

Anti-proliferative effect of astragalosides on human gastric cancer SGC-7901 cells.

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Abstract

To investigate the process conditions for extraction, purification and enrichment of astragalosides with macroporous resin and study its growth inhibitory effect on human gastric cancer SGC-7901 cells. Astragaloside adsorption and desorption capacities of macroporous resins were determined by UV spectrophotometry, and growth inhibitory effect of astragalosides on SGC-7901 cells was measured by MTT assay. Dynamic adsorption performance of D101 adsorption resin was investigated, and dynamic adsorption curve of astragalosides on D101 resin column was plotted. Meanwhile, eluent and elution flow rate were investigated, which revealed that the choice of 80% ethanol as the eluent and a flow rate of 2BV/h could maximize the yield of astragalosides. MTT assay found that astragalosides had a marked anti-proliferative effect on SGC-7901 cells, which strengthened with increasing dose and medication time, showing dose- and time-dependence. D101 resin can effectively enrich astragalosides; astragalosides have a growth inhibitory effect on SGC-7901 cells.

Keywords: Astragaloside; D101 adsorption resin; SGC-7901.

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Introduction

Astragalus membranaceus is the dried root of leguminous plant *Astragalus membranaceus* (Fisch.) Bge. var. *mongholicus* (Bge.) Hsiao or *Astragalus membranaceus* (Fisch.) Bge., which was originally recorded in the Shen Nong's Herbal Classic. As an important qi-benefiting traditional Chinese medicine, it is warm, sweet, and has qi-replenishing, superficies-strengthening, diuresis-inducing, toxin-expelling, sore-astringing, tissue regeneration-promoting and middle warmer-tonifying actions [1-3].

Domestic and foreign scholars have conducted extensive research on *Astragalus membranaceus*, who have confirmed that the major chemical constituents of *Astragalus membranaceus* are polysaccharides, triterpenoid saponins and flavonoids. Besides, it also contains monosaccharides, amino acids, linoleic acid, palmitic acid and linolenic acid. Sufficient studies have demonstrated that *Astragalus membranaceus* also contains protein, riboflavin, folic acid, vitamins, vanillic acid, ferulic acid, isoferulic acid, p-hydroxyphenyl acrylic acid, caffeic acid, chlorogenic acid, daucosterol, lupeol, n-hexadecanol and trace elements [4-7].

Modern pharmacological studies have shown that *Astragalus membranaceus* has anti-tumor, immunoregulatory, anti-viral, anti-aging, anti-oxidant, anti-radiation and anti-stress effects, which has been widely used in areas like medicine and agriculture. Owing to multi-target, multi-link and multi-effect characteristics of *Astragalus membranaceus*, it plays important roles in suppressing and killing tumor cells, improving

symptoms and signs, mitigating adverse chemoradiotherapeutic effects and prolonging patients' survival [8-10]. Meanwhile, *Astragalus membranaceus* can also improve immunity, has small toxic and side effects, and hardly produces drug resistance, which has gradually become the hot topic and focus in clinical immunity enhancement and tumor activity suppression in recent years [11-13]. This study investigates the resin purification process of astragalosides, and explores its inhibitory effect on human gastric cancer cells, in order to lay the foundation for large-scale industrial production and clinical application of *Astragalus membranaceus*.

Materials

Instruments and reagents

UV-2401 UV-Vis spectrophotometer (Shimadzu, Japan); CO-150 CO₂ incubator (NBS, USA); SW-CJ-2F clean bench (Suzhou Purification Equipment Factory); RPMI 1640 medium (Gibco, USA); MTT (Beijing Solarbio Science & Technology Co., Ltd); DMSO, trypsin, and PI (Sigma, USA). Reagents were all of analytical grade.

Cells and drugs

Human gastric cancer SGC-7901 cells were purchased from China Medical University; *Astragalus membranaceus* was purchased from pharmacy; and astragalosides, with a purity of 56.78%, was self-extracted.

Methods and Results

Resin pretreatment

Resin was washed with distilled water, and soaked in 95% ethanol for 24 h. After sufficiently swollen, the resin was loaded onto column by wet method, and flow washed with 95% ethanol till the effluent had similar absorbance with 95% ethanol by UV spectrometry. Then, the resin was washed with distilled water until no ethanol was smelt, and set aside.

