RESEARCH ARTICLE

Ethanol but not Aqueous Extracts of Tubers of *Sauromatum Giganteum(Engl.) Cusimano and Hett* Inhibit Cancer Cell Proliferation

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Abstract

Background: Both alcohol and aqueous extracts of *Sauromatum giganteum(Engl.) Cusimano and Hett*, the dried root tuber of which is named Baifuzi in Chinese, have been used for folklore treatment of cancer in Northeast of China. However, little is known about which is most suitable to the cancer therapy. Materials and Methods: Serum pharmacology and MTT assays were adopted to detect the effects of ethanol and aqueous extracts of *Sauromatum giganteum(Engl.) Cusimano and Hett* , prepared by heat reflux methods, on proliferation of different cancer cells. Results: Cancer cells treated with medium supplemented with 10%, 20%, 40% serum(v/v) containing ethanol extract had a decline in viability, with inhibition rates of 7.69%, 21.8%, 41.9% in MCF-7 cells, 42.8%, 48.1%, 51.8% in SGC-7901 cells, 44.1%, 49.2%, 53.7% in SMMC-7721 cells, 6.8%, 15.2%, 39.8% in HepG2 cells, 7.57%, 16.3%, 36.2% in HeLa cells, 6.24%, 12.5%, 27.4% in A549 cells, and 7.20%, 17.5%, 31.3% in MDA-MB-231 cells, respectively. Viability in the aqueous extract groups was no different with that of controls. Conclusions: An ethanol extract of *Sauromatum giganteum(Engl.) Cusimano and Hett* inhibited the proliferation of SMMC-7721, SGC-7901 and MCF-7 cells, which supports the use of alcoholic but not aqueous extracts for control of sensive cancers, which might include hepatocarcinoma, gastric cancer and breast cancer.

Keywords: *Sauromatum giganteum* - ethanol extract - inhibition - aqueous extarcts - serum pharmacology - cancer cell line

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Introduction

Sauromatum giganteum (Engl.) Cusimano and Hett, a synonym is Typhonium giganteum Engl., the dried root tuber of which is named Baifuzi in Chinese and recorded in Chinese pharmacopoeia as a traditional Chinese medicine (Gao et al., 2007; Chi et al., 2010). It has the effect of "dispelling wind-phelgm" and has been used for the folklore treatment of cancer for a long time in Northeast of China.

About the experimental research of *Sauromatum giganteum (Engl.) Cusimano and Hett* on the anti-tumor activity only Li Q et al reported that SFE-CO_2 extract from Typhonium giganteum Engl. tubers induces apoptosis in human hepatoma SMMC-7721 cells (Li et al., 2011a). Both decoction and vinum of tuber of *Sauromatum giganteum (Engl.) Cusimano and Hett* was used for the folklore treatment of cancer in China. But no any research showed which dosage form is the better for the cancer therapy. The cytotoxicity of decoction and vinum of tuber of *Sauromatum giganteum (Engl.) Cusimano and Hett* on different tumor cell lines has not been revealed. So in this paper serum pharmacology method and MTT assay were adopt to research whether decoction or vinum has better curative effect on cancer, and to which cancer lines the *Sauromatum giganteum (Engl.) Cusimano and Hett* were more sensitive. The results of this investigation might provide a scientific explanation for the folk application of *Sauromatum giganteum (Engl.) Cusimano and Hett* in cancer therapy.

Materials and Methods

Agents

RPMI 1640 culture medium was purchased from GIBCO RBL (USA). Fetal bovine serum (FBS) was purchased from Hyclone company (USA). 3- (4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl- tetrazolium bromide (MTT) and pancreatin was purchased from Sigma-Aldrich. (USA). Adriamycin (ADR) were purchased from Pfizer Inc (USA).

Animals and drug treatment

Kunming mice with both sexes were obtained from Changchun Gao-Xin Experimental Animal Center (Changchun, China) with an initial body weight of 18-

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22 g. The animals were acclimatized to the laboratory conditions for a period of 3 days. They were maintained at an ambient temperature of 20±2°C and 12/12 h of light-dark cycle and given a standard rat feed and water *ad libitum*. The experiments involved with animals were conducted according to the Guide Lines of Animal Experiments from the Committee of Medical Ethics, National Health Department of China (1998), and all the animals experiments was approved by Animal Ethics Committee, Harbin Commerce University.

