The Effect of Ultrafine Process on the Dissolution, Antibacterial Activity, and Cytotoxicity of Coptidis rhizoma

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ABSTRACT

Background: The dosage of herb ultrafine particle (UFP) depended on the increased level of its dissolution, toxicity, and efficacy. Objective: The dissolution, antibacterial activity, and cytotoxicity of Coptidis rhizoma (CR) UFP were compared with those of traditional decoction (TD). Materials and Methods: The dissolution of berberine (BBR) of CR TD and UFP was determined by high-performance liquid chromatography. The antibacterial activity of CR extract was assayed by plate-hole diffusion and broth dilution method; the inhibitory effect of rat serums against bacteria growth was evaluated after orally given CR UFP or TD extract. The cytotoxicity of CR extract was evaluated by 3-(4,5-dimethylthiazol-2-Yl)-2,5-diphenyltetrazolium bromide assay. Results: The dissolution amount of BBR from CR UFP increased 6–8-folds in comparison to TD at 2 min, the accumulative amount of BBR in both UFP and TD group increased in a time-dependent manner. The minimal inhibitory concentrations and minimal bactericidal concentrations of CR UFP extract decreased to 1/2~1/4 of those of TD extract. The inhibitory effect of rat serums against bacteria growth decreased time-dependently, and no statistical difference was observed between two groups at each time point. The 50% cytotoxic concentrations of UFP extract increased 1.66~1.97 fold than those of TD. Conclusions: The antibacterial activity and cytotoxicity of CR UFP extract increased in a dissolution-effect manner in vitro, the increased level of cytotoxicity was lower than that of antibacterial activity, and the inhibitory effect of rat serums containing drugs of UFP group did not improve. Key words: Coptidis rhizoma, Dissolution rate, Minimal inhibitory concentration, Serum pharmacology, Ultrafine

SUMMARY

• Ultrafine grinding process caused a rapid increase of BBR dissolution from CR.
• The antibacterial activity and cytotoxicity of UFP extract in vitro increased in a dissolution-effect manner, but the cytotoxicity increased lower than the antibacterial activity.
• The antibacterial activity of rat serums of UFP group did not improve in comparison to that of TD group.

INTRODUCTION

Coptidis rhizoma (huang lian, CR), the dried rhizome of Coptidis chinensis Franch, Coptidis deltoidea C. Y. Cheng et Hsiao, or Coptidis tetta wall from the family Ranunculaceae, was widely used to treat various diseases by oral intake of the extract, including gastroenteritis, diabetes mellitus, diarrhea, and severe skin diseases. Alkaloids including coptisine, berberine (BBR), palmatine, and jatrorrhizine were the major active components of CR, and BBR is the primary compound. Several pharmacological researches demonstrated that CR extract possessed anti-inflammatory, hypoglycemic, hepatoprotective, anti-Alzheimer, and analgesic. In addition, the previous literature reported that CR had antibacterial activities. In the light of statistics of national variety catalog of proprietary Chinese medicines, CR was used as one of the main ingredients of many Chinese patent medicines. With the demand for CR gradually increased, wild resources have not been able to meet the needs of clinical application; some plantation sites of standardized management for CR have been established, but high cost, labor-consuming, and low income result in decrease of cultivation area, more attention focused on improvement of the bioavailability of CR, and reduced the consumption of CR.

Recently, a new technique has been developed for the production of ultrafine particles (UFPs) (smaller than 70 µm) of medicinal herbs to increase the dissolution, and the UFPs are thought to be more effective than the traditional decoction (TD). Compared with large particles of TD, UFPs improve the therapeutic effects. Currently, there has been considerable interest in the efficacy and toxicity of herb UFP, but studies were just limited to some toxic herbs used in the clinic, and little attention was paid to the commonly consumed herbs.
It was reported that the dissolution rate of BBR in CR UFP significantly increased in comparison to the large particles,[16,17] rats were orally administered of CR UFP extract, and the adsorption of BBR significantly elevated.[18] However, with the increase of dissolution rate, what changes have exactly occurred about the efficacy and toxicity of CR UFP and whether the increase of bioavailability of CR UFP is sufficient to cause its efficacy improved are unknown so far.

The aim of this study was to compare the dissolution, antibacterial activity, and cytotoxicity of CR UFP with those of TD in vitro, evaluate the antibacterial activity of rat serum after orally given a single dose of CR UFP or TD extract, and provide the reference data for further application of CR UFP in the clinic.

