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Original article

Effect of superfine pulverization on physicochemical and medicinal properties of Qili Powder

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ABSTRACT

Qili Powder, a preparation from Traditional Chinese Medicine, commonly used to treat injuries from falling or stumbling, pain caused by bruising, and traumatic hemorrhage. The aim of the present work was to investigate the application of the superfine pulverization on Qili Powder properties. The physicochemical and medicinal properties of fine Qili Powder with D_{90} particle size of 164.5 μm, and superfine Qili Powder with D_{90} particle size of 32.2 μm were investigated. The results showed that with decreasing particle size, the specific surface area and pore volume increased, the fluidity decreased, and the percentage of moisture absorption and the balanced moisture content decreased. Analysis HPLC, XRD and FTIR results indicated that superfine pulverization didn't influence dracorhodin content, nor the molecular structure and crystal form of Qili Powder. The percentage of dissolution of dracorhodin was significantly improved after superfine pulverization. Pharmacokinetics results confirmed that superfine pulverization increases the absorption rate in rats and dracorhodin content of Qili Powder.

Introduction

Traditional Chinese medicine (TCM) has more than 2000 years of history in the prevention and treatment of diseases in China (Li et al., 2008), and it plays an indispensable role in the Chinese healthcare system. In TCM, several different herbs are mixed into a preparation due to their own properties and to be therapeutically effective as a whole. This type of compatible application of herbs in TCM exhibits holistic effectiveness by exerting activities to multiple target organs (organ systems) on the basis of the principles of traditional oriental medicine (Ma et al., 2009). The pharmacological effects of TCM do not only depend on the special chemical components but also the physical states of drugs. In TCM, many medicinal materials are orally administered directly after grounding into a powder. The powders' particle size affects their pharmaceutical behavior, such as solubility; yield of chemical constituents, chemical stability, bioavailability and among others. It was

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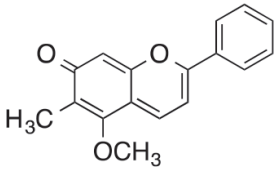
found that the water holding capacity and polysaccharide solubility significantly improved with the decrease in size of *Astragalus membranaceus* particles ($p < 0.05$) (Zhao et al., 2010). It was reported that after superfine pulverization, the plant cell walls of Bai Zhi are broken into pieces and the extraction yield of imperatorin increased by 11.93% compared with the normal particles (Yang et al., 2011). Su (Su et al., 2009) studied the effect of ultrafine grinding on dissolving-out quantity of tanshinone IIA from *Radix Salvia miltiorrhiza*, and the results indicated that the molecular structure of the active ingredients did not change, the dissolving-out quantity of tanshinone IIA increased greatly from 12.77 μg/g to 54.55 μg/g. It was found that ultrafine grinding can obviously increase the absorption rate and degree of absorption of Liuwei Dihuang in rats (Ma et al., 2009). Previous investigations from our group showed that loose intercellular junction without rupture of the wall could increase the dissolution of hydroxysafflor yellow A in *Carthamus tinctorius* L. powders of different sizes (Liao et al., 2010).

Qili Powder (Chinese Pharmacopoeia Commission, 2010), is a TCM preparation, composed of *Sanguis draconis* (*Daemonorops draco* (Willd.) Blume, Arecaceae), olibanum (*Boswellia carteri* Birdw., Burseraceae), myrrh (*Commiphora myrrha* (T. Nees) Engl., Burseraceae), *Flos Carthami* (*Carthamus tinctorius* L., Asteraceae), catechu (*Acacia catechu* (L. f.) Willd., Fabaceae) *Borneolum syntheticum*, musk (Musk berezovskii Flerov) and cinnabar (Red mercuric sulfide). The preparation of Qili Powder consists of the pulverization of the eight traditional Chinese medicines into a fine powder. Qili Powder is well known for its actions of resolve stagnant blood to reduce swelling, as a painkiller and coagulant. It is used for the treatment of injuries from falling or stumbling, pain caused by bruises, and traumatic hemorrhage in China. Chemical analyses show that Qili Powder contains various pharmacologically active ingredients. Dracorhodin (**1**), one of the main ingredients of Qili Powder, is used to estimate the quality of the preparation. Dracorhodin has properties of eliminate blood stasis and to ease pain (Ma et al., 2002; 2005), and the plasma concentration of dracorhodin of Qili Powder is closely related to the efficacy of Qili Powder. Some methods have been used to assess dracorhodin content in plants and rat plasma (Sousa et al., 2008; Zhao et al., 2011; Yi et al., 2012). In this study, a sensitive and simple HPLC and LC-MS methods were developed and validated for the determination of dracorhodin in a sample solution and in rat plasma after oral administration of Qili Powder.

