

A COMPARATIVE STUDY OF ANTI-GASTRIC CANCER ACTIVITY BETWEEN AQUEOUS EXTRACT AND ETHANOL EXTRACT OF *FOLIUM CORDYLINES FRUTICOSAE*

Shaojun Liu¹, Dongbo Cao², Zhiming Xiao¹, Fen Liu¹, Xiaoyan Wang¹, Lian Zhao¹, Li Tian¹,
Shourong Shen^{1*}

¹Department of Digestion Medicine, The Third Affiliated Hospital of Xiang Ya School of Medicine, Central South University, Changsha 410078, Hunan, People's Republic of China

²Department of physiotherapy pain, The First Affiliated Hospital of Hunan university of Chinese medicine, Changsha 410007, Hunan, People's Republic of China.

* E-mail: shenshourong2003@163.com

Abstract

The active components in *Folium Cordylines Fruticosae* were extracted by heat reflux method. The solvents used were distilled water and ethanol. The effects of two types of extracts on gastric cancer cells were compared; dry extract yields were calculated, as well as the inhibition rates of gastric cancer MGC-803 cell proliferation and the colony cell counts. The micro-Kjeldahl method was used to measure the cell protein contents and to make a comprehensive comparison. The results showed that the MGC-803 cell inhibition rates of three different concentrations (32.5, 75 and 150 mg/ml) of ethanol extracts increased with the increase of concentration, which was 48.9% at a concentration of 150 mg/ml; aqueous extract of *Folium Cordylines Fruticosae* had very low inhibitory activity at a low concentration (32.5 mg/ml), which was remained at about 20%. After being affected by two types of extracts, cells had uneven sizes, with very low brightness, while the normal cells presented a uniform full form, with high definition.

Keywords: *Folium Cordylines Fruticosae* Anti-gastric Cancer MGC-803 cell

Introduction

Tie Shu Ye is a plant in the genus *Cycas* in family *Cycadaceae*; its Latin name is *Folium Cordylines Fruticosae*, and it is the leaf of *Cordyline fruticosa* (L.) A. Cheval. (*Agavaceae*). It has good analgesic and anti-cancer activities. So far, there have been few studies on *Folium Cordylines Fruticosae* at home and abroad; therefore, the study of its inhibitory activity and mechanism against gastric cancer MGC-803 cells are a new subject. MGC-803 cells, as a poorly differentiated gastric cancer cell line, has higher tendency to metastasis: the purpose of anti-metastasis of gastric cancer. Chinese medicine Qige Fang can inhibit the activity of MMP-2 and MMP-9, block matrix degradation by MMP-2 and MMP-9, thereby preventing tumour metastasis (Liu et al., 2003) ; Cai's Fuzheng Xiaozheng Decoction can enhance the adhesion between tumour cells, and inhibit the adhesion between tumour cells and endothelial cells, thus achieving the tumour metastasis inhibiting purpose.

Materials and Methods

CO₂ incubator, SANYO; DS-200X inverted microscope, Beijing Cnrico Technology Co., Ltd.; microplate reader, Thermo; SW-CJ-1FD clean bench, Shanghai Boxun Industry & Commerce Co., Ltd; MTT, SIGMA; foetal bovine serum, Hangzhou Sijiqing Bioengineering Co., Ltd.; PRMI1640 and trypsin, GIBCO; reagents were all of analytical grade.

Preparation of drugs

Folium cordylines fruticosae used in this study was purchased from Tong Ren Tang Pharmacy, which was identified as authentic by the Xiang Ya School of Medicine, Central South University. Preparation of aqueous extract: 20g of *Folium cordylines fruticosae* was weighed in duplicate, soaked in 10-fold volume of distilled water for 1 h, and then extracted by heat reflux extraction. The herb was heat reflux extracted three times with 10-fold volume of distilled water respectively, three filtrates were combined, and were then filtered successively using coarse filter paper and 0.8 μm filter paper. The filtrate was concentrated to dry extract, and the yield of dry extract was calculated. After that, the extract was prepared into 150 mg/ml drug solution for later use. Method for obtaining ethanol extract was the same as the aqueous extraction, except that the solvent was changed to ethanol. The dry extract yield was calculated in the same way.