MATERIALS AND METHODS

Plant materials and chemicals

The CR in the study was obtained from the plantation sites of good agricultural practices (Chongqing, China), authenticated by Prof. Kang Si-He from Hubei Institute for Food and Drug Control, Wuhan, China. The TD and UFP samples of CR were processed from the same pitch by Zhongda Pharmaceutical Co., Ltd., (Hubei, China). Chemical standard of BBR was purchased from National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China).

High-performance liquid chromatography

The content of BBR in the CR extract was assayed by high-performance liquid chromatography (HPLC) (Agilent-1,100, Agilent Technologies, Santa Clara, CA, USA) according to China Pharmacopoeia (2010 edition).[19] In brief, the reference substance was accurately weighed and dissolved in methanol to obtain standard solution (80 µg/mL). Standard solution or test solution (5 µL) was injected into the HPLC system for analysis. The separation was achieved through cosmol C18, column (4.6 × 250 mm; 5 µm, Nacalai Tesque Inc., Japan) at a column temperature of 30°C, the elution was monitored spectrophotometrically at 345 nm. The mobile phase contained 40% (V/V) of acetonitrile and 60% (V/V) of 0.05 M potassium dihydrogen phosphate (containing 0.17% sodium dodecyl sulfate); the flow rate was 1.0 mL/min. All test solutions were filtered through a 0.45 µm Millipore filter (Merck Millipore, MA, USA) before being analyzed by HPLC. The content of BBR in the extracts was calculated in accordance with the peak area of the reference substance.

Determination of the dissolution rate

To compare the dissolution rate of CR UFP with that of TD, 2.0 g TD or UFPs was soaked in 500 mL distilled water at 37°C. At 2, 4, 6, 10, 15, 20, 30, 45, and 60 min postincubation, 2 mL solution was accurately obtained, and distilled water was added to the scale. The quantities of BBR were assayed by HPLC as described above; the dissolution rate was expressed as a percentage of the total content of BBR in the UFP or TD.

Preparation of Coptidis rhizoma extracts

Water extract of CR TD was prepared by sustained boiling according to the traditional recipe. Briefly, 50 g of dried material was boiled 0.5 h in 500 mL distilled water at room temperature, the first extraction was collected and the remaining herb material boiled again as described above; two portions were combined and filtered using Whatman No. 1 filter paper, the resulting solution was concentrated to 100 mL; the final concentration of the extract was 0.5 g/mL. The UFP of CR (50 g) was added to 500 mL 90°C distilled water, incubated in a 90°C water bath for 10 min, the solution was centrifuged at 2,000 rpm 30 min, 500 mL distilled water was added to the residue, incubated and centrifuged again, the supernatant was combined, the resulting solution was concentrated to 100 mL, and the final concentration of the solution was 0.5 g/mL.

The content of BBR in the extract of CR UFP and TD was 30.042 and 12.308 mg/g, respectively. Aliquots of both extracts were autoclaved for 20 min at 115°C to sterilize the sample, dispensed, and stored at 4°C for further use.

Antimicrobial assay

Bacterial strains and culture media

Staphylococcus aureus (ATCC25923), Escherichia coli (ATCC25922), Salmonella typhi (ATCC25925), methicillin-resistant S. aureus (MRSA, ATCC33592), and Pseudomonas aeruginosa (ATCC27853) were obtained from China Center for Type Culture Collection (Wuhan, China), β-hemolytic Streptococcus (CMCC32210) was kindly provided by Professor Zhang Chun-Ying (Hubei Institute for Food and Drug Control, Wuhan, China). β-hemolytic Streptococcus was cultivated in brain heart infusion (BHI) agar and broth (Haibo Co., Ltd., Qingdao); other microorganisms were cultivated in nutrition agar and broth (NA and NB, Tianhe Microbial Agents Co., Hangzhou, China).

Growth inhibition by plate-hole diffusion assay

Plate-hole diffusion assay was used to evaluate the antibacterial activity of CR extracts.[20] Logarithmic phase bacteria culture (100 µL) (to a final concentration of approximately 1.5 × 10^7 colony forming units/mL [CFUs/mL]) was inoculated to Mueller-Hinton agar or BHI agar. After dried, wells of 8 mm diameter were made in the agar using a sterile cork borer, and 100 µL of each fraction (0.5 mg/mL) was added to each well, with culture medium as the negative control, penicillin and cefazidime (Sigma) as the positive control. After overnight incubation at 37°C, the diameters of inhibition zones were measured. They were determined in duplicate.

