A preliminary study on the anticancer efficacy of *Caulis spatholobi* compound 1802

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Abstract: In recent years, antitumor and antiviral effect of *Caulis spatholobi* becomes a hot topic of medical drug research. Experiment shows that the water extract of *Caulis spatholobi* compound 1802 showed the effect of inhibiting tumor growth, the difference was statistically significant ($P<0.05$); the rate of tumor inhibition was highest in the high dose group of compound 1802, which could reach 41.99%. Anti tumor drugs generally have high toxicity, therefore, low toxicity is the significant characteristic of *Caulis spatholobi*. In particular the use of *Caulis spatholobi* has certain practical significance for development of tumor patients' daily diet products. In summary, the chemical constituents of *Caulis spatholobi* complex, has extensive pharmacological effects and clinical application.

Keywords: Antitumor, lymphocyte, *Caulis spatholobi*, Compound 1802.

INTRODUCTION

Caulis spatholobi Compound 1802 is a traditional Chinese medicine formula composed of four herbs, with *Caulis spatholobi* as the Ephedra herb, many leaf *Paris rhizome* as the minister herb, Curcuma zedoary as the assistant herb and *Radix bupleuri* as the envoy drug. *Caulis spatholobi* belongs to the Leguminous plant, it has the efficacy of activating blood and enriching blood and relieving meridians and activating collaterals. The research of its anti-tumor effect has become a hot topic in its pharmacological research (Yang *et al*., 2014). The study found that the water extract of *Caulis spatholobi* has inhibitory effect on human lung adenocarcinoma A549 and human intestinal adenocarcinoma HT-29 tumor cells (Pan *et al*., 2011), methods by polyamide column chromatography and gradient ethanol elution purification of flavonoids has an anti-tumor effective component, antitumor effect was enhanced obviously. The separation of *Caulis spatholobi* extract gel column chromatography, the rate of inhibition of A549 can reach 49.9% (Liu *et al*., 2017; Paccez *et al*., 2014). In addition, the use of 60% ethanol, n-butanol and ethyl acetate as solvent extraction of Flavonoids from *Caulis spatholobi*, respectively HT-29, MCF-7, human breast cancer cells of human cervical cancer cells Hela and mouse melanoma B16 cells and human gastric cancer cells, SGC7901 lymphoma cell line P388D1 and mouse leukemia cell L1210 showed inhibition of tumor growth the role (Wang *et al*., 2008). The study also found that the H-103 resin separation and purification products of *Caulis spatholobi*, can inhibit the growth of MCF-7 and A549 cells. We also prove to have serum containing certain antitumor effects. Inhibition of HELA and L1210 cell lines was 20% and 50.48% respectively from the serum of 57.23% (Li *et al*., 2016; Ostojic 2015). Based on these findings, obtained by using different solvent extract of *Caulis spatholobi* has certain inhibitory effect on many tumor cell lines, clear Millettia have antitumor effect *in vitro*.

Anti tumor effect in vivo of *Caulis spatholobi* extraction is mainly manifested in 3 aspects, the first is to inhibit the growth of tumor, the mice S180 sarcoma tumor inhibition rate can reach 30%, and can obviously improve the survival rate of mice (Chen *et al*., 2011); The second is to improve the immunity activity of the mice, the study found that the extract of *Caulis spatholobi* can make NK and LAK cells in mice was significantly increased, suggesting that the antitumor effect of *Caulis spatholobi* was also associated with increased killing mechanism of immune cells (Fu *et al*., 2008; Abu 2017); The third is the attenuated efficiency of *Spatholobus suberectus* flavonoid component inhibit Lewis lung cancer in mice and increased white blood cells and platelets, red blood cells, to the dual role of anticancer, stimulation of hematopoiesis, reduce the chemotherapy induced bone marrow suppression, combined chemotherapy drugs and traditional Chinese medicine clinical application, provide more sufficient evidence (Fang *et al*., 2007; Li 2015; Liu 2017). Due to good growth of *Caulis spatholobi* with tumor inhibition effect, and high safety, so the use of different methods on the effective components of the inhibition of further purification, determine its composition, in order to guide the clinical application and mechanism better (Nayir *et al*., 2015). In this paper the compound *Caulis spatholobi* 1802 is not a single compound, it is an anticancer compound of four kinds of medicinal herbs includes *Caulis spatholobi, Pariphyllin, Zedoary, Bupleurum*. In this paper, the antitumor effect of the compound water extract was studied.

