with predetermined concentration was loaded into a dialysis membrane in PBS at pH 2.5, 5.5, and 7.4. At time intervals, the solution was measured at 425 nm to determine the amount of curcumin released.

### *2.3. Oxidative degradation of CUR@siRNP.*

To simulate oxidative degradation, 2′-azobis (2-amidinopropane) dihydrochloride (AAPH) and hydrogen peroxide ($H_2O_2$) were used in this experiment. A solution of curcumin or CUR@siRNP was added to AAPH or $H_2O_2$, and the reaction was maintained for 30 min at 37 °C.

The level of curcumin degradation was determined by measuring the decrease of curcumin absorption intensity at 425 nm using the Varioskan™ multimode microplate reader.

*2.4. Antioxidant activity of CUR@siRNP by Protein carbonyl assay.*

Protein carbonylation is a type of protein oxidation that can be promoted by ROS. The most often used procedure to detect protein carbonyls is after their derivatization with 2,4-dinitrophenylhydrazine (DNPH). When DNPH reacts with protein carbonyls, the amount of protein-hydrazone produced can also be measured spectrophotometrically at 375 nm [34]. Two grams of mincemeat were taken in a 50 mL beaker and 10 mL of PBS (pH 7.4). The extracted sample was collected by sonicating and then centrifuged at 6000 rpm at 4 °C for 15 min. After that, all mixtures containing the above extract samples, with or without Fenton's reagent (1 mM $Fe^{2+}$ + 10 mM $H_2O_2$) at different concentrations of the test sample (CUR or CUR@siRNP) were incubated at 37 °C for 60 min. 500 µL of mixtures were treated with 250 µL of 10 mM DNPH dissolved in 2 M HCl and allowed to stand in the dark at room temperature for 1 h. After 1 h of incubation, aliquots (750 µL) of the solution were precipitated with 250 µL of 20% trichloroacetic acid and centrifuged at 11000 rpm for 5 min. The precipitates were centrifuged after being washed three times with 500 µL of ethanol:ethyl acetate (1:1 v/v) with resuspension. The washed pellets were dissolved in 400 µL of 6 M guanidine hydrochloride. Carbonyl content was determined from absorbance readings at 375 nm against blank.

*2.5. Antioxidant activity of CUR@siRNP by Thiobarbituric acid reactive substances assay.*

Lipid peroxidation is a process in biological systems initiated by an attack of ROS on polyunsaturated fatty acids. Due to its efficiency and simplicity, the Thiobarbituric acid reactive substances (TBARS) assay is one of the most frequently used methods for measuring lipid peroxidation end product MDA quantification in meats and its products. Spectrophotometric measurement of the MDA reacts with 2-thiobarbituric acid (TBA), forming a pink-colored adduct MDA-$TBA_2$ [35], which is measured at 532-535 nm [36]. MDA levels of the samples were measured by the following procedure. Two grams of mincemeat were taken in a 50 mL beaker and 10 mL of PBS (pH 7.4). The extracted sample was collected by sonicating and then centrifuged at 6000 rpm at 4oC for 15 min. After that, all mixtures containing extract samples, with or without Fenton's reagent (1 mM $Fe^{2+}$ + 10 mM $H_2O_2$) and different test sample concentrations (CUR or CUR@siRNP) were incubated at 37 °C for 60 min. 500 µL of mixture samples were mixed with 200 µL of 10% trichloroacetic acid (TCA) and 200 µL of 0.8% thiobarbituric acid (TBA). All the reaction mixtures were heated in a 95 °C water bath for 60 min to develop a pink color. After cooling, 100 µL of butanol:pyridine (1:1) was added and vortexing vigorously, followed by centrifugation at 5000 rpm for 10 min. The absorbance of the supernatant was measured at 532 nm by spectrophotometer against blank. The TBARS value was expressed in MDA concentration (µg/mL).