The animals were divided into four groups (n=10 per group). Group 1-Control animals were orally administered distilled water. Group 2-animals were orally administered with adriamycin (40 mg/kg·d). Group 3-animals were treated with ethonal extracts of *Sauromatum giganteum (Engl.) Cusimano and Hett* (1200 mg/kg·d) by intragastric administration. Group 4-animals were treated with waters extracts of *Sauromatum giganteum (Engl.) Cusimano and Hett* (1600 mg/kg·d) by intragastric administration. All of groups were administered twice one day for seven times. Sixty minutes (but 3 minutes for Group 2) after the seventh treatment, the blood of animals was extracted by eyeball extirpating one by one under aseptic condition.

Cell lines and cell culture

Human breast adenocarcinoma MCF-7 cell line, human gastric cancer SGC-7901 cell line, human hepatocarcinoma SMMC-7721 cell line, human hepatocarcinoma HepG2 cell line, human cervical cancinoma HeLa cell line, human lung adenocarcinoma A549 cell line, human breast cancer MDA-MB-231 cell line were obtained from Institute of Tumor Research of Harbin Medicine University (Harbin, China). Tumor cells were cultured in RPMI 1640 medium (Gibco, 31800-022) supplemented with 10% (v/v) fetal bovine serum (Gibco, 10099-141), 100 U/mL penicillin, 100 μg/mL streptomycin and 1mM L-glutamine at 37°C in an atmosphere of 5% CO_2. The medium was renewed two or three times/week. Cells in logarithmic growth phase were used for further experiments.

Plant material

Sauromatum giganteum (Engl.) Cusimano and Hett tuber was purchased from Harbin Pharmaceutical Group Co. Ltd (Harbin, China) and identified as the tuber part of *Sauromatum giganteum (Engl.) Cusimano and Hett* by professor D.-L. Zhang (The School of Pharmacy, Harbin Commerce University, Harbin, China). The field studies did not involve endangered or protected species.

Extract preparation and solubility measure

Tubers of *Sauromatum giganteum (Engl.) Cusimano and Hett* were cut into 5 mm length cubic small pieces and kept at room temperature in shade for drying. The tuber (200 g) were extracted three times for 3 h each time with 1600 mL 95% ethanol at 80°C. The extracts were filtered, combined, concentrated using a rotary evaporator to constant weight of dry extract, yielding the 2.7% ethanol extract based on the dry weight of tuber of *Sauromatum giganteum (Engl.) Cusimano and Hett*. Aqueous extract was prepared according to the same procedure but with distilled water. After drying by the vacuum freeze drier, the drugs were collected and weighed, yielding the 21.4% aqueous extract based on the dry weight of tuber of *Sauromatum giganteum (Engl.) Cusimano and Hett*.

0.045, 0.055, 0.065, 0.075, 0.085, 0.095, 0.105 g ethonal extract and 0.025, 0.05, 0.075, 0.10, 0.125, 0.15, 0.175 g aqueous extract was respectively dissolved in 5 mL distilled water, mixed and let stand at 20°C over night, the next day we observed whether the extract was completely dissolved. The max concentration of no precipitation solution was used for the experiment.

Acute toxicity study in vivo

Acute toxicity *in vivo* studies with different concentrations of ethanol extract or aqueous extract were carried out to determine the median lethal dose (LD50). Deaths or adverse effects were detected during the 24 hour observation period in mice treated with up to the max concentration of ethanol extract (1200 mg/kg·bw) or aqueous extract (1600 mg/kg·bw). The animals were observed for general behavioral changes, signs of toxicity and mortality continuously for 1 h after treatment, then intermittently for 4 h, and thereafter over a period of 24 h. Further, the mice were observed for up to 14 days following the treatment for any lethality and death (Wang et al., 2013b).

Serum preparation

Sixty minutes (three minutes for group 2) after the last treatment, the blood of animals was extracted by eyeball extirpating one by one under aseptic condition, and let stand at 4°C for twelve hours, then centrifuged at 3000×g for 15 min. Serum were collected and inactivated (heat by water at 56°C for 30min), then filtered through a 0.22 μm micropore film (Millipore, USA), and stored at -80°C until use (Zhang et al., 2008).

