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Study on Rheology and Release Kinetics of Chuanqi Ophthalmic Microemulsion *in situ* Gel

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Abstract **[Objectives]** To study the rheology and release kinetics of Chuanqi ophthalmic microemulsion *in situ* gel, so as to provide references for its future study and development. **[Methods]** The fluid properties and linear viscoelastic regions of this preparation were investigated by MCR 102 rheometer. The release kinetics of Chuanqi ophthalmic microemulsion *in situ* gel was evaluated by modified Franz diffusion cell method, the ligustrazine and ligustilide were selected as the indictors, and semi-permeable membrane was used as a barrier, sampling time point was 0.5, 1, 2, 4, 6, and 8 h respectively. **[Results]** Chuanqi ophthalmic microemulsion *in situ* gel was a pseudoplastic fluid and it had a linear viscoelastic region. Taking the shear stress as the indicator, the linear viscoelastic region was 0 - 302.74 Pa; taking the strain as the indicator, the linear viscoelastic region was 0 - 7.45%. At the critical point, the storage modulus (G') = the loss modulus (G'') = 2 976.60 Pa, critical shear stress was 302.74 Pa and critical strain was 7.45%. The average cumulative release of ligustrazine of 6 samples within 8 h was 33.71 μg, the average cumulative release rate reached 90.08%, and the release kinetics followed Higuchi equation. The average cumulative release of ligustilide of 6 samples within 8 h was 68.46 μg, the average cumulative release rate reached 84.32%, and the release kinetics followed the zero-order kinetics equation. **[Conclusions]** Chuanqi ophthalmic microemulsion *in situ* gel has excellent viscoelasticity and its strain is reversible in a certain range. The release kinetics of ligustrazine is the result from synergistic effect of its physicochemical properties and matrix skeleton, while the release kinetics of ligustilide is mainly affected by its physicochemical properties.

Key words Rheology, Linear viscoelastic region, Ophthalmic *in situ* gel, Microemulsion, Chuanqi prescription, Ligustrazine, Ligustilide

1 Introduction

Chuanqi ophthalmic microemulsion *in situ* gel is clinical effective prescription, consists of CHUANXIONG RHIZOMA (Chuanxiong) and ASTRAGALI RADIX (Huangqi), has functions of nourishing blood and invigorating qi, and is clinically used for treating the age-related macular degeneration (AMD). At present, drugs for the treatment of AMD [1] include Lucentis, Avastin, Pegaptanib Sodium, Conbercept, Ziv-aflibercept/Zaltrap, and Esculin and Digitalisglycosides Eye Drops. All these are chemical drugs. Except Esculin and Digitalisglycosides Eye Drops, all the other preparations are intravitreal injection. Because of directly acting on the administration site, at the same time of taking effect, intravitreal injection has relatively high side effects. Besides, intravitreal injections are expensive, each piece of injection costs a few thousand or tens of thousand yuan; the use of intravitreal injections is frequent, generally, it needs injection one time monthly, or one time every 2 - 3 months, patients have poor tolerance; the use of intravitreal injections may have a certain risk of bringing certain damage to the eyeball. Esculin and Digitalisglycosides Eye Drops belong to local administration preparation, and it overcomes the high cost and potential damage of intravitreal injection to the eyeball; however, it has poor bioadhesive, it can be retained in the eyes only for a short time and it is easy to be removed, and it is difficult to reach the posterior eye position due to its physiological structure of penetrating the eyeball.

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At present, there is no traditional Chinese medicine available for the treatment of AMD, thus our team intended to prepare Chuanqi prescription into an ophthalmic microemulsion *in situ* gel, to solve the problems in treatment of AMD and provide an attempt for treatment of AMD by traditional Chinese medicines. *In situ* gel is a kind of special gel, and it can undergo reversible phase transformation; according to the difference in transformation factors, the *in situ* gels can be divided into temperature-sensitive gels [2], ion-sensitive gels [3] and pH-sensitive gels [4]. Rheology is the science that studies the behavior of fluids subject to a flow or a strain, and this concept was proposed by Bengham in 1929 [5]. The preparation of suspensions, emulsions, colloids, ointments, and gels involve the rheology theory. Fluid is divided into Newtonian fluid and non-Newtonian fluid. Characteristics of Newtonian fluid: generally, it is a pure liquid or dilute solution with low molecular weight; at a certain temperature, the viscosity (η) of Newtonian fluid is a constant which is only a function of temperature; the viscosity of Newtonian fluid drops with the rise of temperature. Most fluids do not conform to Newton's law and are called non-Newtonian fluids. According to the difference of flow curves, non-Newtonian fluids can be divided into plastic flow, pseudoplastic flow, dilatant flow and thixotropic flow. Chuanqi ophthalmic microemulsion *in situ* gel is a temperature-sensitive gel. It is of great practical value to study the fluid type and linear viscoelastic range after strain.

