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ESOPHAGEAL CANCER

Antitumor effect and mechanism of *Gecko* on human esophageal carcinoma cell lines *in vitro* and xenografted sarcoma 180 in Kunming mice

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Abstract

AIM: To investigate the anti-tumor effect of Chinese medicine *Gecko* on human esophageal carcinoma cell lines and xenografted sarcoma 180 in Kunming mice and its mechanism.

METHODS: The serum pharmacological method was used *in vitro*. The growth rates of the human esophageal carcinoma cells (EC9706 or EC1) were measured by a modified 3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. The transplanted tumor model of the mouse S180 sarcoma was established. Fifty mice were randomly divided into five groups (n = 10). Three *Gecko* groups were treated respectively with oral administration of *Gecko* powder at a daily dose of 13.5 g/kg, 9 g/kg, and 4.5 g/kg. The negative group (NS group) was treated with oral administration of an equal volume of saline and the positive group (CTX group) was treated with 100 mg/kg Cytoxan by intraperitoneal injection at the first day. After 2 wk of treatment, the anti-tumor activity was evaluated by tumor tissue weighing. The impact on immune organ was detected based on the thymus index, spleen index, phagocytic rate and phagocytic index. The protein expression of vascular endothelin growth factor (VEGF) and basic fibroblast growth factor (bFGF) were detected by immunohistochemistry. The cell apoptotic rate was detected by terminal deoxynucleotidyl transferase (TdT)-mediated dUTP nick end labeling (TUNEL) assay.

RESULTS: The A value in each group treated with *Gecko* after 72 h was reduced significantly in EC9706 and in EC1. The tumor weight in each group of *Gecko* was decreased significantly (1.087 ± 0.249 *vs* 2.167 ± 0.592; 1.021 ± 0.288 *vs* 2.167 ± 0.592; 1.234 ± 0.331 *vs* 2.167 ± 0.592; P < 0.01, respectively). However, the thymus index and Spleen index of mice in *Gecko* groups had no significant difference compared with the NS group. The immunoreactive score of VEGF and bFGF protein expression of each *Gecko* group by immunohistochemical staining were lowered significantly. The apoptosis index (AI) of each group was increased progressively with increase of dose of *Gecko* by TUNEL.

CONCLUSION: *Gecko* has anti-tumor effects *in vitro* and *in vivo*; induction of tumor cell apoptosis and the down-regulation of protein expression of VEGF and bFGF may be contributed to anti-tumor effects of *Gecko*.

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Key words: Chinese medicine *Gecko*; Human esophageal carcinoma cells; S180 sarcoma of mouse; Vascular endothelin growth factor; Basic fibroblast growth factor; Apoptosis

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INTRODUCTION

Malignant tumor is always a great menace to human health. The incidence and mortality of tumor keep ascending all over the world. Anti-tumor therapies contain surgery, radiotherapy and chemotherapy, and it depends on synthetic agents to a great extent in chemotherapy. Although chemical anti-neoplastics have definite effect; they cause severe adverse effects. Moreover, many chemotherapy drugs bring about multiple drug resistance. Great efforts have been made to develop new anti-cancer pharmaceuticals from Chinese herbal medicine[1-4].

Gecko is a traditional Chinese medicine found to restrain inflammation and allergic response, detumescence and alimentation. *Gecko* is also called *Tian Long*, *Shou Gong*. It contains *Gekko swinhonis Gunther*, *Gekko japonicus*, *etc*.. It is cold in nature and flavor. The dosage forms are pill, powder and mastic[5]. Although the *Gecko* was not recorded in a medical dictionary, there have been reports that *Gecko* and its compound prescriptions could treat malignant tumors, tuberculosis, osteomyelitis and syrinx. In clinical practice, it has definite effect against malignant tumors, especially digestive system tumors, such as esophagus cancer, gastric cancer and liver cancer[6-8]. Research on *Gecko* mainly focused on the anti-tumor compound prescriptions, but there have been few pharmacological studies of *Gecko* and its mechanisms of anti-tumor action.