Effect of mice serum on cell proliferation

MCF-7, SGC-7901, SMMC-7721 cells were plated at a density of 3×10^4, 4×10^4, 3×10^4 cells/well in 96-well plate, respectively, which was in 100 μL RPMI 1640 medium containing 10% (v/v) fetal bovine serum for 24 h incubation. After 24 h, the medium were discarded, and the medium supplemented with mice serum was added into the wells and adjusted the final concentrations of mice serum to 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100% (v/v), respectively. At the end of 72 h incubation, the medium were discarded and 100 μL of MTT stock solution (1 mg/mL) were added to each well and the plates were further incubated. Four hours later, DMSO (150 μL) was added to each well to solubilize the water-insoluble purple formazan crystals. The amount of MTT-formazan is directly proportional to the number of living cells and was determined by measuring the optical density (OD) at 570 nm using microplate reader (Bio-Rad). The percentage of cytotoxic activity compared to the untreated cells was determined using the following equation:

$$\text{Cell inhibitory rate (\%)} = \frac{\text{OD of control cells-OD of treated cells}}{\text{OD of control cells}} \times 100$$

Effect of extracts of Sauromatum giganteum (Engl.) Cusimano and Hett on cancer cell proliferation

Cell viability was measured by the MTT assay (Gao

SY, 2014), which was based on the conversion of MTT to formazan crystals by mitochonfrial dehydrogenases (Mosmann, 1983). Briefly, MCF-7, SGC-7901, SMMC-7721, HepG2, HeLa, A549, MDA-MB-231 cells were plated at a density of 3×10^4, 4×10^4, 3×10^4, 4×10^4, 4×10^4, 5×10^4, 5×10^4 cells/well in 96-well plate, respectively, which was in 100μL RPMI 1640 medium containing 10% (v/v) fetal bovine serum for 24 h incubation. After 24 h, the medium were discarded, and mice serum containing drug was added into the wells and adjusted the final concentrations to 10%, 20%, 40% (v/v), respectively. Similarly, negative group was added into mice serum and positive control group was mice serum containing ADR. At the end of 72 h incubation, the medium was discarded and 100 μL of MTT stock solution (1 mg/mL) was added to each well and the plates were further incubated. Four hours later, DMSO (150 μL) was added to each well to solubilize the water-insoluble purple formazan crystals. The amount of MTT-formazan is directly proportional to the number of living cells and was determined by measuring the optical density (OD) at 570 nm using microplate reader (Bio-Rad). The percentage of cytotoxic activity compared to the untreated cells was determined using the following equation:

$$\text{Cell inhibitory rate (\%)} = \frac{\text{OD of control cells-OD of treated cells}}{\text{OD of control cells}} \times 100$$

Statistical analysis

All the data were represented as the mean±SD. Statistical significance was calculated using student's t-test. P-values of 5% or less were considered statistically significant.

Results

Saturation concentration of ethonal extract and aqueous extract

To detect the solubility of extracts, aqueous extract or ethanol extract of different weights was dissolved in 5 mL of water, repectively. The results showed that no precipitation appeared when 0.045, 0.055, 0.065, 0.075 g ethanol extract or 0.025, 0.050, 0.075, 0.100 g aqueous extract was respectively dissolved in 5mL distilled water and let stand at 20°C over night, but precipitation appeared when 0.085, 0.095, 0.105 g ethanol extract or 0.125, 0.150, 0.175 g aqueous extract dissolved in 5 mL distilled water, repectively. So 0.015 g/mL ethanol extract or 0.02 g/mL aqueous extract is the saturation concentration, which is the reference to the administration in the experiment. The results were shown in Figure 1 and Figure 2.

Acute toxicity in vivo

No deaths or adverse effects were detected during the 24 hour observation period in mice treated with up to the max administration dose of ethanol extract (1200 mg/kg.bw) or aqueous extract (1600 mg/kg.bw). Tested mice of each group did not show any overt signs of toxicity during 24 h and 14 days observation. No mortality was recorded throughout 14 days monitoring. Based on these results, the dose was the reference for the experiments.

Effect of mice serum on MCF-7, SGC-7901 and SMMC-7721 cell proliferation

The effect of the mice serum on proliferation of MCF-7, SGC-7901, SMMC-7721 cell line assessed using the MTT assay are shown in Table 1 and Figure 3. The results showed that proliferation of the cancer cell culturled in medium supplemented with 10% (v/v) mice serum was equivalent to that in medium supplemented with 10%

