

Full Length Research Paper

Study on mock strawberry extracts *in-vitro* anti-tumor activity

Zhang Lili¹, Zhang Lan^{1#}, Wang Xiangming², Gu Jinxia¹, Wang Xiaoyun¹, Li Xueqi¹ and Xia Hongyuan^{1*}

¹Department of Cardiology, the Fourth Affiliated Hospital of Harbin Medical University, Harbin 150001, People's Republic of China.

²People's Hospital in Hegang City, Hegang 154100, People's Republic of China.

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This study was aimed at investigating the anti-tumor effects of mock strawberry extracts on S180 tumor-bearing mice and exploring its action mechanism. Solid tumor mice models were made and drugs were administered based of grouping. The tumor bodies were used to measure the anti-tumor rate, the blood was used to measure the interleukin-2 (IL-2) and tumor necrosis factor- α (TNF- α) contents in the serum; while the thymus and spleen were obtained to measure their respective indexes. The mock strawberry extracts can inhibit tumor to some extent, increase the contents of IL-2 and TNF- α in the serum and improve the thymus and spleen indexes in some measure. Mock strawberry extracts may act by improving the immunity of the organism to achieve *in-vitro* anti-tumor activity.

Key words: Mock strawberry, S180 sarcoma, IL-2; interleukin-2 (IL-2), tumor necrosis factor- α (TNF- α), immunity.

INTRODUCTION

First written in Miscellaneous Records of Famous Physicians—a famous medical science book of ancient China, and nicknamed cockscomb berry, myrica rubra, arbutus etc., mock strawberry is the whole grass for Rosaceae plant - *Duchesnea indica* (Andr.) Focke, used for curing febrile disease, cough, hematemesis, swollen sore throat and eczema (New Jiangsu Medical School, 1997; Hong-wei, 2002). The chemical compositions of mock strawberry are triterpene and glycosides, coumarins, flavonoid, sterols etc.

It has been reported that mock strawberry water extract has cytotoxicity effect on human hepatoma (7721),

gastric cancer (7901) and esophageal cancer (Eca109) (Jing-yun et al., 1998). Mock strawberry total phenol inhibits proliferating tumor cells in a dose-dependence manner, so it acts obviously in *in-vitro* anti-tumor (Bo et al., 2007). Mock strawberry aqueous extracts act well in inhibiting the growth of lung cancer (LLC), pancreatic cancer (Panc02) and mammary cancer (MC-NeuA) cells of the mice, with IC₅₀ of 217, 206 and 311 μ g/ml, respectively (Shoemaker et al., 2005). Sarcoma 180 (S180) solid tumor animal model was adopted in this paper to study the anti-tumor mechanism of mock strawberry extracts.

*Corresponding author. E-mail: xhy197112@126.com.

#Authors contributed equally to this work.

MATERIALS AND METHODS

Drug and reagent

Cyclophosphamide was purchased from Jiang Su Heng Rui Co. Ltd., while IL-2 and TNF radio immunoassay kits were purchased from Beijing Furui Biological Technology Co., Ltd., Beijing.

Experimental animal and S180 Tumor strain

Male and female Kunming mice, each weighing 18 to 22g, were purchased from the Experimental Animal Centre of Harbin Medical University, with license no. 2011-8-56. The S180 tumor strain was purchased from Nanjing KeyGen Biotech. Inc., Nanjing, China.

Preparation of mock strawberry extract

To obtain mock strawberry extract, appropriate amount of mock strawberry materials were crushed and 60% ethanol was added. Three 30 mm ultrasonic extraction was conducted and combine the extracting solutions. After concentrating in low temperature, polyamide resins was purified, and the mock strawberry extract powders (polyphenols) were obtained after the outflow liquid was concentrated in low temperature and dried. When needed, these were prepared into solutions of proper density for use.

Cell culture

Cells were prepared into single cell suspension, and were adjusted to 1×10^6 . 0.2 ml of the suspension was transplanted into the peritoneal cavity of each mouse under the conditions of asepsis, and was observed for the expansion of the abdomen every day. After seven days, seroperitoneum of the mice were extracted.

