Research Article

Different Proportions of Huangqi (Radix Astragali Mongolici) and Honghua (Flos Carthami) Injection on \( \alpha \)-Glucosidase and \( \alpha \)-Amylase Activities

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Received 23 September 2014; Accepted 3 March 2015

Objective. To study the effect of different proportions of Huangqi (Radix Astragali Mongolici) and Honghua (Flos Carthami) injection on \( \alpha \)-glucosidase and \( \alpha \)-amylase activity simultaneously.

Methods. The injections were prepared according to the standards of the China Food and Drug Administration. The assay for potential \( \alpha \)-glucosidase inhibitors was based on the hydrolysis of 4-methylumbelliferyl-\( \alpha \)-D-glucopyranoside (4-MUG). The \( \alpha \)-amylase EnzChek assay kit was used to determine potential \( \alpha \)-amylase inhibitors. Acarbose was the positive control.

Results. The half maximal (50%) inhibitory concentration (IC_{50}) of acarbose against \( \alpha \)-glucosidase and \( \alpha \)-amylase was \((1.8 \pm 0.4) \mu g/mL\) and \((227 \pm 32) \mu g/mL\), respectively. Honghua showed significant inhibition of \( \alpha \)-glucosidase activity compared with Huangqi \((P < 0.01)\). Honghua inhibited \( \alpha \)-amylase activity, but Huangqi did not. IC_{50}s for \( \alpha \)-glucosidase inhibition by mixtures at 10:1, 5:1, and 2:1 were significantly lower than those at the 20:1 mixture \((P < 0.01)\). \( \alpha \)-Amylase inhibition by the 2:1 mixture was significantly higher than that by the 20:1, 10:1, and 5:1 mixtures at 500 \( \mu g/mL\) and 1000 \( \mu g/mL\) \((P < 0.01)\), with 5:1 significantly higher than 20:1 and 10:1 at 1000 \( \mu g/mL\) \((P < 0.01)\).

Conclusion. Honghua significantly inhibited \( \alpha \)-glucosidase activity compared with Huangqi \((P < 0.01)\). For simultaneous inhibition of \( \alpha \)-glucosidase and \( \alpha \)-amylase activities, the mixtures at 2:1 and 5:1 exhibited significant effects compared with those at 20:1 \((P < 0.01)\).

1. Introduction

Carbohydrates in foods are digested into oligosaccharides under the action of salivary and pancreatic \( \alpha \)-amylases and then digested into monosaccharides by \( \alpha \)-glucosidase at the brush borders of small intestinal mucosal cells. The monosaccharides are absorbed by small intestinal epithelial cells in the blood circulation. To seek new opportunities in the treatment of diabetes mellitus, a number of studies have been carried out in China and abroad on inhibitors of the two enzymes. Currently, microorganisms and plants are the main sources of inhibitors of the two enzymes [1, 2]; for example, acarbose, which is commonly used clinically, is derived from microbial metabolites.

In this study we sought \( \alpha \)-glucosidase and \( \alpha \)-amylase inhibitors from the traditional Chinese medicines commonly used for treating diabetes mellitus [2], based on the modern Chinese medicine theory that the underlying pathogenesis of diabetes mellitus and its complications is blood stasis due to qi deficiency [3] and on clinical reports that Huangqi (Radix Astragali Mongolici) injection was usually combined with Honghua (Flos Carthami) injection to treat diabetes mellitus and its complications [4]. In clinical usage, there are different reports about the mixture proportions of Huangqi and Honghua injection, ranging from 4:1 to 10:1 [4–6]. In this research, Huangqi and Honghua injection were extracted according to the national standards established by the China Food and Drug Administration (CFDA). The contents of astragaloside IV in Huangqi injection and total flavonoids in Honghua injection were the quantitative requirements in the CFDA standards, and we determined these two active ingredients separately. The aim of this research was to...
study the effects of Huangqi and Honghua injection on \( \alpha \)-glucosidase and \( \alpha \)-amylase activity and to determine their optimum mixture proportions to inhibit these two enzymes simultaneously.