The influence of superfine pulverization on the stability of Qili Powder was investigated, and the results showed that at high temperatures dracorhodin (**1**) content in the fine powder decreased even though there was no other significant change in the superfine powder (Wang et al., 2013; Zhao et al., 2013). Few studies have reported any other difference between fine powder and superfine powder regarding the physical-chemical and medicinal characteristics of Qili Powder. The objective of the present work was to investigate the effect of the superfine pulverization in Qili Powder.

A comparative study of physicochemical properties (specific surface area, morphology analysis, bulk density, moisture absorption) and the medicinal characterization (dracorhodin dissolution, pharmacokinetics) of superfine and fine Qili powder was performed. Through the study to explore the application of superfine pulverization technology in Qili Powder.



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Materials and methods

Materials

Sanguis draconis was bought from China National Corp. of Traditional and Herbal Medicine (Beijing, China) and was kept in a cool dry room. Seven other medicinal materials were purchased at a local drugstore (Nanchang, China). All medicinal materials were identified by Professor Shouwen Zhang, at JiangXi University of Traditional Chinese Medicine, and the medicinal materials met all requirements from "identification" in 2010 Pharmacopoeia of the People's Republic of China. The voucher specimens of these eight plants of *Sanguis draconis* (N° 063416), olibanum (N° 085127), myrrh (N° 085128), *Flos Carthami* (N° 105412), catechu (No. 063480), *Borneolum syntheticum* (N° 074501), musk (N° 050233), and cinnabar (N° 078425) were deposited in the Herbarium of the Department of Pharmacognosy, JiangXi University of Traditional Chinese Medicine. Reference substance, dracorhodin perchlorate, was obtained from the National Institute for the Control of Pharmaceuticals and Biological Products (Nanchang, China).

Male Sprague-Dawley rats were bred at the central animal facility of JiangXi University of TCM (Nanchang, China) was used. The animals were housed under standard conditions with free access to food and water. All the animal studies were done in accordance with the approved protocols and guidelines of the Institutional Animal Ethical Care Committee (Sharma et al., 2005) and were approved by the local ethics committee for animal experimentation (No. 49101595-1-10). All efforts were made to minimize the number of animals used and their suffering.

Preparation of fine Qili powder (FP)

Cinnabar was grounded to a very fine powder (part I). *Sanguis draconis*, olibanum, myrrh, *Flos Carthami* and catechu were subjected to coarse grinding using a 6202 type high speed disintegrator (Huanya-Tianyuan Machine Technology Co., Beijing, China) and screened through a sieve (80 mesh) to separate granulates (d < 180 μm) (part II). Musk and *Borneolum syntheticum* were triturated into a fine powder (part III). These three parts were sifted and mixed thoroughly.

Preparation of superfine Qili powder (SP)

The powders part I, part II and part III were put into the close delivery kettle of a SYFM-8 II type vibrating ultrafine pulverizer (Powder Technology Engineering Co., Ltd, Jinan,

China), and processed. The temperature was kept at below 5°C to prevent musk and *Borneolum syntheticum* of Qili Powder from volatilizing.

Determination of powder size

The average particle size of the FP and SP was determined by dry laser diffraction. Mastersizer 2000 (Malvern instruments Ltd., UK) which equipped with Scirocco dry module was applied in the test. The intake air pressure and feed-rate of operating parameters of the experiment are 2.5 bars and 55%, respectively.

Specific surface area and pore volume

The specific surface area and pore volume of the FP and SP were determined by nitrogen gas absorption based on the BET method (Sousa et al., 2002) using TriStar3000 surface area and pore volume analyzer (Micromeritics Instrument Corp., USA).

Morphology analysis

The morphology of the FP and SP was evaluated using a MD_{50} polarizing microscope (Ming-Mei Technology Co., Ltd, Guangzhou, China).

Bulk density and the angle of repose

The bulk density and angle of repose were determined using appropriate methods validated previously, by Taser et al. (2005) and Bai et al. (2006), respectively.

Moisture absorption

According to the guiding principle of moisture absorption medicine testing of the Pharmacopoeia of the People's Republic of China (Chinese Pharmacopoeia Commission, 2010) to determine the moisture absorption of the FP and SP.

Fourier Transform Infrared Spectroscopy (FTIR) and X-ray Diffraction (XRD)

FTIR spectra of the FP and SP from 400 to 4000 cm^{-1} were collected by a Vertex70 FTIR (Bruker spectrometer company, Germany). The FP and SP XRD was performed using a D8 ADVANCE X-ray diffraction (Beuker-axs, Germany), with Cu Kα radiation, voltage 40 kV, current 40mA and scanning range of 2θ is 5 -50°.

