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A STUDY ON ANTI-TUMOUR EFFECT OF *SOLANUM LYRATUM* THUNB. EXTRACT IN S_{180} TUMOUR-BEARING MICE

Yin Guan[1], Hong Zhao[1]*, Xiang Yan[1], Jing Meng [1],Weilan Wang[2]

[1]Medical Oncology Department of PLA General Hospital,Beijing,China

[2]Pharmaceutical Care Department of PLA General Hospital,Beijing,China

***E-mail:** zhh301@yeah.net

Abstract

The objective of the study was to investigate the anti-tumour effect of ethanol extract of *Solanum lyratum* Thunb. in S_{180} tumour-bearing mice, and to preliminarily explore its mechanism of action. Methods: Mice were made into S_{180} solid tumour model, grouped and administered. Tumour inhibition rate was measured by harvesting the tumours. Serum IL-2, TNF-a contents were measured by taking blood samples, and thymus index and spleen index were measured by harvesting the thymus and spleen. The results showed that the *Solanum lyratum* Thunb. extract had certain tumour inhibitory effect, which can elevate the serum IL-2, TNF-a contents, and increase the thymus and spleen indices to a certain extent. The study concluded that *Solanum lyratum* Thunb. extract has certain *in vivo* anti-tumour effect which may be exerted through enhancing the body immunity.

Key words: *Solanum lyratum* Thunb.; S_{180} sarcoma; tumour inhibition rate

Introduction

Solanum lyratum Thunb. is the dried whole plant of *Solanum lyratum* Thunb., which belongs to the family Solanaceae. It was originally recorded in the "Shen Nong's Herbal Classic" (Xu, 1994) as top grade, bitter and pungent in taste, slightly cold in nature, and entering the liver and gall bladder meridians. Currently, it is mainly used in the clinical treatment of colds, fever, hot and humid jaundice, dysentery, nephritis, oedema, cholecystitis, cholelithiasis, cervical erosion, leucorrhoea, etc. Recently, the compounds that have been isolated from *Solanum lyratum* Thunb. by researchers at home and abroad mainly include the following categories: saponin compounds (Kotaro et al, 1985; Kotaro et al, 1981; Shoji et al, 1985; Stero et al, 1986), organic acids (Yang et al, 2002; Yin et al, 2010), flavonoids compounds (Li, 2006; Wang, 2004; Yin et al, 2010), terpenoids, etc. And the studies on its pharmacological activities are mainly focused on aspects such as anti-cancer (Dan et al, 2001; Ren et al, 2006; Yu et al, 2008), anti-oxidation (Wei et al, 2009), and anti-bacteria (Cui et al, 2004). In this paper, animal model of S180 solid tumour was used to study the tumour inhibitory mechanism of extract of *Solanum lyratum* Thunb.

Materials and Methods

Drugs and Reagents

Solanum lyratum Thunb. decoction pieces were identified by Professor Wang Ping, and was deposited to the Pharmacy Centre of PLA General Hospital. (They were purchased from Jiangxi Nanhua Medicine Co., Ltd.). CTX was purchased from Jiangsu Hengrui Medicine Co., Ltd.). Trypan blue staining solution was also used.

Main Instruments

The main instruments used in the study included the following: FM-2000A dual probe γ immune counter (Xi'an Kaipu Electromechanical Co., Ltd.); AE31 inverted phase contrast microscope (Motic); SW-CJ-IF clean bench (Suzhou Purification Equipment Factory); low-temperature refrigerated centrifuge (Eppendorf, Germany); electronic balance (Beijing Sartorius Instrument System Co., Ltd.); cell counting chamber (Shanghai Qiujing Biochemical Instrument Factory).

Experimental Animals

Kunming mice, half male and half female, weighing 18~22 g, were purchased from the Laboratory Animal Center of PLA General Hospital. All experimental procedures were approved by the Animal Research Ethics Committee of Yunnan Medical College University

S180 Tumour Lines

S180 tumour lines were purchased from the KeyGEN Biotech Co., Ltd.