2. Methods

2.1. Preparation of Samples. Huangqi (Radix Astragali Mongolici) and Honghua (Flos Carthami) were purchased from Shanxi Double-Crane Pharmaceutical Co., Ltd., and were identified by the Department of Pharmacy of Shanxi Provincial People's Hospital. The Huangqi injection was prepared as follows [7]. Huangqi (2000 g) was added into water and decocted three times, for 1.5 h each time. The decocted solutions were combined and filtered. The filtrate was concentrated three times, for 12 h each time. The solution was collected and filtered. Ethanol was added from the filtrate, which was then concentrated to a solution containing 1.6 g raw herb per mL. Ethanol was added twice: first to achieve an alcohol content of 75% and then alcohol content of 85% in the second time; the filtrate was kept at 4 °C–8 °C for 12 h each time. The solution was collected and filtered. Ethanol was removed from the filtrate, which was then concentrated to a solution containing 10 g raw herb per mL, and then was diluted with water for injection to 0.87 g raw herb per mL. The solution was refrigerated, set aside for 12 h, and then filtered. The filtrate was concentrated to a solution containing 5.6 g raw herb per mL and was adjusted to pH 7.5 with 20% sodium hydroxide solution. Activated carbon (0.125%) was added to the filtrate and it was boiled for 5 min and the mixture was stirred well, filtered, ultra-filtered, and then was adjusted to pH 7.5 again with 20% sodium hydroxide solution. Water for injection was added to a total volume of 1000 mL, thereby formulating an extract solution containing 2 g of raw herb per mL.

The Honghua injection was prepared as follows [8]. Honghua (500 g) was added into water and decocted three times: for 1 h, then for 50 min, and lastly for 30 min. The decocted solutions were combined and filtered. The filtrate was concentrated to a relative density of 1.20, and ethanol was added to achieve a alcohol content of 70%. After being refrigerated at 4 °C for 48 h, the solution was filtered. Ethanol was removed from the filtrate, which was then concentrated to a relative density of 1.10. Additional ethanol was added to achieve an alcohol content of 80%, and the solution was filtered after it was refrigerated and set aside for another 48 h. Ethanol was removed from the filtrate, which was then concentrated to a relative density of 1.16. Thereafter, a 10-fold volume of water was added, and the solution was refrigerated, set aside for 20 h, and then filtered. The filtrate was concentrated to a relative density of 1.02 and was adjusted to pH 7.5 with 20% sodium hydroxide solution. It was heated at 115 °C for 15 min, refrigerated, and set aside for 120 h. An appropriate amount of activated carbon was added, and the mixture was stirred well, filtered, ultra-filtered, and then was adjusted to pH 7.5 again with 20% sodium hydroxide solution. Water for injection was added to a total volume of 1000 mL, thereby formulating an extract solution containing 0.5 g of raw herb per mL. A 10 mL aliquot of the above Huangqi injection solution was accurately pipetted and dried in vacuo to a constant weight. The resultant extract was weighed as 208.60 mg and the yield was calculated as 10.43 mg of extract per gram of raw herb. A 5 mL aliquot of the above Honghua injection solution was accurately pipetted and dried in vacuo to a constant weight. The resultant extract was weighed as 260.90 mg and the yield was calculated as 104.36 mg of extract per gram of raw herb. The extract solution and the dried extracts were both stored at 0 °C–4 °C until use.

2.2. Reagents and Instruments. \( \alpha \)-Amylase EnzChek Assay Kit and fluorescein-conjugated corn starch (DQ starch) were from PerkinElmer (Boston, Mass, USA). Porcine pancreatic \( \alpha \)-amylase (type VI-B), yeast \( \alpha \)-glucosidase (EC3.2.1.20), 4-methylumbelliferyl-\( \alpha \)-D-glucopyranoside (4-MUG), kaempferol, and astragaloside IV were from Sigma-Aldrich China (Shanghai, China). Acarbose (Glucobay, 50 mg/tablet) was obtained from Bayer Health Care Company Ltd. (Beijing, China). Methanol and aluminium muriate, 50 mM sodium acetate buffer (pH 5.5), 100 mM sodium glycinate buffer (pH 10.6), and 50 mM 3-morpholinopropanesulfonic acid buffer (MOPS, pH 6.9) were all prepared in-house from reagents purchased from Wako Chemicals USA Inc. (Richmond, VA, USA). The Wallac 1420 Victor2 plate reader, the 96-well polystyrene microplates, black 96-well microplates and the DELFA 1296-003 plate shaker were purchased from PerkinElmer (Boston, MA, USA).