According to the guiding principles of drug china food and drug administration (CFDA) pharmacodynamics, we analyzed the anti-tumor and immune regulation activity of

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the aqueous extract of *Caulis spatholobi* Compound 1802, and provide references for further development and application.

MATERIALS AND METHODS

Experimental animals and feeding conditions

Cell strain: Lewis lung cancer cell line (from stem cell bank of Chinese Academy of Sciences), resuscitated and cultured, and was used in the axillary test of only 10^7/ in C57 mice.

Experimental animal: SPF grade C57 mice were reared and managed by people who had obtained the accreditation of laboratory animals. 4 weeks old, 50 males and the breeding unit was the East Campus of Zhongshan University experimental animal center. The certificate number of experimental animals was NO. 44008500014488. The animal quarantine observation was observed for 7 days. During this period, the animal's physical signs, behavioral activities, fecal traits, weight and diet were observed. The number is marked by ear mark method to show different numbers. The completed label card is marked on the front side of the cage.

Feeding conditions: The room is the SPF barrier environment animal room of the laboratory animal center of Zhongshan University. The laboratory animal uses the license number: SYXK (Guangdong) 2016-0112; Temperature: 20~25 C; humidity: 40%~70%; air change times more than 10 times per hour. Lighting time: 12 hours (7:00 a.m. to 7:00 p.m.).

Feed: SPF grade mice feed, production unit: feeding method of Guangdong Medical Laboratory Animal Center: free intake. The index of routine nutritional composition of feed was detected by Guangdong experimental animal monitoring Institute (referring to People's Republic of China national standard GB14924.3-2010) and the detection frequency was two times a year. Keep it in the special feed room and keep it ventilated, clean and dry.

Animal carcass treatment: The animal corpse is temporarily stored in the temporary storage room of the animal - 20°C special refrigerator, which is concentrated on the innocuous treatment center of the living environment of Guangdong.

Experimental method

We compound 1802 aqueous extract of *Caulis spatholobi* anti-tumor and immune regulation preliminary verification, and provide references for further development and application. The 5 tube of Lewis lung cancer cells was resuscitation, and the number of each tube was about 1×10^7. It was inoculated in 5 C57 mice under the axilla, and grew to about 14d, and the diameter was about more than 1cm. Selected the tumor bearing mice with high growth and no break. The tumor bearing mice were killed by cervical dislocation, under aseptic conditions (clean bench, or vaccination cover) out skin disinfection with iodine, alcohol or animal Bromogeramine, then cut the skin, subcutaneous tumors. The tumor tissue was cut into about $1.5mm^3$ and the needle was inoculated subcutaneously on one side of the animal or bilateral axillary. 50 C57 mice were inoculated into 5 groups on the next day after inoculation, with 10 rats in each group. They were model control group, compound 1802 low dose group, compound 1802 medium dose group, compound 1802 high dose group and positive drug control group, respectively.

At the same time, the corresponding drug was given to the mice. The drug was continuously administered for 14 d. After weighing the drug, the weight and material were collected. The levels of INF-γ, IL-4, thymus index, spleen index, tumor inhibition rate and T lymphocyte subsets in the peripheral blood were detected respectively. The animal experiment plan is approved by the laboratory animal ethics committee, numbered 44008500014488, which is consistent with animal protection, animal welfare and ethical principles, and is consistent with the relevant provisions of laboratory animal welfare.

Drug dosage design

See table 1, the experiment was divided into five groups. Each group consisted of 10 mice. The positive drug group was administered intraperitoneally, 0.2mL/10g, and the other groups were administered by gavage, 0.25mL/10g. The resuscitation of rat lung cancer cell line Lewis was 5 tubes, the number of each tube was about 1×10^7, inoculated 3 C57 mice under axillary hypoderma, and allowed it to grow naturally, about 14d, to a diameter of 1cm or more.