Content of dracorhodin

The content of dracorhodin (**1**) in the FP and SP was determined by HPLC analysis (Wang et al., 2013).

Dissolution testing

The dissolution of the FP and SP was tested using Pharmacopoeia of the People's Republic of China (Chinese Pharmacopoeia Commission, 2010) type 2 dissolution testing apparatus (paddle method). ZRS-8G Intelligent Dissolution Tester (Tianda Tianfa Technology Co., Ltd., China) was used in this study. The weights of the FP and SP were accurately weighed (about 1 g) and put into the vessels with 250 ml double distilled water (n = 6); paddle speed was 100 g and temperature was 37 ± 0.5°C. Samples were taken at 5, 10, 15, 30, 45, 60, 90, 120 min, and replaced with an equal volume of the same fresh medium. An aliquot of 1.5 ml was filtered through a 0.22 μm filter, and the concentration of dracorhodin was determined according to the above-mentioned chromatogram condition.

Pharmacokinetics

LC and MS/MS parameters

The quantitative analysis was performed on a 6410 Triple Quad LC-MS/MS coupled with an Agilent 1200 HPLC system (Agilent, USA). The chromatography was performed using a Kromasoil C18 column (150 × 4.6 mm, 5 μm). The mobile phase consisted of acetonitrile and 0.1% formic acid in double distilled water (55:45 in volume). The flow rate of the mobile phase was set at 0.4 ml/min, the column temperature was 35°C, and the injection volume was 20 μl.

The mass spectrometer was operated at ESI positive ion mode and detection was performed in the multiple reaction monitoring (MRM) modes. The analytes were first characterized by Q1 MS (Q1) scan and enhanced product ion (EPI) scan to determine the precursor ions and product ions used in MRM mode. The MS/MS transitions were: *m/z* $[M+H]^+$ 267.2 precursor ion to the *m/z* 252 product ion. The ion spray voltage was set at 3,500 V; ionization temperature was set at 350°C.

Oral administration

FP/SP suspension was administered orally at a dose of 12.15 g/kg to rats. Blood samples of about 0.5 ml were collected in heparinized test tubes via the postorbital venous plexus at 0.08, 0.17, 0.25, 0.50, 0.75, 1, 1.5, 2, 3 , 4, 6, 8, 12, 24, 36, 48, 72 h after administration. Blood was separated by centrifugation at 4000× *g* for 10 min, the supernatant was collected and stored at -20°C until analysis.

Preparation of the plasma sample

Plasma samples (50 μl) were transferred to centrifuge tubes. 400 μl 6% perchloric acid in water and 400 μl methanol were added to the plasma. The mixture was mixed on a vortex for 2 min, and then centrifuged at 8000× *g* for 15 min to precipitate protein. The supernatant was transferred to another tube, and the methanol was eliminated by evaporation at 40°C under a gentle stream of nitrogen. The residue I was extracted twice with 400 μl chloroform. The chloroform layer of the residue I was collected and evaporated to dryness at 40°C under a gentle stream of nitrogen. The residue II was dissolved in 200 μl of 50% methanol solution and vortex-mixed for 2 min. The tubes were centrifuged at 18000× *g* for 10 min. The supernatants were transferred to vial inserts and 20 μl was injected into LC-MS/MS.

Calibration curve

The stock solution was diluted to give solutions of 0.01, 0.50, 1.00, 2.51, 3.51 and 5.01 μg/ml; respectively. Each concentration

was injected in triplicate and the mean peak area was used for the calibration plot.

Precision

Dracorhodin (**1**) standard plasma solution (2.51 μg/ml) was sampled for LC-MS analysis. The work was repeated five times, and to calculate the accurate relative standard deviation (RSD %).

Repeatability

Five 2.51 μg/ml dracorhodin standard plasma solutions were sampled for LC-MS analysis, to explore the repeatability of the analysis method.

Stabilization

The FP plasma solution after oral administration was sampled for LC-MS analysis respectively after being prepared for 0, 4, 8, 12, and 24 h to ensure stabilization of the solution. The concentration of dracorhodin was obtained by to the above-mentioned chromatogram conditions.

Statistical analysis

All pharmacokinetic parameters were calculated using DAS software (Ver. 2.0, Mathematical Pharmacology Professional Committee of China).