Preparation of extract of *Solanum lyratum* Thunb.

Dried crude *Solanum lyratum* Thunb. was crushed and 50 g was weighed. It was then added with a 20-fold volume of 70% ethanol, and ultrasonically extracted three times with 30 min each time. After the extracts were combined and cryogenically concentrated, NKA-9 resin was applied, and column was washed with 50% ethanol. After the column effluent was cryo-concentrated and freeze-dried, *Solanum lyratum* Thunb. extract powder was obtained and was diluted to a certain concentration when using.

Determination of tumour cell concentration

According to the Response Evaluation Criteria in Solid Tumours, if the average weight of tumours in mice of negative control group is less than 1 g or tumour weights of 20% of the mice are less than 400 mg, it indicates the tumour growth is poor. During the therapy, if mortality of mice in administration group exceeds 20%, or more than 15% of the mice has a decrease (self control) of average weight (after tumour removal), then it indicates the drug is toxic and the dose should be reduced. The inoculum concentration of tumour cells is the key to this experiment. When the amount of inoculated tumour cells is too small, there will be no sarcoma growth or tumour weight will be too light, thus drug efficacy could not be evaluated. When the amount of inoculated tumour cells is too high, the drug in the experimental group could not inhibit the growth of tumour, and pharmacodynamic effect could not be manifested. Therefore, the concentration of inoculated tumour cells must be carefully selected. Tumour cells with tumour mass weight of about 2 g 10 days after inoculation were selected as the final inoculum concentration.

Under sterile conditions, a certain amount was inoculated into healthy Kunming mice. After about 7 days, 0.1 mL of S180 ascites were diluted to 100-fold with normal saline. 0.5 mL of the tumour cell dilution was taken and added with 0.5 mL of 0.2% trypan blue dye, mixed well, and then counted under a microscope using cell counting chamber (leukocytes counting method), and the number of leukocytes in original ascites were obtained.

The original ascites was prepared into tumour cell suspension with number of tumour cells of 10^5, 10^6, 10^7, 10^8 cells/mL^{-1} with normal saline, and inoculated into the forelimb armpit of three groups of Kunming mice respectively. Each mouse was inoculated with 0.2 mL, and each group contained eight mice. 10 days later, the mice were sacrificed, and tumours were harvested and weighed from each group of animals.

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Cell Culturing

The cells were made into single cell suspension, and the number of cells was adjusted according to the data obtained from the above experiment. The cell suspension was implanted into the abdominal cavity of mice with 0.2 mL per mouse under sterile conditions, and abdominal distension status of mice was observed daily. 7 days later, peritoneal effusion was extracted from mice.

Animal Model Building (Li et al, 2006)

7 days after the inoculation, the mice whose abdominal circumference was increased to the maximum were sacrificed by cervical dislocation. After abdomen disinfection, abdominal cavity was cut open, and ascites were extracted with 1 mL sterile syringe. The ascites were diluted with PBS, centrifuged at 1000 rpm/min for 10 min, and then the supernatant was discarded. Living cells were counted by trypan blue staining, and the number of tumour cells was adjusted to 1×10^7 cells/mL. 50 mice were picked and their right armpit skin was disinfected. Tumour cell suspension was subcutaneously injected to the right forelimb armpit of mice using 1 mL sterile syringe with 0.2 mL per mouse to induce the solid tumour model.

Grouping and Treatment

24 hours after inoculation, the 50 mice were randomly divided into 5 groups, namely the model group, CTX group, and *Solanum lyratum* Thunb. extract high-, medium-, and low-dose groups. Each group contained 10 mice. All mice were given enough food and water, and each group of mice was weighed and the weights were recorded. Administration method and dose were determined by referencing the "Experimental Methodology of TCM Pharmacology" (Li et al, 2006). *Solanum lyratum* Thunb. high-, medium-, and low-doses were introduced. Mental state, activity and feeding status of the mice were observed daily. On the 11th day after administration and weighing, blood was sampled from the eyeballs, and centrifuged to obtain serum, followed by measurement of serum IL-2, TNF-a contents by radioimmunoassay. After blood collection, the mice were sacrificed. Tumours were harvested and weighed. Tumour inhibition rate was calculated according to the following formula:

Tumour inhibition rate = (average tumour weight of control group - average tumour weight of experimental group) / average tumour weight of control group × 100%

Mouse thymus and spleen were harvested and weighed. The ratio of thymus or spleen weight (mg) to body weight (10g) was taken as the thymus or spleen index.

