Iranian Red Crescent Medical Journal

ORIGINAL ARTICLE

Antitumor Effects of Ethanolic Extracts from *Sophora moorcroftiana* Seeds in Mice

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Abstract

Background: *Sophora moorcroftiana* (Wallich) is an endemic shrub species in Tibet, China, and is a plant with a great value in folk medicine. The previous studies had shown that the ethanolic (95%) extracts were much more effective than other extracts from Sophora moorcroftiana seeds on antiproliferative effects, inducing apoptosis in the human stomach cancer SGC-7901 cell line *in vitro*.

Methods: To investigate the antitumor activity of the ethanolic extracts from *Sophora moorcroftiana* seeds, various doses of ethanolic extracts (200 mg/kg/d, 400 mg/kg/d, 800 mg/Kg/d) were administered via gavage to tumor-bearing mice with S_{180} sarcoma cell for ten days. The weights of tumor tissue stripped from the left flank, TNF-α and IL-2 in blood-serum were tested and analyzed for photochemical composition, using standard procedures.

Results: The weight of tumor tissue was significantly decreased upon the treatment with ethanol extracts, but the decrease was more prominent in the group receiving ethanol extracts treatment at 800 mg/kg/d (1.35±0.21mg) and the inhibition rate on the growth of tumor tissue was higher (28.1%). The structural changes of post-treatment in the tumor tissue by the ethanolic extracts at a dose of 800 mg/kg/d showed larger areas of necrosis and more significantly invaded lymphocytes. IL-2 and TNF-α in serum from the treated mice significantly increased in ethanolic extract-treated groups, compared with the untreated control animals.

Conclusion: This suggested that the ethanolic extracts from *Sophora moorcroftiana* seeds had a weak antitumor role and in high concentration to tumor-bearing mice *in vivo*, the 95% ethanolic extracts was rather effective.

Keywords: *Sophora moorcroftiana*; Sarcoma; Cytokine; Ethnobotany; Therapeutics

Introduction

Medicinal plants play a key role in human health care. About 80% of the world population rely on the use of traditional medicine, which is predominantly based on plant materials.[1] Scientific studies indicate that the promising phytochemicals can be developed from the medicinal plants for many health problems.[2] Moreover, the herbal drugs have gained importance in recent years because of their efficacy and cost effectiveness. These drugs are invariably single plant extracts or fractions thereof or mixtures of fractions/extracts from different plants, which have been carefully standardized for their safety and efficacy.[2]

Sophora moorcroftiana (Wallich) Benth ex Baker is an endemic shrub species in Tibet, China, and is mainly distributed in the wide valleys and the middle reaches of several main tributaries of Yalu Tsangbo River (Nianchu and Lhasa Rivers).[3] *Sophora moorcroftiana* seeds have been used for a long time in Chinese folk medicine. The decoction of the seeds is used in Chinese folk medicine for dephlogistication, detoxication, emetic, infectious diseases and verminosis.[3] Alkaloids from *Sophora moorcroftiana* have a protoscolicidal, anti-inflammatory effect[4] and induce apoptosis of human stomach cancer cell line SGC-7901

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Received: September 6, 2008 Accepted: November 6, 2008

in vitro.[3] The sophorastilbene A and (+)- α -viniferin from *Sophora moorcroftiana* inhibited copper-induced protein oxidative modification in vitro.[5] Prenylflavanones from *Sophora tomentosa* and *Sophora moorcroftiana* show tumor-specific cytotoxic activity and antimicrobial activity.[6]

We have previously examined the antimicrobial activity and cytotoxicity of chloroform, 95% alcohol, 75% alcohol, and water extracts from *Sophora moorcroftiana* seeds on human stomach cancer cells in vitro.[7] The most interesting discovery of the study was that the 95% ethanolic extracts is much more effective than other extracts of *Sophora moorcroftiana* seeds against human stomach cancer cell line SGC-7901 and can induce apoptosis of human stomach cancer cell line SGC-7901 in vitro.[7] However, the antitumor effects in vivo of the ethanolic extracts from *Sophora moorcroftiana* seeds have not been studied. Hence we investigated the anticancer activity and pertinent mechanisms of the ethanolic extracts in mice and report the results of our study in this paper.

Material and Methods

The 95% ethanolic extracts were extracted from *Sophora moorcroftiana* seeds by our laboratory as described previously.[7] The 95% ethanolic extracts were tested for the presence of carbohydrates, proteins, amino acids, alkaloids, flavonoids, glycosides, saponins, quinones, fats and oils, using standard procedures.[8] *Sophora moorcroftiana* ethanolic extracts were diluted with distilled water into the required concentrations.