Cell cultivation

Poorly differentiated gastric cancer MGC-803 cells were purchased from the Shanghai Cell Bank of Chinese Academy of Sciences. The cells were placed in RPMI1640 complete medium (containing 10% FBS), and cultured routinely in a CO₂ incubator set at 37 °C, 5% CO₂ with saturated humidity.

Determination of cell proliferation inhibitory activity (Huang et al., 2012)

Cells in the logarithmic phase were collected, seeded in 96-well plates at 1×10^4 cells/well, and cultured for 24 h under conventional conditions. The status of cells was observed under the inverted microscope. Then the negative control, positive control and sample groups were set up, and sample was added. In addition, gradient groups consisting of three concentrations were set up for the sample group by doubling dilution method. At the same time, four different times of 12, 24, 36 and 48 h were set up to determine the inhibition rates. After each well was added with 10 μl of drug and cultured for 24 h, the cells were removed from the incubator and processed successively according to the MTT assay. Then OD values were measured at 570 nm, and inhibition rates were calculated by the average OD value. Inhibition rate = $1 - \text{average OD value of the sample group} / \text{average OD value of the control group}$. The results are shown in Table 1, 2 and Figure 1, 2.

Results

Dry extract yield (Tang et al., 2012)

After the dry paste obtained by aqueous extraction was dried in water bath, its weight was 14.1g. So, the dry extract yield was calculated to be 70.5% according to the formula in the Methods section. After the dry paste obtained by ethanol extraction was dried in water bath, its weight was 10.2g; therefore, the dry extract yield was calculated to be 51.0% according to the formula in the Methods section.

Activity assay results

Results for the inhibitory activity of ethanol extract

As can be seen from Figure 1 and Table 1, the inhibition rate of ethanol extract gradually increased between 12~36 h, regardless of the concentration; at 36 h and 150 mg/ml, the maximum inhibition rate was 48.9%. Furthermore, inhibition rate was found to be directly proportional to the concentration; however, between 36~48 h, the inhibition rate gradually decreased to the level near the value at 12 h.

<http://dx.doi.org/10.4314/ajtcam.v10i4.13>

Table 1: Inhibition rate of ethanol extract against MGC-803 cells at different times

Time(h)	Concentration (mg/ml)		
	150	75	32.5
12	23.4	19.4	17.3
24	35.2	25.8	22.1
36	48.9	36.7	25.6
48	33.1	20.7	18.2

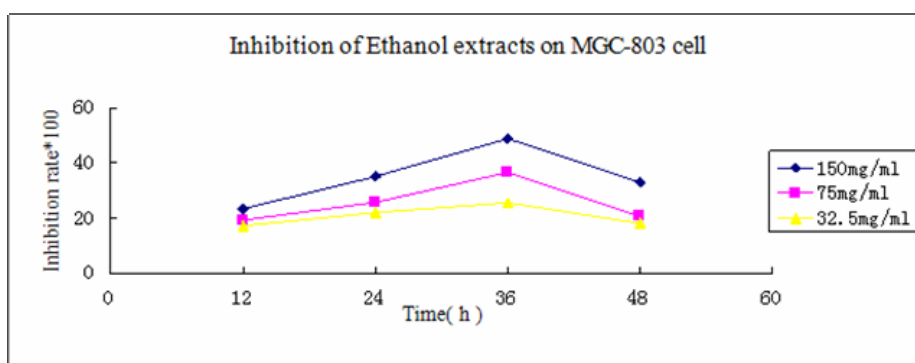


Figure 1: Diagram of inhibition rate of ethanol extract against MGC-803 cells at different times

Results for the inhibitory activity of aqueous extract

As can be seen from Figure 2 and Table 2, the inhibition rate reached 56.1% at 150 mg/ml and 36 h, which was higher than that of the ethanol extract group. At a concentration of 32.5 mg/ml, inhibition rate did not change over time, which fluctuated in the range of 18~20%.