Results and discussion

Powder size distribution

The particle size distribution of the FP and SP can be seen in Fig. 1. The average particle size, D_{90} and D_{50} of the FP were 60.7 μm, 164.5 μm and 28.9 μm, respectively. The average particle size, D_{90} and D_{50} of the SP were 30.1 μm, 32.2 μm and 5.2 μm, respectively.

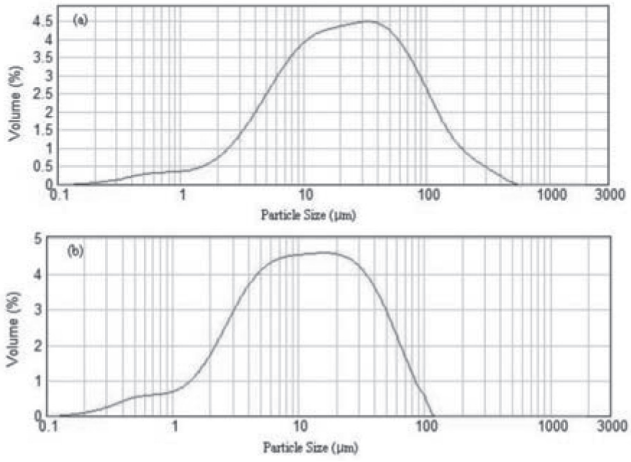

Figure 1 – Particle size distribution of fine Qili powder (a) and superfine Qili powder (b).

Specific surface area and pore volume

The specific surface area (SSA) and pore volume of SP were significantly different ($p < 0.05$) compared with FP. The SP was found to have higher SSA and larger pore volume. Conversely, the FP had lower SSA and smaller pore volume (Table 1). Since higher SSA and pore volume may increase contact area with dissolution medium, they will have effect indirectly on drug dissolution from solid dosage forms (Riley et al., 2008).

Morphology analysis

The optical characteristics of Qili Powder are showen in Fig. 2. The eyepiece lens had a magnification of 20× and the objective lens was 40×. The magnification of polarizing microscope was 800 times. In the FP, yellowish-brown long tubular secretory cell of *Flos Carthami* (a), ellipsoidal pollen grains of *Flos Carthami* (b), irregular sanguineous pieces around with bright yellow liquid of *Sanguis draconis* (c), irregular minute granules dark brownish-red lustrous and with dark black edges of cinnabar (d) were clearly observed. For SP (e), the cell walls were broken into pieces and optical microstructure characteristics of *Sanguis draconis* and cinnabar were disappeared.

Bulk density and the angle of repose

The bulk density (ρ_b) was the density including pores and interparticle voids. The ρ_b of the FP and SP were 0.3604 and

Table 1
Specific surface area and Pore volume of fine Qili powder (FP) and superfine Qili powder (SP).

Sample	Specific surface area (m^2/g)	Pore volume (cm^3/g)
FP	0.2998 ± 0.0051	0.000849 ± 0.000030
SP	2.0309 ± 0.0471*	0.007062 ± 0.000056*

Mean ± S.D., $n = 3$; *$p < 0.05$ compared to FP.

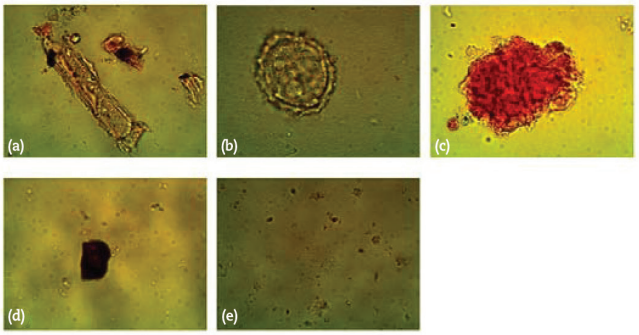

Figure 2 – Optical microstructure of long tubular secretory cell of Flos Carthami in fine Qili powder (a), pollen grains of *Flos Carthami* in fine Qili powder (b), pieces of *Sanguis draconis* in fine Qili powder (c), granules of cinnabar in fine Qili powder (d), superfine Qili powder (e).

0.3732 g/ml, respectively. The ρ_b varied significantly between the FP and SP ($p < 0.05$). This might be due to the fact that particle size decreases leading to bulk density increase.

The angle of repose (θ) could reflect the change in the fluidity of the powders (Ileleji et al., 2008). The θ of FP and SP was 54.64° and 56.85°, respectively (Table 2). The results confirmed that superfine-treatment had a significant effect on θ ($p < 0.001$). As θ increased, the fluidity of powder bulk could diminish.

