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A STUDY ON THE INHIBITORY EFFECT OF *SOLANUM LYRATUM THUNB* EXTRACT ON LEWIS LUNG CARCINOMA LINES

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Abstract

The objective of this paper was to observe the effects of *Solanum lyratum Thunb* extract on tumour inhibition, immune function and survival time of tumour-bearing mice. Lung carcinoma-bearing mouse model was established, the tumour-bearing mice were divided into model group, CTX group, *Solanum lyratum Thunb* extract high-dose group and low-dose group. By the examination of tumour inhibition rate of *Solanum lyratum Thunb* extract in Lewis lung carcinoma-bearing mice and determination of the number of NK cells and T cell subsets, the survival rate of tumour-bearing mice was observed. *Solanum lyratum Thunb* extract had some anti-tumour effect in Lewis tumour-bearing mice. The tumour inhibition rate of high-dose group reached 46.28%, and the tumour inhibition rate of low-dose group was 31.42%. *Solanum lyratum Thunb* extract can improve the NK cell activity of Lewis tumour-bearing mice, increase the number of CD4 cells in the tumour-bearing mice, and significantly increase the survival rate of tumour-bearing mice. The study concluded that *Solanum lyratum Thunb* extract has some anti-tumour effect and can improve immune function and survival rate of tumour-bearing mice.

Keywords: *Solanum lyratum Thunb*; tumour-bearing mice; anti-tumour effect

Introduction

Solanum lyratum Thunb is the dried whole plant of *Solanum lyratum Thunb* in the genus *Solanum* of the family Solanaceae (Ming, 1985), which is mainly grown in places such as Anhui, Zhejiang and Jiangsu, on the roadsides and in the mountains and bushes. It has the effects of clearing heat and detoxifying, dispelling wind and resolving phlegm, eliminating dampness and removing jaundice. The chemical constituents of *Solanum lyratum Thunb* include saponins, steroidal alkaloids, flavonoids, polyphenols, organic acids, etc. (Ming, 1985; Chinese Materia Medica., 2000). As a traditional Chinese medicine, *Solanum lyratum Thunb* has a long history, with a variety of pharmacological activities such as anti-tumour effect. Studies have shown that *Solanum lyratum Thunb* has inhibitory effects on mouse sarcoma S180, uterine cervix carcinoma 14 and Ehrlich ascites carcinoma cells (Zhang, 2000), as well as anti-allergic effect. Bookyung et al.'s (1997) study showed that the inhibition rate of aqueous extract of *Solanum lyratum Thunb* on compound 48/80-induced anaphylactic shock is 100% bacteriostatic effect (Wu et al., 2001; Li et al., 2001). Sun et al.'s (2003) experimental study showed that *Solanum lyratum Thunb* has a relatively strong antibacterial effect on a variety of bacteria such as streptococci and staphylococci; anti-inflammatory effect; hepatoprotective effect (Sun et al., 2003; Lu et al., 2003) and immune enhancing function (Kang et al., 1998; Sang et al., 1998). Clinically, it was commonly used in combination with other drugs for the treatment of various cancers such as lung cancer, stomach cancer, pancreatic cancer and liver cancer (Wu et al., 2004; Yin et al., 2005).

Materials and methods

Drugs

Solanum lyratum Thunb was purchased from the Nepstar Drugstore and was identified by Professor Jinshan Liu (Zhengzhou University).

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Reagents

The reagents used for the study included Mouse CD4+, CD8+, antibody, Lianke Biotech Co., Ltd., Shanghai; newborn bovine serum (NBS), Sijiqing Bioengineering Co., Ltd., Hangzhou; CTX, Hengrui Medicine Co., Ltd., Jiangsu.

Instruments

Other instruments were Olympus inverted microscope, OLYMPUS, Japan; low-temperature refrigerated centrifuge, Eppendorf, Germany; CO_2 incubator, SANYO, Japan; DYQ2 multi-head cell collector, Shaoxing Medical Instrument Factory, Zhejiang; electronic balance, Precision Instrument Co., Ltd., Shanghai.

Experimental animals

Animals and tumour cell lines: ICR mice, weighing 18~22 g, males, were provided by the Animal Center of China Medical University; Lewis lung carcinoma lines were provided by the Cancer Laboratory of China Medical University. All experimental procedures were approved by the Animal Research Ethics Committee of Yunnan Medical College University**.**

Preparation of *Solanum lyratum Thunb* extract

Referring to the method in the literature, 20 g of dried *Solanum lyratum Thunb* was taken, soaked in 500 ml of water for 60 min, and heated to boiling for 60 min. It was then filtered through a 200 mesh sieve to discard dregs. The filtrate was centrifuged at a speed of 3000 rpm (revolutions/minute) for 20 min × twice, and the supernatant fluid was taken and concentrated to 20 ml, adjusted to pH of 7.0-7.2, and filter sterilised with a 0.22 μm filter. The resulting liquid is the drug solution with crude drug content of 1.0 g/ml, which was stored at -20℃ for later use.