Cyclophosphamide (Cy) was purchased from Shanghai Huanlian Pharmaccuticals Limited Company in China. RPMI 1640 medium, fetal calf serum and dimethyl solfoxide (DMSO) were obtained from Sino-American Biotechnology Company in China. An IL-2 and TNF-α radioimmunoassay kit were purchased from Beijing North Institute of Biotechnology, Beijing, China.

Murine S_{180} sarcoma cell line was obtained from the Medical Experiment Center of Lanzhou University, in China. S_{180} sarcoma cells were maintained in RPMI 1640 medium, being supplemented with heat inactivated 10% fetal calf serum. Normal inbred female Kunming mice were purchased from the Medical Experiment Center of Lanzhou University, in China. All the mice, at 6 weeks of age (20±2 g in body weight), were housed in a standard condition with food and water ad libitum.

The method has been described by Indap et al.[9] All mice were inoculated subcutaneously in the left flank with 5 x 10^6 viable S_{180} sarcoma cells in 0.2 ml saline water. After 3 days, the tumor-bearing mice were divided into 5 groups of eight mice each. Mice in 3 groups were treated by intragastric gavage (ig) with the ethanolic extracts in 0.2 ml distilled water at 200 mg/kg/d, 400 mg/kg/d and 800 mg/kg/d, respectively. The administration of cyclophosphamide singly at a dose of 20 mg/kg/d, as a positive control group was initiated (day 1) and continued until days 5 and 9. The negative control group was given the same volume of 0.2 ml distilled water by the same route. The body weights of both control and treated animals were recorded on days 1, 3, 5, 7 and 10 to determine the toxicity of the ethanolic extracts from *Sophora moorcroftiana* seeds.[9] After a 10-day treatment period, all the tumor-bearing mice were anesthetized with pentobarbital sodium in accordance with the institutional guidelines, then blood samples were collected from eyeballs, and the tumor tissue was stripped from the left flank. The weight of the tumor tissue was measured immediately to examine the S_{180} sarcoma cell growth, the inhibition ratio was calculated as described in the literature,[10] and then the tumor tissue was fixed in 10% formalin for several days, being sliced and stained with hematoxylin-eosin (HE).[10] IL-2 and TNF-α in the serum were measured by radioimmunoassay for determination of anticancer mechanisms via immunoregulation efficacy.

Data were expressed as Mean±SD. Statistical significance was determined by variance analysis and one-way ANOVA with SPSS (Statistical Package for the Social Sciences, 10.0) for Windows (SPSS Incorporation in Chicago, IL, USA).

Results

The 95% ethanolic extracts showed the presence of 3.1% carbohydrates, 1.6% alkaloids, 4.9% proteins, amino acids, quinones, fats and oils, which also showed the inexistence of flavonoids and saponins.

The body weight of all animals increased during the treatment days. Especially, the body weight of the negative control group was higher than that of the other group. The in vivo efficacy was determined by measuring the weights of tumor tissue from tumor-bearing mice. The weight of tumor tissue significantly decreased upon the treatment with each drug. The ethanolic extracts, at a dose of 800 mg/kg/d,

significantly decreased the tumor tissue growth compared with that in the untreated mice, although the efficacy was still not as good as the cyclophosphamide at the standard dose rate (20 mg/kg/d) (Table 1).

Microscopically, the tumor tissue from the control mice, which had been treated with distilled water for 10 days, showed no alteration in their structure (Figure 1A). However, the structural changes of post-treatment

Table 1: The weight and inhibition rate of tumor tissue and levels of serum cytokines in mice

Group	Tumor weight (g)	Inhibition rate (%)	IL-2 (ng/ml)	TNF-α (fmol/ml)
Negative control	1.88±0.39		1.79±0.29	12.01±1.21
Cy control 20 mg/kg/d	0.42±0.19[b]	77.6	1.71±0.42	11.91±2.31
Ethanolic extracts 200 mg/kg/d	1.64±0.44[c]	12.0	1.90±0.47	12.21±1.61
Ethanolic extracts 400 mg/kg/d	1.67±0.36[c]	11.1	2.07±0.89	12.16±1.49
Ethanolic extracts 800 mg/kg/d	1.35±0.21[ac]	28.1	2.15±0.47[ac]	12.39±1.69[c]

Statistical data are reported as mean±SD: [a]P<0.05; [b]P<0.01 vs negative control group; P<0.05 vs cyclophosphamide control group