The release kinetics studies the amount of drug released from the matrix and its release pattern. The release kinetics is generally determined using the Pepps equation. Pepps equation is generally expressed in $\ln Q = n\ln t + b$, where n is the release parameter,

which is the characteristic parameter of the release mechanism, related to the shape of the matrix of the preparation. When $n < 0.45$, it is the drodible matrix; when $0.45 < n < 0.89$, it is non-Fick diffusion; when $n > 0.89$, it is Fick diffusion, namely, drug diffusion [6]. Besides, the Pepps equation can reflect the drug release kinetics. When $n > 0.66$, the drug release is mainly zero-order kinetics, and when $n = 1$, the drug release completely presents zero-order kinetics [7]. This experiment studied the rheology and release kinetics of Chuanqi ophthalmic microemulsion *in situ* gel, to obtain the characteristic parameters, and so as to provide references for future study and development of this preparation.

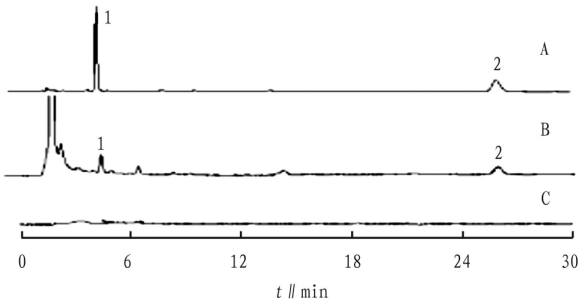
2 Materials

Waters 2487 High Performance Liquid Chromatograph (Waters Corporation, USA), TK-24BL Transdermal Diffusion Tester (Shanghai Kaikai Technology Trade Co., Ltd.), MCR 102 rheometer and PP50 rotor [Austria Anton Paar China) GmbH]. Chuanqi ophthalmic microemulsion *in situ* gel (self made), ligustrazine and ligustilide reference substance (Wuhan Tianzhi Biooloigcal Technology Co., Ltd., lot number CFS201601 and CFS201602, and purity ≥98%), dialysis bag (Beijing Jingke Hongda Biological Technology Co., Ltd., retained the relative molecular mass 7 kDa, and stored at 10 – 29℃), CHUANXIONG RHIZOMA and ASTRAGALI RADIX decoction pieces (Beijing Qiancao Chinese Herbal Medicine Co., Ltd., identified by assistant researcher Du Maobo from Institute of Chinese Materia Medica, China Academy of Chinese Medical Sciences as dry roots of *Ligusticum chuanxiong* Hort. and *Astragalus membranaceus* (Fisch.) Bge. var. mongholicus (Bge.) Hsiao), water was purified water, methanol and acetonitrile were chromatography reagents (CR), and other reagents were analytical reagents (AR).

3 Methods and results

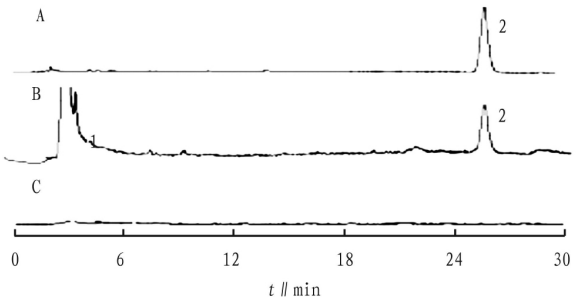
3.1 Determination of ligustrazine and ligustilide content

3.1.1 Chromatographic conditions [8]. Eclipse XDB-C_{18} chromatographic column (4.6 mm × 250 mm, 5 μm); the detection wavelength of ligustilide and ligustrazine was 328 nm and 295 nm, respectively; column temperature was 30℃; mobile phase was methanol-water (60∶40), the flow rate was 1 mL/min, as shown in Fig. 1 and Fig. 2.