With the decreasing of particle size, the θ of Qili Powder significantly increased ($p < 0.001$). This result is not in agreement with what the reports stated (Santomaso et al., 2003; Zhao et al., 2009; 2010). The reason might be due to the fact that *Sanguis draconis* is the main component of Qili Powder, and is a cohesive medicinal material. *Sanguis draconis* in SP might have been exposed after superfine pulverization, which may be cause of the surface attachment of the SP was higher than that of FP, so the θ of SP significantly increased and the fluidity diminished.

Moisture absorption

As shown in Figure 3, the powders with different particle size displayed a different curve of moisture absorption. The percentage of moisture absorption of FP and SP slowly increased before 36 h, and reached to balance after 36 h. It was interesting to note that both percentage of moisture absorption and balanced moisture content (BMC) decreased with the decrease of particle size and the increase of SSA. The reason might be related to the cohesive characteristic of *Sanguis draconis*, a main component in Qili Powder. *Sanguis draconis* of SP might have been exposed after superfine-treatment. A compact film had formed on the surface of SP after moisture absorption, which blocked up diffusion path of moisture that finally led to a decreased percentage of moisture absorption and BMC.

FTIR and XRD analysis

The FTIR spectra and XRD patterns of Qili Powder are shown in Figure 4. From Figure 4A we can see that no molecular structure change occurred between the SP and FP, because the characteristic peaks didn't show any shifts. There were no differences between the characteristic diffraction peaks of FP and that of SP in Figure 4B, because the old characteristic peaks didn't disappear and the new characteristic peaks didn't appear. It meant that superfine pulverization didn't change the molecular structure and crystal form of Qili Powder.

Content of dracorhodin

The identification and quantification of the active ingredient are very important for quality control and efficacy of Qili Powder. The HPLC analysis showed that the dracorhodin (**1**) did not cause alterations in the chromatographic profile in relation to superfine pulverization (Fig. 5). The contents of dracorhodin in the FP and SP were 1.12 ± 0.02 mg/g and 1.13 ± 0.01 mg/g, respectively. There was no significant difference in dracorhodin content between FP and SP. It means that superfine pulverizing didn't influence the content of dracorhodin in Qili Powder.

Table 2
Bulk density and angle of repose of fine Qili powder (FP) and superfine Qili powder (SP).

Sample	Bulk density (g/ml)	Angle of repose (°)
FP	0.3604 ± 0.0160	54.64 ± 0.60
SP	0.3732 ± 0.0133*	56.85 ± 0.36***

Mean ± S.D., $n = 3$; *$p < 0.05$ compared to FP; ***$p < 0.001$ compared to FP.

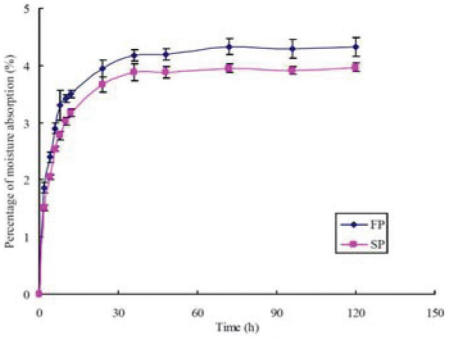

Figure 3 – Curve diagram of moisture absorption of fine Qili powder (FP) and superfine Qili powder (SP).

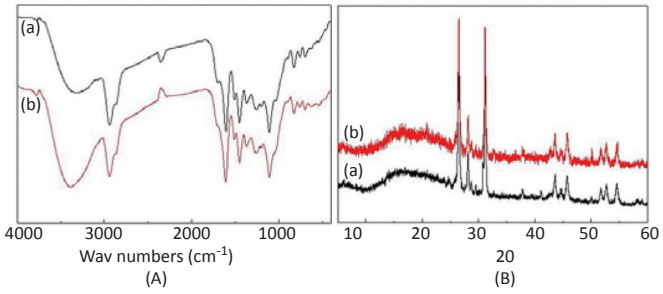

Figure 4 – FTIR spectra (A) and XRD patterns (B) of fine Qili powder (a) and superfine Qili powder (b).

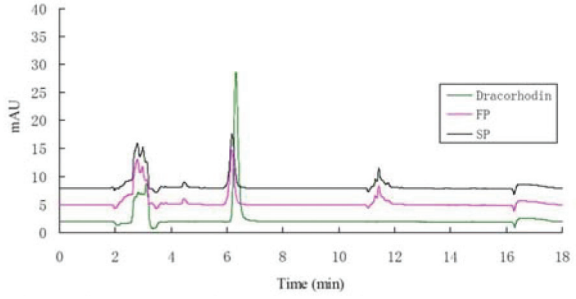

Figure 5 – HPLC chromatogram of dracorhodin (**1**), fine Qili powder (FP) and superfine Qili powder (SP).