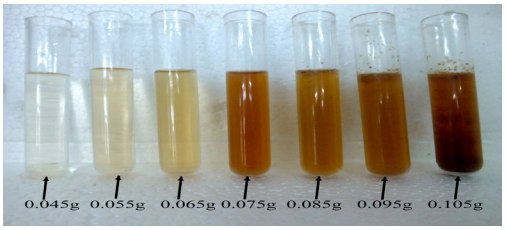

Figure 1. Solubility of Ethanol Extract in Water

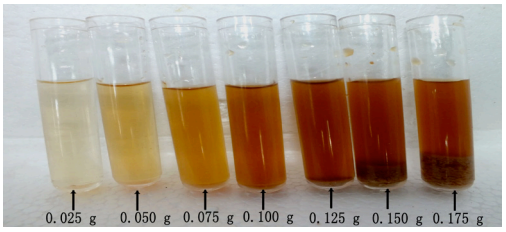

Figure 2. Soulbility of Aqueous Extract in Water

Table 1. Effect of Mice Serum on MCF-7, SGC-7901 and SMMC-7721 Cells Proliferation(n=6)

Groups	Serum volume/ medium(v/v) (%)	MCF-7		SGC-7901		SMMC-7721	
		OD	Inhibition rate (%)	OD	Inhibition rate (%)	OD	Inhibition rate (%)
Fetal bovine serum	10	0.9062±0.0137	0	0.8548±0.0422	0	0.8818±0.0663	0
Mice serum	5	0.7766±0.0633**	14.30	0.7888±0.0359**	7.72	0.7588±0.0530**	13.95
Mice serum	10	0.9224±0.0447	-1.79	0.8596±0.0401	-0.56	0.8766±0.0574	0.59
Mice serum	20	0.9688±0.0530**	-6.91	0.8866±0.0155	-3.72	0.9056±0.0275**	-2.70
Mice serum	30	0.9836±0.0456**	-8.54	0.9348±0.0587**	-9.36	0.9348±0.0482**	-6.01
Mice serum	40	1.0166±0.0499**	-12.18	0.9690±0.0439**	-13.36	0.9800±0.0347**	-11.14
Mice serum	50	0.8330±0.0444**	8.08	0.6762±0.0429**	20.89	0.8330±0.0457**	5.53
Mice serum	60	0.7222±0.0339**	20.30	0.5284±0.0309**	38.18	0.6078±0.0448**	31.07
Mice serum	70	0.4936±0.0284**	45.53	0.3906±0.0160**	54.31	0.4796±0.0357**	45.61
Mice serum	80	0.2036±0.0437**	77.53	0.2104±0.0398**	75.39	0.3040±0.0439**	65.53
Mice serum	90	0.1698±0.0320**	81.26	0.1858±0.0364**	78.26	0.2786±0.0249**	68.41
Mice serum	100	0.1628±0.0379**	82.03	0.1598±0.0199**	81.31	0.2596±0.0374**	70.56

Note: *P<0.05, **P<0.01 compared with fetal bovine serum group

Table 2. Effect of 10 %, 20 % and 40 % Serum Containing Ethanol Extract or Aqueous Extract on MCF-7 Cell Proliferation (n=6)

Groups	Oral administration dose (mg/ kg•d)	Administration dose in MTT assay (μg/mL)	10 % Serum containing drug Serum volume/ medium(v/v)(%)	OD	Inhibition rate(%)	20 % Serum containing drug Serum volume/ medium(v/v)(%)	OD	Inhibition rate(%)	40 % Serum containing drug Serum volume/ medium(v/v)(%)	OD	Inhibition rate(%)
Febal bovine serum control	—	—	10	1.1138±0.0548	0	10	1.1138±0.0548	0	10	1.1138±0.0548	0
ADR	—	0.1862	10	0.4905±0.0218**	55.96	10	0.4905±0.0218**	55.96	10	0.4905±0.0218**	55.96
Mice serum containing ADR	40	—	10	0.7880±0.0479##	21.79	20	0.6820±0.0336##	31.99	40	0.5928±0.0215##	40.35
Mice serum control	—	—	10	1.0075±0.0352	0	20	1.0028±0.0141	0	40	0.9938±0.0183	0
Mice serum containing ethanol extract	1200	—	10	0.9300±0.0187#	7.69	20	0.7840±0.0137##	21.82	40	0.5768±0.0388##	41.96
Mice serum containing aqueous extract	1600	—	10	0.9674±0.0361	3.98	20	0.9168±0.0364	8.57	40	0.8903±0.3820	10.42

Note: *P<0.05, **P<0.01 compared with Febal bovine serum control; #P<0.05, ##P<0.01 compared with mice serum control

Table 3. Effect of 10 %, 20 % and 40 % Serum Containing Ethanol Extract or Aqueous Extract on SGC-7901 Cell Proliferation (n=6)