Note: A. Reference substance; B. Test sample; C. Blank sample; 1. Ligustrazine; 2. Ligustilide

Fig. 1 HPLC of Chuanqi ophthalmic microemulsion *in situ* gel at 295 nm



Note: A. Reference substance; B. Test sample; C. Blank sample; 2. Ligustilide

Fig. 2 HPLC of Chuanqi ophthalmic microemulsion *in situ* gel at 328 nm

3.1.2 Preparation of reference solution. Precisely weighed 1 mg of ligustrazine and ligustilide reference substance separately, placed in a 50 mL volumetric flask, precisely added methanol to prepare 20 mg/L of the mixed reference solution.

3.1.3 Preparation of test sample solution. Precisely weighed 0.2 g of samples of Chuanqi ophthalmic microemulsion *in situ* gel, placed in a 50 mL volumetric flask, precisely added 25 mL methanol, ultrasonic treatment 30 min, filtered with 0.22 μm porous filter membrane, and the filtrate was the test sample solution.

3.2 Preparation of Chuanqi ophthalmic microemulsion *in situ* gel [9]

Weighed proper amount of the ethanol extract of Chuanqi (self made, Chuanxiong and *Astragalus* extracted by 95% ethanol), added the oleic acid, polyoxyethylene hydrogenated castor oil RH40, polyethylene glycol 400 and propylene glycol, to make into microemulsion for use; weighed proper amount of water extract of Chuanqi self made. Chuanxiong and Astragalus extracted by water, added Poloxamer 407, gelatin and water, to make into the *in situ* gel for use; uniformly mixed proper amount of microemulsion and *in situ* gel, to make into Chuanqi ophthalmic microemulsion *in situ* gel. In accordance with the method in Section **3.1**, the measured mass fraction of ligustrazine and ligustilide in Chuanqi ophthalmic microemulsion *in situ* gel was 74.86 μg/g and 162.38 μg/g respectively.

3.3 Rheological behavior study

3.3.1 Fluid properties. The steady-state shear can reflect the evolution of heterogeneous internal structure over a wide range. Generally, ophthalmic gel needs higher yield stress in the static state, so as not to run away from the eye surface. In the process of use, it needs good fluidity (shear thinning), which is the popular pseudoplastic fluid. Using the flow curve module provided with the MCR 102 rheometer, placed the samples of Chuanqi ophthalmic microemulsion *in situ* gel on the plate, stabilized 5 min at 32℃, set the shear rate at 1 – 500 per second, measured one time every 6 s, and plotted the shear rate-viscosity curve (Fig. 3). The results indicated that the viscosity of Chuanqi ophthalmic microemulsion *in situ* gel declines with the increase in the shear rate, the *in situ* gel belongs to pseudoplastic fluid and has the characteristics of shear thinning.

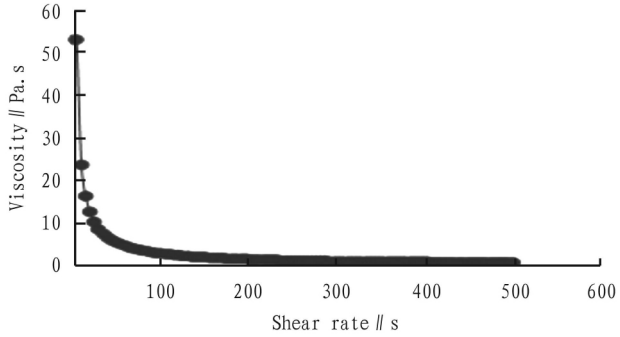


Fig. 3 Shear rate – viscosity curve for Chuanqi ophthalmic microemulsion *in situ* gel