The present study was to observe the anti-tumor activities of *Gecko in vivo* and *in vitro* using the transplanted tumor model of the mouse S180 sarcoma on mice and the human esophageal carcinoma cells and investigate the effects of *Gecko* on cell apoptosis and the expression of (vascular endothelin growth factor) VEGF and (basic fibroblast growth factor) bFGF of S180 mice.

MATERIALS AND METHODS

Materials

Chinese medicine *Gecko* was purchased from Anhui Bozhou Yonggang Co. Ltd. They were identified *Gekko japonicus*. The whole dry *Gecko* were ground to fine powder and diluted in suspension using 0.2% carboxymethyl cellulose (CMC). Cytoxan (CTX) was purchased from Jiangsu Hengrui Medicine Co. Ltd. (Batch No. H32020857). Fifty Kunming mice and ten SD rats were provided by the Medical Experimental Animal Center, Henan Province. All were female. The code number of the animals was 0001350. The human esophageal carcinoma EC9706 cell line was of esophageal carcinoma of fungating type which is well-differentiated squamous cell carcinoma, EC1 cell line was isolated from esophageal carcinoma cell line EC9706 and one subline, and the S180 mouse sarcoma cell line was kindly provided by the Medical College, Zhengzhou University.

Cell culture and establishment of S180 model

The cells were grown in a monolayer culture containing humidified 5% CO_2 in air at 37℃. They were cultured in RPMI-1640 (Sigma, USA) medium supplemented with 10% fetal calf serum, 100 U/mL and penicillin and 100 mg/L streptomycin. The S180 model was established by subcutaneous injection as previously described[9]. Briefly, the S180 mouse sarcoma cells with ascites were harvested, diluted with sterilized saline at a ratio of 1:8 (cell concentration was adjusted to 1.0×10^6/mL), and inoculated subcutaneously into the right armpit region of Kunming mice.

Preparation of the serum with medicine

Ten SD rats (aged 10-12 wk and weighing 280 ± 30 g) were randomly divided into five groups: the negative group (NS group), the positive group (CTX group) and three *Gecko* groups. The CTX group received 50 mg/kg intraperitoneally once a day. Three *Gecko* groups were treated respectively with oral administration of *Gecko* at a dose of 13.5 g/kg, 9 g/kg and 4.5 g/kg, twice a day. The NS group received the equivalent amounts of normal saline in the same way. After 1 wk of treatment, according to the method principle of Li Yikui[10,11], blood was collected aseptically and serum was collected by centrifugation. It was deactivated at 56℃ for 30 min and filtrated for sterilization. Medical serum was stored at -20℃ for use.

3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay

Cell growth was measured by a modified MTT assay[12,13]. The logarithmic cells (EC9706, EC1) were dispersed with 0.02% (w/v) EDTA to prepare 1×10^4/mL cell suspension, and partitioned into 96-well plates at 180 μL/well for 24 h culture in a 5% CO_2 incubator under 37℃. Five groups were divided, the negative group, the CTX group and the three *Gecko* groups. Each group had 6 wells. Each well was treated respectively with 20 μL medical serum prepared as above. Cells were incubated for 24, 48 and 72 h. Then 20 μL MTT (Sigma, USA) was added to each well and the cells were further incubated at 37℃ for 4 h. The supernatant was removed and 200 μL DMSO (Sigma, USA) was added into each well to solubilize the formazan product. The absorbance also known as A value at wavelength of 470 nm was measured by a microplate reader (Sigma, USA). Triplicate experiments were performed in a parallel manner for each concentration point and the results were presented as mean ± SD. Cell inhibitory rate was calculated by the following formula: inhibitory rate (%) = (1-A treated/A control) × 100%.

Viable cell number counting and growth curve

EC9706, EC1 cells (1×10^4/mL cell suspension) were plated onto 96-well plates at 180 μL/well. Each well was treated respectively with 20 μL medical serum prepared as above and cultured for 7 d. Viable cells were counted under the inverted light microscope by trypan blue dye (Sigma, USA) exclusion method and growth curves were drawn.

