J Pharm Biopharm Res, 2019, 1(1): 15-20
DOI: 10.25082/JPBR.2019.01.002

RESEARCH ARTICLE

The preparation of Garcinia Glycosides solid dispersion and intestinal absorption by rat in situ single pass intestinal perfusion

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Abstract: Garcinia Glycosides is a candidate drug obtained by structural modification of Gambogic Acid (GA), which was acquired through High Throughput Screening(HTS). As Garcinia Glycosides is an effective but insoluble anti-tumor drug, the aim of this study was to obtain a solid dispersion form Garcinia Glycosides by using solvent-melt method so that improve the solubility and dissolution rate. The solid dispersion was characterized by High Performance Liquid Chromatography (HPLC), infrared spectroscopy and evaluated the intestinal absorption of the drug by rat in situ single pass intestinal perfusion. The results showed the increase of solubility, dissolution velocity and absorption compared to other forms. This indicated that solid dispersion could greatly improve the relative bioavailability of Garcinia Glycosides *in vivo*.

Keywords: Garcinia Glycosides, solid dispersion, intestinal perfusion, in situ single-pass perfusion method

1 Introduction

Garcinia Glycosides is synthesized by structural modification of gambogic acid, which is a natural product found in gamboges. As a highly effective and low toxicity anticancer drug, the effect of anti-tumor is accomplished through different mechanisms, including induction of cell cycle arrest and cell apoptosis, inhibition of telomerase and topoisomerase activity, inversion of multidrug resistance and so on.[1] Animal experimental studies show that the half-life is 2.2 h following oral administration, the metabolic rate is rapid, multiple continuous administration is not conducive to clinical treatment of patients, while produces lots of toxicity.[2,3]

Drug release is a crucial and limiting step for oral drug bioavailability, particularly for drugs with low gastrointestinal solubility and high permeability.[4] Given Garcinia Glycosides's poor solubility in water and short half-life, Garcinia Glycosides is prepared into solid dispersion to increase the solubility and the dissolution rate of the drug, thereby increasing the relative bioavailability, so that solid dispersion can not only retain the immediate release of onset, but also extend the delivery time, while reduce the toxic and side effects.

The term solid dispersion (SD) is used to describe a solid system in which the drug is dispersed in a biologically innocuous hydrophilic carrier. These systems are generally centered on the conversion of a candidate pharmaceutical carrier mix from liquid to solid state.[5] The solid dispersion technology has been used extensively to enhance the solubility, dissolution and bioavailability of poorly water soluble drugs by water soluble carriers.[6] As we known, solid dispersion preparations using hydrosoluble carriers lead to significantly enhanced dissolution rates of poorly water-soluble drugs.[7–9] So in the present study, we choose water-soluble carrier material Polyethylene Glycol for Garcinia Glycosides solid dispersion, based on drug release and production many other aspects adventages of SD. Then we established HPLC to examine the basic properties of Garcinia Glycosides, to determine the drug release and the content. And studied the kinetic characteristic of Garcinia Glycosides solid dispersion in the rats in vivo intestinal absorption through single-pass perfusion technology. The paper provided biopharmaceutical basis for the research of Garcinia Glycosides.

Intestinal absorption characteristics of drugs are very important for oral drug delivery system. In situ perfusion experiment is under the condition of not cutting animal blood vessels and nerves, the drugs will be transported

Received: December 25, 2018 **Accepted:** January 25, 2019 **Published:** January 30, 2019

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Citation: Li S, Ji J, Chen Y, *et al*. The preparation of Garcinia Glycosides solid dispersion and intestinal absorption by rat in situ single pass intestinal perfusion. *J Pharm Biopharm Res*, 2019, **1**(1):15—20

in blood after penetrating the intestinal epithelial cells to avoid the effect of gastric emptying and digestive tract inherent movement and other factors on the experimental results. The drug concentration of the circulating fluid in absorbing parts is so low that sink conditions can be formed, and can be excluded liver first-pass effect.[10–12]