*2.6. Cytotoxicity evaluation.*

Depending on changes in cellular metabolic activity, the cytotoxicity was assessed, using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT; St. Louis, MO) assay. MTT

assay was performed on a mouse embryonic cell line (L929 from JCRB Cell Bank, Japan) with slight modifications from the previous report [37]. Dulbecco's modified Eagle's medium (DMEM, Sigma-Aldrich, St. Louis, MO) containing 10% fetal bovine serum (Sigma-Aldrich, St. Louis, MO), and 1% antibiotics (penicillin/ streptomycin/neomycin; Invitrogen, Carlsbad, CA) with approximately $10^4$ L929 cells were seeded in a 96-well plate. After 24 h incubation, different concentrations of curcumin and CUR@siRNP were added. After another 24 h of incubation, 50 µL of MTT solution was added to each well. Each well received 100 µL of dimethyl sulfoxide after 4 h of incubation, which was followed by 15 min of incubation. The absorbance of each well was measured at 540 nm.

*2.7. Gastrointestinal distribution study in mice model and data analysis.*

The adult Swiss mice weight 30 g (Institute of Drug Quality Control Ho Chi Minh City) fasted overnight before the experiments. The experimental animal protocol was approved by the School of Biomedical Engineering, International University, VNU-HCM. Two groups of Swiss mice were divided at random (n = 3), including curcumins aqueous suspension in carboxymethylcellulose sodium (CMC) 0.5% and CUR@siRNP solution with the single oral dose of 50 mg/kg.

After the mice sacrifice, the stomach, small intestine, and colon were collected and cleaned with water. The organs were homogenized in PBS pH 7.4, followed by centrifugation at 15,000 rpm at 4 °C for 10 min. The concentration of curcumin in the supernatant was measured by the fluorescence intensity at wavelengths of 425 nm and 548 nm for excitation and emission, respectively.

*2.8. Statistical Analysis.*

All experiments were conducted three times, and the data represent the mean ± SEM. One-way analysis of variance (ANOVA) was used to perform statistical comparisons. The results were analyzed and considered significant differences with *p < 0.05, **p <0.01, ***p< 0.001.

## 3. Results and Discussion

*3.1. Characteristic of CUR@siRNP.*

Pure curcumin remained insoluble in distilled water, while CUR@siRNP appeared in a dark brown transparent solution (Figure 1a), suggesting the solubility of curcumin was improved. Characteristics evaluation determined nanoparticles size and PDI, which were evaluated siRNP before and after encapsulating free curcumin by the DLS measurement. As shown in Figure 1b, the size of siRNP slightly increased from 71.26 ± 0.973 nm (siRNP) to 99.135 ± 2.099 nm (CUR@siRNP), suggesting that curcumin was encapsulated into the core of nanoparticles.

The release of curcumin from CUR@siRNP was evaluated under different pH conditions. As shown in Figure 1c, CUR@ siRNP was slowly released at pH 7.4 while at the pH 5.5 and 2.5 conditions, higher drug release was observed, indicating the pH-sensitive of siRNP. Although silica moieties improved the stability of siRNP, the amino group in the hydrophobic segment was protonated under acidic pH, leading to the release of curcumin.

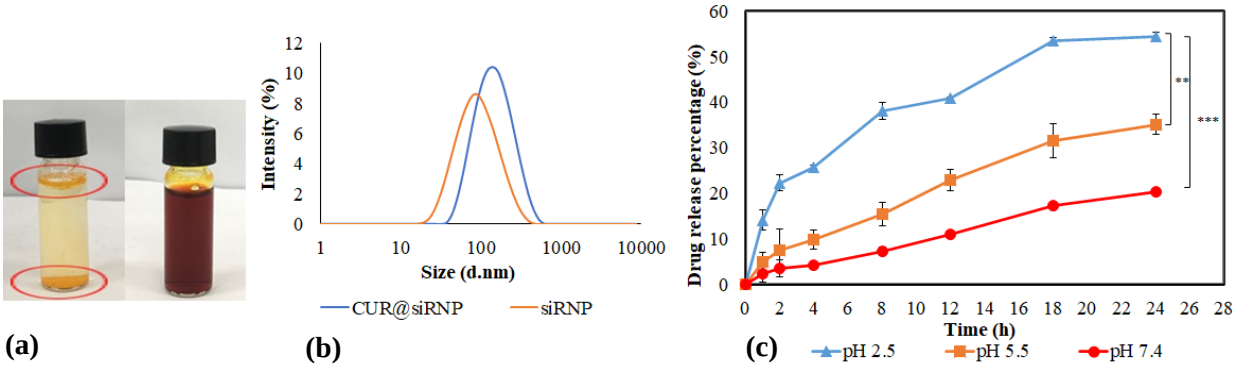


**Figure 1.** Characteristic of CUR@siRNP. (**a**) The solubility of 1 mg/mL Curcumin and CUR@siRNP in distilled water; the red circles indicate the unsoluble curcumin; (**b**) Size distribution of siRNP and CUR@siRNP; **(c)** Drug released profile of CUR@siRNP in different pH conditions.