3.3.2 Determination of linear viscoelastic range [10-12]. To determine the linear viscoelastic range of the gel, it is necessary to introduce the concept of dynamic rheology. Dynamic rheology is a small strain rheology, its process is non-destructive and will not affect or damage the material. Slight changes in the morphology of the gel system will have a clear response. It can provide information about gel viscoelastic properties such as the gel's storage modulus (G'), loss modulus (G''), and linear viscoelastic regions. When the gel is in the linear viscoelastic region, $G' > G''$; when the gel is at the critical point of the linear viscoelastic region, $G' = G''$, at this time, it is able to estimate both values and estimate the maximum deformability of the gel, calculate the maximum shear stress at which the linear viscoelasticity of the gel can be kept; when the gel is beyond the linear viscoelastic region, $G' < G''$. Oscillatory stress sweeping is one of the oscillatory sweeping modes in the MCR 102 rheometer and it can determine the linear viscoelastic range, critical modulus, minimum strain range, and maximum shear of gel. Placed samples of Chuanqi ophthalmic microemulsion *in situ* gel on the plate, stabilized 5 min at 32℃, and carried out the test with Oscillatory Amplitude Sweep module. The angular frequency (ω) is 100 r/s, and the amplitude is the strain range of 0.1 – 1 000%, |Slope| = 5Pt./dec, and results are shown in Fig. 4 and Fig. 5.

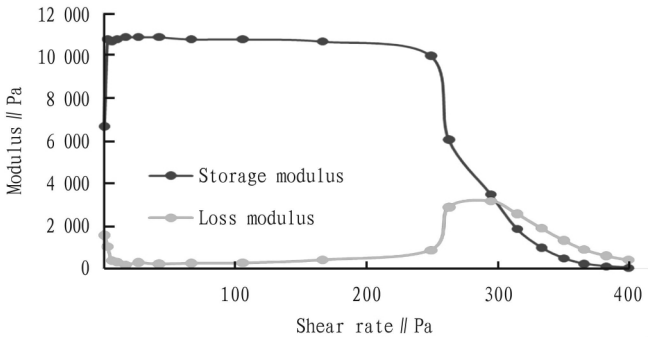


Fig. 4 Modulus – shear stress curve for Chuanqi ophthalmic microemulsion *in situ* gel

From Fig. 4, it can be known that Chuanqi ophthalmic microemulsion *in situ* gel has the linear viscoelastic region of shear stress, and there is related information of changes of G' and G″ with the shear stress. When the shear stress is small, G' drops slowly with the increase of shear stress, and G'' increases slowly with the increase of shear stress; when the shear stress reaches 251 Pa, G' drops significantly with the increase of shear stress, and G'' increases significantly with the increase of shear stress until the two become equal. Outside the linear viscoelastic region, G'' drops with the increase of shear stress. Taking the first point where the shear stress is lower than 300 Pa as the starting point, conducted the equation fitting of G' and G″ separately, obtained the fitting equation $Y = 1 \times 10^9 e^{-0.042X}$ ($r = 0.996\ 2$) and $Y = 0.127X^2 - 116.56X + 26\ 596$ ($r = 0.998$), through calculation, the critical stress of Chuanqi ophthalmic microemulsion *in situ* gel was 302.74 Pa, at this time $G' = G'' = 2\ 976.60$ Pa, and the range of critical shear stress was 0 – 302.74 Pa. The critical value of Chuanqi ophthalmic microemulsion *in situ* gel was much higher than the shear stress 0.2 Pa reported in the literature [13-14], indicating that Chuanqi ophthalmic microemulsion *in situ* gel can keep its state after phase transition in normal condition. Strain occurs under the action of external stress; when the external stress is removed, it will quickly restore.