Dissolution testing

The percentage of dissolution of dracorhodin in the FP and SP was studied and the results are shown in Fig. 6. The results showed that lower percentage of dracorhodin dissolution in both FP and SP. This was due to the characteristic liposoluble nature of dracorhodin. However, it was clear that the percentage of dissolution of dracorhodin significantly increased with the decrease of particle size ($p < 0.05$). There was no difference in the content of dracorhodin between FP and SP therefore, the main factor affecting the dissolution of dracorhodin was proven to be the particle size and the SSA of Qili Powder.

Pharmacokinetics

The calibration plots were linear in the range of 0.01-5.01 μg/ml. The mean of six different calibration plots yielded the equation Y = 34633X + 132710, R = 0.9909. The precision and repeatability of dracorhodin (**1**) standard plasma solution were 4.5 and 4.8%, respectively. These results showed that the precision and repeatability were favorable. The RSD of stabilization of FP plasma solutions after oral administration was 1.59%, which showed that the solution was stable in 24 h.

The mean plasma dracorhodin concentration–time curve after oral administration of the FP and SP was shown in Figure 7. All pharmacokinetic parameters were calculated by DAS software. The major pharmacokinetic parameters were presented in Table 3. After superfine pulverization, the maximal plasma concentration (C_{max}) of dracorhodin increased from 0.28 mg/ml to 3.06 mg/ml, while the peak time (T_{max}) decreased from11.95 h to 2.10 h. This showed that the SP was absorbed faster than that of FP. The mean residence time (MRT) and the total body clearance rate (CLz/F) of dracorhodin of SP were 31.09 ± 6.14 h and 0.47 ± 0.14 l/h/kg, respectively, which was significantly different from FP ($^{*}p < 0.05$, $^{**}p < 0.01$). This indicated that the duration of drug effect has been prolonged after superfine pulverization. Therefore, the AUC(0-t) of dracorhodin in SP was 26.84 ± 7.77 mg/l*h, which was 3.75 times of the FP's ($^{**}p < 0.01$). This showed that the absorbption capacity of the medicine in rats was increased by superfine pulverization. The reason might be due to that the effective contact area of the medicine with the body fluid is increased when the medicine is pulverized to superfine powder. The results of the pharmacokinetics analysis showed that superfine powder has the advantage of faster absorption and higher bioavailability.

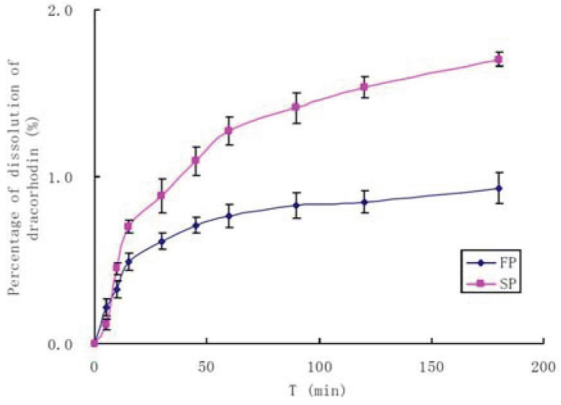

Figure 6 – Curve diagram of dracorhodin dissolution (**1**) in fine Qili powder (FP) and superfine Qili powder (SP).

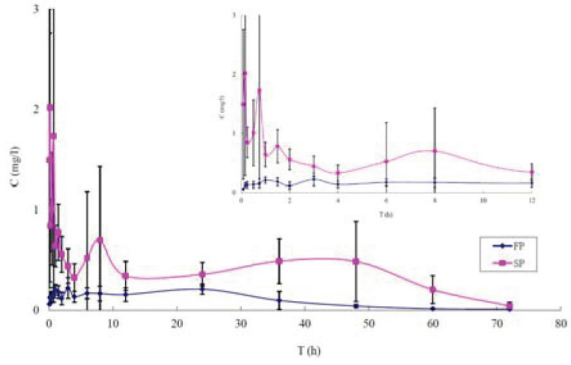

Figure 7 – Plasma dracorhodin (**1**) concentration-time curves after oral administration of fine Qili powder (FP) and superfine Qili powder (SP) ($n = 6$)

Table 3
Pharmacokinetic Paramaters of dracorhodin (**1**) after oral administration of fine Qili powder (FP) and superfine Qili powder (SP)