The validation calibration curves were used to measure the amount of curcumin by absorption at wavelength 425 nm. The EE and LC of CUR@siRNP were 91.93 ± 3.27 % and 9.193 ± 0.327 %, which are higher than in the previous study [16]. In this study, the addition of TEOS increased the silica nanoparticle in the core of siRNP, improving the absorption of curcumin in the core of siRNP.

*3.2. Oxidative degradation of CUR@siRNP.*

As shown in Figure 2a, the degradation effect by AAPH treatment showed that the absorption intensity of curcumin was significantly reduced, while the curcumin in the CUR@siRNP was not degraded compared with free curcumin.

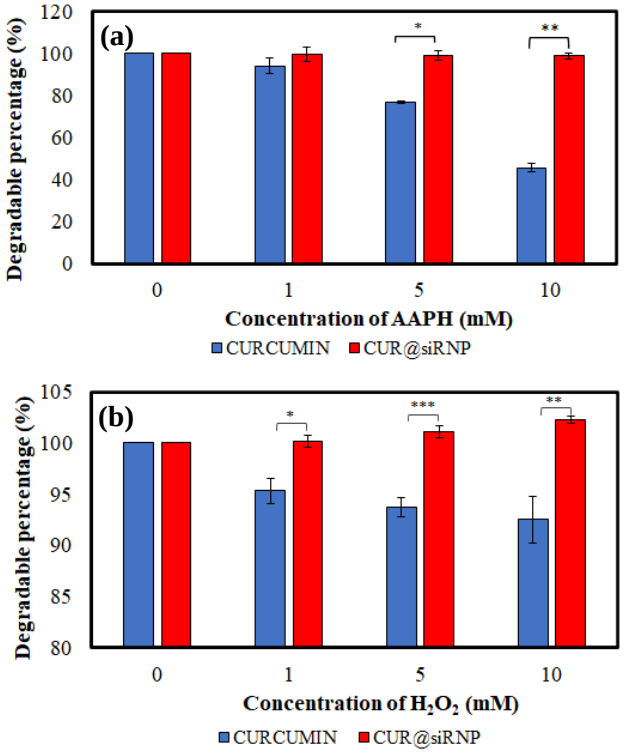


**Figure 2.** Suppression of ROS-induced Curcumin degradation by siRNP. (**a**) siRNP inhibits the degradation of curcumin by AAPH; (**b**) siRNP inhibits the degradation of curcumin by $H_2O_2$.

It demonstrated that the TEMPO moiety with the ROS scavenger function of siRNP reacted with the oxidizing radical, suggesting that a nanoparticle shell and the ROS scavenging activity of siRNP protected curcumin. As shown in Fig. 2b with $H_2O_2$ treatment, the degradation of pure curcumin and CUR@siRNP have tended to be related similarly to the AAPH treatment. This study showed that the ROS moiety in the structure of the nanoparticles effectively protects the therapeutic agents against oxidative stress environment.

*3.3. Antioxidant activity of CUR@siRNP by Protein carbonyl assay.*

Protein oxidation and lipid oxidation assays were performed to investigate the antioxidant capacity of curcumin and CUR@siRNP. The analysis of protein oxidation in CUR and CUR@siRNP-treated samples is shown in Figure 3. The amount of protein carbonyl concentration in CUR and CUR@siRNP-treated samples decreased significantly ($p < 0.05$), with the decrease being more pronounced in CUR@siRNP than CUR. The carbonyl content concentration decreased from 5.39 to 2.81 mg/mL in CUR-treated samples and from 3.66 to 0.90 mg/mL in CUR@siRNP-treated samples, indicating that the siRNP improved the antioxidant activity of curcumin by inhibition in protein oxidation (PO) products.