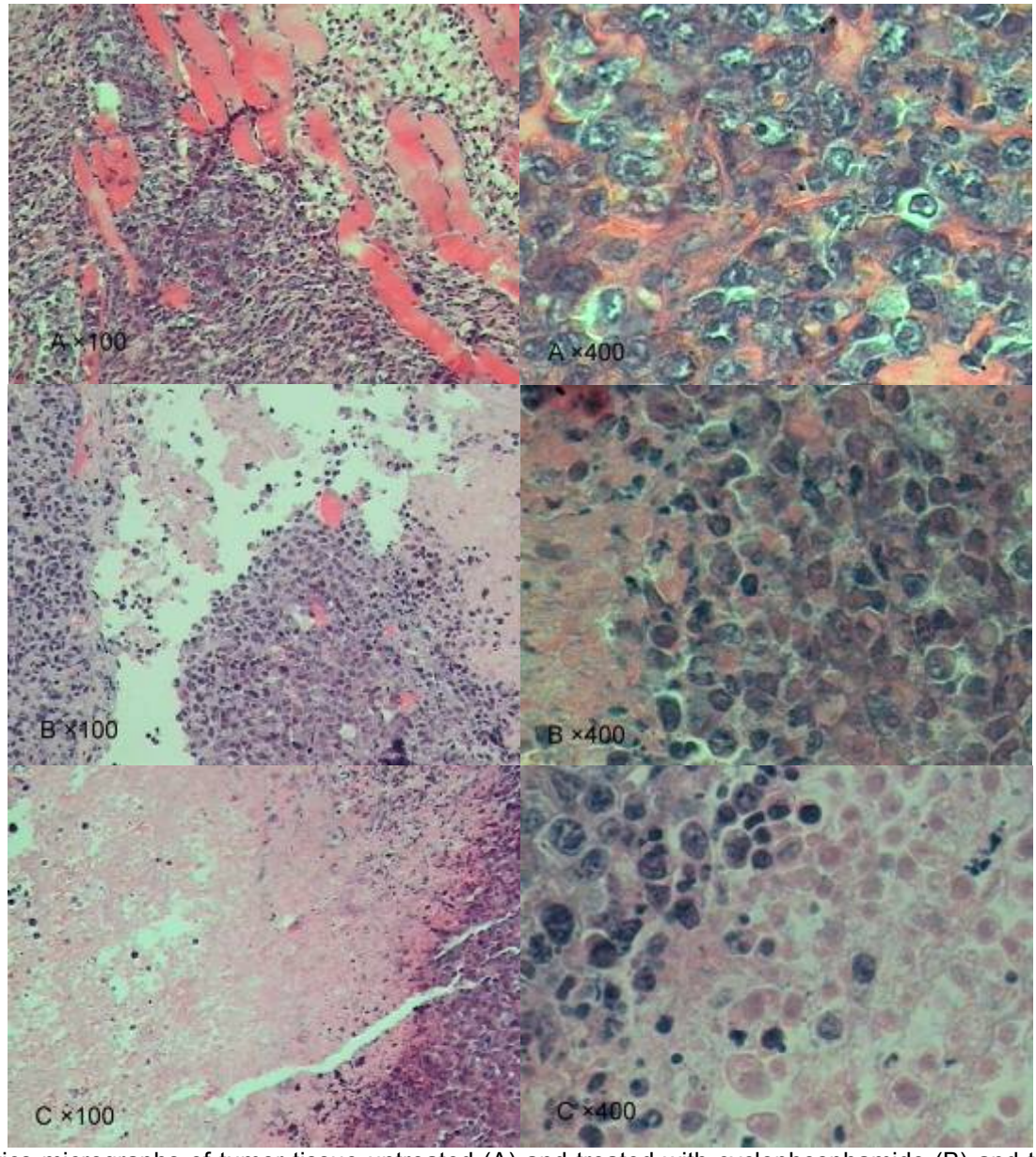

Fig 1: Optics micrographs of tumor tissue untreated (A) and treated with cyclophosphamide (B) and the ethanolic extracts at 800 mg/Kg/d (C) in tumor- bearing mice

in the tumor tissue by the ethanolic extracts at a dose of 800 mg/kg/d showed larger areas of necrosis (Figure 1C) and also many smaller areas of necrosis in the tumor tissue by the cyclophosphamide (Figure 1B).

The levels of serum IL-4 and IL-2 alteration in tumor-bearing mice with S_{180} sarcoma cell are shown in Table 1. IL-2 and TNF-α in serum from the treated mice increased in ethanolic extract-treated groups, compared with the untreated control animals ($P<0.01$). The ethanolic extracts may potentially enhance the self antitumor response, and then stimulate the secretion of TNF-α and IL-2 by immune cells in tumor-bearing mice.