Table 2: Inhibition rate of aqueous extract against MGC-803 cells at different times

Time(h)	Concentration (mg/ml)		
	150	75	32.5
12	29.3	22.5	18.2
24	38.7	28.1	18.5
36	56.1	38.9	19.1
48	35.4	28.3	16.4

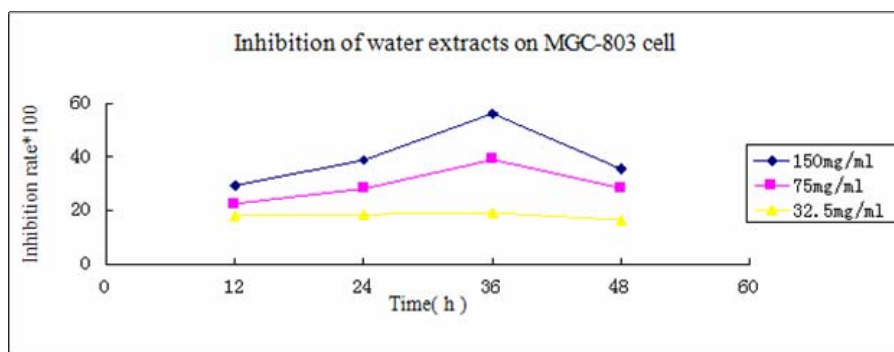


Figure 2: Diagram of inhibition rate of aqueous extract against MGC-803 cells at different times

Cell morphology, live & dead cell counts (Sun et al., 2001)

Cell count of the aqueous extract group was 83; ethanol extract group was 62, while the control group was 153. Therefore, the inhibition rate of aqueous extract group was 46.8%; inhibition rate of ethanol extract group was 59.5%, as shown in Table 3.

Table 3: MGC-803 cell morphology and live & death cell counts

	Aqueous extract group	Ethanol extract group	Control group
Cell morphology	Uneven sizes, very low brightness	Uneven sizes, low brightness	Uniform and full, clearly visible
Live cells	39	32	85
Dead cells	9	7	3

Discussion

The metastasis of gastric cancer must meet two conditions: penetration of basement membrane by gastric cancer cells and tumour angiogenesis, while the extracellular matrix degradation effect of matrix metalloproteinases (MMPs) creates the conditions for the gastric cancer cells to pass through the basement membrane and invade adjacent or distant tissues and organs (Xu & Zhou.,2011). In this paper, two types of extracts were obtained by heat reflux method separately using water and ethanol, and differences between them were compared by testing their anticancer activity on gastric cancer MGC-803 cells. MTT assay results showed that the anticancer activity of aqueous extract was slightly higher than that of the ethanol extract, indicating that the aqueous extract may contain some active component(s). The difference between the two extracts was further illustrated by the number of colonies and the number of live and dead cells.

At present, classical chemotherapy drugs used for gastric cancer include 5-fluorouracil, mitomycin, cisplatin, etc. Clinical practices have found that some patients tolerated the chemotherapy drugs; therefore, Chinese medicine adjuvant therapy seems to be a good choice (Dong., 2012) Morphine, as an analgesic, also has a good inhibitory effect on gastric cancer cells. The mechanism of morphine inhibiting gastric cancer progression in vitro might be associated with activation of caspase-9 and caspase-3 and inhibition of surviving and NF- κ B (Yi et al., 2012). Some researchers also produced monoclonal antibodies (McAb) – biological missiles against gastric cancer cells. This research area has good prospects. While for the exploration of the reversal of malignant phenotype of gastric cancer cells by inducers, from the standpoint of developmental biology, cancer is the cell differentiation disorders, which means that the tumour cells can be induced to differentiate to

normal mature cells.

There have been a number of studies on the inhibition of MGC-803 cells by Chinese medicines. Some studies used the cell affinity chromatography to study the biological affinity between bufadienolides (Bu) and tumour cells, and analysed its correlation with the anti-tumour activity. These studies found that the Bu had varying degrees of affinity with MGC-803, and the degree of affinity and affinity rate of tumour cells or cell lysates were significantly correlated with the anti-tumour activity (Li et al., 2006; Jiang et al., 2011; Yi et al., 2012). Another study used fluorescence spectrophotometry to demonstrate that other Chinese patent medicines, including Jianpi Bushen Prescription and Yangwei Kangliu Granules, also had very good inhibitory effect on MGC-803 cells (Tang et al., 2005). 2', 4'-Dihydroxychalcone (TFC), a main component in Herba Oxytropis, is grouped under flavonoids, and it significantly increases caspase-3 activity but decreases surviving mRNA expression (Hu et al., 2010). The above examples all indicate that effective new drug lead compounds are likely to be found from Chinese medicines which inhibit MGC-803 gastric cancer cells.