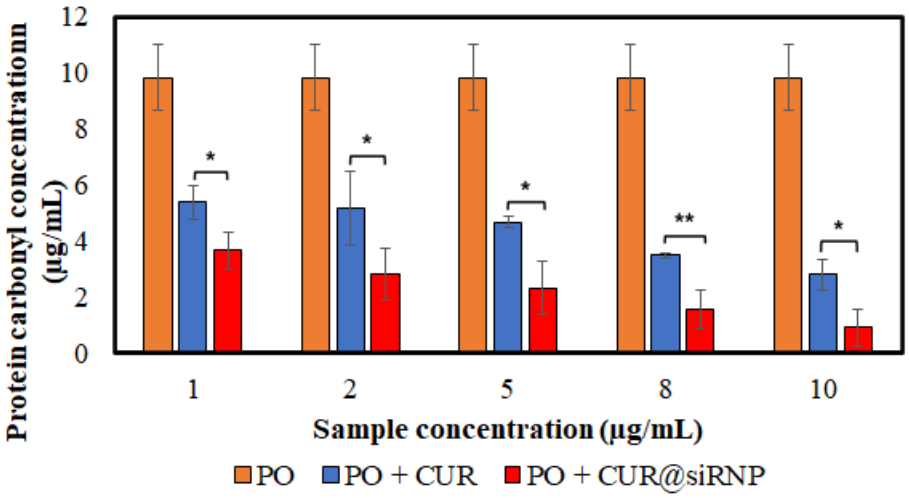


**Figure 3.** Suppression of protein carbonyl by curcumin and CUR@siRNP.

*3.4. Antioxidant activity of CUR@siRNP by Thiobarbituric acid reactive substances assay.*

The measurement of MDA provides a convenient index of lipid peroxidation, a well-established mechanism of oxidative damage caused by ROS [18]. This study shows that hydroxyl radical-induced lipid peroxidation causes an increase in MDA. As expected, samples treated with CUR as well as the CUR@siRNP-treated samples, had decreased MDA values than lipid oxidation samples (LO) (Figure 4). This was probably due to the natural antioxidative effect of CUR. There were significant differences in MDA values among samples added with CUR and CUR@siRNP throughout the procedure. All the CUR@siRNP-treated samples decreased MDA content drastically since siRNP enhances antioxidation processes in CUR, the decrease being significant ($p < 0.05$). Therefore, it demonstrated the promising ability of siRNP in the prevention of MDA formation under lipid peroxidation.

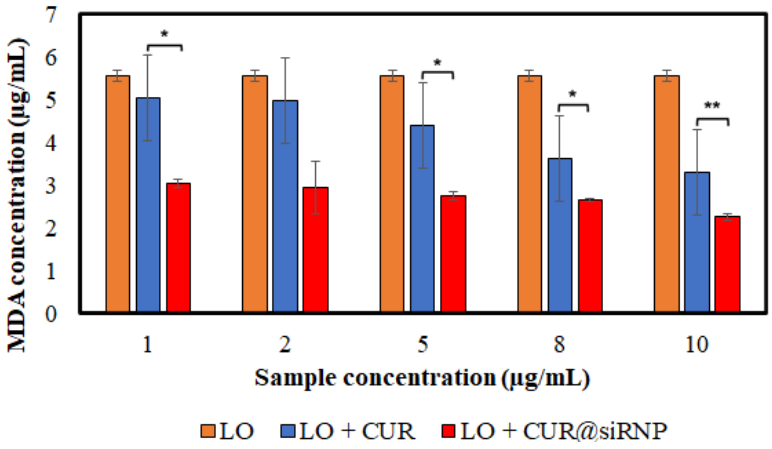


**Figure 4.** Suppression of MDA by curcumin and CUR@siRNP.

*3.5. Drug cytotoxicity.*

To evaluate the cytotoxicity of curcumin and CUR@siRNP, the MTT assay was used on the normal fibroblasts L929 cells. The optical images of the cells after the MTT assay were observed under the microscope (Figure 5a). The optical images showed that the morphology of the cells between CUR and CUR@siRNP significantly changed from 20 - 60 µg/mL concentration of curcumin. At high concentrations (>20 µg/mL), the shrinkage of cells treated with curcumin was observed, indicating cell death, which was not observed in CUR@siRNP-treated cells. As shown in Fig. 5b, CUR@siRNP revealed no noticeable difference in cell viability in comparison at low concentrations to CUR-treated cells. CUR-treated samples were highly toxic for L929 cells at higher concentrations, while CUR@siRNP-treated samples demonstrated less toxicity.