2.3. Determination of Astragaloside IV in Huangqi Injection [7]. Preparation of the control solution: an appropriate amount of astragaloside IV as a control was weighed accurately, and then methanol was added to prepare a solution containing 1 mg of astragaloside IV per mL as a control solution. Preparation of the test solution: 25 mL of Huangqi injection was measured accurately, dried to a constant weight, and then supplemented with methanol to 5 mL, 8, 12, and 16 \( \mu \)L of the control solution and 10 \( \mu \)L of the test solution were accurately pipetted and injected into a liquid chromatograph, respectively. The concentration of the test sample was obtained by calculating the common logarithm of the concentration based on the common logarithms of the peak areas and concentrations of the control solutions by the external standard method and converting it into the content of the test sample.

2.4. Determination of Total Flavonoids in Honghua Injection [8]. Preparation of reference solution: a reference solution of kaempferol was prepared in methanol at 0.1 mg/mL. Preparation of standard curve: 1.0, 2.0, 3.0, 4.0, 5.0, and 6.0 mg of the reference solution were added into 25 mL volumetric flasks and made up to volume with methanol. After shaking, 2.0 mL of each of the above solutions was added into a 10 mL test tube with a stopper. Then 1.0 mL of 0.1 M aluminium trichloride solution and 2.0 mL of methanol were added into the test tube, shaken well, and heated in a water bath at 40 °C for 20 min. The test tube was removed from the water bath and cooled down to room temperature. With a corresponding reagent as the blank, the absorbance at wavelength of 422 nm was measured by UV-Vis spectrophotometry as described in
Determination of total flavonoids: 0.5 mL of the Honghua injection extract solution was added into a 50 mL volumetric flask, made up to volume with methanol, and shaken well (test solution). 2.0 mL of the test solution was added into a 10 mL test tube with a stopper and its absorbance was measured according to the method as described in the preparation of standard curve. The blank solution was prepared by the same steps as above except that it contained 2.0 mL of the test solution and 3.0 mL of methanol. The total flavonoid content, as kaempferol equivalents in the test solution, was calculated after comparison with the kaempferol standard curve.

2.5. Stability of Different Mixtures of Huangqi Injection and Honghua Injection. According to the normal descriptions of the different mixtures’ proportions based on the raw herbs and the range from 4:1 to 10:1 in clinical reports [4–6], we tested the stability of different mixture proportions of Huangqi injection and Honghua injection as follows: 20:1, 10:1, 5:1, and 2:1. Proportion 20:1 was 10 mL Huangqi injection mixed with 2 mL Honghua injection, mixed well, and tested for the pH value, particulate determination, and UV spectrum at 0, 1, 2, 3, and 5 h. Other proportions were mixed on an orbital shaker for 30 s and incubated for further 30 s, and the fluorescence measured at $\lambda_{ex}$ 355 nm and $\lambda_{em}$ 460 nm.

For the test samples and positive control, instead of 5 $\mu$L buffer, 5 $\mu$L samples or acarbose was added to wells and subsequent assay steps were carried out as above. The test concentrations of Huangqi were as follows: 375, 750, 1500, and 2000 $\mu$g/mL and Honghua: 15.6, 31.3, 62.5, and 125 $\mu$g/mL. The concentrations of mixtures with Huangqi and Honghua proportions 20:1, 10:1, 5:1, and 2:1 were all as follows: 31.3, 62.5, 125, and 250 $\mu$g/mL. Acarbose was the positive control, and its concentration was 0.3, 0.6, 1.3, and 2.5 $\mu$g/mL. Sodium acetate buffer (50 $\mu$L/well) was used as a negative control. Each concentration was assayed 6 times

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\alpha\text{-glucosidase inhibition} (\%) = 100 \times \frac{A(\alpha\text{-glucosidase}) - A(\text{samples or acarbose})}{A(\alpha\text{-glucosidase}) - A(\text{negative})}.
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The 50% inhibitory concentrations (IC$_{50}$ values) of the samples and acarbose on $\alpha$-glucosidase activity were calculated based on the active concentrations and the inhibitory effects.