Preparation of Astragalus membranaceus sample solution

1 kg of crude Astragalus membranaceus powder was weighed, and extracted for 2 h twice with 80% ethanol. After filtration, extracts were combined, and ethanol was removed until no ethanol was smelt. The concentrate was then dissolved in distilled water, filtered, and diluted to 1,000 ml to give Astragalus membranaceus sample solution.

Determination of total astragaloside content

2.5 mg of astragaloside standard substance was accurately weighed, and diluted to 10 ml. 0.1, 0.2, 0.3, 0.4, 0.5, 0.6 and 0.7 ml of the solutions were drawn, respectively, added precisely with anhydrous ethanol to 0.5 ml, and then with 0.5 ml of 8% vanillin anhydrous ethanol, shaken well, placed in an ice bath, and added with 5 ml of sulfuric acid test solution (volume fraction of 72%). After shaken well, the solutions were kept warm in 60water bath for 20 min, removed, shaken well, and cooled to room temperature. Within 30 min, absorbance was measured at 540 nm by spectrophotometry, standard curve was plotted with absorbance as the ordinate and mass concentration as the abscissa, and the resulting linear regression equation was $y=0.0288x-0.0231$ ($r=0.9993$).

Resin type selection

Practicality of different types of resins was investigated by dynamic adsorption method. Approximately 5 g of different types of resins were accurately weighed, and loaded onto column by wet method. 0.858 mg/ml total astragaloside sample solutions were slowly added to different resin columns while controlling volume flow rate at 1.5 ml/min. Fraction was collected once per 10 ml, astragalosides in ethanol elution fraction were measured, and dynamic astragaloside adsorption performances of various model resins were determined.

Dynamic adsorption results showed that the astragaloside adsorption capacity and elution rate of D101 macroporous resin reached 27.2 mg/g and 93.3%, respectively, both of which were superior to the other four resin types. Thus, D101 macroporous resin was selected for enrichment and purification of astragalosides, as shown in Figure 1.

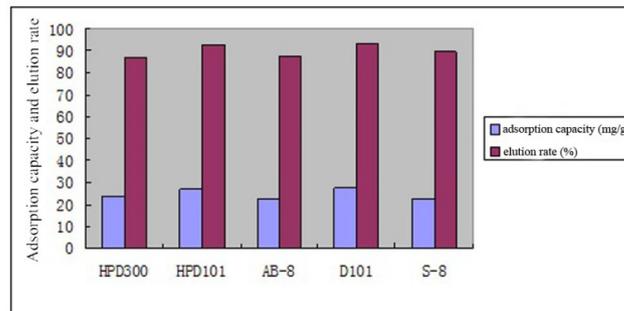


Figure 1: Comparison of astragaloside adsorption performance between various macroporous resins.

Investigation on enrichment and purification processes of D101 macroporous resin

Influence of adsorption rate on adsorption efficiency: 0.858 mg/mL total astragaloside sample solutions were taken, whose outflow rates were controlled at 1, 2, 3 and 4BV/h. The samples were eluted with 5BV of water first, and then desorbed with 5BV of 75% ethanol. Ethanol desorption solutions were collected, respectively, for determination of total astragaloside contents in desorption solutions, followed by calculation of desorption rates, as shown in Figure 2.

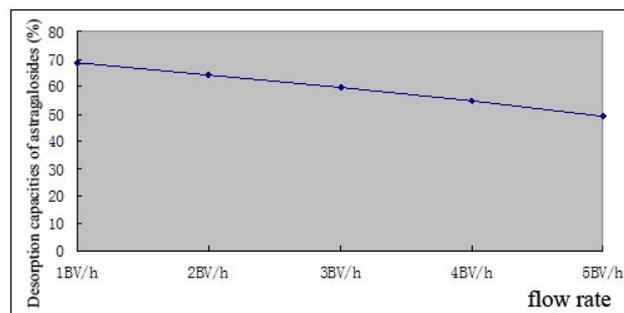


Figure 2: Influence of different adsorption rates on adsorption efficiency.