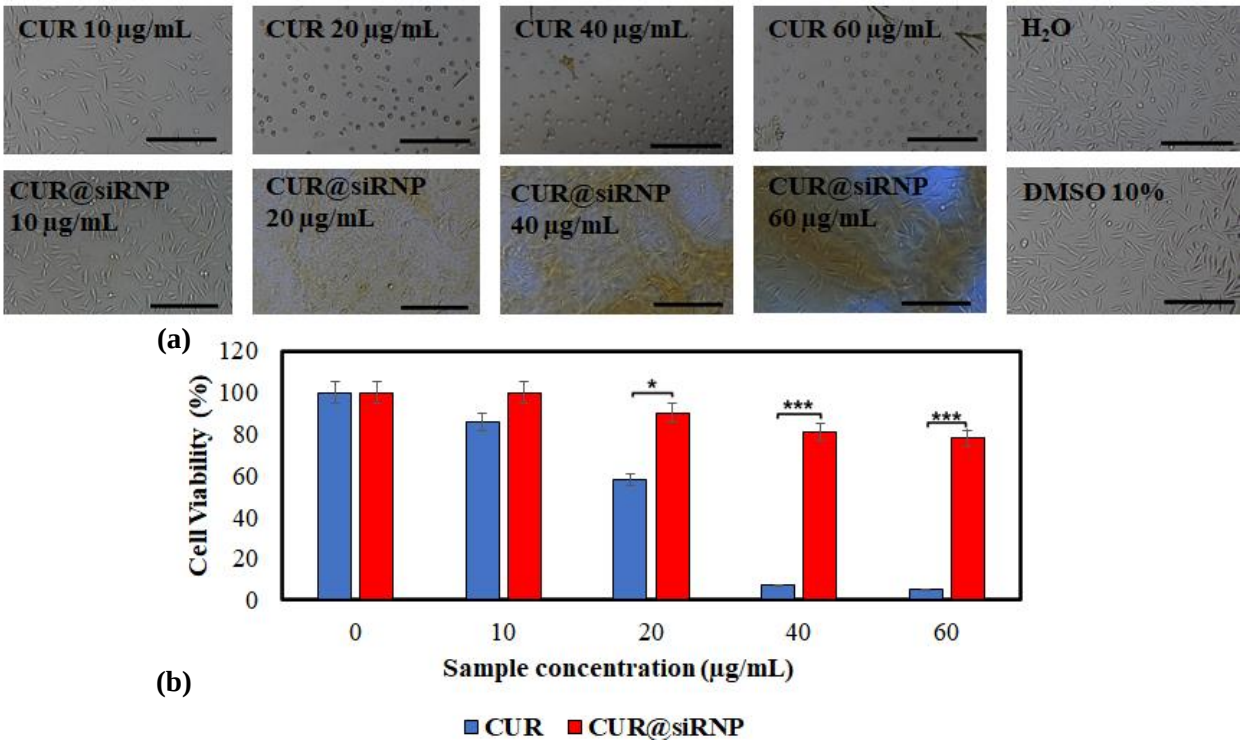


**Figure 5.** Drug cytotoxicity of CUR and CUR@siRNP. **(a)** Morphology of L929 cells cultured with CUR and CUR@siRNP samples. Scale bar = 100 µm; **(b)** Cell viability of L929 was determined by MTT assay.

Additionally, even at high concentrations (60 µg/mL), all CUR@siRNP obtained cell viability of 78% or above, suggesting low toxicity of CUR@siRNP against L929 cells. Free curcumin has been demonstrated that induces a very rapid and significant ROS generation in L929 cells, leading to multiple apoptotic signals [37]. Moreover, antioxidants like siRNP prevent CUR-induced cell death by preventing the production of ROS. A previous study proposed that ROS-scavenging nanoparticles would lower the toxicity of the nanomaterial itself [30]. Hence, an individual siRNP sample needs to be tested to evaluate an accurate L929 cell viability value. While improving the beneficial biological effects of free curcumin, CUR@siRNP was found to have low cytotoxicity, which is extremely valuable and promising as a method of treating inflammation.