Table 1 Inhibition effect of *Gecko*-contained serum on EC9706 by MTT assay (mean ± SD, *n* = 6)

Groups	24 h		48 h		72 h	
	A	Inhibition rate (%)	*A*	Inhibition rate (%)	*A*	Inhibition rate (%)
NS	0.472 ± 0.05		0.97 ± 0.118		1.63 ± 0.224	
CTX	0.286 ± 0.06[a]	39.1	0.55 ± 0.09[a]	42	0.82 ± 0.163[a]	49.6
Gecko 1	0.355 ± 0.04[a]	24.8	0.65 ± 0.136[a]	32.5	0.99 ± 0.154[a]	39.1
Gecko 2	0.372 ± 0.07[a]	21	0.721 ± 0.09[a]	25.1	1.15 ± 0.925[a]	29.8
Gecko 3	0.393 ± 0.03[a]	16.4	0.756 ± 0.07[a]	22.4	1.27 ± 0.07[a]	22.3

[a]$P < 0.05$ *vs* control.

Inhibitory effect of Gecko on S180 in vivo

Twenty-four hours after establishment of S180 model, the fifty transplanted mice (aged 8-10 wk and weighing 26 ± 2 g) were randomly divided into five groups: the NS group, the CTX group (CTX was proved to have definite effect against mouse S180 sarcoma and frequently used in experiment), and the three *Gecko* groups. They were treated respectively with oral administration of saline once a day, intraperitoneal injection of CTX 100 mg/kg only once, and oral administration of *Gecko* at a dose of 13.5 g/kg, 9 g/kg and 4.5 g/kg once a day. After 2 wk of treatment, the anti-tumor activity was evaluated by tumor tissue weighing. The following formula was used: Tumor inhibitory rate (%) = (1-average tumor weighing of administration team/average tumor weighing of the control) ×100%[9].

Immune function

According to the above-mentioned methods, at the 14th day, 5% chicken-red cells were injected intraperitoneally into each group. After 12 h, the mice were killed and 1 mL peritoneal fluid was drawn for glass slide. After incubated for 30 min, peritoneal fluid was fixed with the mixture of acetone/methanol (1:1, v/v) and dyed with 4% Giemsa stain. Peritoneal macrophages were counted under microscope. The effect of *Gecko* on phagocytosis of enterocoelia macrophage was evaluated by the chicken-red cell phagocytic index and phagocytic rate[14]. At the same time, thymus and spleen were taken from mice. The impact on immune organ was evaluated based on the thymus index and spleen index[15].

Immunohistochemistry

The tumor tissues were fixed with 10% neutral formalin at room temperature for 24 h. The paraffin-embedded specimens were cut into sections with a thickness of 5 μm. The detection procedure was done as described in Kit protocol (Wuhan Boster Biological Technology Co. Ltd). PBS instead of the first antibodies was used in the negative control. The VEGF and bFGF positive cells were defined when there was an aggregation of brown particles in the cytoplasm of the tumor cells. The immunoreactive score was determined by the sum of extension and intensity as reported previously[16]. The intensity of staining was scored on a scale of 0 to 3 (0 = negative staining, 1 = weakly positive staining, 2 = moderately positive staining, and 3 = strongly positive staining). The extent of positivity ("extent of distribution" of positive cells) was estimated on a scale of 0 to 4 (0 = negative, 1 = positive staining in 1%-25% of cells, 2 = positive staining in 26%-50%; 3 = positive staining in 51%-75%; and 4 = positive staining in 76%-100%). The combined staining score (extension + intensity) was considered as positive staining.