2 Materials and methods

2.1 Materials

Garcinia Glycosides (Lot#: 0404901) was made by School of pharmacy, Liaoning Unversity. (Shenyang, China). Hydroxy propyl methyl cellulose (HPMC) and ethyl cellulose(RT-N-10) were obtained from Ruitai Chemical Group Co., Ltd. (Shandong, China). Lauryl sodium sulfate was purchased from Sinopharm Chemical Reagent Co., Ltd (Beijing, China). Methanol of HPLC grade was provided from Yuwang Chemical Co. (Shandong, China). Polyethylene Glycol (4000,6000) of Chinese Pharmacopoeia grade was purchased from Shanghai Chineway Pharmaceutical Tech.Co., Ltd. (Shanghai, China). All other chemicals and solvents used were of AR grade.

2.2 Preparation of Solid Dispersion

As a result of solvent-melt method using less solvent, simple process, requiring short time and remaining less residue, the solid dispersion was prepared by solvent-melt method.

2.2.1 Effect of carrier species on drug dissolution

Prepared different solid dispersions with different molecular weights PEG as carrier material, investigated the effects of glycosides in the case of the solid dispersion of drug dissolution. Determined drug-carrier mass ratio of 1:8, prepared different solid dispersions with PEG4000, PEG6000 and PEG4000-EG6000(1:1w/w) as carrier material separately. Then the cumulative release percentage of the three batches solid dispersions were measured, and drawed the release curve in Figure 1.

2.2.2 Effect of drug / carrier ratio on drug dissolution

PEG4000-PEG6000 (3:10, w/w) was used as the carrier material to prepare the solid dispersion as the drug/carrier ratio was 1:8, 1:13 and 1:18 separately. The cumulative release percentage of these three batches of solid dispersion was determined, and the release curve was plotted in Figure 2.

2.2.3 Effect of solvent ratio on drug dissolution

Respectively of Garcinia glycosides dissolved in anhydrous ethanol as the proportion was 1:10, 1:20 and 1:30, (w/V), fixed drug/carrier ratio was 1:8, water bath temperature of 80 °C, stirring time was 2h of the preparation of solid dispersion. Observed the effects of both ratio of drug dissolution. The cumulative release percentage of these three batches of solid dispersion was determined, and the release curve was plotted in Figure 3.

2.2.4 Effect of agitation time on drug dissolution

Mixing makes the carrier materials with the molten state of the drug fully exposed, so that the solid dispersion can be uniformly and stably. The ratio of the fixed drug/carrier was 1:8, the stirring time was 0.5h, 1h, 2h and 3h respectively. Prepared solid dispersion respectively, and the cumulative release percentage of these four batches of solid dispersion is determined, and the release curve chart was drawn in Figure 4.

2.2.5 Effect of bath temperature on drug dissolution

The melting point of PEG4000 and PEG6000 were about 60 °C, to ensure the carrier's completely molten and organic solvent volatilization, stirring time was 2h, fixed drug/carrier ratio was 1:8, with anhydrous ethanol (1:10, w/v) as solvent, respectively in 60 °C, 70 °C, 80 °C and 90 °C water bath temperature of four batches of solid dispersions were obtained, and the cumulative release percentage of these four batches of solid dispersion was determined, and the drug release curve was plotted in Figure 5.

2.2.6 Effect of cooling rate on drug dissolution

Fixed drug /carrier ratio 1:8, anhydrous ethanol (1:10, w/v) as solvent, stirring time was 2h, the water bath temperature of 80 °C. The two batches of solid dispersion were prepared, and a batch of the method of rapid cooling was used, while the other group was cooled at room temperature. The cumulative release percentage of these two batches of solid dispersion was determined, and the drug release curve was plotted in Figure 6.

2.2.7 The process of preparation

Weighed medicament and carrier in a 1:8 proportion, while the relative proportions of the carrier material PEG4000:PEG6000 was 3:10. Garcinia Glycosides was dissolved in bit anhydrous ethanol (1:10, w/v) with heat, then placed the carrier material in 80 °C water bath heated to molten state, added Garcinia Glycosides solution into the carrier material until completely melted. The mixing time was 2h under mechanical agitation and evaporated the solvent, the resultant rapidly poured onto -20 °C steel plates and severely stirred to make it cool.