Select the tumor bearing mice with high growth and no break. The tumor bearing mice were killed by cervical dislocation, under aseptic conditions (clean bench, or vaccination cover) out skin disinfection with iodine, alcohol or animal Bromogeramine, then cut the skin, subcutaneous tumors. The tumor tissue was cut into about $1.5mm^3$, and the needle was inoculated subcutaneously on one side of the animal or bilateral axillary. The C57 mice were inoculated and were randomly divided into weight groups on the next day after inoculation. At the same time, the drug was given, each group one time a day, continuous 14d.

Data processing

The experimental data were analyzed by GraphPad Prism 6.o Biostatistics software. The measurement data is expressed in ± s. Analysis was carried out with analysis of variance and Dunnett's multiple comparison, and the count data were analyzed by Kruskal-Wallis rank sum test.

Table 1: Dose design

Group	Animal number	Dose (/kg weight)	Clinical dose
Model control group	10	-	Solvent (0.2mL/10g)
Low dose group of compound 1802	10	6.25mL original liquid /kg, equivalent to 20mg raw material /kg	1 times the clinical equivalent dose
Medium dose group of compound 1802	10	12.5mL original liquid /kg, equivalent to 40mg raw material /kg	2 times the clinical equivalent dose
High dose group of compound 1802	10	25mL original liquid /kg, equivalent to 80mg raw material /kg	4 times the clinical equivalent dose
Positive drug group	10	30 mg/kg	1-2 times the clinical equivalent dose

Table 2: The changes of body weight of mice (g, $\bar{x}$±s, n = 10)

Group	0 d	7 d	14 d
Model control group	17.26±1.21	22.24±1.93	23.68±2.55
Low dose group of compound 1802	17.16±1.12	21.15±1.04	22.73±2.02
Medium dose group of compound 1802	17.03±1.10	20.02±1.52**	22.22±1.87
High dose group of compound 1802	17.04±0.89	20.14±1.70*	21.62±2.59*
Positive drug group	17.02±1.00	19.51±0.81**	19.91±1.51**

Note: compared with the model control group, *:P < 0.05, **:P < 0.01.

Table 3: Changes of IFN-γ and IL-4 in the serum of mice in each group (x± s, n = 10)

Group	IFN -γ(pg/mL)	IL-4(pg/mL)
Model control group	89.35±15.39	3.36±1.11
Low dose group of compound 1802	96.7±32.95	3.63±1.08
Medium dose group of compound 1802	113.18±33.24	3.97±1.45
High dose group of compound 1802	111.31±39.36	4.08±1.36
Positive drug group	49.95±3.49**	1.76±0.44*

Table 4: Changes of thymus index and spleen index in each group (x± s, n = 10)

Group	Thymus weight (mg)	Thymus index (mg/g)	Spleen weight (mg)	Spleen index (mg/g)
Model control group	28.80±8.63	1.21±0.32	130.70±29.93	5.53±1.17
Low dose group of compound 1802	26.50±8.19	1.18±0.39	125.90±20.86	5.55±0.87
Medium dose group of compound 1802	27.60±8.37	1.23±0.33	128.10±19.59	5.80±0.96
High dose group of compound 1802	27.9±10.16	1.27±0.36	123.70±21.03	5.72±0.72
Positive drug group	13.20±4.12**	0.67±0.21**	85.80±19.18**	4.34±1.09

Table 5: The changes of tumor weight and tumor inhibition rate ($\bar{x}$± s, n = 10)

Group	Tumor weight (g)	Tumor suppressor rate (%)
Model control group	2.82±0.77	-
Low dose group of compound 1802	1.97±0.54*	30.23
Medium dose group of compound 1802	1.9±0.42**	32.62
High dose group of compound 1802	1.64±0.67**	41.99
Positive drug group	1.31±0.44**	53.65

