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Improved stability and oral bioavailability of Ganneng dropping pills following transforming lignans of herpetospermum caudigerum into nanosuspensions

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[ABSTRACT] The present study was designed to improve storage stability and oral bioavailability of Ganneng dropping pills (GNDP) by transforming lignans of *Herpetospermum caudigerum* (HL) composed of herpetrione (HPE) and herpetin (HPN) into nanosuspension (HL-NS), the main active ingredient of GNDP, HL-NS was prepared by high pressure homogenization and lyophilized to transform into solid nanoparticles (HL nanoparticles), and then the formulated HL nanoparticles were perfused into matrix to obtain NS-GNDP by melting method. For a period of 3 months, the content uniformity, storage stability and pharmacokinetics test *in vivo* of NS-GNDP were evaluated and compared with regular GNDP at room temperature. The results demonstrated that uniformity of dosage units of NS-GNDP was acceptable according to the criteria of Chinese Pharmacopoeia 2015J. Physical stability of NS-GNDP was investigated systemically using photon correlation spectroscopy (PCS), zeta potential measurement, and scanning electron microscopy (SEM). There was a slight increase in particles and PI of HL-NS re-dispersed from NS-GNDP after storage for 3 months, compared with new formulated NS-GNDP, which indicated a good redispersibility of the NS-GNDP containing HL-NS after storage. Besides, chemical stability of NS-GNDP was studied and the results revealed that HPE and HPN degradation was less when compared with that of GNDP, providing more than 99% of drug residue after storage for 3 months. In the dissolution test *in vitro*, NS-GNDP remarkably exhibited an increased dissolution velocity compared with GNDP and no distinct dissolution difference existed within 3 months. The pharmacokinetic study showed that HPE and HPN in NS-GNDP exhibited a significant increase in AUC_{0-t}, C_{max} and decrease in T_{max} when compared with regular GNDP. These results indicated that NS-GNDP possessed superiority with improved storage stability and increased dissolution rate and oral bioavailability.

[KEY WORDS] Ganneng dropping pills; Lignans; *Herpetospermum caudigerum*; Nanosuspensions; Stability; Oral bioavailability

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Introduction

As a superior folk medicine, *Herpetospermum caudigerum* is the dry, mature seeds of *H. caudigerum* Wall [1], which has been widely used for the treatments of liver diseases, cholic diseases, and dyspepsia [2-3] in Tibet. In previous chemical studies, a large number of lignans were isolated and identified from *H. caudigerum* [4-6], in which both herpetrione (HPE) and herpetin (HPN) (Fig. 1) are considered as major active components with significant anti-hepatitis B virus and hepatoprotective effects [6-7]. However, HPE and HPN are poorly soluble in water, resulting in poor oral bioavailability and thus restricting its application. Considering further pharmaceutical use of the two components, the fifth class of new Chinese medicine, Ganneng dropping pills (GNDP) containing lignans of *H. caudigerum* (HL) composed of HPE and HPN has been produced and randomized clinical trials

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have exhibited positive activity in hepatitis B virus inhibition test [8]. Nevertheless, there is another inevitable problem: although the application of dropping pills increases the solubility of HL effectively, the pharmaceutical production of GNDP is still limited, primarily due to stability concerns of dropping pills [9].

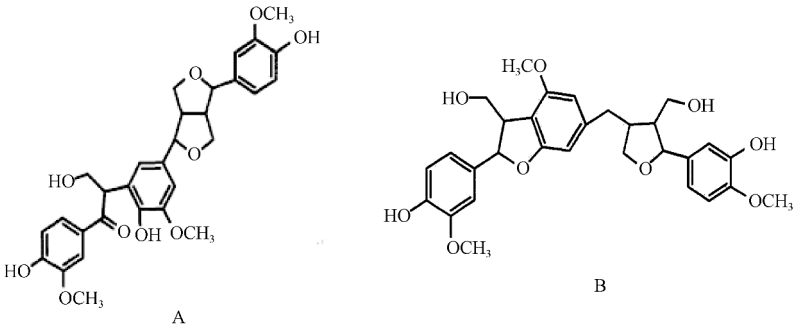

Fig. 1 Structures of herpetrione (A) and herpetin (B)