2.3 Characterization of Garcinia Glycosides solid dispersion

2.3.1 High-Performance Liquid Chromatography

The exact content of the compounds was determined using Waters 1525 Brnary HPLC Pump, and a Waters 2487 Dual λ Absorbance Detector (Waters, Amer-

ica). A C18 reversed-phase chromatographic column (150mm3.9 mm; 5m particle size) was used. The column was kept at 30 °C throughout the elution process, which used a mobile phase consisting of 5% Phosphoric acid solution and Methanol at a total flow rate of 1.0 mL/min and the detection wavelength set to 360 nm; injection volume: 20μL.

The method was fully validated for specificity, lower limit of quantification (LLOQ), accuracy, precision and linearity Control samples were assessed by the procedure as described above to evaluate specificity of the method.

2.3.2 Phase identification of the preparation

There are plenty of dispersion states of drugs in solid dispersion, like the molecular state, no fixed patterns, colloidal microcrystalline or micronized state, *etc*. Therefore, identification of drug dispersion state is primary to analysis solid dispersion. The experiment used infrared spectroscopy to identify the present state in Garcinia Glycosides of solid dispersion.

2.3.3 Evaluation of solubility

The SD of Garcinia Glycosides were dispersed in a distilled water solution, 0.5% Sodium dodecyl sulfate; maintained horizontally with agitation at a temperature of 37±0.5 °C. Sampling 5ml per predetermined time interval, and promptly supplemented with an equal volume of fresh medium. After this stage, the samples were first filtered with qualitative paper filters and subsequently passed through a filter with 0.45 mm pores. The quantity of Garcinia Glycosides dissolved was determined by spectrometry. Substituting the corresponding standard curve equation to calculate the concentration, obtained the drug cumulative release percentage Q:

$$Q(100\%) = \frac{C \times V \times D}{W \times F \times 1000} \times 100\%$$

where the drug cumulative release percentage Q is determined by C, the concentration of the dissolution liquid g/ml; V, the volume of the dissolution medium ml; D, dilution ratio; W, the weight of solids; F, the percentage of the drug in the formulation. Then, plot a graph showing the dissolution calculated by time as X-axis and drug cumulative release percentage as Y-axis.

2.4 Evaluation of intestinal absorption

Adopt in situ single-pass perfusion method to evaluate the SD of Garcinia Glycosides in intestinal absorption. This experiment was conducted using male adult rats and the animals were deprived of solid food for 12 hours (Free access to water) before the start of the experiment. Then intraperitoneal injection of 10% chloral hydrate(3.4 ml/kg), fixed and maintained them body temperature. A midline abdominal incision was made and the small intestine was exposed. The intestine was rinsed by physiological saline at 37 °C until the washing appeared clear and ligated after cannulated into intestine. Then kept it thermal insulated under infrared lamps.

After that, cannulated to the constant flow pump, Garcinia Glycosides solution of 100ml at 37 °C was perfused as 1ml/min flow rate for 2 hours. Sampling 1ml to 10ml volumetric flasks at times 0, 10, 30, 60, 90 and 120 minutes and immediately filtered through membranes with 0.45 mm pores. The perfusate was diluted to 10 ml and was detected by HPLC. The volume of the dissolution medium was maintained constant by the addition of 1ml of Garcinia Glycosides solution. Then took gambogic acide 0.5% CMC-Na suspension as a control, operated the same method.(Table 1, Table 2)

Table 1. Gambogic acid reference substance

Time (min)	Area (Microvolt * s)	Concentration (mg/ml)	Percent Absorption (%)
0	137389	0.005279	0.00
10	137310	0.005276	0.0568
30	132432	0.005084	3.646
60	112486	0.004322	18.13
90	96982	0.003726	29.42
120	90137	0.003463	34.40

Table 2. Garcinia Glycosides SD

Time (min)	Area (Microvolt * s)	Concentration (mg/ml)	Percent Absorption (%)
0	175119	0.0132	0.00
10	165800	0.0125	5.383
30	91946	0.006932	47.47
60	75561	0.005696	56.85
90	63131	0.004764	63.95
120	42420	0.003198	75.77

3 Results

3.1 Effect of carrier species on drug dissolution

The figure showed the type of carriers was a significant factor. Drug release rate increased with the increase of polyethylene glycol (PEG) molecular weight. Its order was PEG6000>PEG4000-PEG6000>PEG4000. However, the solid dispersion prepared by PEG6000 was used as the carrier to make the preparation process difficult. Therefore, PEG4000-PEG6000 was used and adjusted the proportion of the PEG6000. Determined PEG4000-PEG6000 (3:10, w/w) as the carrier.