Determination of minimal inhibitory concentrations and minimal bactericidal concentrations

Minimal inhibitory concentrations (MICs) and minimal bactericidal concentrations (MBCs) were determined by a 2-fold dilution method as recommended by the Clinical and Laboratory Standards Institute.[21] The MIC was defined as the lowest concentration that yielded no visible growth. The test medium was Mueller-Hinton broth (MHB, Tianhe Microbial Agents Co., Hangzhou, China) or BHI broth, and the density of bacteria was 5 × 10^5 CFUs/mL. Cell suspensions (1 mL) were inoculated into sterile test tubes in the presence of each fraction with different final concentrations (0, 0.12, 0.24, 0.49, 0.98, 1.95, 3.91, 7.82, 15.63, 31.25, 62.50, 125, and 250 mg/mL), penicillin and cefazidime were used as positive control. The inoculated test tubes were incubated at 37°C for 24 h before being read. The MBCs were determined by subculturing the media from each tube showing no visible growth onto Mueller-Hinton agar or BHI agar plates, the plates were incubated at 37°C for 24 h and the MBC was defined as the lowest concentration that produced subcultures growing no more than five colonies on each plate.

Antibacterial activity of rat sera containing drug Animals

Male Wistar rats (180–220 g) were purchased from Hubei Research Center of Experimental Animals (Wuhan, China). Room temperature was maintained at 22 ± 1°C with 67% humidity at 12 h dark/light cycle. Food and water access were allowed ad libitum. They were kept for 1-week prior to use in experiments. All work was carried out in accordance with the international ethical guidelines.
**Experimental design**

The rats were fasted, but free access to drink water 12 h before the test, randomly divided into two groups; TD and UFP group (n = 9). Under anesthesia with 10% chloral hydrate solution, blank blood was collected from jugular vein of all animals at zero time (0 h) to serve as control. TD group rats were orally administered of RC TD extract at a dose of 2 g/kg containing 24.62 mg BBR/kg according to body weight while UFP group rats were orally administered of UFP extract at a dose of 2 g/kg containing 60.08 mg BBR/kg (W/W). Blood samples were collected from the jugular vein at 0.5, 1.0, and 1.5 h postadministration, and immediately centrifuged at 3,000 rpm for 15 min. The serums were obtained and stored at 4°C until analysis.

**Determination of antibacterial activity of rat serums**

The antibacterial activity of rat serums was evaluated by micro-dilution with some modifications. Briefly, each well of flat-bottomed polystyrene 96-well plates was loaded with 100 µL of the S. aureus logarithmic phase culture (containing 1.5 × 10^5 CFUs/mL), and 100 µL of rat serum collected at different time was added to the wells in triplicate. The controls on each plate were the bacterial inoculums without serum used for determining the bacteria growth and medium for sterility control, the plates were incubated at 37°C for 24 h, 10 µL of serial 10-fold dilution of the subculture from each well was plated on Mueller-Hinton agar plates in triplicate, after overnight incubation at 37°C, the plates containing 30–300 CFUs were selected for counting. The number of viable cells in the subculture was determined by the mean of CFUs, multiplied by the dilution factor. The results were expressed as an inhibition rate of bacteria inoculums control, and calculated as the following formulae.

\[
\text{Inhibition rate (\%)} = \frac{\text{Lg Mean CFUs of bacteria inoculum control} - \text{Lg Mean CFUs of rat serums}}{\text{Lg Mean CFUs of bacteria inoculum control}} \times 100\%
\]

**Cytotoxicity**

**Cell culture**

HepG2, MCF-7, and HL-7,702 cells were obtained from Chinese Typical Culture Collection (Wuhan, China). The cell lines were cultured at 37°C in a complete Dulbecco’s modified Eagle’s medium (DMEM) containing 10% fetal calf serum, penicillin G, streptomycin, nonessential amino acids, L-glutamine.

**3-(4,5-dimethylthiazol-2-Yl)-2,5-diphenyltetrazolium bromide assay**

To determine the 50% cytotoxic concentration (CC<sub>50</sub>) of UFP or TD pieces of CR extract, monolayer of confluent HepG2, MCF-7, and HL-7,702 cells were exposed to various concentrations of CR extract in 2% DMEM, and the dilution medium was used as the control. After 72 h of incubation, cell viability was examined by the 3-(4,5-dimethylthiazol-2-Yl)-2,5-diphenyltetrazolium bromide (MTT) assay. The CC<sub>50</sub> was the concentration (mg/mL) required to reduce cell viability by 50%. Assays were performed in triplicate on three independent experiments.