Prepared by melting, solvent or melting solvent method, dropping pills are defined as a solid dispersion of one or more active ingredients in matrix at the solid state, which mainly present in molecules, colloidal or microcrystalline and can improve the bioavailability of poorly soluble active ingredients effectively [10]. However, following solvent evaporating or matrix condensing after long-term storage, the ability of matrix to dissolve drug is decreased and then the drug in dropping pills would precipitate partly, which could reduce the drug's solubility, bioavailability, and inherent efficacy [11]. Therefore, new technologies are needed in the pharmaceutical development. Recently, the surfactants in combination with matrix, such as sodium lauryl sulfate/Polysorbate 80 [12], gelucire 44/14 [13-15] and poloxamer 407 [16], have been employed in preparation of solid dispersion with the aim of increasing the wettable property of solid dispersion particles and the rheological property of the mixture of drugs and matrix, thus inhibiting the drug precipitation and enhancing bioavailability *in vivo*. However, in the previous experiments, a number of interesting phenomena have been observed: numerous large particles did not dissolve completely, while HL raw material dispersed in the matrix during the GNDP preparation. Ostwald ripening phenomena shows that smaller particles become smaller and larger particles grow up gradually. It is of interest to determine whether the precipitated large particles during long-term storage in dropping pills are mainly caused by the undissolved drug particles? If yes, the most urgent problem to solve is the particle size of the drug of interest.

In the last few years, nanosuspensions (NS) has become a promising and effective method in reducing particle size of active pharmaceutical ingredient to nano-sized range (typically between 200 to 500 nm) [17]. Nano-sized particles could increase the surface area-to-volume ratio, and thus better interact with surrounding aqueous media to improve dissolution [18]. In addition to better solubility and higher dissolution rate of poorly water-soluble drugs, NS has some other advantages, such as high drug loading, low incidence of side effects by excipients, and low cost [19]. Besides, NS preparation technology is relatively simple; NS could be produced by top-down process or bottom-up process or a combination of the two methods [20-22]. Therefore, the use of NS in the formulation of GNDP filled with HL raw material may be a potential strategy to provide uniform and small particles, while minimizing granular changes in the polymeric carrier and obtaining stable GNDP.

The purpose of the present study was to improve the stability and oral bioavailability of GNDP by transforming HL into HL-NS. HL-NS was prepared by high pressure homogenization; liquid HL-NS was transformed into solid HL nanoparticles by lyophilization to prepare the new NS-GNDP. The particle size distribution of HL nanoparticles re-dispersed from NS-GNDP after storage for 3 months was investigated by photon correlation spectroscopy (PCS), and the prepared NS-GNDP was characterized using scanning electron microscopy (SEM). The content uniformity and stability were also tested. To verify the advantages of the NS-GNDP containing HL nanoparticles, the dissolution behavior and the pharmacokinetic profiles were also investigated.

Materials and Methods

Chemicals and reagents

A mixture of two lignans of HL were prepared in the laboratory of Dr YUAN (302 Military Hospital of China, Beijing, China), including HPE (the purity is up to 67%) and HPN (the purity is up to 31%); sodium dodecyl sulfate (SDS) and polyvinypyrrolidone kollidon 30 (PVPK-30) were purchased from BASF Corp (Ludwigshafen, Germany). PEG-4000 and PEG-6000 were obtained from Guangdong Guanghua Sci-Tech Co., Ltd. (Guangdong, China); high-performance liquid chromatography (HPLC) grade acetonitrile was purchased from Fisher (Fair Lawn, NJ, USA). All other chemicals used in the present study were of analytical grade and commercially available.

Preparation of HL coarse suspensions (HL-CS)

HL coarse suspensions were prepared by dispersing HL