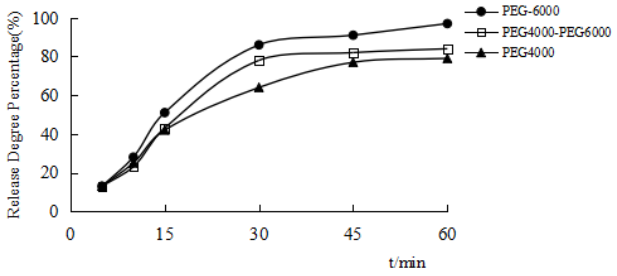

Figure 1. The release curve of different carrier types of the Garcinia Glycosides solid dispersion and the carriers were PEG4000, PEG4000-6000 and PEG6000 respectively

3.2 Effect of drug /carrier ratio on drug dissolution

By the graph, the proportion of the drug carriers could be increased, but the effect was small. Considering the amount of solid dispersion containing the amount of the solid dispersion, the initial determination of the drug / carrier ratio was 1:8.

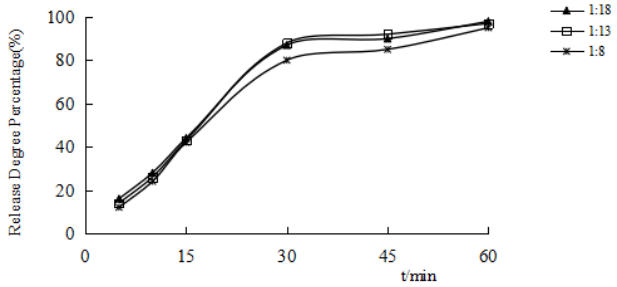

Figure 2. The release curve of the proportion of different carrier of the solid dispersion and the ratio of drug and carrier were 1:8, 1:13and 1:18 respectively

3.3 Effect of solvent ratio on drug dissolution

The figure showed the dissolution rate of the solid dispersions increased with the increase of absolute ethanol ratio, but the difference was not obvious. In the case of the full dissolution of the drug, the proportion of the solvent was not the main factor affecting the drug dissolution. So determination of solvent ratio of drug / ethanol (1:10, w/v).

3.4 Effect of agitation time on drug dissolution

As shown in Figure 4, the time of stirring had a great influence on the drug dissolution. However the increase of the mixing time after 2h had little effect on the drug dissolution. Therefore, in order to ensure the uniform and stable dispersion of solid dispersion and the organic solvent, the stirring time should be 2h.

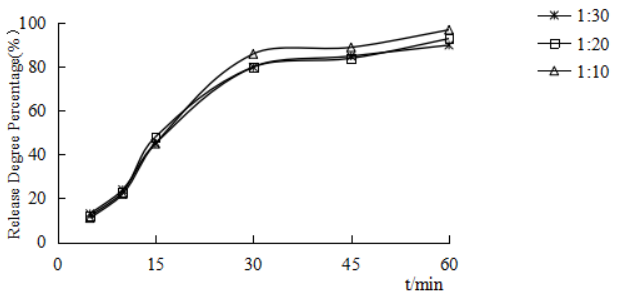

Figure 3. The release curve of the solid dispersion of the proportion of the different solvents and the solvent ratio of drug and ethanol were 1:10, 1:20 and 1:30 respectively