Groups	Oral administration dose (mg/ kg•d)	Administration dose in MTT assay (μg/mL)	10 % Serum containing drug Serum volume/ medium(v/v)(%)	OD	Inhibition rate(%)	20 % Serum containing drug Serum volume/ medium(v/v)(%)	OD	Inhibition rate(%)	40 % Serum containing drug Serum volume/ medium(v/v)(%)	OD	Inhibition rate(%)
Febal bovine serum control	—	—	10	0.9368±0.0535	0	10	0.9368±0.0535	0	10	0.9368±0.0535	0
ADR	—	1.0655	10	0.4845±0.0387**	48.28	10	0.4845±0.0387**	48.28	10	0.4845±0.0387**	48.28
Mice serum containing ADR	40	—	10	0.6523±0.0306##	31.07	20	0.5675±0.0162##	42.75	40	0.4748±0.0481##	52.17
Mice serum control	—	—	10	0.9463±0.0410	0	20	0.9913±0.0203	0	40	0.9925±0.0149	0
Mice serum containing ethanol extract	1200	—	10	0.5415±0.0290##	42.77	20	0.5145±0.0196##	48.10	40	0.4785±0.0503##	51.79
Mice serum containing aqueous extract	1600	—	10	0.9353±0.0399	1.16	20	0.9300±0.0090	6.18	40	0.9013±0.0237	9.19

Note: *P<0.05, **P<0.01 compared with Febal bovine serum control; #P<0.05, ##P<0.01 compared with mice serum control

Table 4. Effect of 10 %, 20 % and 40 % Serum Containing Ethanol Extract or Aqueous Extract on SMMC-7721 Cell Proliferation (n=6)

Groups	Oral administration dose (mg/ kg•d)	Administration dose in MTT assay (μg/mL)	10 % Serum containing drug Serum volume/ medium(v/v)(%)	OD	Inhibition rate(%)	20 % Serum containing drug Serum volume/ medium(v/v)(%)	OD	Inhibition rate(%)	40 % Serum containing drug Serum volume/ medium(v/v)(%)	OD	Inhibition rate(%)
Febal bovine serum control	—	—	10	0.9140±0.0217	0	10	0.9140±0.0217	0	10	0.9140±0.0217	0
ADR	—	0.1862	10	0.4910±0.0418**	46.28	10	0.4910±0.0418**	46.28	10	0.4910±0.0418**	46.28
Mice serum containing ADR	40	—	10	0.5465±0.0434##	39.11	20	0.4838±0.0348##	48.80	40	0.4120±0.0418##	58.25
Mice serum control	—	—	10	0.8975±0.0143	0	20	0.9448±0.0455	0	40	0.9868±0.0427	0
Mice serum containing ethanol extract	1200	—	10	0.5018±0.0315##	44.09	20	0.4798±0.0370##	49.22	40	0.4568±0.0435##	53.71
Mice serum containing aqueous extract	1600	—	10	0.8595±0.0393	4.24	20	0.8463±0.0403	10.43	40	0.8745±0.0442	11.38

Note: *P<0.05, **P<0.01 compared with Febal bovine serum control; #P<0.05, ##P<0.01 compared with mice serum control

Table 5. Effect of 10 %, 20 % and 40 % Serum Containing Ethanol Extract or Aqueous Extract on HepG2 Cell Proliferation (n=6)

Groups	Oral administration dose (mg/ kg•d)	Administration dose in MTT assay (μg/mL)	10 % Serum containing drug Serum volume/ medium(v/v)(%)	OD	Inhibition rate(%)	20 % Serum containing drug Serum volume/ medium(v/v)(%)	OD	Inhibition rate(%)	40 % Serum containing drug Serum volume/ medium(v/v)(%)	OD	Inhibition rate(%)
Febal bovine serum control	—	—	10	0.9732±0.0427	0	10	0.9732±0.0427	0	10	0.9732±0.0427	0
ADR	—	0.7527	10	0.4936±0.0324**	49.28	10	0.4936±0.0324**	49.28	10	0.4936±0.0324**	49.28
Mice serum containing ADR	40	—	10	0.7540±0.0464##	18.84	20	0.8228±0.0409##	21.20	40	0.7072±0.0413##	28.49
Mice serum control	—	—	10	0.9290±0.0108	0	20	1.0442±0.0301	0	40	0.9890±0.0297	0
Mice serum containing ethanol extract	1200	—	10	0.8658±0.0472#	6.80	20	0.8856±0.0297##	15.19	40	0.5952±0.0492##	39.82
Mice serum containing aqueous extract	1600	—	10	0.8990±0.0393	3.23	20	0.9824±0.0230	5.92	40	0.9158±0.0375	7.40