Model building

After seven days of vaccination, the mice were executed by cervical dislocation when their abdominal circumference increases to the largest. After disinfecting their abdomen, they were then cut-off and the seroperitoneum was extracted with 1 ml sterilized syringe. The seroperitoneum was diluted with PBS solution, centrifuged (10 min) at 1000 rpm/min to give up the supernatant, and the cells were counted with trypan blue dye and the number of cells was adjusted to 1×10^6 ml⁻¹. In a circle of 50 mice, their subaxillary cutaneous was disinfected and infected with 0.2 ml tumor cell suspension into the right forelimb subaxillary with 1 ml sterilized syringe making them into solid tumor models (Yi-kui et al., 2006).

Grouping and processing

After 24 h of vaccination, the mice were classified randomly into model group, cyclophosphamide group, and group with high, medium and low dose of mock strawberry extract; all in 5 groups with ten mice in each group). All the mice were supplied with enough water and food, and were weighed and the record taken. The work of Jing-yun et al. (1998) and the methods in the methodology of pharmacological experiment by Yi-Kui et al. (2006) were used to determine the administration and dose of the drugs, for high, medium, and low doses of mockery extract to be 13.5, 9.0 and 4.5 g/kg, respectively. The mental states, activities and eating of the mice were observed daily. At the 11th day after drug administration, blood was extracted from the eyeball after weighing and serum was extracted after centrifugation. IL-2 and TNF- α

contents in the serum were detected with radioimmunoassay. The mice were executed after extracting the blood, exfoliating the tumor bodies, weighing them and calculating the tumor inhibition rates according to the following formula:

$$\text{Tumor inhibition rate} = (\text{average tumor weight of the control group} - \text{average tumor weight of the experiment group}) / \text{average tumor weight of the control group} \times 100\%$$

The thymus and spleen of each mouse were removed and weighed. The weight (mg) of the thymus and spleen divided by the weight (10 g) of the mice was taken as thymus and spleen indexes.

Statistical methods

The experimental data were analyzed by Statistical Package for Social Sciences (SPSS)13.0 software. Comparison between two groups was done using t-detection and the comparison between many groups was done using one-way analysis of variance (ANOVA).

RESULTS

Effects of mock strawberry extracts on the weight of the mice

The weight changes before and after the experiments are shown in Table 1. The weight of the experimental groups of mice increased obviously except that of the Cyclophosphamide group, but there was small comparative increase for the model group, in which the increase and the dose form a negative correlation.

Effects of mock strawberry extracts on tumor weight and anti-tumor rate

Effects of mock strawberry extracts on tumor weight and anti-tumor rate are shown in Table 2. Compared with the weight of model group, the tumor weight of group with high dose of mock strawberry extracts was significantly different ($P < 0.01$), and the tumor weight of group with medium dose of mock strawberry extracts is also obviously different ($P < 0.01$). The anti-tumor rates of both groups were more than 30%, indicating that the mock strawberry extracts have apparent inhibition on S180 tumor-bearing mice solid tumor. Compared with the model Group, the group with high dose of mock strawberry extracts had micro smaller weight.

Effects of mock strawberry extracts on IL-2 and TNF- α contents in mice serum

Effects of mock strawberry extracts on IL-2 and TNF- α contents in mice serum are shown in Table 3. Compared with that of model group, the IL-2 level of Cyclophosphamide group was lower ($P < 0.01$), and that of TNF- α was higher but not obviously. IL-2 and TNF- α

Table 1. Effects of mock strawberry extracts on the weight of s180 tumor-bearing mice before and after experiment (mean \pm SD, n=10).

Group	Dose (g /Kg)	No. of animals	Average weight of the mice before experiment (g)	Average weight of the mice after experiment (g)
Model group	-	10	22.98 \pm 2.51	30.46 \pm 4.23
Cyclophosphamide group (g)	0.02	10	22.42 \pm 2.78	25.24 \pm 3.77
Group with high dose of mock strawberry extracts	13.5	10	22.74 \pm 2.39	28.69 \pm 4.45
Group with medium dose of mock strawberry extracts	9.0	10	22.36 \pm 2.88	29.88 \pm 4.75
Group with low dose of mock strawberry extracts	4.5	10	22.53 \pm 2.13	30.23 \pm 5.12

Table 2. Effects of mock strawberry extracts on tumor weight and anti-tumor rate.