As can be seen from the experimental results, different flow rates have a marked influence on the adsorption results of astragalosides. With the increasing flow rate, astragaloside adsorption efficiency of D101 macroporous resin decreased gradually, which dropped to 48.93% at a 5BV/h flow rate. Based on actual production conditions and comprehensively considering various factors, the flow rate was set as 2BV/h.

Influence of different volume fractions of ethanol on astragaloside extraction efficiency: 20 g of pretreated D101 macroporous resin was loaded onto column, and then 20 ml of 0.858 mg/ml astragaloside sample solution was injected into the column. 5 BV of 10%, 30%, 50%, 70%, 80% and 95% ethanol were used for elution of astragalosides, respectively, at a 2BV/h flow rate. Total astragaloside content was determined as per the method in 3.3., as shown in Figure 3.

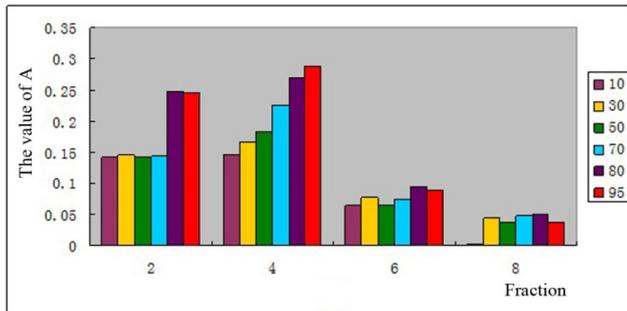


Figure 3: Influence of different concentrations of ethanol on astragaloside extraction efficiency.

With increasing concentration of ethanol, astragaloside desorption efficiency also increased. Total astragaloside

Table 1: Influence of different eluent volumes on total astragaloside content.

Ethanol volume	Total astragaloside content (mg/10 ml)	Cumulative percentage content (%)
1 BV/h	5.98	63.74
2 BV/h	3.45	89.65
3 BV/h	1.48	96.48
4 BV/h	0.34	99.87
5 BV/h	-	-

Data showed that when 3BV of ethanol was used to elute 1.48 mg/10 ml astragalosides, cumulative percentage content was 96.48%, which basically reached the elution end, as shown in Table 1.

Cell cultivation

Gastric cancer SGC-7901 cells were cultured statically in a 37, 5% CO₂ incubator with RPMI1640 medium containing 10% FBS, penicillin (100 ml/L) and 100 ku/L penicillin and streptomycin (1 mg/ml).

Well growing exponential phase cells were used in the experiment. The monolayer cultured SGC7901 cells were digested with 0.25% trypsin, prepared into a 1×10⁵/ml cell suspension, seeded in a 96-well plate at 1×10⁴ cells per well, and treated by adding astragalosides (concentrations of 5, 10 and 20 μmol/L). 72 h later, 20 μl of MTT solution (5 mg/ml) was added to each well, and the incubation was continued for an additional 4 h. After discarding the supernatant, each well was added with 150 μl of DMSO, shaken on horizontal shaker for 10 min, and then a value was measured at 490 nm with ELISA reader. The experiment was repeated three times, and the growth inhibition (CI) rate was calculated.

$$CI = \frac{(A \text{ value of negative control group} - A \text{ value of treatment group})}{A \text{ value of negative control group}} \times 100\%$$

The results showed that different concentrations of astragalosides had marked anti-proliferative effects on gastric cancer SGC7901 cells, which were enhanced with increasing

content reached its highest in the 4th fraction, and A value of total astragaloside content reached 0.287 when eluted with 95% ethanol. 80% ethanol had enrichment efficiency slightly worse than 95% ethanol, but no statistical difference was noted. After comprehensive consideration, 80% ethanol was selected as the optimal enrichment concentration for total astragaloside content.

Determination of optimal elution volume: 10 ml of the above astragaloside solution was accurately weighed, absorbed by D101 macroporous resin column for 30 min, then washed with 4BV of distilled water, and later eluted with 5BV of 80% ethanol at a flow rate of 2BV/h. With 1BV as one fraction, a total of 5 fractions were collected.

astragaloside dose and prolonging treatment time, showing dose- and time-dependence. The differences were significant between varying astragaloside dose groups and untreated group (P<0.05), as shown in Figure 4.