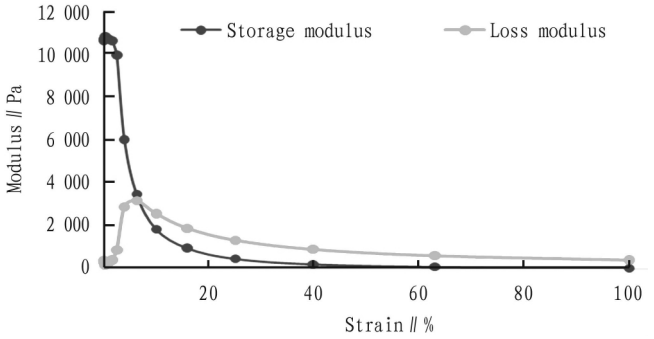


Fig. 5 Modulus – strain curve for Chuanqi ophthalmic microemulsion *in situ* gel

According to Fig. 5, in the linear viscoelastic region, G' declines with the deterioration of strain, while G″ increases with the deterioration of strain, till both reach the critical value and becomes equal; outside the linear viscoelastic region, $G' < G''$, both G' and G″ decline with the deterioration of strain. The intersection of G' and G'' is consistent with the intersection of critical shear stress, and it found that 6.32% is the starting point of fitting. The equation fitting was carried out for G' and G'' separately, obtained the fitting equation $Y = 68\ 351X^{-1.562}$ ($r = 0.998\ 8$) and $Y = 14\ 462X^{-0.764}$ ($r = 0.995\ 7$), estimated that the critical strain value of Chuanqi ophthalmic microemulsion *in situ* gel was 7.45%, $G' = G'' = 2\ 976.60$ Pa, and critical strain range was 0 – 7.45%.

3.4 *In vitro* release experiment [15-16] Took the dialysis bag, cut it into 3 cm × 3 cm pieces, soaked in water for 24 h, rinsed with water 3 times, placed in 50% ethanol to soak for 24 h, then rinsed with water 3 times again, placed in water to heat for 30 min, cool down to room temperature, cut into a single layer, rinsed with water 3 times, placed on absorbent paper to dry for use. Using a vertical Franz diffusion cell, the receiving solution was polyethylene glycol 400 – 95% ethanol-water (1∶3∶6). Fixed the treated semipermeable membrane in the area between diffusion chamber and the receiving chamber of the diffusion device, weighed 0.5 g Chuanqi ophthalmic microemulsion *in situ* gel on

the semipermeable membrane, evenly distributed it, filled the receiving chamber with the receiving solution, and emptied the bubbles. Set the magnetic stirrer speed at 400 r/min, the water bath temperature of 32℃, the volume of diffusion cell of 18 mL, and effective diffusion area of 2.834 cm^2. Sampling was carried out at 0.5, 1, 2, 4, 6 and 8 h respectively; during sampling, poured out all the liquid in the receiving chamber, and added new receiving solution with equal temperature and volume; filtered the poured liquid with 0.22 μm porous filter membrane, measured the content of ligustrazine and ligustilide, calculated the cumulative release, and plotted the curve for *in vitro* release (Fig. 6).

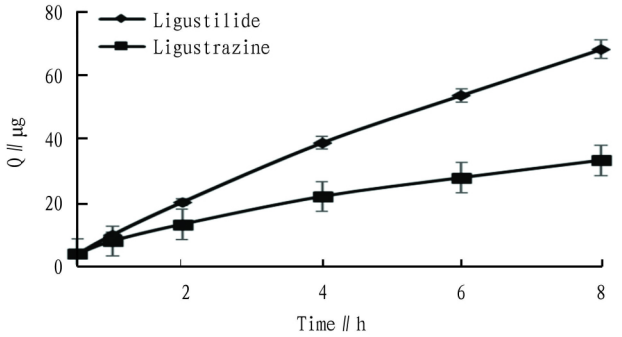


Fig. 6 Curve for *in vitro* release of Chuanqi ophthalmic microemulsion *in situ* gel ($\bar{x} \pm s$, $n = 6$)

According to Fig. 6, the average cumulative release of ligustrazine in 6 samples within 8 h was 33.71 μg, and average cumulative release rate was 90.08%. The release curve of ligustrazine in 6 samples was consistent, and the discrete degree of cumulative release of ligustrazine in each sampling point was relatively ideal and evenly distributed in two sides of the mean value. The average cumulative release of ligustilide in 6 samples within 8 h was 27.38 μg with an average cumulative release rate of 33.73%. The cumulative release rate of ligustilide was not ideal, possibly because the condition of the cell for the receiving solution was not ideal. According to conditions reported in the literature [17] and results of pre-experiment, changed the conditions of ligustilide receiving solution into polyethylene glycol 400-ethanol-water (1∶4∶5) composite system, and carried out the *in vitro* release experiment in accordance with conditions under Section **3.4**. The average cumulative release of ligustilide in 6 samples within 8 h was 68.46 μg with an average cumulative release rate of 84.32%. The release curve of ligustilide in 6 samples was consistent, and the discrete degree of cumulative release of ligustilide in each sampling point was relatively ideal and evenly distributed in two sides of the mean value. Taking cumulative release (Q) of ligustrazine and ligustilide in 6 samples, equation fitting was carried out for the time t, as listed in Table 1. The results showed that the release kinetics of ligustrazine conformed to Higuchi equation. In addition, the correlation coefficient of Pepps equation fitting was also ideal. The release kinetics of ligustilide conformed to zero-order equation, and the correlation coefficient of Pepps equation fitting was also ideal.