Observation of the antitumour effect of *Solanum lyratum Thunb* aqueous extract on Lewis lung carcinoma-bearing mice

Under sterile conditions, the tumour tissues of Lewis lung carcinoma-bearing mice were harvested, homogenised and made into a 1×10^7/ml tumour cell suspension. The number of viable cells >95% was counted. The prepared cell suspension was subcutaneously transplanted into the right axilla of ICR mice at 0.2 ml/mouse, and the mouse model of Lewis lung carcinoma metastasis was replicated. 24 h after inoculation, the mice were randomised into the model group, CTX group, *Solanum lyratum Thunb* extract high-dose group and low-dose group. Each group contained 10 mice. *Solanum lyratum Thunb* extract high- and low- dose groups were intragastrically administered with 100, 25 mg/(kg·d) of drug for 10 consecutive days respectively. Positive control group was administered through intraperitoneal injection of 20 mg/ (kg·d) of CTX every other day, and the negative control group was intraperitoneally injected with the same amount of sterile saline for 10 consecutive days.

24 h after the last administration, the mice were weighed, and then sacrificed by cervical dislocation. Tumour masses were harvested, and the tumour inhibition rate was calculated. The formula is as follows:

tumour inhibition rate = (average tumor weight of the negative control group - average tumor weight of the experimental group) / average tumour weight of the negative control group × 100%.

Observation of the effect of *Solanum lyratum Thunb* extract on immune status of Lewis lung carcinoma-bearing mice

Determination of NK cell activity (Du et al., 2001)

The subcultured L929 cells were prepared into a cell suspension of 2×10^5 cells/mL, added to a 96-well plate at 100 μL per well, and cultured for 12 h. Under sterile conditions, spleens were removed and ground. After lysis of red blood cells, they were adjusted into a single cell suspension of 1×10^7 cells/mL with 1640 medium containing 10% NBS and added to each well at 100 μL per well. They were then incubated at 37℃ with 5% CO_2 for 20 h. Six replicate wells were set up for each specimen. Next, 100 μL of 0.1% neutral red dye was added, and the incubation was continued at 37℃ for another 30 min. After washing in PBS, each well was added with 100 μL of cell lysis buffer and shaken gently. The absorbance value (A value) of each well was measured using a micro-plate reader (λ = 492nm).

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NK cytotoxic activity % = [1 - (average A value of experimental group - average A value of effector cells) / average A value of target cell control group] × 100%.

Determination of T lymphocyte subsets (Chen, 1994)

Blood samples were collected from eyeballs of mice. After anticoagulation with heparin, lymphocytes were separated using lymphocyte separation medium. After washing twice in PBS, fluorescently labelled anti-CD4 and -CD8 antibodies were added and reacted at 4℃ under dark conditions for 30 min before the lymphocytes were washed in PBS twice. The lymphocytes were then fixed with 400 μL of 1% paraformaldehyde, and CD4+, CD8+ cell numbers were counted using flow cytometry.

Observation of the effect of *Solanum lyratum Thunb* extract on survival rate of tumour-bearing mice

Modelling and grouping methods were as described above. Administration was continued for 15 consecutive days. After drug withdrawal, the survival time of each mouse in each group was recorded, and the life prolongation rate was calculated according to the following formula:

Life prolongation rate (%) = [(average survival time of mice in the drug treatment group - average survival time of mice in the negative control group) / average survival time of mice in the negative control group] × 100.

Statistical analysis

All data were processed using SPSS 11.0 statistical software. The experimental data were expressed as $\bar{X} \pm s$, comparisons of means among groups were analysed using one-way ANOVA. If the variance was homogeneous, pairwise comparisons among groups were performed using LSD test; if the variance was heterogeneous, rank-sum test was used. $P<0.05$ was considered statistically significant.

Results

Observation of the anti-tumour effect of *Solanum lyratum Thunb* aqueous extract on Lewis lung carcinoma-bearing mice

The growth of transplanted lung tumours in mice of *Solanum lyratum Thunb* aqueous extract high- and low-dose groups was evidently suppressed, compared with the model group. Tumour weights of tumour-bearing mice in CTX group and *Solanum lyratum Thunb* aqueous extract high- and low-dose groups were significantly reduced. Tumor inhibition rate of each *Solanum lyratum Thunb* aqueous extract dose group was lower than that of the CTX group, which were 46.28% and 31.42%, respectively. The results are shown in Table 1.