Note: *P<0.05, **P<0.01 compared with Febal bovine serum control; #P<0.05, ##P<0.01 compared with mice serum control

Table 6. Effect of 10 %, 20 % and 40 % Serum Containing Ethanol Extract or Aqueous Extract on HeLa Cell Proliferation (n=6)

Groups	Oral administration dose (mg/ kg•d)	Administration dose in MTT assay (μg/mL)	10 % Serum containing drug Serum volume/ medium(v/v)(%)	OD	Inhibition rate(%)	20 % Serum containing drug Serum volume/ medium(v/v)(%)	OD	Inhibition rate(%)	40 % Serum containing drug Serum volume/ medium(v/v)(%)	OD	Inhibition rate(%)
Febal bovine serum control	—	—	10	0.8590±0.0286	0	10	0.8590±0.0286	0	10	0.8590±0.0286	0
ADR	—	0.4300	10	0.4160±0.0172**	51.57	10	0.4160±0.0172**	51.57	10	0.4160±0.0172**	51.57
Mice serum containing ADR	40	—	10	0.5692±0.0464##	29.82	20	0.5582±0.0459##	36.80	40	0.4546±0.0289##	51.54
Mice serum control	—	—	10	0.8110±0.0227	0	20	0.8832±0.0266	0	40	0.9380±0.0292	0
Mice serum containing ethanol extract	1200	—	10	0.7496±0.0376##	7.57	20	0.7396±0.0281##	16.26	40	0.5986±0.0245##	36.18
Mice serum containing aqueous extract	1600	—	10	0.8000±0.0415	1.36	20	0.8361±0.0340	5.33	40	0.8338±0.0382	11.11

Note: *P<0.05, **P<0.01 compared with Febal bovine serum control; #P<0.05, ##P<0.01 compared with mice serum control

(v/v) fetal bovine serum, but the proliferation of the cancer cell culturled in medium supplemented with 10% to 40% (v/v) mice serum was promoted comparing with 10% (v/v) fetal bovine serum, and 50% to 100% (v/v) was inhibited. So 10%-40% mice serum could be chosen for the experiments.

Effect of ethanol extract or aqueous extract on cancer cells proliferation

The effects of ethanol extract-containing serum or

Table 7. Effect of 10 %, 20 % and 40 % Serum Containing Ethanol Extract or Aqueous Extract on A549 Cell Proliferation (n=6)

Groups	Oral administration dose (mg/ kg•d)	Administration dose in MTT assay (μg/mL)	10 % Serum containing drug Serum volume/ medium(v/v)(%)	OD	Inhibition rate(%)	20 % Serum containing drug Serum volume/ medium(v/v)(%)	OD	Inhibition rate(%)	40 % Serum containing drug Serum volume/ medium(v/v)(%)	OD	Inhibition rate(%)
Febal bovine serum control	—	—	10	0.7034±0.0294	0	10	0.7034±0.0294	0	10	0.7034±0.0294	0
ADR	—	0.0658	10	0.3688±0.0199**	47.57	10	0.3688±0.0199**	47.57	10	0.3688±0.0199**	47.57
Mice serum containing ADR	40	—	10	0.5900±0.0216##	17.41	20	0.5418±0.0326##	24.52	40	0.4116±0.0256##	49.58
Mice serum control	—	—	10	0.7144±0.0304	0	20	0.7178±0.0428	0	40	0.8164±0.0443	0
Mice serum containing ethanol extract	1200	—	10	0.6698±0.0510	6.24	20	0.6282±0.0373##	12.48	40	0.5926±0.0251##	27.41
Mice serum containing aqueous extract	1600	—	10	0.7072±0.0354	1.01	20	0.6792±0.0287	5.38	40	0.7440±0.0395	8.87

Note: *P<0.05, **P<0.01 compared with Febal bovine serum control; #P<0.05, ##P<0.01 compared with mice serum control

Table 8. Effect of 10 %, 20 % and 40 % Serum Containing Ethanol Extract or Aqueous Extract on MDA-MB-231 Cell Proliferation (n=6)