*3.6. Gastrointestinal distribution study.*

Since CUR@siRNP was developed for oral administration to treat intestinal inflammation, curcumin distribution in the gastrointestinal tract should be investigated. According to Fig. 6a and 6b, the curcumin accumulation in the stomach at the first 30 min of CUR@siRNP was higher than pure curcumin. After that, the amount of curcumin in the stomach dramatically decreased, suggesting the short retention of siRNP in the stomach and rapid delivery to the intestine. The speed at which the drug went quickly out of the stomach contributed to limiting the degradation of curcumin under the acidic environment of the stomach [38]. As shown in Figures 6c and 6d, the amount of curcumin accumulation in the intestine of mice treated with CUR@siRNP was significantly higher than those treated with curcumin. Nanoparticles could enhance the drug accumulation on the mucus layer on the surface of the small intestine by the PEG tail in the nanoparticle structure [14]. Long retention of nanoparticles in the intestine leads to easier absorption of curcumin into the bloodstream [39]. According to Fig. 6e and 6f, the curcumin levels in the colon were similar in the first two hours when taken in both formulas. After 3 h of administration, the amount of curcumin in the colon of mice treated with CUR@siRNP was higher than mice treated with curcumin, suggesting the longer retention of CUR@siRNP in the colon. The obtained data of GI distribution indicated that CUR@siRNP shows a higher accumulation of curcumin in the intestine and colon, promising for treating intestinal inflammation, including IBD.

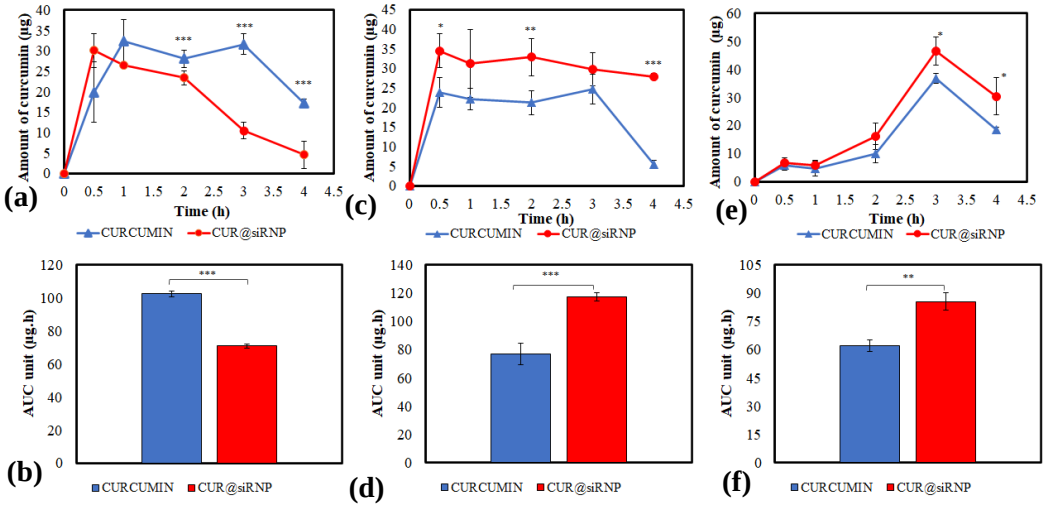


**Figure 6.** Curcumin distribution in GI tract after oral administration. **(a)** Curcumin in the stomach; **(b)** AUC of curcumin in the stomach; **(c)** Curcumin in the small intestine; **(d)** AUC of curcumin in the small intestine; **(e)** AUC of curcumin in the colon; **(f)** AUC of curcumin in the colon.

## 4. Conclusions

In this study, silica-containing redox nanoparticle siRNP as a nanocarrier has been demonstrated to improve the stability of curcumin and loading encapsulation capacity. CUR@siRNP exhibited a better effect in preventing curcumin degradation than free curcumin under oxidative conditions. Furthermore, CUR@siRNP showed significantly high antioxidant activity against protein oxidation and lipid oxidation. Additionally, the cellular toxicity of CUR@siRNP was much lower than free curcumin *in vitro*. CUR@siRNP significantly improved the curcumin distribution in the GI tract after oral administration. Taken together, the combination of curcumin and siRNP is promising for an oral anti-inflammatory drug and has the potential as a new antioxidant nano-formulation for treating IBD.

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## Conflicts of Interest

The authors declare no conflict of interest.

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