Paramaters	FP	SP
AUC(0-t)(mg/l*h)	7.15 ± 1.53	26.84 ± 7.77**
AUC(0-∞)(mg/l*h)	7.26 ± 1.51	27.42 ± 7.76**
MRT(0-t)(h)	22.25 ± 2.72	29.75 ± 5.67*
MRT(0-∞)(h)	23.37 ± 2.39	31.09 ± 6.14*
t1/2z(h)	10.69 ± 3.56	9.39 ± 4.36
CLz/F(l/h/kg)	1.75 ± 0.46	0.47 ± 0.14**
C_{max}(mg/l)	0.28 ± 0.06	3.06 ± 1.50*
T_{max}(h)	11.95 ± 11.31	2.10 ± 3.31*
F(%)	100	375.38

Mean ± S.D., $n = 6$; $^{*}p < 0.05$ compared to FP; $^{**}p < 0.01$ compared to FP.

Conclusions

SSA and the pore volume of Qili Powder after superfine pulverization increased. The fluidity of powders decreased with the decreasing of particle size. The percentage of moisture absorption and the balanced moisture content of SP decreased, which may result in a slow agglomeration of SP that finally leads to increased storage stability and prolonged period of guaranteed quality of Qili Powder. Compared with FP the superfine pulverization did not influence the content of dracorhodin nor the molecular structure and crystal form of Qili Powder, but significantly improved the percentage of dissolution and absorption of dracorhodin in rats. The superfine pulverization of Qili Powder showed some physicochemical and medicinal properties that might be of potential use in the drug industry.

According to related works, the general view is that after superfine pulverization SSA of superfine powder increases, which results in easy moisture absorption. With the increase of water content, the stability of TCM preparation decreases, such as charging of chemical component, midewing and so on, and guaranteed quality period was shortened. However,

after superfine pulverization, the rate of moisture absorption and BMC of SP decreased. These results showed that maybe superfine pulverization could increase storage stability of Qili Powder and prolong guaranteed quality period of Qili Powder.

Our results suggested that fluidity was negatively related to the particle size of Qili Powder. The research group has studied *Flos Carthami*, *Angelicae sinensis radix*, *Radix et rhizoma Notoginseng*, *Drynariae rhizoma*, *Radix et rhizoma Rhei*, catechu, *Sanguis draconis* separately and got the same result. Bad fluidity was not conducive to subsequent processing of superfine powders, and may lead to a bigger load difference during the packing process of superfine powders.

To improve bioavailability of SP, the efficacy of superfine powders will be enhanced, which means that dosage can be reduced as well as the raw material, thus minimizing TCM materials, especially the tense situation of endangered TCM biological materials such as musk.

The Pharmacopoeia of the People's Republic of China (2010 Edition) contains about 1036 kinds of TCM preparations. Among them, 742 kinds are used directly as a medicine in powder form after pulverization, which are about 72% of TCM preparations. It is clear now that a TCM powder is an important intermediate product or end product of TCM preparation. The physicochemical and medicinal properties of a TCM powder is directly related to the particle size of powders. Thus, the physicochemical and medicinal properties of TCM preparations may improve by making TCM powders into superfine powders. Therefore, this study will not only helpful to the application of superfine pulverization in Qili Powder, but also provide a possible method to prepare other TCM powders.

Authors' contributions

GZ contributed in collecting materials, preparation of powder, and analysis of the data and drafted the paper. CW contributed in preparation of powder and physicochemical studies. XL contributed to pharmacokinetics studies and critical reading of the manuscript. ZL contributed in the identification of biological materials and running the laboratory work. ZX and ZL contributed to chromatographic analysis and critical reading of the manuscript. All the authors have read the final manuscript and approved the submission.

Conflicts of interest

The authors declare no conflicts of interest.

REFERENCES

Bai, Y.X., Y.F. Li, 2006. Preparation and characterization of crosslinked porous cellulose beads. Carbohyd. Polym. 64, 402-407.

Chinese Pharmacopoeia Commission, 2010. *Pharmacopoeia of the People's Republic of China*. Beijing: China Medical Science Press.

Ileleji, K.E., Zhou., B., 2008. The angle of repose of bulk corn stover particles. Powder Technol. 28, 110-118.

Li, W.F., Jiang, J.G., Chen, J., 2008. Chinese medicine and its modernization demands. Arch. Med. Res. 39, 246-251.

Liao, Z.G., Zhang, X.H., Zhao, G.W., Yang, Y.F., Liang X.L., Yang, L.W., Yang, M., Gai, G.S., 2010. Comparative study of physicochemical properties of *Carthamus tinctorius* L. powders with different sizes. Chin. Pharm. J. 145, 602-607.