Table 1: Effect of *Solanum lyratum Thunb* aqueous extract on tumour growth in Lewis lung carcinoma-bearing mice ($\bar{X} \pm s$)

Group	Dose mg/(kg·d)	Average tumour weight (g)	Tumour inhibition rate (%)
Model group	—	2.96±0.52	—
CTX group	20	1.33±0.15*	55.06
Solanum lyratum Thunb aqueous extract high-dose group	100	1.59±0.35*	46.28
Solanum lyratum Thunb aqueous extract low-dose group	25	2.03±0.38*	31.42

Note: comparison with the model group * $P<0.05$

Effects of *Solanum lyratum Thunb* aqueous extract on NK cells and T cell subsets of Lewis lung carcinoma-bearing mice

Compared with the model group, NK cell counts increased in mice of *Solanum lyratum Thunb* aqueous extract high- and low-dose groups. Compared with the normal group, CD4 decreased in tumour-bearing mice of model group and CTX group, while the *Solanum lyratum Thunb*

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aqueous extract high- and low-dose groups can improve the decreased CD4 counts in tumour-bearing mice. CD8 counts of tumour-bearing mice in each *Solanum lyratum Thunb* aqueous extract dose group decreased compared with the normal group. The results are shown in Table 2.

Table 2: Effects of *Solanum lyratum Thunb* aqueous extract on NK cells and T cell subsets of Lewis lung carcinoma-bearing mice ($\bar{X} \pm s$)

Group	NK cells (%)	CD4 (%)	CD8 (%)	CD4/CD8
Normal group	62.59±4.74	63.04±4.85	18.99±3.22	3.44±1.26
Model group	54.92±3.53	50.38±4.38	15.84±2.85	3.31±1.05
CTX group	49.38±3.10	45.73±3.26	17.76±3.54	2.73±1.24
Solanum lyratum Thunb aqueous extract high-dose group	80.32±5.21*	60.54±5.47*	13.43±3.55	4.59±1.44
Solanum lyratum Thunb aqueous extract low-dose group	71.50±4.43*	57.28±4.84*	14.58±3.28	4.07±1.32

Note: comparison with the model group * P<0.05

Effect of *Solanum lyratum Thunb* aqueous extract on survival rate of Lewis lung carcinoma-bearing mice

Compared with the model group, survival time of mice in CTX group and *Solanum lyratum Thunb* aqueous extract groups were all significantly prolonged. The survival time of *Solanum lyratum Thunb* aqueous extract high- and low-dose groups were 24.8 ± 1.9 d and 19.5 ± 2.1 d, and life prolongation rates were 61.0% and 26.6%, respectively. The results are shown in Table 3.

Table 3: Effect of *Solanum lyratum Thunb* aqueous extract on survival rate of tumour-bearing mice ($\bar{X} \pm S$)

Group	Dose mg/(kg·d)	Survival time (days)	Life prolongation rate (%)
Model group		15.4±1.9	
CTX group	20	27.4±2.4**	77.9
Solanum lyratum Thunb aqueous extract high-dose group	100	24.8±1.9**	61.0
Solanum lyratum Thunb aqueous extract low-dose group	25	19.5±2.1*	26.6

Note: comparison with the model group * P<0.05, ** P<0.01

Discussion

Cellular immune dysfunction is closely related to the incidence and development of tumours. Tumour incidence significantly increases in the event of immune hypo function or immune suppression. When tumour is in progressive growth, immune function of cancer patients can also be suppressed (Ji et al., 2012; Luo, 2010). The body's anti-tumour immune response relies mainly on the T-lymphocyte-mediated cell immunity (Yu et al., 2011). T lymphocytes can generally be divided into two subsets: CD4+ and CD8+. NK cells are another killer cells in the human and mouse lymphocytes, which mainly exert immune surveillance function and anti-tumour effect. Therefore, cytotoxic activity of NK cells can also effectively reflect the body's immune function (He and Xi, 2002). This experimental study showed that the aqueous extract of *Solanum lyratum Thunb* has an anti-tumour effect in Lewis lung carcinoma-bearing mice. In the experiment on the effect of immune status of Lewis lung carcinoma-bearing mice, it was found that the *Solanum lyratum Thunb* aqueous extract can increase the number of NK cells, and the number of CD4 cells in tumour-bearing mice, thereby increasing the CD4/CD8 ratio. Compared with the model group, *Solanum lyratum Thunb* aqueous extract can evidently increase the survival time of tumour-bearing mice. It is thus obvious that the anti-tumour effect of *Solanum lyratum Thunb* aqueous extract is related to its capacities to improve immune system status and enhance the body’s anti-tumour capacity. The exact mechanism needs further study nonetheless.

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