**Statistic analysis**

The results were expressed as mean ± standard deviation, and statistical comparisons were made by means of a one-way ANOVA test, P < 0.05 was
RESULTS

The dissolution rate of Coptidis rhizoma ultrafine particle

The content of BBR in the aqueous solution was assayed by HPLC, and the dissolution time curve of BBR from 2 to 60 min is shown in Figure 1. Ultrafine grinding process caused a rapid increase of BBR dissolution at 2 min, the dissolution percentage of BBR of UFP was 42.18%, while that of TD was 6.16%, and the dissolution amount of BBR in CR UFP increased more than 6-fold in comparison to TD. The dissolution amount of BBR from both UFP and TD increased in a time-dependent manner, the slopes of both dissolution curves for BBR were similar, and the cumulative dissolution amount of BBR from UFP was higher than that from TD at each time point. At 60 min, the dissolution percentage of BBR from CR UFP was 50.76%, whereas that from TD was 16.33%; compared with TD, the accumulative dissolution amount of BBR from CR UFP increased 3-fold at 60 min.

The antibacterial activity of Coptidis rhizoma ultrafine particle

To compare the antibacterial activity of CR UFP with that of TD, penicillin (1 mg/mL) and ceftazidime (1 mg/mL) was used as positive control. As the results shown in Figure 2, compared with TD extract, stronger antibacterial activity of UFP extract against all test bacteria strains was observed \((P < 0.05\) vs. TD), the inhibition zones of UFP against \(S.\) aureus, MRSA, \(\beta\)-hemolytic Streptococcus were 29.8, 28.8, and 27.3 mm, while that of TD against \(S.\) aureus, MRSA, and \(\beta\)-hemolytic Streptococcus were 27.7, 26.2, and 25 mm; the inhibition zones of penicillin against Gram-positive bacteria were more than 31 mm. The inhibition zones of UFP against \(S.\) typhi, \(P.\) aeruginosa, \(E.\) coli ranged from 12.5 to 14.5 mm \((P < 0.05,\) vs. TD), whereas those of TD ranged from 7.0 to 9.3 mm; ceftazidime exerted a significant inhibition effect against the Gram-negative bacillus, the inhibition zones of ceftazidime against them were above 27.5 mm.

As the results shown in Table 1, the MICs of CR UFP against Gram-positive bacteria strains decreased to 1/2~1/4 of that of TD, the MICs of TD against \(S.\) typhi, \(P.\) aeruginosa, and \(E.\) coli were in the range of 62.5–250 mg/mL, whereas the MICs of UFP against these bacteria strains were in the range of 15.63–62.50 mg/mL; the MICs of ceftazidime against Gram-negative bacteria ranged from 0.19 to 3.13 μg/mL.

| Bacterium strain | UFP (μg/mL) | TD (μg/mL) | U/T | Ceftazidime (μg/mL) | Penicillin (μg/mL) |
|------------------|-------------|------------|-----|--------------------|-------------------|
| \(S.\) aureus    | 0.98        | 1.95       | 1:2 | -                  | 0.12              |
| MRSA             | 1.95        | 7.82       | 1:4 | -                  | 3.91              |
| \(\beta\)-hemolytic Streptococcus | 1.95 | 7.82 | 1:4 | - | 0.12 |
| \(Escherichia coli\) | 15.63 | 62.50 | 1:4 | 0.78 | - |
| \(Salmonella typhi\) | 15.63 | 62.50 | 1:4 | 0.19 | - |
| \(Pseudomonas aeruginosa\) | 62.50 | 250.00 | 1:4 | 3.13 | - |

UFP: Ultrafine particle; TD: Traditional decoction; MICs: Minimal inhibitory concentrations; CR: Coptidis rhizoma; MRSA: Methicillin-resistant S. aureus; \(S.\) aureus: \(Staphylococcus\) aureus