Ma, J.J., Song,Y., Jia, M., Li, C.L., 2002. Effect of total flavone in *Sanguis draconis* on platelet aggregation, thrombus formation and myocardial ischemia. Chin. Traditional and Herbal Drugs. 33, 1008-1010.

Ma, P.Y., Fu, Z.Y., Su, Y.L., Zhang, J.Y., Wang, W.M., Wang, H., Wang, Y.C., Zhang, Q.J., 2009. Modification of physicochemical and medicinal characterization of Liuwei Dihuang particles by ultrafine grinding. Powder Technol. 191, 194-199.

Ma, Q.S., Yin, S.J., Zhang, F., Lan, T.H., Liu, X.M., 2005. Concentration-dependent inhibition of dragon's blood total flavone on the peak of tetrodotoxin-resistance boltage-gated sodium currents in trigeminal ganglion cells: Unility part of drangon's blood analgesia action. Chin. J. Clin. Rehabilitation. 9, 108-111.

Riley, C.K., Adebayo, S.A., Wheatley, A.O., Asemota, H. N., 2008. Surface properties of yam (*Dioscorea* sp.) starch powders and potential for use as binders and disintegrants in drug formulations. Powder Technol. 185, 280-285.

Santomaso, A., Lazzaro, P., Canu, P., 2003. Powder flowability and density ratios: the impact of granules packing. Chem. Eng. Sci. 58, 2857-2874.

Sharma, P., Varma, M.V.S., Chawla, H.P.S., Panchagnula, R., 2005. *In situ* and *in vivo* efficacy of peroral absorption enhancers in rats and correlation to *in vitro* mechanistic studies. IL Farmaco 60, 874-883.

Sousa, J.J., Sousa, A., Podczeck, F., Newton, J.M., 2002. Factors influencing the physical characteristics of pellets obtained by extrusion-spheronization, Int. J. Pharm. 232, 91-106.

Sousa, M.M., Melo, M.J., Jorge Parola, A., Sérgio Seixas de Melo, J., Catarino, F., Pina, F., Cook, F.E.M., Simmonds M.S.J., Lopes, J.A., 2008. Flavylium chromophores as species markers for dragon's blood resins from *Dracaena* and *Daemonorops* trees. J. Chromatogr. A, 1209, 153-161.

Su, Y.L., Fu, Z.Y., Ma, P.Y., Wang, W.M., Zhang, J.Y., 2009. Effect of ultrafine grinding on dissolving-out quantity of tanshinone IIA from *radix Salvia miltiorrhiza*. J. Wuhan University Technol. Mater. Sci. Ed. 24, 973-976

Taser, O.F., Altuntas, E., Ozgoz, E., 2005. Physical properties of Hungarian and common vetch seeds. J. Appl. Sci. 5, 323-326.

Wang, C.L., Zhao, G.W., Liao, Z.G., Liang X.L., Cao, Y.C., Zhu, M.L., 2013. Influence of ultrafine grinding on stability of Qili Powders. Chin. J. Exp. Traditional Med. Formulae. 19, 1-5.

Yang, L.W., Sun, P., Gai, G.S., Yang, Y.F., Wang, Y.R., 2011. Physicochemical characterization of Baizhi particles by ultrafine pulverization. Int. J. Miner. Metall. Mater. 18, 247-251

Yi, T., Tang, Y.N., Zhang, J.Y., Zhao, Z.Z., Yang, Z.J., Chen, H.B., 2012. Characterization and determination of six flavonoids in the ethnomedicine "Dragon's Blood"by UPLC-PAD-MS. Chem. Central J. 6, 116-122.

Zhao, G.W., Wang, C.L., Liao, Z.G., Liang X.L., Cao, Y.C., Chen, X.L., 2011. Comparative study of physicochemical properties of dragon's blood powders. Adv. Mater. Res. 311-313, 560-565.

Zhao, G.W., Wang, C.L., Liao, Z.G., Liang X.L., Cao, Y.C., Zhu, M.L., 2013. Influence of superfine grinding on stability of hydroxysafflor yellow A in Qili Powder. Chin. Traditional Patent Med. 35, 1427-1432.

Zhao, X.Y., Du, F.L., Zhu, Q.J., Qiu, D.L., Yin, W.J., Ao, Q., 2010. Effect of superfine pulverization on properties of *Astragalus membranaceus* powder. Powder Technol. 203, 620-625.

Zhao, X.Y.,Yang, Z.B., Gai, G.S., Yang Y.F., 2009.Effect of superfine grinding on properties of ginger powder. J. Food Eng. 91,217-222.