Statistical Methods

Experimental data were analysed using the SPSS 13.0 software, and pairwise comparisons among groups were performed using one-way ANOVA.

Results

Selection of concentration of S_{180} sarcoma cells

The results for selection of S_{180} sarcoma cell concentration are shown in Table 1. The results show that when the tumour cell concentration is 10^5 cells/mL, tumour weight is very light, which only has a few tiny granules. It is more appropriate to select tumour cell concentration with tumour mass weight of about 2 g according to the requirements of the animal experimental methodology. Therefore, 10^7 cells/mL were selected as the final

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inoculum concentration.

Table 1: Investigation of inoculum concentration of S_{180} sarcoma cells

Cell concentration (cells/mL)	Tumour weight (g)						Mean (g)
10^5	0.05	0.08	0.13	0.04	0.02	0.14	0.08
10^6	0.87	0.96	1.22	1.08	0.68	1.05	0.98
10^7	1.95	1.35	1.44	2.39	2.01	1.78	1.82
10^8	3.78	2.67	3.54	2.31	3.14	3.63	3.18

Effect of *Solanum lyratum* Thunb. extract on body weight of mice

The changes in body weight of mice before and after the experiment are shown in Table 2. Except for the CTX group where weight gain is not obvious, body weights were significantly increased in all of the experimental groups. But the amount of increase is less when compared with the model group, and the amount of increase is basically negatively correlated with the dose.

Table 2: Effect of *Solanum lyratum* Thunb. extract on change in body weight of S_{180} tumour-bearing mice before and after experiment ($\overline{X}\pm S$, n=10)

Group	Dose (g crude drug/Kg)	Number of animals	Average body weight (g) of mice before experiment	Average body weight (g) of mice after experiment
Model group	-	10	22.87±2.53	30.34±4.15
CTX group	0.02g	10	22.37±2.64	25.18±3.66
Solanum lyratum Thunb. high-dose group	28	10	22.65±2.28	28.71±4.37
Solanum lyratum Thunb. medium-dose group	14	10	22.29±2.75	29.73±4.54
Solanum lyratum Thunb. low-dose group	7	10	22.51±2.21	30.31±5.11

Effect of *Solanum lyratum* Thunb. extract on tumour weight and tumour inhibition rate

The results for the effect of *Solanum lyratum* Thunb. extract on tumour weight and tumour inhibition rate are as shown in Table 3. There is a highly significant difference ($P<0.01$) in tumour weight of the *Solanum lyratum* Thunb. high-dose group compared with the model group. Tumour weight of *Solanum lyratum* Thunb. medium-dose group also showed a significant difference ($P<0.05$) compared with the model group, and the tumour inhibition rates of the two groups were both greater than 30%, suggesting that the *Solanum lyratum* Thunb. extract has a significant inhibitory effect on solid tumour in S_{180} tumour-bearing mice. In the *Solanum lyratum* Thunb. low-dose group, tumour weight was also reduced compared with the model group, but there was no significant difference.

Effect of *Solanum lyratum* Thunb. extract on serum IL-2 and TNF-a contents in mice

The effect of *Solanum lyratum* Thunb. extract on serum IL-2 and TNF-a contents in mice is as shown in Table 4. Compared with the model group, IL-2 level was decreased (P<0.01) and TNF-a was slightly increased but not significant in CTX group. IL-2, TNF-a contents were increased greatly (P<0.01) in *Solanum lyratum* Thunb. high-dose group. IL-2, TNF-a contents were also increased significantly (P<0.05 and P<0.01) in *Solanum lyratum* Thunb. medium-dose group. IL-2 was not changed obviously in *Solanum lyratum* Thunb. low-dose group. From this result, we can see that the *Solanum lyratum* Thunb. extract can increase IL-2, TNF-a contents, thereby enhancing the body's ability to kill tumour cells, and exerting the inhibitory effect.