Detection of apoptosis by terminal deoxynucleotidyl transferase (TdT)-mediated dUTP nick end labeling (TUNEL)

Apoptotic cells were detected *in situ* using TUNEL method according to the manufacturer's instructions[17]. Slices were treated with proteinase K and 0.3% H_2O_2, labeled with fluorescein dUTP (Wuhan Boster Biological Technology Co.Ltd) in a humid box for 1 h at 37℃, then combined with POD-horseradish peroxidase, stained with DAB and counterstained with methyl green. Controls received the same management except the labeling fluorescein dUTP. Dark brown nucleus represented the positive apoptotic cells. In each section, 5 high -power fields were chosen and the apoptosis index (AI), the percentage of positive cells in the total cells, was calculated. The AI was calculated as follows: AI = number of apoptotic cells/total number × 100%.

Statistical analysis

Data were presented as mean ± SD and analyzed with SPSS 11.0 software. The one-way ANOVA or Student's *t* test was used to analyze data. All comparisons were made with untreated controls and significance of difference is indicated as $P < 0.05$ and $P < 0.01$.

RESULTS

Effects of Gecko on cell proliferation

As shown in Tables 1 and 2, compared with control group, the growth of cells treated with serum of different concentrations of mice with medicine *Gecko* was inhibited significantly in a concentration and time-dependent manner. Seventy-two h after treatment, the inhibitory rates were 22.3%-39.1% (EC9706) and 18%-34.1% (EC1). Compared with the NS group, the differences were significant among the groups of *Gecko* ($P < 0.05$).

Growth curves of EC9706 and EC1

To investigate anti-tumor activity of the *Gecko in vitro*, tumor cells treated with serum medicine were cultured

Table 2 Inhibition effect of *Gecko*-contained serum on EC1 by MTT assay (mean ± SD, *n* = 6)

Experiment	24 h		48 h		72 h	
	A	Inhibition rate (%)	*A*	Inhibition rate (%)	*A*	Inhibition rate (%)
NS	0.282 ± 0.09		0.506 ± 0.05		1.329 ± 0.73	
CTX	0.19 ± 0.03[a]	32.6	0.298 ± 0.124[a]	41.1	0.673 ± 0.08[a]	48.7
Gecko 1	0.215 ± 0.08[a]	24.8	0.368 ± 0.03[a]	27.2	0.865 ± 0.08[a]	34.1
Gecko 2	0.234 ± 0.04[a]	16.6	0.378 ± 0.03[a]	25.1	0.94 ± 0.112[a]	30.5
Gecko 3	0.255 ± 0.05[a]	9.5	0.426 ± 0.08[a]	15.8	1.075 ± 0.132[a]	18

[a]$P < 0.05$ *vs* control.

Table 3 Inhibitory effects of *Gecko* on transplanted sarcoma 180 in mice (mean ± SD, *n* = 10)

Groups	Dose (g/kg)	Weight (g)		Tumor weight (g)	Inhibitory rate (%)
		Pre-treatment	Post-treatment		
NS		26.1 ± 2.57	28.1 ± 2.64	2.167 ± 0.592	
CTX	0.1	26.2 ± 2.13	24.3 ± 2.91[a]	0.548 ± 0.135[b]	74.6
Gecko 1	13.5	26.5 ± 2.44	28.8 ± 3.01	1.087 ± 0.249[b]	49.8
Gecko 2	9.0	26.1 ± 2.11	26.6 ± 2.18	1.021 ± 0.288[b]	52.8
Gecko 3	4.5	25.4 ± 1.90	27.8 ± 3.74	1.234 ± 0.331[b]	43.1

[a]$P < 0.05$, [b]$P < 0.01$ *vs* control.

Table 4 Influence of *Gecko* on immune organs of transplanted sarcoma 180 in mice (mean ± SD, *n* = 10)

Groups	Dose (g/kg)	Thymus index (× 10^{-3})	Spleen index (× 10^{-3})	Phagocytic rate(%)	Phagocytic index
NS		2.662 ± 0.131	8.143 ± 0.294	30.2	0.414
CTX	0.1	1.939 ± 0.981[a]	5.376 ± 0.570[a]	7.0[b]	0.085[b]
Gecko 1	13.5	2.260 ± 0.092	6.943 ± 0.306	16.8[a]	0.206[a]
Gecko 2	9.0	2.680 ± 0.064	7.418 ± 0.209	19.0[a]	0.218[a]
Gecko 3	4.5	2.376 ± 0.051	7.354 ± 0.236	20.5[a]	0.231[a]

[a]$P < 0.05$, [b]$P < 0.01$ *vs* control.