Table 2: The MBCs of CR UFP extract in comparison to those of TD (mg/mL)

| Bacterium strain | UFP | TD | U/T | Ceftazidime | Penicillin |
|------------------|-----|----|-----|-------------|------------|
| \(Staphylococcus aureus\) | 3.91 | 15.63 | 1:4 | - | 0.49 |
| MRSA             | 31.25 | 31.25 | 1:1 | - | 15.63 |
| \(\beta\)-hemolytic Streptococcus | 31.25 | ≥250.00 | ≤1:8 | - | 0.98 |
| \(Escherichia coli\) | 31.25 | 125.00 | 1:4 | 12.5 | - |
| \(Salmonella typhi\) | 62.50 | ≥250.00 | ≤1:4 | ≥50 | - |
| \(Pseudomonas aeruginosa\) | 250.00 | ≥250.00 | ≤1:4 | ≥50 | - |

UFP: Ultrafine particle; TD: Traditional decoction; MBCs: Minimal bactericidal concentrations; CR: Coptidis rhizoma; MRSA: Methicillin-resistant \(S.\) aureus; \(S.\) aureus: \(Staphylococcus\) aureus

Figure 2: The antibacterial activity of aqueous solution of Coptidis rhizoma herb ultrafine particle in comparison to that of traditional decoction

Figure 3: The inhibitory effect of rat serums containing drugs against bacteria growth
whereas the MBCs of TD against these bacteria strains were ≥15.63 mg/mL. The MBCs of penicillin against Gram-positive bacteria ranged from 0.49 to 15.63 μg/mL. The MBCs of CR UFP against *S. typhi*, *P. aeruginosa*, and *E. coli* ranged from 31.25 to 250 mg/mL, whereas the MBCs of TD against these bacteria strains were ≥125 mg/mL; the ratios of MBCs between TD and UFP against most test bacteria were higher than 1:1. The MBCs of cefazidime against Gram-negative bacteria were higher than 12.50 μg/mL.

The antibacterial activity of rat serums containing drug

The inhibitory effect of rat serums against bacteria growth was determined as described in materials and methods. As the results shown in Figure 3, the inhibitory effect of rat serums containing CR metabolites decreased in a time-dependent manner. At 0.5, 1.0, and 1.5 h, the inhibition rates of rat serums of UFP group were 50.32, 39.21, and 28.78% (*P* < 0.05 vs. 0.5 h), whereas those of TD group were 46.53, 42.18 (*P* < 0.05 vs. 0.5 h), and 30.42% (*P* < 0.05 vs. 0.5 h), all rat serums containing CR metabolites showed weaker inhibition than the blank rat serums (*P* < 0.05 vs. blank serum control), the inhibition rates of blank serum of UFP and TD group were 62.87, and 58.29%, respectively. The antibacterial activity of rat serums showed no significant difference between two groups at each time point.

The cytotoxicity of *Coptidis rhizoma* ultrafine particle

The results assayed by MTT as shown in Table 3, compared with CR TD, the cytotoxicity of UFP extract increased 1.66–1.97-fold, the CC₅₀ of UFP extract for HepG-2, MCF-7, and HL-7702 cells ranged from 220.07 to 672.85 μg/mL, whereas that of TD ranged from 434.27 to 1197.15 μg/mL.

### DISCUSSION

Previous studies mainly focused on the dissolution rate of active ingredients, pharmaceutical properties, and the bioavailability of CR UFP.[16,17] CR consisted of a variety of ingredients, the alkaloids of CR were always thought to be poorly absorbed,[8] even with the dissolution and adsorption of CR UFP elevated, whether the efficacy and toxicity of CR UFP increased or not is still unknown. In this study, the dissolution rate, antibacterial activity, and cytotoxicity of CR UFP was compared with those of TD *in vitro*; rat serums were collected at 0.5, 1, and 1.5 h postoral administration of CR UFP or TD extract, the inhibitory effect of rat serums against bacteria growth was determined.

Firstly, the TD and UFP samples were processed from the same pitch of CR to guarantee the reliability of experiment results, and our results demonstrated that ultrafine grinding process accelerated the dissolution of BBR at convenient way. It has been reported that the BBR concentration in the serums increased in a time-dependent manner from 0.5 to 2 h after rats were orally administered of CR extract.[18,27] so rat serums were collected at 0.5, 1.0, and 1.5 h postadministration in the present study. Interestingly, our results indicated that rat blank serums exhibited strong antibacterial effect against *S. aureus*. Therefore, conventional methods such as plate-hole diffusion or micro-dilution assay cannot be suitable to evaluate the antibacterial activity of rat serums in this study. In order to evaluate the inhibitory effect of rat serums against bacteria growth, the log mean of CFUs was calculated in our study, and the results were expressed as an inhibition rate of bacteria inoculums control.