2.7. Determination of Effect of Huangqi Injection, Honghua Injection, and Mixtures of These Two on Yeast $\alpha$-Amylase Activity. The measurement method as described by Omichi and Ikenaka [10] and the product information brochure for Molecular Probes EnzChek Amylase Assay Kit (E-11954) were used with a minor modification. A mammalian $\alpha$-amylase enzyme was used instead of the bacterial enzyme provided in the kit. The reaction is based on the principle that the substrate fluorescein-conjugated corn starch (DQ starch) is not fluorescent but hydrolysis of DQ starch by $\alpha$-amylase can produce degraded fragments with high fluorescence. The presence of $\alpha$-amylase inhibitors can reduce the hydrolysis of DQ starch, thereby decreasing the production of the fluorescent fragments. The effects of the samples on $\alpha$-amylase can be reflected by the changes in fluorescence before and after addition of the test samples.

The “$\alpha$-amylase group” ($\alpha$-amylase with no inhibitors present) was obtained by adding 95 $\mu$L of 125 U/mL $\alpha$-amylase solution, 10 $\mu$L of 50 mM MOPS buffer, and finally 95 $\mu$L of 1 mg/mL DQ starch, vortexing the above mixture for

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Table I: Different mixture proportions of Huangqi (Radix Astragali Mongolici) and Honghua (Flos Carthami).

| Proportions of raw herb | Huangqi injection* (mL) | Honghua injection* (mL) | Proportions of extract | Proportions of astragaloside IV and total flavonoids |
|------------------------|-------------------------|-------------------------|------------------------|--------------------------------------------------|
| 20:1                   | 10                      | 2                       | 2:1                    | 1:1:1                                             |
| 10:1                   | 10                      | 4                       | 1:1                    | 0.5:1                                             |
| 5:1                    | 10                      | 8                       | 0.5:1                  | 0.25:1                                            |
| 2:1                    | 10                      | 20                      | 0.2:1                  | 0.1:1                                             |

Notes: * Huangqi injection contained 0.5 g of raw drug per mL. * Honghua injection contained 2 g of raw drug per mL.
30 s and incubating it at 37°C for 30 min. The fluorescence values were measured at $\lambda_{ex}$ 505 nm and $\lambda_{em}$ 512 nm.

Each test group was prepared by the same procedure as above except for replacing the buffer with 10 μL of the test samples. The test concentrations of Huangqi and different proportions were all at 500 and 1000 μg/mL, and Honghua was at 125, 250, 500, and 1000 μg/mL. For the acarbose positive control, buffer was replaced with 10 μL acarbose, and the test concentrations were 62.5, 125, 250, and 500 μg/mL. The negative control was prepared by the same procedure as above except for replacing α-amylase with 95 μL of buffer. The measurement was repeated six times

$$\alpha$$-amylase inhibition (%)  
$$= 100 \times \frac{A (\alpha\text{-amylase}) - A (\text{samples or acarbose})}{A (\alpha\text{-amylase}) - A (\text{negative})}$$  

(2)

2.8. Statistics. Statistical analysis was done using SPSS software (version 12.0, SPSS Inc., Chicago, IL, USA). All data were expressed as mean ± standard deviation. Student’s t-test was used for intergroup comparison. $P < 0.05$ was considered statistically significant.

3. Results

3.1. Content of Astragaloside IV in Huangqi Injection and Total Flavonoids in Honghua Injection. According to the standards of CFDA, Huangqi injection solution contains 2 g of raw herb per mL and the content of astragaloside IV is no less than 0.08 mg per mL. In our research, astragaloside IV was 0.127 mg per mL, equivalent to 6.1 μg per mg extract and 63.5 μg astragaloside IV equivalents per g dried raw herb.

According to the new standards established in 2013, Honghua injection solution contains 0.5 g of raw herb per mL and the content of total flavonoids in 1 mL injection is 0.20–0.70 mg. Our result showed that the total flavonoids were 0.57 mg per mL, equivalent to 10.9 μg kaempferol per mg extract and 1.14 mg total flavonoid equivalents per g dried raw herb.

3.2. Stability of Different Mixtures of Huangqi Injection and Honghua Injection. The results showed that there were no significant differences in pH value, particulate determination, and UV spectrum among the different proportions and different time points (data not shown). Corresponding injection solution, extract, and active ingredients were also listed in Table 1.