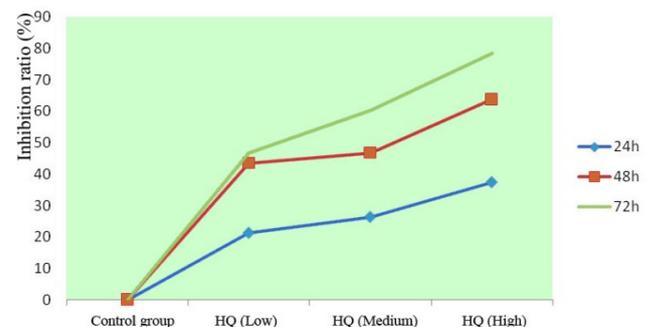


Figure 4: Anti-proliferative effect of astragalosides on gastric cancer SGC7901 cells.

Discussion

With the increase of social pressure and the changes in living environment and lifestyle, tumor is increasingly becoming a common disease seriously harming human health. According to relevant statistics, there are 1.6 million new cases of cancer, 1.3 million deaths and more than 2 million active cancer patients in China annually, which has brought heavy disasters and losses to families and society. Gastric cancer, as a

malignancy with high incidence, high metastasis and high mortality, has a tremendous impact on people's lives.

Currently, radiotherapy and chemotherapy are considerably effective for cancer treatment, but they have large side effects, which seriously affect the patients' quality of life. In comparison, Chinese medicine has its unique advantage in improving the quality of life of patients. Characterized by low toxicity and high efficiency, Chinese medicine differs from the awkward situation of Western medicine where single drug corresponds to single disease. In the treatment process of diseases, Chinese medicine can reasonably combine traditional Chinese drugs through syndrome differentiation from a holistic approach to achieve better outcomes. Combined with radiotherapy and chemotherapy, traditional Chinese medicine can achieve toxicity attenuating and efficacy enhancing effects, not only inhibiting tumors, but also improving immunity. Active exploration of effective Chinese medical treatment regimens has important clinical significance to improving the quality of life for patients with advanced gastric cancer and prolonging their survival.

Cao Liping et al.'s [14] study showed that many tumor cells can increase the synthesis of prostaglandins, especially PGE₂, both in vitro and in vivo. Excessively increased PGE₂ has the ability to promote tumor cell proliferation, migration and tumor angiogenesis and inhibit tumor cell apoptosis and host immunity, which is thus closely associated with tumorigenesis, development and metastasis. Chinese herbal drug *Astragalus membranaceus* can effectively inhibit PGE₂, whose inhibitory effect is stronger than the chemical drug celecoxib. Besides, *Astragalus membranaceus* can also prevent the occurrence of tumor immune escape, and thereby prevent the tumorigenesis through inhibiting COX-2 expression and reducing PGE₂-induced inflammation and immune responses.

Conclusion

This study investigates the process conditions for extraction, purification and enrichment of astragalosides with different macroporous resin types. The results show that D101 macroporous resin has astragaloside adsorption capacity and elution rate of 27.2 mg/g and 93.3%, respectively, both of which are superior to the other four resin types. Astragaloside adsorption results at different flow rates reveal that with the increase in flow rate, astragaloside adsorption efficiency of D101 macroporous resin decreases gradually, which drops to 48.93% at a 5BV/h flow rate. Based on actual production conditions and comprehensively considering various factors, the flow rate is set as 2BV/h. In the investigation of extraction solvent concentration, with the increasing concentration of ethanol, astragaloside desorption efficiency also increases. A value of total astragaloside content reaches maximum when eluted with 95% ethanol. 80% ethanol has an enrichment efficiency slightly worse than 95% ethanol, but no statistical difference is noted. After comprehensive consideration, 80% ethanol is selected as the optimal enrichment concentration for total astragaloside content.

MTT results demonstrate that different concentrations of astragalosides have marked anti-proliferative effects on gastric cancer SGC-7901 cells, which strengthen with increasing dose and medication time, showing dose- and time-dependence.

Conflict of Interests

The authors declare that they have no conflict of interests.

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