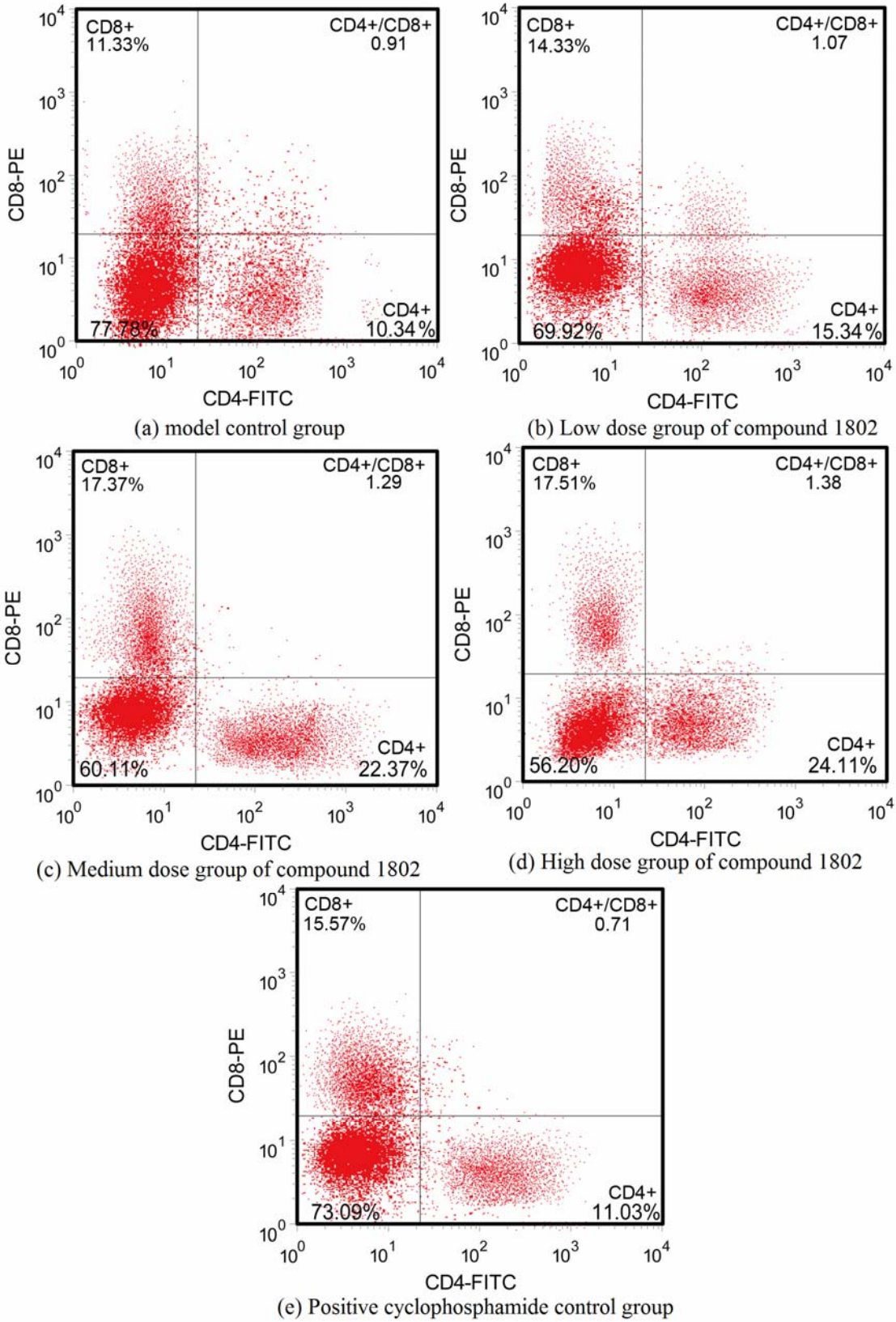

Fig. 1: Effect of *Caulis spatholobi* compound 1802 on T lymphocyte subsets in peripheral blood

RESULTS

Effect of Caulis spatholobi compound 1802 on weight

As shown in table 2, compared with model control group, after administration of 7days and 14days (d), the weight of each group was reduced in varying degrees. After 7d of the drug, the weight loss of the compound 1802 medium dose group, the compound 1802 high dose group and the positive drug group were statistically significant (P<0.05 or P<0.01). After 14 d of the drug, the weight loss of the 1802 high dose group and the positive group was statistically significant. (P<0.05 or P<0.01).

Effect of Caulis spatholobi compound 1802 on immune cells in mice serum factor IFN –γ and IL-4

As shown in table 3., compared with the model group, the content of IFN –γ and IL-4 in serum of mice in the reagent were increased to varying degrees, of which 1802 compound middle dose group, high dose group of compound 1802 was the most significant, but the differences shows no statistically significant (P>0.05).