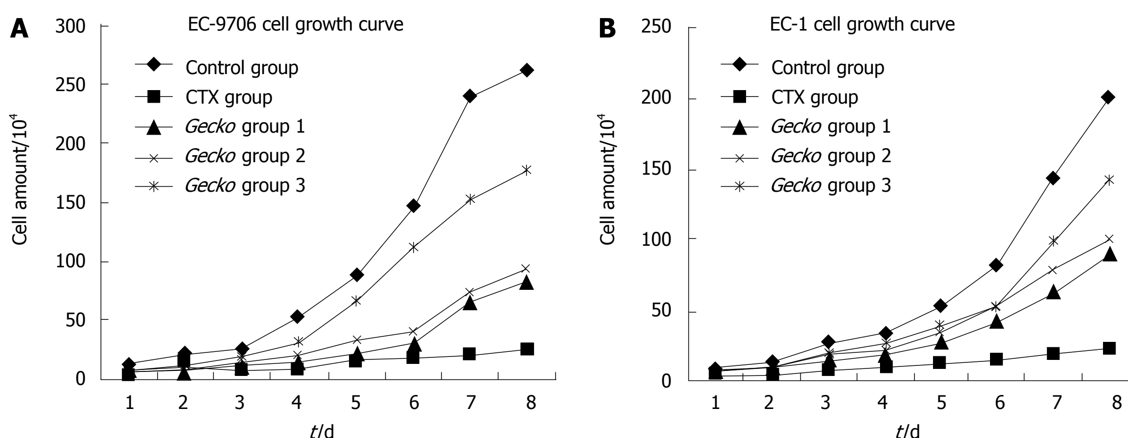

Figure 1 Growth curves of EC9706 (**A**) and EC1 (**B**) in all groups.

for 7 d, and then the cell growth curve was drawn. Under the inverted light microscope, obvious difference was observed in the cell morphology among the five groups of cells. Compared with the control group, growth curves of the three *Gecko* groups gradually moved down in a dose-dependent manner (Figure 1). These results indicated that the serum with medicine *Gecko* could inhibit EC9706 and EC1 growth and proliferation *in vitro*.

Anti-tumor effects of Gecko on S180 mice

As shown in Table 3, compared with the NS group, the tumors of the *Gecko* and CTX groups shrank significantly ($P < 0.01$), but the tumor weight of three *Gecko* groups had no difference statistically compared with the CTX group ($P > 0.05$). The tumor inhibitory rates of the CTX group and *Gecko* groups were 74.6%, 49.8%, 52.8% and 43.1%, respectively. After experiment, there no significant difference was found in mouse weight between the *Gecko* groups and the NS group ($P > 0.05$). Compared with the NS groups, the weight of mice in the CTX group decreased significantly ($P < 0.05$).

Influence of Gecko on immune organs

As shown in Table 4, compared with the NS group, the thymus index and Spleen index of mice in the CTX group decreased significantly ($P < 0.05$). However, there was no significant difference between the *Gecko* groups and the NS group; CTX could decrease the phagocytic index and phagocytic rate very significantly ($P < 0.01$); *Gecko* could decrease the phagocytic index and phagocytic rate significantly ($P < 0.05$). These results indicated that *Gecko* could not enhance the immune functions.