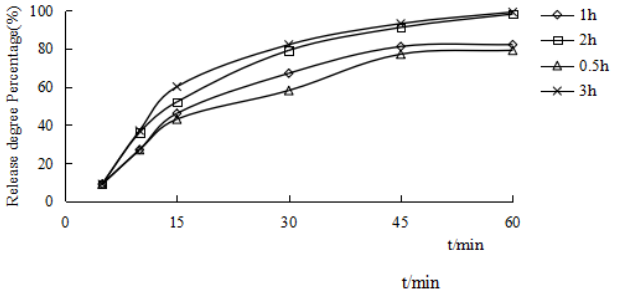

Figure 4. The release curve of the solid dispersion of different stirring time and the stirring time were 0.5 h, 1 h and 2 h respectively

3.5 Effect of bath temperature on drug dissolution

The diagram showed with the increase of the water bath temperature, the drug dissolution rate increased, but after temperature more than 80 °C, drug dissolution rate decreased. Probably because of the high temperature accelerated solvent evaporation speed, so the solvent evaporate uneven, bring about shell encapsulated drug, the drug release rate decreased. So the bath temperature was 80 °C.

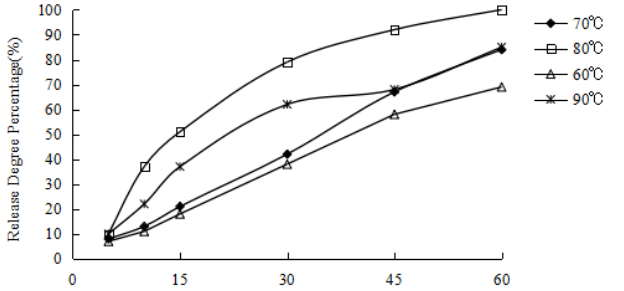

Figure 5. The release curve of Garcinia glycosides solid dispersions in different water bath temperature and the bath temperature were 70 °C, 80 °C and 90 °C respectively

3.6 Effect of cooling rate on drug dissolution

It was found that the cooling rate had a great effect on the dissolution of the drug, which may be due to the dissolution of the solid dispersion, which was caused crystallization by the slow cooling of the drug. In this experiment, the solid dispersion was prepared by rapid cooling method.

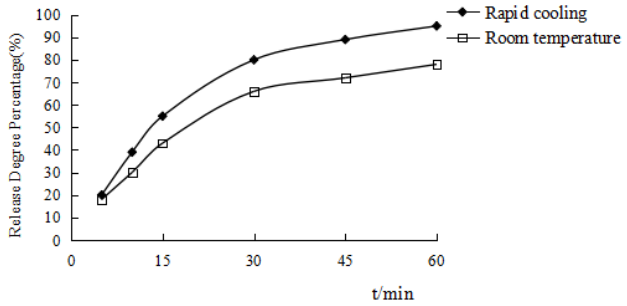

Figure 6. The release curve of the solid dispersion with different cooling methods and the cooling methods were rapid cooling and cooling with room temperature

3.7 Phase identification of the solid dispersion

If Garcinia Glycosides existed in complex molecular, forming hydrogen bond, the absorption peak of some drugs and carriers disappeared or displaced between 2800-3200. IR spectra shown in Figure 7 did not show the absorption peak change, so the solid dispersion may not be in the form of complex molecule, it may exist in microcrystallines or nano-particles.

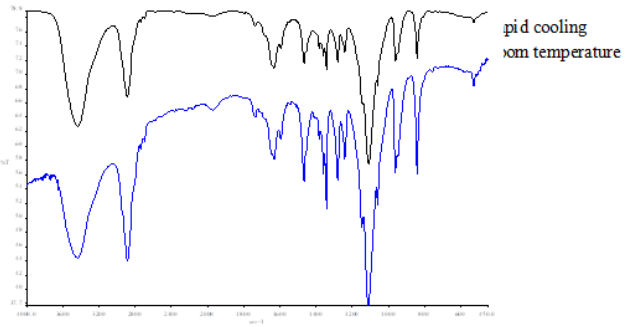

Figure 7. The infrared spectroscopy of the THS SD and physical mixtures of PEG4000 and PEG6000 with THS

3.8 Evaluation of solubility

The result showed that the release rate of the sample reached the standard and the release rate was good.