3.3. Inhibitory Effect of Huangqi Injection, Honghua Injection, and Mixtures on Yeast α-Glucosidase Activity. In Table 2, the IC$_{50}$ value of acarbose was (1.8 ± 0.4) μg/mL, Huangqi was (1686 ± 810) μg/mL, and Honghua was (32.8 ± 5.7) μg/mL. There was a significant difference among them by Paired t-test ($P < 0.01$). IC$_{50}$ values of the different mixtures, 20 : 1, 10 : 1, 5 : 1, and 2 : 1, were all significantly lower than Huangqi alone ($P < 0.01$) and significantly higher than Honghua alone ($P < 0.01$). IC$_{50}$ values of 10 : 1, 5 : 1, and 2 : 1 did not show any significant difference among them by Paired t-test ($P > 0.05$), but they all were significantly lower than those of the 20 : 1 mixture ($P < 0.01$).

3.4. Inhibitory Effect of Huangqi Injection, Honghua Injection, and Mixtures on α-Amylase Activity. Acarbose showed an inhibitory effect on α-amylase activity from 62.5 μg/mL to 500 μg/mL, with an IC$_{50}$ value of (227 ± 32) μg/mL. Honghua had an inhibitory effect on α-amylase activity within the concentration range (125–1000) μg/mL, but Huangqi did not show any inhibitory effect on α-amylase at the tested

| Group          | Concentration (μg/mL) | Percentage of inhibition (%) | IC$_{50}$ (μg/mL) |
|----------------|-----------------------|------------------------------|-------------------|
| Acarbose       | 2.5                   | 70.2 ± 3.2                   | 1.8 ± 0.4         |
|                | 1.3                   | 34.0 ± 2.7                   |                   |
|                | 0.6                   | 24.4 ± 1.7                   |                   |
|                | 0.3                   | 12.9 ± 1.2                   |                   |
| Huangqi injection | 2000               | 54.1 ± 5.6                   | 1686 ± 810        |
|                | 1500                 | 43.6 ± 3.7                   |                   |
|                | 750                  | 38.1 ± 2.6                   |                   |
|                | 375                  | 9.7 ± 1.1                    |                   |
| Honghua injection | 125                | 89.8 ± 5.6                   | 32.8 ± 5.7        |
|                | 62.5                 | 66.9 ± 5.1                   |                   |
|                | 31.3                 | 46.6 ± 3.7                   |                   |
|                | 15.6                 | 22.8 ± 3.1                   |                   |

Notes: *P < 0.001, compared with acarbose control. **P < 0.001, Honghua injection and different proportions compared with Huangqi injection. $^*$P < 0.01, proportions 10 : 1, 5 : 1, and 2 : 1 compared with 20 : 1. IC$_{50}$: half maximal (50%) inhibitory concentration on yeast α-glucosidase activity.
concentrations (500–1000) µg/mL. The inhibitory effect of Honghuawas significantly lower than acarbose at 500 µg/mL (16.5 ± 5.7% vs. 81.3 ± 7.2%, P < 0.01). The inhibitory effect on α-amylase at 2:1 was significantly higher than that at 20:1, 10:1, and 5:1 at 500 µg/mL and 1000 µg/mL (P < 0.01), and 5:1 showed a significant higher inhibition effect compared with 20:1 and 10:1 at 1000 µg/mL (P < 0.01).

4. Discussion

Acarbose is the first hypoglycemic agent mainly used for controlling postprandial blood glucose and also the first α-glucosidase inhibitor approved by the United States Food and Drug Administration (FDA). It is considered to be more suitable for Chinese patients with abnormal glucose metabolism on a carbohydrate-dominated diet and has become one of the most common oral hypoglycemic agents used clinically in China [11]. The main effect of acarbose is to postpone the breakdown of disaccharides and oligosaccharides into glucose, thereby reducing postprandial blood glucose.

China was one of the first countries to recognize diabetes mellitus. The book “Huangdi Neijing,” written about 2,000 years ago, has systematically described diabetes mellitus and its complications. A variety of herbs and extracts have been clinically proven to be effective against diabetes mellitus [12]. Some progress has been made in the search for α-glucosidase inhibitors from traditional Chinese herbs [13]. This current study aimed to further this investigation.

The treatment of diabetes mellitus with traditional Chinese medicines attaches importance to treatment upon syndrome differentiation so as to provide a guide for clinical medication. According to some research, qi deficiency and blood stasis are the main causes and mechanism of diabetes [14]. The traditional Chinese medicines which can supplement qi, activate blood circulation, and remove blood stasis are considered to play an important role in the treatment of diabetes mellitus [3].