Effects of Gecko on VEGF and bFGF expressions

As shown in Figures 2 and 3, the VEGF and bFGF

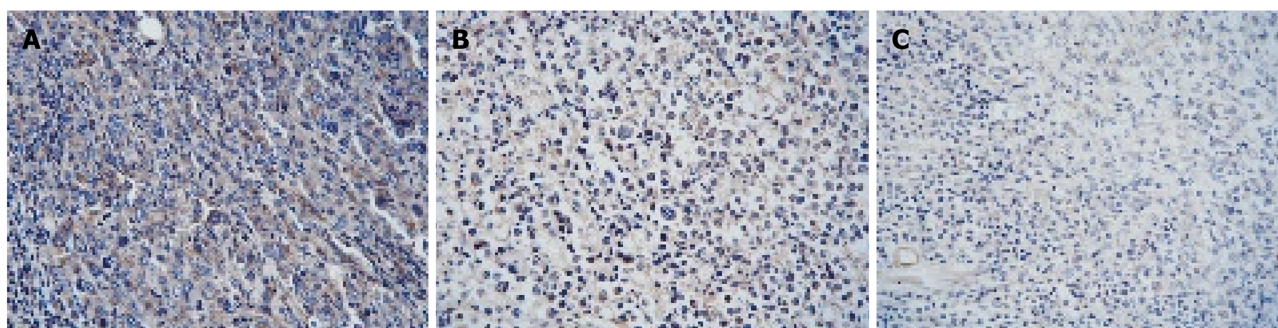

Figure 2 Expression of VEGF on S180 tissues in mice (DAB, x 400). NS group (**A**), *Gecko* group (**B**), CTX group (**C**).

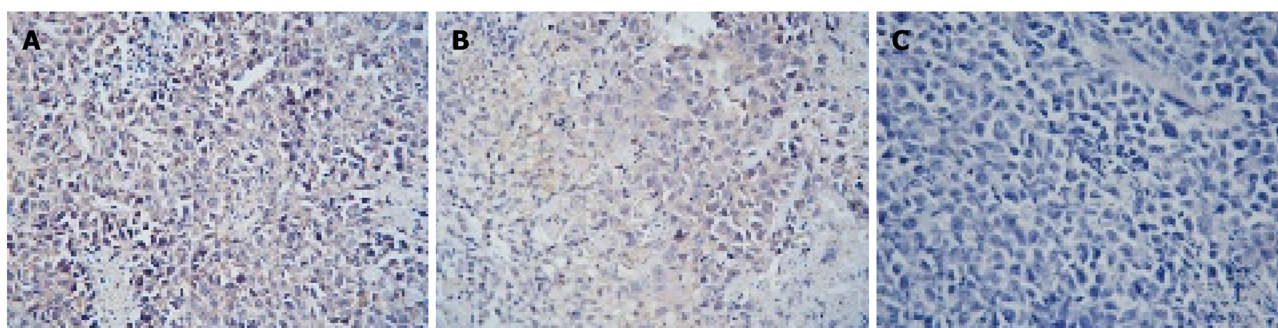

Figure 3 Expression of bFGF on S180 tissues in mice (DAB, x 400). NS group (**A**), *Gecko* group (**B**), CTX group (**C**).

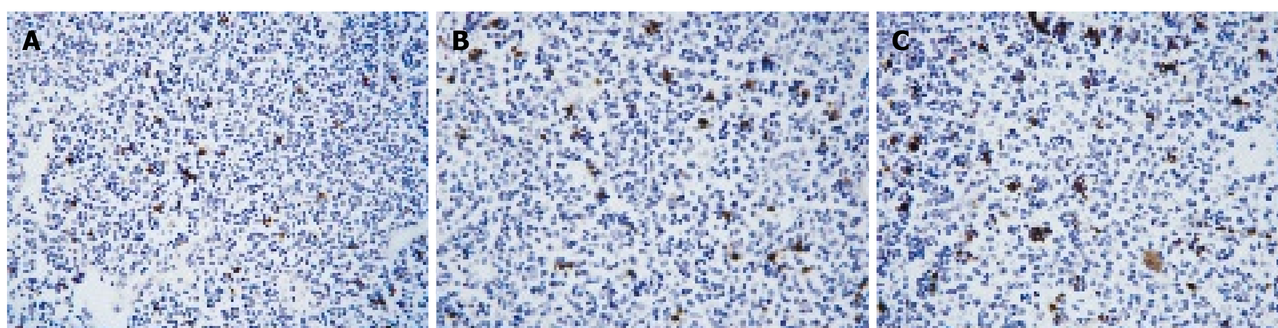

Figure 4 Apoptotic cells on S180 the tissue in mice (DAB, x 400). NS group (**A**), *Gecko* group (**B**), CTX group (**C**).