Groups	Oral administration dose (mg/ kg•d)	Administration dose in MTT assay (μg/mL)	10 % Serum containing drug Serum volume/ medium(v/v)(%)	OD	Inhibition rate(%)	20 % Serum containing drug Serum volume/ medium(v/v)(%)	OD	Inhibition rate(%)	40 % Serum containing drug Serum volume/ medium(v/v)(%)	OD	Inhibition rate(%)
Febal bovine serum control	—	—	10	0.7842±0.0492	0	10	0.7842±0.0492	0	10	0.7842±0.0492	0
ADR	—	0.3329	10	0.3710±0.0301**	52.69	10	0.3710±0.0301**	52.69	10	0.3710±0.0301**	52.69
Mice serum containing ADR	40	—	10	0.6042±0.0210##	20.02	20	0.5644±0.0291##	27.62	40	0.5022±0.0442##	31.97
Mice serum control	—	—	10	0.7554±0.0426	0	20	0.7798±0.0336	0	40	0.7382±0.0270	0
Mice serum containing ethanol extract	1200	—	10	0.7010±0.0346#	7.20	20	0.6432±0.0486##	17.52	40	0.5074±0.0311##	31.27
Mice serum containing aqueous extract	1600	—	10	0.7262±0.0297	3.87	20	0.7272±0.0341#	6.75	40	0.6604±0.0135##	10.54

Note: *P<0.05, **P<0.01 compared with Febal bovine serum control; #P<0.05, ##P<0.01 compared with mice serum control

Table 9. Effectt of 10 %, 20 %, 40 % Serum Containing Ethanol Extract on Cancer Cell Proliferation

Cancer cell lines	Serum additive volume 10%	Serum additive volume 20%	Serum additive volume 40%
SMMC-7721	44.09 %	49.22 %	53.71 %
SGC-7901	42.77 %	48.10 %	51.79 %
MCF-7	7.69 %	21.82 %	41.96 %
HepG2	6.80 %	15.19 %	39.82 %
HeLa	7.57 %	16.26 %	36.18 %
MDA-MB-231	7.20 %	17.52 %	31.27 %
A549	6.24 %	12.48 %	27.41 %

Table 10. Effectt of 10%, 20%, 40% Serum Containing Aqueous Extract on Cancer Cell Proliferation

Cancer cell lines	Serum additive volume 10%	Serum additive volume 20%	Serum additive volume 40%
SMMC-7721	4.24	10.43	11.38
SGC-7901	1.16	6.18	9.19
MCF-7	3.98	8.57	10.42
HepG2	3.23	5.92	7.40
HeLa	1.36	5.33	11.11
MDA-MB-231	3.87	6.75	10.54
A549	1.01	5.38	8.87

aqueous extract-containing serum on proliferation of MCF-7, SGC-7901, SMMC-7721, HepG2, HeLa, A549, MDA-MB-231 cell line assessed using the MTT assay were shown in Table 2-8. Compared with control group (control of fetal bovine serum or control of mice serum), treated cells with 10%, 20%, 40% serum (v/v) containing ethanol extract had a decline in viability, inhibition rate was 7.69%, 21.82%, 41.96% on MCF-7 cell, 42.77%, 48.10%, 51.79% on SGC-7901 cell, 44.09%, 49.22, 53.71 on SMMC-7721 cell, 6.8%, 15.19%, 39.82% on HepG2 cell, 7.57%, 16.26%, 36.18% on HeLa cell, 6.24%, 12.48%, 27.41% on A549, 7.20%, 17.52%, 31.27% on MDA-MB-231, respectively. Viability of aqueous extract-containing serum groups had no different with that of control group. The positive control ADR or

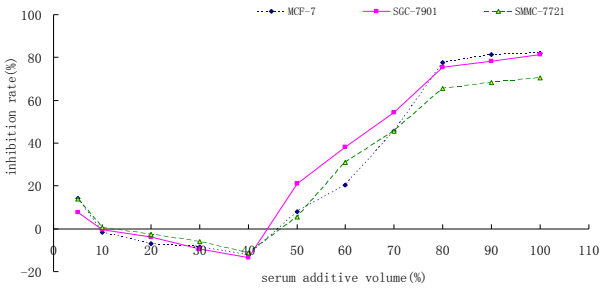

Figure 3. Inhibitory Rate of Mice Serum on MCF-7 Cell, SGC-7901 Cell and SMMC-7721 Cells

serum containing ADR was very toxic with a significant inhibition rate.

The order of cytotoxicity on cancer cell from strong to weak is SMMC-7721>SGC-7901>MCF-7>HepG2>HeLa>MD-MB-231>A549 (show in Table 9).