Table 3: Effect of *Solanum lyratum* Thunb. extract on tumour weight and tumour inhibition rate

Group	Dose (g crude drug/Kg)	Number of animals	Tumour weight (g)	Tumour inhibition rate (%)
Model group	-	10	2.15±0.44	-
CTX group	0.02g	10	0.76±0.31**	64.65
Solanum lyratum Thunb. high-dose group	28	10	1.21±0.34**	43.72
Solanum lyratum Thunb. medium-dose group	14	10	1.41±0.38*	34.42
Solanum lyratum Thunb. low-dose group	7	10	1.91±0.52	11.16

Comparison with the model group, *P<0.05; **P<0.01

Table 4: Effect of *Solanum lyratum* Thunb. extract on serum IL-2 and TNF-a contents in S_{180} tumour-bearing mice ($\bar{X}$±S)

Group	Dose (g crude drug/Kg)	Number of animals	IL-2 (ng/mL)	TNF-a (ng/mL)
Model group	-	10	12.418±4.531	0.521±0.072
CTX group	0.02g	10	9.557±1.548**	0.579±0.063
Solanum lyratum Thunb. high-dose group	28	10	16.198±3.144**	0.754±0.087**
Solanum lyratum Thunb. medium-dose group	14	10	15.475±2.9794*	0.711±0.108**
Solanum lyratum Thunb. low-dose group	7	10	14.495±2.129	0.657±0.078*

Comparison with the model group, *P<0.05; **P<0.01

Effect of *Solanum lyratum* Thunb. extract on immune organ thymus index and spleen index in S_{180} tumour-bearing mice

The effect of *Solanum lyratum* Thunb. extract on immune organ thymus index and spleen index in S_{180} tumour-bearing mice is shown in Table 5. Compared with the model group, thymus and spleen weights were reduced. And thymus index and spleen index were both decreased in CTX group, while thymus index and spleen index were all increased in each of the *Solanum lyratum* Thunb. extract does groups, suggesting that the extract can

enhance the body's immune function, improving the tumour cell inhibitory effect to some extent.

Table 5: Effect of *Solanum lyratum* Thunb. extract on immune organ thymus index and spleen index in S_{180} tumour-bearing mice

Group	Thymus index (mg/10 g weight)	Spleen index (mg/10 g weight)
Model group	19.3±3.4	54.7±4.6
CTX group	16.9±4.3	52.5±5.8
Solanum lyratum Thunb. high-dose group	24.4±5.1	62.7±4.9
Solanum lyratum Thunb. medium-dose group	21.4±3.6	61.2±6.8
Solanum lyratum Thunb. low-dose group	20.5±3.3	57.5±5.2

Discussion

As an anticancer drug, CTX has side effects such as leucopoenia, and decreased immune function (Jia and Lu, 1996). Body weight is the most direct reflection of drug toxicity in the S_{180} sarcoma experiment. After tumour weight of mice in CTX group and negative group was removed respectively, the body weight of mice in CTX group was 3-5 g lighter than that of the negative group, while the body weight of mice in experimental group was 3-5 g heavier than negative group after removal of tumour weight of mice in experimental group and negative group. Therefore, although *Solanum lyratum* Thunb. is toxic, its toxicity is far less than CTX. Inhibition of tumour growth and shrinkage of tumour size is an important indicator in evaluating the efficacy of anticancer therapy of antineoplastic drugs. This experiment demonstrated that the *Solanum lyratum* Thunb. extract can exert inhibitory effect on growth of tumours in S180 tumour-bearing mice. And based on its in vivo antitumour effect, its effect on immune function was further explored, and it was initially confirmed that the extract has the effect of enhancing immune function. Its exact mechanism of action still needs more in-depth studies.

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