Discussion

Anticancer drugs have well known therapeutic limitations and this has stimulated the search for new agents with enhanced therapeutic efficacy. Considerable efforts have been directed towards medicinal plants, which have been reported to be effective in the treatment of human cancers. Therefore, search for new drugs is required for the treatment of cancers. [9,11] In China, especially the Tibet areas, *Sophora moorcroftiana* seeds are commonly used for the treatment of dephlogistication, detoxication, emetic, infectious diseases and verminosis in folk.[3] In this study, we confirmed these previous findings and further showed, for the first time, that *Sophora moorcroftiana* has obviously antitumor effects on S_{180} sarcoma cells in animals. Moreover, our results showed evidently the immunoregulation efficacy of the ethanolic extracts in tumor-bearing mice.

The antitumor effects of the ethanolic extracts were studied in murine tumour models. After 10 days, although the antitumor efficacy was not as good as the cyclophosphamide at the standard dose rate, the inhibition rate of the tumor tissue growth that had been treated with ethanolic extracts (800 mg/kg/d), was 28.1%. In addition, we detected clear changes of post-treatment with the ethanolic extracts in the tumor tissue, and even the appearance of larger areas of necrosis and more significantly invaded lymphocytes could be considered as the indirect effects of the present treatment. In view of the present findings with regard to the weight and biopsy alteration of the tumor tissue, we conclude that the ethanolic extracts treatment at a dose of 800 mg/Kg/d has a marked inhibiting effect on S_{180} sarcoma development in vivo.

The human immune system and inflammatory response are regulated by a large group of proteins called cytokines,[12] such as Interleukin-2 (IL-2) and tumor necrosis factor-alpha (TNF-α) plays a key role in the development and function of the immune system.[13] IL-2, an immune modulator, is a protein made by the body. T-helper cells, a kind of white blood cell, produce IL-2 when they are stimulated by an antigen and has been approved by the FDA for the treatment of some types of cancer in clinic.[11] IL-2 is the primary cytokine responsible for proliferative stimulation of activated T-cells and can improve the body's natural response to disease. Patients who use IL-2 have large increases in their CD4+T-cell counts.[12] However, TNF-α, produced chiefly by activated monocytes and macrophages, is a pleiotropic cytokine with a wide range of biological effects. One of these effects is the capability of killing many tumor cells.[14] TNF can also bind to TNF receptor 1 (TNFR1) and TNFR2, leading to receptor homotrimer formation and consecutive triggering of various biological responses such as cytotoxicity and apoptosis in target cells.[15] Therefore, IL-2 and TNF, two of the most important non-specific immune factors, as demonstrated by numerous experiments and clinical applications, playes a positive and estimable role in treating tumors.[12,16] It can promote the induction, activation, and reproduction of all kinds of effector cells, including T-cell, as well as enhancing their activity. At the same time, examination of serum IL-2 and TNF levels in patients has been suggested as to be helpful in monitoring therapeutic effects.[12,14-16] In this study, we found an increase of serum IL-2 and TNF-α levels in tumor-bearing mice that were treated with the ethanolic extracts for 10 days. In comparison with the other test groups, a decrease of serum IL-2 and TNF-α levels in tumor-bearing mice with cyclophosphamide treatment. Cyclophosphamide, an alkylating agent, has widely been used as a cytotoxic antitumor drug in clinical medicine, and as an immunosuppressive drug, which can suppress immune cell functions such as T cell, monocytes and macrophages.[17] However, it is possible that, after treatment with ethanolic extracts, the growth and structure of tumor may be inhibited and thus, the inhibition of tumor on immune cells is attenuated. In comparison with the control group, it is suggested that the ethanolic extracts from *Sophora moorcroftiana* seeds have a weak antitumor role and in high concentration to tumor-bearing mice in vivo, the 95% ethanolic extracts was rather effective. The ethanolic extracts can

enhance the self antitumor response and stimulate the secretion of TNF-α and IL-2 by immune cells in tumor-bearing mice, and this may be one of mechanism of the ethanolic extracts in vivo. Furthermore, it is well known that extracts, plant sources, isolated constituents from plants such as polysaccharide and alkaloids possess immunoregulative and antitumor activities, which may be the main biochemical constituents of the antitumor immunity effects in tumor-bearing mice. However, how they inhibit the growth, induce apoptosis, necrosis and antitumor immunity in tumor-bearing mice remain unknown. Further studies are needed to address these important questions.

These results suggest that ethanolic extracts may exert their antitumor effects in vivo associated with four fundamental processes: (a)suppression of tumor tissue growth, (b) induction of apoptosis[7] and (c) necrosis on tumor cell, and (d) enhancement of the self antitumor response in tumor-bearing mice. Therefore, it is concluded that the ethanolic extracts from *Sophora moorcroftiana* seeds possess potent antitumor fraction(s) in animals.

Acknowledgment

This work was supported by Gansu Provincial Administration Bureau of Chinese Medical Sciences Fund (No:2003-GZK-17) and Gansu Provincial Natural Sciences Fund (No: 0708WCGA150).

Conflict of interest: None declared.

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