References

1. Dong Chun-lu. (2012). Efficacy and Safety of Intraoperative Mesenchymal Chemotherapy with 5-Fluorouracil Release Formulation in Radical Gastrectomy of Advanced Gastric Cancer . Lanzhou University. 34-45.
2. Huang Lu-fen, Huang Xiu-rong, Lin Yao. (2012). Research Progress on Cell Proliferation Inhibition Mechanism of Resveratrol . Contemporary Medicine, **24**: 13-14.
3. Hu An-la, Li Li, Hu Chuan-lai, Bian Jian, Bo Qing-li, Ruan Liang. (2010). Effect of theanine on in vitro proliferation of human gastric cancer MGC803 cells and its combined effects with adriamycin. Acta Nutrimenta Sinica, **01**: 60-63.
4. Jiang Jie-jun, You Fen-qiang, Ma Hong-yue, Zhou Jing, Zhang Jun-feng, Zhan Zhen, Tang Yu-ping, Duan Jin-ao, Ding An-wei. (2011). Cell continuous extraction-HPLC determination of biological affinity of 8 bufadienolides on MGC-803 cells and their correlation with anti-tumor activities . China Journal of Chinese Materia Medica, **02**: 205-208.
5. Li Jie, Sun Gui-zhi, Lin Hong-sheng, Pei Ying-xia, Qi Xin, Hua Bao-jin. (2006). Effects of yangwei kangliu granule and its disassembled prescription on the invasion potency of gastric cancer cell lines MGC-803/BGC823 to reconstitute basement membrane and the mechanism . Chinese Journal of Clinical Rehabilitation, **35**: 126-129.
6. Liu Ya-xian, Li Ji-jun, Fu Xiao-mei, et al., (2003). Experimental Study on Induction of Apoptosis by Qige Fang in Leucocythemia Cell Line K562. Chinese Journal of Information on Traditional Chinese Medicine, **10 (10)**: 24-26.
7. Sun Ling-ling, Bi Fu-yong, Ling Lie-feng, Shi Bi-zhi.(2001). Inhibiting Effect of Sago Cycas Leaves on Proliferation of Leukemia K₅₆₂ and HL-60 Cells. Jiujiang Medical Journal, **04**: 192-193.
8. Tang Xiao-po, Sun Gui-zhi, Pei Ying-xia, Qi Xin, Guo Jian-you, Li Jie.(2005). Effect of Jianpi Bushen Formulation on the Level of Mitochondrial Membrane Potential and Endocellular Reactive Oxygen Species in MGC-803 Cells . Chinese Journal of Experimental Traditional Medical Formulae, **06**: 63-66.
9. Tang Wan-he, Sun Yi-na, Zhao Lei, Tang Si-si, Wang Hong-rong, Zhou Yong, Yu Zhi-yong, Cai Ang, Wang Xi. (2012). Effect of Yangqi decoction patch on apoptosis of gastric cancer cells in animal model. Journal of Hubei University of Chinese Medicine, **05**: 24-26.
10. Xu Wen-jie, Zhou Jian-jiang. (2011). Research Progress of Gastric Cancer Metastasis. Chinese Journal of Clinicians (Electronic Edition), **01**: 170-173.
11. Yi Qin&, Jing Chen&, Li Li &, Chun-Jie Liao, Yu-Bing Liang, En-Jian Guan, Yu-Bo Xie. (2012). Exogenous Morphine Inhibits Human Gastric Cancer MGC-803 Cell Growth by Cell Cycle Arrest and Apoptosis Induction. Asian Pacific Journal of Cancer Prevention, **13**:1377-1382.