Table 1 Fitting equation of cumulative release and time of active components in Chuanqi ophthalmic microemulsion *in situ* gel

Fitting equation	Ligustilide	Ligustrazine
Zero order	$Q = 3.409\,7t + 0.908\,4$ ($r = 0.997\,0$)	$Q = 3.835\,5t + 4.727\,8$ ($r = 0.988\,7$)
First order	$\ln Q = 0.328\,9t + 1.021\,3$ ($r = 0.916\,6$)	$\ln Q = 0.241\,8t + 1.832\,9$ ($r = 0.919\,9$)
Higuchi	$Q = 12.154\,0t^{1/2} - 7.938\,9$ ($r = 0.996\,5$)	$Q = 13.832\,0t^{1/2} - 5.506\,8$ ($r = 0.999\,7$)
Pepps	$\ln Q = 0.986\,4\ln t + 1.335\,4$ ($r = 0.996\,6$)	$\ln Q = 0.722\,9\ln t + 2.065\,7$ ($r = 0.997\,3$)

The ligustrazine Pepps equation fitting obtained $n = 0.722\,9$, indicating that its release was non-Fick diffusion and the release behavior was the results of the drug composition and substrate matrix erosion, in other words, physicochemical properties of ligustrazine and matrix types will have influence on its release behavior. The Pepps equation reveals that the release pattern is consistent with the Higuchi equation because the Higuchi equation is one of the equations that reflect the interaction of the drug with the matrix. Ligustilide Pepps equation fitting obtained $n = 0.986\,4$, indicating that its release was Fick diffusion and its release behavior was the result of drug action, in other words, physicochemical properties of ligustilide play a decisive role in its release. In the release kinetics, $n > 0.66$, close to $n = 1$, indicating that its release kinetics was zero-order kinetics. The rules of release kinetics reflected by Pepps equation are consistent with that revealed by the zero-order equation fitting.

4 Discussions

In the study of Chuanqi ophthalmic microemulsion *in situ* gel, first, artificial tear and physiological saline were used as receiving solution. Results indicated that the cumulative release rate of ligustrazine and ligustilide within 8 h was 25% and 21% respectively, and the cumulative release rate in physiological saline was 19% and 17% respectively. According to the literature, polyethylene glycol 400 – 95% ethanol-water (1∶3∶6) was taken as the receiving solution [15-16], results indicated that the cumulative release rate of ligustilide in this receiving solution within 8 h reached 90.08%, and the cumulative release rate of ligustrazine in this receiving solution within 8 h reached 33.73%. According to conditions reported in the literature [17] and results of pre-experiment, polyethylene glycol 400-ethanol-water (1∶4∶5) was taken as the receiving solution for study of ligustilide release, and the results indicated that the cumulative release rate of ligustilide in this receiving liquid reached 84.32%.

Through this study of kinetics of Chuanqi ophthalmic microemulsion *in situ* gel through the *in vitro* release experiment, it found that the rules of release of ligustrazine and ligustilide in 6 samples were close. The release kinetics of ligustrazine conformed to the Higuchi equation, and physicochemical properties of ligustrazine and the matrix determined its release behavior; the ligustilide release kinetics conformed to the zero-order equation and its release

behavior was determined by its physicochemical properties. The average cumulative release of ligustrazine and ligustilide in Chuanqi ophthalmic microemulsion *in situ* gel system reached 90.08% and 84.32% respectively, indicating that active components in this gel can be fully released from this system. Kinetic equation fitting showed that the release kinetics of ligustrazine and ligustilide were close to zero-order release, indicating that the active components in the gel can be released from the system continuously and stably. In summary, Chuanqi ophthalmic microemulsion *in situ* gel has good viscoelasticity and relatively ideal release behavior. This experiment is expected to lay a foundation for the follow-up study of the preparation.

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no significant effect on the development of gonads of rats. Therefore, clinically, it is feasible to take low dose *P. ginseng* and *C. pilosula* for short period, or use *P. heterophylla* to take the place of *P. ginseng* and *C. pilosula*, to avoid precocious puberty of children.

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