In traditional Chinese medicine, qi and blood, two essential substances for life activities, originate from the viscera and flow constantly inside the body. One role of qi is propelling. Qi can stimulate and maintain the physiological functions of the viscera and other organs [15]. Deficiency of qi in promotion will lead to reduced function and cause various deficiency problems. One class of Chinese medicines is called restoratives for invigorating qi, which can tonify the qi of the general body to strengthen the functional activity of the body. These restoratives are mainly used for symptoms due to qi deficiency. There are many herbs in this class, such as Radix Ginseng (Ren-shen), Radix Panacis Quinquefoli (Xiyangshen), and Huangqi [16]. Huangqi is the root of Astragalus membranaceus (Fisch.) Bunge var. mongolicus (Bunge) Hsiao and A. membranaceus (Fisch.) Bunge, family Leguminosae. It mainly grows in Inner Mongolia, Shanxi, Gansu, and Heilongjiang. Its main action is to replenish qi [16]. Some research reported that herbs with the action of “invigorating qi” are effective in increasing the content of the hepatic cell membrane insulin mediator and improving the sensitivity to serum insulin in diabetic rat models with spleen-qi deficiency syndrome [17]. Further research showed that high Huangqi dosages in Buzhong Yiqi decoction can improve the anaerobic oxidative metabolism and regulate the glucose level of rats with qi deficiency [18].

As qi acts directly to facilitate blood circulation, deficiency of qi makes it difficult for qi to propel the blood, eventually causing blood stasis [15]. Huangqi with the function of “replenishing qi” is always used with medicine with the function for “invigorating the blood and removing blood stasis,” such as Honghua [16]. Huangqi combined with Honghua has been used clinically to treat diabetes mellitus and its complications [4]. Honghua is from the flower of Carthamus tinctorius L., family Compositae, and medicinal material is mainly produced in the areas of Henan, Hubei, Sichuan, and Zhejiang [16]. It was reported that some serotonin derivatives found in the seed of Carthamus tinctorius L. acted as α-glucosidase inhibitors [19]. Our previous research showed that Honghua had an inhibitory effect on yeast α-glucosidase activity [20].

Clinical data confirm that acarbose can ameliorate the tendency to blood hypercoagulability in patients, and this study provides evidence that cardiovascular benefits can be obtained from the postprandial blood glucose [21]. Huangqi and Honghua are both commonly used clinically to supplement qi and activate blood circulation. In this study the effects of mixtures of different proportions of these two on α-glucosidase activity were observed using acarbose as a positive control.

Using 4-MUG as a reaction substrate, the product 4-MU obtained after hydrolysis of 4-MUG by yeast α-glucosidase was determined by fluorescence analysis. The results showed that acarbose inhibited yeast α-glucosidase activity within the concentration range of 0.3–2.5 µg/mL, with an IC$_{50}$ value of 1.8 ± 0.4 µg/mL. Both Huangqi and Honghua alone inhibited α-glucosidase activity; the IC$_{50}$ value of Honghuawas 32.8 ± 5.7 µg/mL, significantly lower than the IC$_{50}$ value of Huangqi of 1688 ± 810 µg/mL.

Studies have found that the glycohydrolase inhibitors from Chinese herbs tend to inhibit both α-glucosidase and α-amylase [13]. Studies showed that a coffee extract that had an inhibitory effect on α-amylase could inhibit the increase in blood glucose due to high starch load in rats, and the extract also had a strong inhibitory effect on α-glucosidase [22]. It was reported that acarbose could inhibit pancreatic α-amylase, but the inhibitory effect was relatively weak [23]. This study also showed an inhibitory effect of acarbose on porcine pancreatic α-amylase, with an IC$_{50}$ value of 227±32 µg/mL, which was significantly higher than that for inhibiting α-glucosidase, with a 126-fold difference between them. A similar result was obtained for Honghua: at the same active concentration of 125 µg/mL it showed 89.8 ± 5.6% inhibition of α-glucosidase, but only 5.8 ± 2.1% inhibition of α-amylase (Tables 2 and 3). Huanggidid not show any effect on α-amylase in the tested concentration range of 500–1000 µg/mL. In terms of simultaneously inhibiting α-glucosidase and α-amylase activity, Honghua, which activates blood circulation and removes blood stasis, seemed significantly better than Huangqi, which supplements qi.
Table 3: Inhibitory effect of Huangqi (Radix Astragali Mongolici) injection, Honghua (Flos Carthami) injection, and different proportions on α-amylase activity (X ± s).