3.9 Evaluation of intestinal absorption

The Figure 9 showed the intestinal absorbability of Garcinia Glycosides SD apparently higher than gambogic acid. Probably due to the Garcinia Glycosides made into solid dispersion, the drug highly dispersed in the carriers as microcrystalline or amorphous, so that greatly improved the dissolution and absorption of the drug.

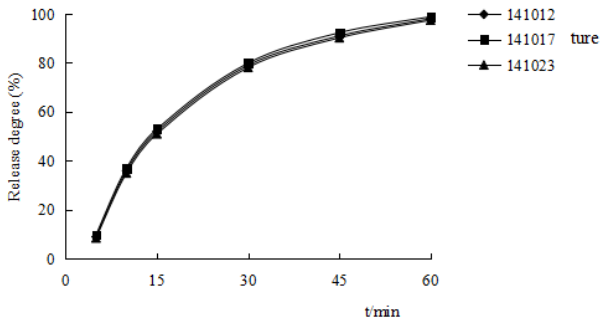

Figure 8. The drug release curves of 3 batches solid dispersion

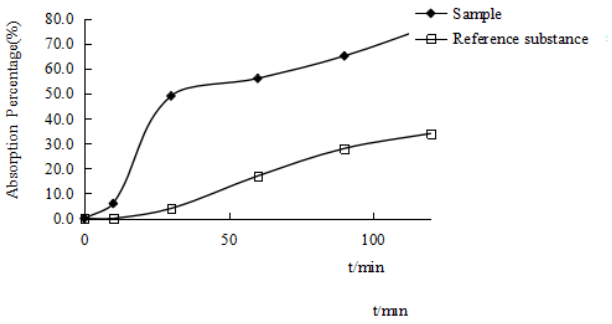

Figure 9. The intestinal absorption of solid dispersion and reference substance

4 Discussion

The properties of the solid dispersion carrier directly affect the properties of the solid dispersion, so the carrier should have the following conditions: physiological inertia, no carcinogenic, no toxic, no opposite effect with drug treatment purposes; no chemical reaction with the drug, do not affect the chemical stability of the main drug; can get the best dispersion state; get the source easily and cheap price, *etc.*. PEG is a crystalline polymer with stable properties and heat resistance, which can be compatible with many drugs, and the melting point is low (60 °C). Also solid dispersion can be crushed and stored easily. Therefore, PEG polymer was used as the carrier material.

In the present study, the characterization results indicated that Garcinia Glycosides was prepared into solid dispersions with the carrier of PEG. The dissolution rate of the drug was not determined by the change of the molecular weight of PEG, instead depended on the drug

specific. With the increase of the PEG molecular weight, the dissolution rate increased slightly; with the increase of the proportion of the carrier, the dissolution rate was significantly improved. PEG6000 was used alone as the carrier, which makes the preparation process complex and difficult. So the experiment selects PEG4000 and PEG6000 as the joint carrier.

In this paper, the method of single-pass perfusion was used in the study of intestinal absorption. Compared with the circulation method, the experimental conditions of this method were close to the intestinal circumstances after the drug delivery, so that avoided the measurement error caused by the high flow rate of the Injury of intestinal mucous membrane.

5 Conclusions

Oral drug delivery is the simplest and commonest way of administering drugs. Actually, most drugs are poorly water soluble drugs, not well absorbed after oral administration.[13] So Garcinia Glycosides was prepared into solid dispersion. By improving the drug release profile of these drugs, it is possible to enhance their bioavailability and reduce side effects.[14, 15] This series of results show that SD is a useful strategy for increasing the bioavailability of Garcinia Glycosides and have conducive to intestinal absorption.The single-pass intestinal perfusion studies in rats also confirmed that Garcinia Glycosides solid dispersion displayed a good absorption in intestinal.

6 Acknowledgements

This study was supported by grants from the Drug Discovery Initiative of the National 11th Five Year Plan (2009ZX09103-030) and Innovation Team Project (No: LT2015011) from the Education Department of Liaoning Province. Gareinia glycosides was provided by College of Pharmacy of Liaoning University, New Drug R&D Key Laboratory of Liaoning Province.

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