Group	Dose(g /Kg)	No. of animals	Tumor Weight (g)	Anti-tumor rate (%)
Model group	-	10	2.12 \pm 0.48	-
Cyclophosphamide group (g)	0.02	10	0.78 \pm 0.35**	63.21
Group with high dose of mock strawberry extracts	13.5	10	1.13 \pm 0.39**	46.70
Group with medium dose of mock strawberry extracts	9.0	10	1.35 \pm 0.34*	36.32
Group with low dose of mock strawberry extracts	4.5	10	1.85 \pm 0.44	12.73

Compared with the Model Group, * P<0.05; ** P<0.01

Table 3. Effects of mock strawberry extracts on il-2 and tnf-a contents in mice serum (X \pm S)

Group	Dose(g /Kg)	No. of animals	IL-2 (ng/mL)	TNF-a (ng/mL)
Model group	-	10	12.412 \pm 4.523	0.518 \pm 0.083
Cyclophosphamide group	0.02	10	9.563 \pm 1.554**	0.597 \pm 0.071
Group with high dose of mock strawberry extracts	13.50	10	16.216 \pm 3.158**	0.746 \pm 0.098**
Group with medium dose of mock strawberry extracts	9.00	10	15.492 \pm 2.984*	0.709 \pm 0.114**
Group with low dose of mock strawberry extracts	4.50	10	14.516 \pm 2.135	0.664 \pm 0.089*

Compared with the Model Group, * P<0.05; ** P<0.01

contents of the group with high dose of mock strawberry extracts rise tremendously (P<0.01), those of Group with Medium Dose of Mock Strawberry Extracts also rise obviously, and IL-2 of Group with Low dose of Mock Strawberry Extracts rises little. We can find from this that mock strawberry extracts can add the contents of IL-2 and TNF-a, thereby improving the lethality of organism to tumor cells and inhibiting tumor.

Effects of mock strawberry extracts on the thymus index and spleen index of the S180 tumor-bearing mice immune organs

Effects of mock strawberry extracts on the thymus index and spleen index of the S180 tumor-bearing mice immune

organs are shown in Table 4. Compared with Model Group, the thymus and spleen weights of Cyclophosphamide Group decrease, and the thymus and spleen indexes lower, while the thymus and spleen indexes of the groups of mock strawberry extracts rise, indicating that the extracts can improve the immunity of organisms, enhancing the inhibition on tumor cells.

As an anti-tumor drug, Cyclophosphamide has the side effects of decreasing organism leukocytes and deteriorating immune functions (Bo-qi and Yunlan, 1996). With the advantages of few side effects and being acceptable to patients, Chinese medicine is focused by researchers home and abroad in the tumor researching field. Many researchers have expanded their research from the activities of anti-tumor to the level of anti-tumor action mechanism (Xiao and Rui-xin, 2011). The occurrence and

Table 4. Effects of mock strawberry extracts on the thymus index and spleen index of the s180 tumor-bearing mice immune organs.

Group	Thymus Index (mg/10g mouse weight)	Spleen Index (mg/10g mouse weight)
Model group	19.5±3.8	54.6±5.2
Cyclophosphamide group	17.3±4.5	53.4±6.3
Group with high dose of mock strawberry extracts	24.5±5.3	64.3±5.6
Group with medium dose of mock strawberry extracts	22.1±4.3	60.8±6.3
Group with low dose of mock strawberry extracts	20.2±3.1	58.1±5.7

and development of tumor are closely related to the deteriorating immunity of the whole organism. If the immunity of human can be improved by certain drugs, there will be positive effects on curing tumors. Currently, many drugs have been found to be able to improve human immunity (Xiao-Ming et al., 2009; Hai-Zhen et al., 2008; Jing-Tao, 2004). Inhibiting tumor development and shrinking tumor bodies is a key indicator to judge whether the anti-tumor drugs act on curing tumor. It has been proved in the paper that mock strawberry extracts can inhibit tumor of S180 tumor-bearing mice, and on the basis of *in-vitro* anti-tumor, effects on immunity of organism are further discussed. Moreover, the effects of mock strawberry extracts on improving organism immunity are primarily proved, of which the exact action mechanism is to be further discussed.

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