It has been reported that the BBR concentration in the serums increased in a time-dependent manner from 0.5 to 2 h after rats were orally administered of CR extract.[18,27] so rat serums were collected at 0.5, 1.0, and 1.5 h postadministration in the present study. Interestingly, our results indicated that rat blank serums exhibited strong antibacterial activity against *S. aureus*; Merchant et al. obtained a similar result that alligator (*Alligator mississippiensis*) and human serums can inhibit the growth of *E. coli*,[28] all these suggesting that an active serum complement system might directly inhibit bacteria growth. Compared with blank serum control, the inhibitory effect of rat serums containing drug against bacteria growth decreased in a time-dependent manner [Figure 3], but no significant difference was observed between two groups, suggesting that the inhibitory effect of rat serums of UFP group did not significantly enhance, serum concentrations of CR active components in both UFP and TD group might be similar. We deduced that the serum concentration of CR active components and its metabolites was very low; the inhibition effect against bacteria growth was solely due to the role of rat serums rather than CR active components. Thus, with the increase of serum concentrations of CR active components, serum proteins with antibacterial activity were gradually bound with them, and resulting in the inhibition activity of blank serums decreased in a time-dependent manner.

### Table 3: The cytotoxicity of CR UFP extract in comparison to that of TD

| Cell strain | UFP (μg/mL)* | TD (μg/mL) | T/U |
|-------------|--------------|------------|-----|
| HepG-2      | 672.85±16.15 | 1197.15±37.20 | 1.78:1 |
| MCF-7       | 220.07±12.11 | 434.27±33.06 | 1.97:1 |
| HL-7702     | 459.50±9.64  | 763.61±23.38 | 1.66:1 |

*Mean±SD (n=3). UFP: Ultrafine particle; TD: Traditional decoction; CR: *Coptidis rhizoma*; CC₅₀: 50% cytotoxic concentration; SD: Standard deviation.
The results of Kheir et al. suggested that there might have a limit in oral administration of BBR for mice, when the dosage exceeded this point, the blood concentration of BBR would not continue to increase.\[29\] We considered there probably had a similar limit of dosage for rats, the BBR content of CR UFP extract given to rats is 2.44-fold higher than that of TD extract, but the antibacterial activity of rat sera from two groups were similar at each time point, we deduced that the concentration of alkaloids in both CR UFP and TD extract perhaps exceeded the limit of absorption, so serum concentrations of the active components in two groups showed a similar increase. Previous investigation indicated that the Tmax was 1.33 h after rats were orally administered of CR extract,\[14\] suggesting that although serum concentrations of CR active components in both groups would reach the peak from 1 to 1.5 h, they were still below the minimum effective concentration. Our results indicated that the inhibition rate of rat sera collected at 1.0 h showed statistical difference with that of 0.5 h for UFP group ($P<0.05$), while no statistical difference was observed for TD group, suggesting that serum concentration of CR active components of UFP group reached the threshold earlier than TD group, this is consistent with the results of pharmacokinetic.\[16\]

The $CC_{50}$ values of both extracts for MCF-7 cells are the smallest, followed by HL-7,702 cells, and those of HepG-2 cells are the largest, suggested that the cytotoxicity of CR extract was different for different cell lines. The ultrafine grinding process increased the cytotoxicity of CR, but the ratios of $CC_{50}$ values between TD and UFP were less than 2-fold, and lower than those of dissolution amount and antibacterial activity, suggested that the antagonistic effects among variety components caused toxicity-reduced effect of CR UFP extract. The currently recommended dose of CR TD proposed in the Chinese Pharmacopoeia is 2–5 g;\[19\] in the light of our results of antibacterial activity, cytotoxicity, and dissolution rate, we propose 2.5 g is the highest curative dose for CR UFP, which is also relatively safe.

**CONCLUSIONS**

The ultrafine grinding process had no effect on the quality of CR. Ultrafine grinding process caused a rapid increase of BBR dissolution; the antibacterial activity and cytotoxicity of UFP extract in vitro increased in a dissolution-effect manner, but the toxicity increased lower than the antibacterial activity; we first reported that after given a single dose of CR extract, rat serum containing drug showed weak inhibitory effect against bacteria growth, even weaker than the blank serum control, the antibacterial activity of rat sera of UFP group did not improve. Further investigations needed to study the toxicity of CR UFP in vivo to provide the basis for the safe application of CR UFP.

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**Conflicts of interest**

There are no conflicts of interest.

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