positive cells were defined when there was an aggregation of brown particles in the cytoplasm of the tumor cells. The proteins of VEGF and bFGF had high expression in the tissue of transplanted sarcoma 180 in mice. Compared with the NS group, the immunoreactive score of expression VEGF and bFGF expression of CTX group and *Gecko* groups decreased significantly (Table 5), (P < 0.05). It indicated *Gecko* could decrease VEGF and bFGF protein expression in the tissue of transplanted sarcoma 180 in mice.

Effects of Gecko on AI

As shown in Figure 4, dark brown nucleus represented the positive apoptotic cells on the tissue of transplanted sarcoma 180 in mice. As shown in Table 5, compared with the NS group, AI of the CTX group and the *Gecko* groups increased very significantly (P < 0.01), indicating that *Gecko* could increase AI in the tissue of transplanted sarcoma 180 in mice.

Table 5 Expressions of VEGF, bFGF and AI on S180 tissues in mice (mean ± SD, n = 10)

Groups	Dose (g/kg)	AI (%)	Immunoreactive score VEGF	Immunoreactive score bFGF
NS		3.58 ± 0.87	5.80 ± 0.73	4.81 ± 0.82
CTX	0.1	17.95 ± 2.14[b]	4.00 ± 0.38[a]	3.00 ± 0.63[a]
Gecko 1	13.5	11.28 ± 1.03[b]	4.62 ± 0.52[a]	3.34 ± 0.77[a]
Gecko 2	9.0	13.56 ± 2.13[b]	4.75 ± 0.68[a]	3.46 ± 0.56[a]
Gecko 3	4.5	11.05 ± 1.69[b]	4.83 ± 0.72[a]	3.52 ± 0.62[a]

[a]P < 0.05, [b]P < 0.01 *vs* control.

DISCUSSION

The processing of *Gecko* introduced in the "Holy Benevolent Prescription" is to "grind it into powder", while according to "The Medical Science Outline", the processing is "drying by baking"[18]. Basically two

processing methods, living and drying, are used in clinical practice. For fresh *Gecko*, there are a few studies describing the mechanisms of lyophilized powder of fresh *Gecko* in inhibiting H22 hepatocarcinoma and C6 glioma cells[19-22], but there has been no experimental study on anti-tumor of drying *Gecko*. We therefore, selected drying *Gecko* as a study object.

Serum pharmacological method was first introduced by Japanese authors. It is a good approach to study traditional Chinese medicine(TCM). The serum pharmacology points out that the serum collected from animals treated with traditional Chinese medicine can be used in experiments *in vitro*. This kind of experimental methods can expel various interference of traditional Chinese medicine and they are close to the true process of the pharmacological effect inside the body[10-11,23]. So we used the method of serum pharmacology *in vitro* experiments and observed the effect of *Gecko*-contained serum on growth of the tumor cells. In this study, MTT showed the growth of EC9706, EC1 cells treated with different concentrations of the *Gecko*-contained serum was inhibited significantly in a concentration and time-dependent manner. Growth curves of the three *Gecko* groups gradually moved down, being obviously dose dependent. These results indicated that the *Gecko*-contained serum could inhibit the growth and proliferation of human esophageal carcinoma cells. *In vivo*, the transplanted tumor model of the mouse S180 sarcoma was established. In the *Gecko* groups at dosage of 13.5, 9 and 4.5 g/kg, the inhibitory rate was 49.8%, 52.8% and 43.1%, respectively. The differences of the groups of *Gecko* were very significant from the control group ($P < 0.01$). These results indicated that *Gecko* could inhibit growth of solid tumor of S180 mice.