Discussion

In Chinese folklore, decoction and vinum of *Sauromatum giganteum (Engl.) Cusimano and Hett* tuber are used to treat many kinds of cancer, such as gastric carcinoma and liver cancer et al. Experimental studies found that SFE-CO_2 extract from *Sauromatum giganteum (Engl.) Cusimano and Hett* Tuber could induce tumor cell apoptosis (Li et al., 2011a). The up-regulation of TRAIL/TRAIL-R1 and TRAIL-R2 by Lignans of Rhizoma Typhonii could be involved in the induction of apoptosis (Ma, 2010). But it was unclear whether decoction or vinum had better curative effect, so in this paper, with the method of serum pharmacology, we research which dosage form work better for the treatment of cancer and which cancer lines are more sensitive to it.

Study on traditional Chinese medicine or Chinese formulation is very difficult because the chemical components of a Chinese formulation are very complex. "Serum pharmacology" was first presented by Tashino

(Tashino, 1988), a Japanese scholar, in 1984. The theory believed that only chemical components of Chinese herb absorbed in blood could exert their activity on diseases (Wang et al., 2005), therefore, the serum pharmacology method provides a good research approach for traditional Chinese medicine (Wang et al., 2012; Dang et al., 2013; Liu et al., 2013; Yin et al., 2013), which could avoid the interference of the chemical components not absorbed in blood (Cao et al., 2008; Zhang et al., 2009). And in recent years, it is widely applied in the research of the natural medicine in China (Tang et al., 2009; Li et al., 2011b; Jiang et al., 2012; Wang et al., 2013a) and East Asian countries (Kurokawa et al., 1996).

First in current study, ethanol extract and aqueous extract were prepared by heat reflux method and the dry extract yields were 2.7% and 21.4%, respectively. The solubility and acute toxicity of extract were determined, the max solubility of ethanol extract and aqueous extract was 15 mg/mL and 20 mg/mL, respectively. The aim of the "Acute toxicity study *in vivo*" is that determining the median lethal dose (LD50) (Zhang et al., 2014) or the largest drug concentration and providing the basis for dosage regimen in the follow-up research (Sakthivel et al., 2012). The result showed that no deaths or adverse effects were detected during the 24 hour observation period in mice treated with up to the max administration dose of ethanol extract (1200 mg/kg.bw=15mg/mL×0.8mL/20g·bw×twice/day×50) or aqueous extract (1600 mg/kg.bw=20mg/mL×0.8mL/20g·bw×twice/day×50). So ethanol extract of 1200 mg/kg.bw and aqueous extract of 1600 mg/kg.bw could be chosen for the administration dose of the experiments.

Secondly, serum pharmacology method in the experiment was adopted to research the antitumor activity of *Sauromatum giganteum (Engl.) Cusimano and Hett*. Unfortunately, the higher concentration of serum could inhibit the growth of the tumor cells, so the effect of culture medium supplemented with different percent mice serum on cancer cell proliferation was measured. The results showed that the proliferation of the cancer cell culturled in medium supplemented with 10% (v/v) mice serum was equivalent to that in medium supplemented with 10% (v/v) fetal bovine serum, but the proliferation of the cancer cell culturled in medium supplemented with 10% to 40% (v/v) mice serum was promoted comparing with 10% (v/v) fetal bovine serum, and 50% to 100% (v/v) was inhibited (Table 1, Figure 3). So 10%-40% mice serum could be chosen for the experiments.

Based on above data, effect of culture medium supplemented with 10%, 20%, 40% (v/v) serum containing ethanol extract or aqueous extract on proliferation of seven kinds of tumor cells was measured, which would tell us that whether ethanol extract or aqueous extract could inhibit the tumor proliferation, namely, whether decoction or vinum is more suitable for the treatment of cancer, and which cancer line is more sensitive to the *Sauromatum giganteum (Engl.) Cusimano and Hett*. The results showed that the serum containing ethanol extract exhibited inhibiting effect on cancer cells proliferation, but aqueous extract none (show in Table 2-8). The order of cytotoxicity of medium supplemented with 40% serum containing ethanol extract on cancer cell from strong to weak is SMMC-7721>SGC-7901>MCF-7>HepG2>HeLa>MD-MB-231>A549, the order of medium supplemented with different percent serum containing ethanol extract is not exactly the same because of the experimental error (Table 9, 10), but the order of SMMC-7721>SGC-7901>MCF-7 is exactly the same.

In conclusion, our results indicated that the ethanol extract of *Sauromatum giganteum (Engl.) Cusimano and Hett* inhibited the proliferation of SMMC-7721, SGC-7901 and MCF-7 cell, which supports the use of vinum not decoction of this plant in traditional medicine for the treatment of caner, and the sensive cancers are hepatocarcinoma, gastric cancer and breast cancer, respectively. It maybe helpful to the cancer treatment for the patients in China and other countries.

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