| Group                | Concentration (μg/mL) | Percentage of inhibition (%) |
|----------------------|-----------------------|------------------------------|
| Acarbose             |                       |                              |
| 500                  | 81.3 ± 7.2            |                              |
| 250                  | 65.7 ± 7.1            |                              |
| 125                  | 32.1 ± 4.7            |                              |
| 62.5                 | 24.4 ± 1.9            |                              |
| Huangqi injection    | 1000                  | 0.9 ± 0.4                    |
| 500                  | 0.8 ± 0.5             |                              |
| Honghua injection    | 1000                  | 16.6 ± 1.4^c                 |
| 500                  | 16.5 ± 5.7^b          |                              |
| 250                  | 6.9 ± 1.3             |                              |
| 125                  | 5.8 ± 2.1             |                              |
| Different proportions|                       |                              |
| 20:1                 | 1000                  | 4.5 ± 1.7                    |
| 500                  | 0.7 ± 0.5             |                              |
| 10:1                 | 1000                  | 5.4 ± 1.8                    |
| 500                  | 0.8 ± 0.5             |                              |
| 5:1                  | 1000                  | 14.5 ± 3.2                   |
| 500                  | 2.1 ± 1.0             |                              |
| 2:1                  | 1000                  | 21.3 ± 4.6                   |
| 500                  | 10.2 ± 2.1            |                              |

Notes: ^cP < 0.01, Honghua injection compared with acarbose at 500 μg/mL. ^bP < 0.01, Honghua injection compared with Huangqi injection at 500 μg/mL. ^aP < 0.01, Honghua injection compared with Huangqi injection at 1000 μg/mL.

Studies have shown that the main components in traditional Chinese medicines for treating diabetes mellitus belong to four categories: saponins, flavonoids, polysaccharides, and alkaloids [24]. The saponin astragaloside IV in Huangqi injection and total flavonoids in Honghua injection were quantitatively monitored as the main active ingredients in the respective preparations. According to the national standards established by CFDA, Huangqi injection should contain no less than 0.08 mg of astragaloside IV per mL [7], and the content of astragaloside IV in this study was 0.127 mg/mL; Honghua injection should contain 0.20–0.70 mg of total flavonoids on the basis of kaempferol equivalents per mL [8], and the content in this study was 0.57 mg/mL. In this study, their proportions for raw herbs and injections in clinical applications were based on the ratios of their active ingredients (Table 1).

In clinical applications, proportions of Huangqian and Honghuainjection ranging from 4:1 to 10:1 on the basis of raw herbs have been reported [4–6]. In this study, we designed a wider range of preparations, that is, four groups of different proportions, 20:1, 10:1, 5:1, and 2:1. The results showed that the yield of the Honghuainjection extract was 10.44%, which was 10 times greater than the extraction yield of Huangqi, 1.04%, and the extraction yield of total flavonoids in Honghua injection was 20 times greater than that of astragaloside IV in Huangqi injection. This probably was one of the reasons why the clinical usage amount of Huangqi injection was greater than that of Honghua injection.

IC50s on α-glucosidase at 10:1, 5:1, and 2:1 were significantly lower than IC50 at 20:1 (P < 0.01). The inhibitory effect on α-amylase at 2:1 was significantly higher than that at 20:1, 10:1, and 5:1 at 500 μg/mL, 1000 μg/mL separately (P < 0.01) (Figures 1 and 2). Proportion 5:1 showed a significantly higher inhibitory effect compared with 20:1 and 10:1 at 1000 μg/mL (P < 0.01) (Figure 2). In terms of simultaneously improving the above two indicators, the raw herbs proportions of 2:1 and 5:1 exhibited significant effects compared to those of 20:1.

In this study, the four groups of different proportions were superior to Huangqianonebut inferior to Honghua alone for the inhibition of α-glucosidase activity. The combination of Huangqi and Honghua injections was reported to be more effective than Honghua injection alone for inhibiting apoptosis of nerve cells around a cerebral hemorrhage in rats [25]. There did not appear to be a synergistic effect in this research. In vivo experiments may be required to better understand and optimize the herbal formulation.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.
Acknowledgment

This paper was supported by Shanxi Scholarship Council of China (2010-102).

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