The effect on anti-tumor of TCM is related to pathways and targets. Most studies on anti-tumor mechanisms of TCM showed that TCM could inhibit tumors though supporting the healthy energy and strengthening the body resistance[1,2]. In our study, the thymus index, Spleen index, phagocytic index and phagocytic rate showed that *Gecko* could not reinforce immunity of organism. These results indicated the anti-tumor mechanism of TCM might not be related to reinforcement of immune organs.

With the development of modern molecular technology, the anti-angiogenic effect and inducing apoptosis by TCM has become a new area for the research and development of TCM. Apoptosis is a fundamental cellular activity to maintain the physiological balance of the organism and plays a necessary role as a protective mechanism against carcinogenesis by eliminating damaged cells or cells that proliferate excessively[24]. Furthermore, the apoptotic cell death plays an important role in the regulation of pathological conditions as well, such as development and progression of malignant tumors[25,26]. Among the methods that are used to identify cells undergoing the apoptotic process, the TUNEL technique is one of the most successfully utilized[26]. Angiogenesis, the growth of new blood vessels from pre-existing capillaries, is necessary for solid tumor growth and metastasis. Angiogenesis is initiated by the release of certain angiogenic factors from tumor cells. VEGF and bFGF which have been shown to be the most potent angiogenic factors are associated with tumor-induced angiogenesis[27,28]. To investigate the mechanisms of anti-tumor action of *Gecko*, we detected the protein expression of VEGF and bFGF using immunohistochemical method and detected apoptotic index by the TUNEL. The results indicate *Gecko* can decrease VEGF and bFGF protein expression in tumor tissues and induce tumor cell apoptosis.

In conclusion, this study has demonstrated that traditional Chinese medicine *Gecko* has anti-tumor activity *in vitro* and *in vivo*; its mechanism might be related to the induction of tumor cell apoptosis and down-regulation of protein expression of VEGF and bFGF.

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COMMENTS

Background

Malignant tumor is always a great menace to human health. Chemical anti-neoplastics have definite effect, but they cause much severe adverse effects. *Gecko* is a traditional Chinese medicine. It could treat malignant tumors, especially in the tumor of digestive tract. Research on *Gecko* mainly focuses on anti-tumor compound prescriptions, but the pharmacological studies of *Gecko* and its mechanisms of anti-tumor action are rare.

Research frontiers

Basically, two processing methods of *Gecko*, living and drying, are used in clinical practice. Questions still remain to be answered such as what is the difference of anti-tumor effect between fresh *Gecko* and dry *Gecko*? What kind of tumors can *Gecko* treat? What is the mechanism of *Gecko* in treating tumors?

Innovations and breakthroughs

This article describes the effect of the *Gecko* on esophageal carcinoma cell lines (EC9706 and EC1) *in vitro* and xenografted sarcoma 180 *in vivo*. Serum pharmacological method *in vitro* was used to study the anti-tumor mechanism of *Gecko* in the aspect of the anti-angiogenic and apoptosis inducing effect and investigate if *Gecko* had impact on immune organs.

Applications

The incidence and mortality of tumors keep ascending all over the world. The study on anti tumor effect and mechanisms of anti-tumor action of *Gecko* can provide the theoretical evidence in clinical practice. Furthermore, the results may lay a foundation for further research in *Gecko*'s effective constituent.

Terminology

Serum pharmacological method is a good approach to study traditional Chinese medicine *in vitro*. It can expel various interference of traditional Chinese medicine as it is close to the true process of the pharmacological effect inside the body

Peer review

This is a well-conducted study. This manuscript describes the effect of the Chinese medicine *Gecko* on esophageal carcinoma cell lines (EC9706 and EC1) and in xenografted sarcoma 180 Kunming mice. The studies have provided data that support the conclusions of the authors. This is an interesting study.

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