Effect of Caulis spatholobi compound 1802 on the thymus and spleen index

As shown in table 4, compared with the model control group, there was no significant difference in the weight of the thymus and the weight of the spleen. Compared with the model control group, except for compound 1802 low dose group, the thymus index and spleen index increased slightly, but the difference was not statistically significant (P>0.05). Compared with the model control group, the spleen index of the positive group had a downward trend (P>0.05) and thymus weight, thymus index and spleen index all decreased significantly (P<0.05).

Effect of Caulis spatholobi compound 1802 on tumor weight and tumor inhibition rate

As shown in table 5, compared with the model control group, the tumor weight of the mice in each group decreased in varying degrees. Among them, compound 1802 low dose, compound 1802 middle dose, compound 1802 high dose group and positive drug group significantly reduced tumor weight, the difference was statistically significant (P<0.05 or P<0.01). The high dose of compound 1802 has the highest rate of tumor suppressor, which can reach 41.99%.

Effect of anti cancer compound of traditional Chinese medicine on T lymphocyte subsets in peripheral blood

The effect of T on murine peripheral blood lymphocytes of 1802 compound *Caulis spatholobi* subsets was further studied as shown in table 6 and fig. 1. Compared with the model group, high dose of compound *Caulis spatholobi* 1802, group of CD4+ cell percentage was significantly increased, the difference was statistically significant (P<0.05);Compared with the model control group, the percentage of CD8+ cells in each group was increased, but there was no statistical significance (P> 0.05). Compared with the model control group, the medium and high dose CD4+/CD8+ ratio of compound 1802 increased significantly and the difference was statistically significant (P<0.05 or P<0.01).Compared with the model control group, the levels of CD4+, CD8+ and CD4+/CD8+ in the positive drug groups were all decreased (P>0.05).

DISCUSSION

In summary, by inserting block inoculation method can successfully replicate the mice tumor model of Lewis lung cancer, in this model, the result shows *Caulis spatholobi* Compound 1802 can inhibit tumor growth (Chen *et al*., 2011; Shi 2015). The anti-tumor effect of high-dose group was the best, and its mechanism may be through increasing the ratio of CD4 + / CD8+ ratio, Or by raising Th1 immune cytokine IFN-γ, lowering the Th2 immune cytokine IL-4 to regulate the body's immune status, which has anti-tumor ability (Sun *et al*., 2015; Tang *et al*., 2017).

Modern pharmacological studies have shown that can promote bone marrow hematopoietic Spatholobi and hemodynamics, blood pressure, regulate immune function, antiviral effect (Tural *et al*., 2015; Takahashi 2017). Studies have reported that the spatholobus stem transplantation H22 mice hepatocellular carcinoma, Lewis lung cancer and S180 sarcoma had certain inhibition (Yang *et al*., 2014), and spatholobus stem in anti tumour at the same time, with increased albumin levels, enhance immunity, reduce bone marrow suppression (Chen *et al*., 2011; Udagawa *et al*., 2012; Vekov *et al*., 2015). CTX is a common chemotherapeutic drug in clinic. When it is killing tumor cells, it will also cause different degrees of adverse reactions to human body (Liu *et al*., 2017). The most important factors are myelosuppression and immune dysfunction, resulting in leukocyte reduction. The experimental results also show that although the inhibitory effect of compound *Caulis spatholobi* was weaker than that of CTX group, but it can improve the immunity function.

CONCLUSION

In recent years, antitumor and antiviral effect of *Caulis spatholobi* has become the research focus of scholars at home and abroad. Anti tumor drugs generally have high toxicity and low toxicity of *Caulis spatholobi* very significant characteristics, especially the use of *Caulis spatholobi* has certain practical significance for development of tumor patients' daily diet products. In summary, the chemical constituents of *Caulis spatholobi* complex, has extensive pharmacological effects and clinical application. The chemical and pharmacological research has been partially elucidated between jixueteng

chemical composition and biological activity of contact, but there are a lot of active components have not been found. Therefore, further research on its chemical composition and pharmacological action will further clarify the relationship between its chemical composition and clinical application, which is very necessary for guiding clinical medication and developing new drugs.

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