THE MOST AUTHORITATIVE RECOMMENDATION OF ANTI－CANCER CHINESE MEDICINE——"9405"

The experiment performed by
the Institute of Materia Medica, Chinese Academy of Medical Sciences

"9405" Anti－cancer Chinese Medicine" is the best anti－cancer Chinese medicine testified by scientific experiments!

COMPOSITION: Ginsenoside Rh2, Radix Astragali, Radix Salviae Miltionhizae, Cordyceps Sinensis Fomes japonica, Fructus Lycii, Citrus reticulata Blanco, Glycyrrhiza uratensis, Vitamin C Vitamin E.

"**9405**" was created on the basis of traditional Chinese Medicine that have been passed down for hundreds of years and have had utstanding success in treating lung cancer, other forms of eancer and lung abscess. Thanks to the efforts of a group of well－known Chinese pharmacological specialists－－the high－level cancer medicine research team organized by Professor Han Rui of the Institute of Materia Medica, Chinese Academy of Medical Science－and the clinical experience of famous spcialists in Chinese traditional medicine the efficacy of 9405 has been put on a modern scientific basis.

"**9405**": 1. Significant anti－tumor effect
2. Outstanding anti－invasion and antimetastasis effect
3. Excellent protective effect of bone marrow inhibition induced by chemotherapeutic agents
4. Activativation of lymphoblast cells and enhance the cellular immunity function
5. Preventing cancer metastasis, serving as the pre－and post－surgery adjunct therapy, alleviating the side effects of chemotherapy
6. Protection & treatment of high－risk patients of cancer (such as heavy smoker, patients with hepatitis, liver cirrhosis...)
7. Prevention & treatment of chronic hepatitis, liver cirrhoosis
8. Auxilliary treatment for chronic renal failure

DOSAGE: 3 times a day, 4－5 capsules once(500mg /cap)
3 times a day, 2g－2.5g once (powder)

PHARMACOLOGICAL STUDIES OF "9405"

9405 is a composite prescription of traditional Chinese medicine. The results of animal experiments are presented below in terms of antitumor effects, protecting of hematopoiesia and bone marrow in mice and the effect of 9405 on lipid peroxidation.

1. Effect of 9405 on the growth of sarcoma 180 in mice.
2. Effect of 9405 on the growth of Walker carcinosarcoma 256 in rats.
3. Effect of 9405 on the growth of hepatoma H22 in mice.
4. Effect of 9405 on lipid peroxidation of liver microsome.
5. Protective effect of 9405 on peripheral leukopenia induced by cytoxan in mice.
6. Protective effect of 9405 on nucleated cell number and DNA content of bone marrow cells i nduced by cytoxan in mice.
7. Protective effect of 9405 on the proliferation inhibition of stem cells of bone marrow induced by cytoxan (cyclophosphamide).

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Chinese Academy of Medical Sciences

Date of Experiments: Jan. 1995－April 1995

EFFECT OF 9405 ON THE GROWTH OF SARCOMA 180 IN MICE

A. MATERIALS AND METHODS

ANIMALS: Kunming Mice, male 18－22g, provided by the Experimental Animal Center, Chi nese Academy of Medical Sciences.

TRANSFER OF TUMORS: Under aseptic conditions, ascites of Day 7 were taken from a tu mor－bearing mouse and diluted with normal sterile physiological saline(1:3), 0.2ml of diluted ascitic fluid were injected subcutaneously in axillary region.

GROUPING AND EXPERIMENT SIZE: The animals transplanted with tumor were randomized into five groups, namely, high, middle and low dosage group, control group and positive control group.

DRUG ADMINISTRATION SCHEDULE: The drug was administered orally 24 hours after th tumor transplantation and for eight days. At the 10th day all animals were killed and Weighed and the tumor weights are also determined. The tumor inhibition rate was calculated according to the formula and statistical analysis was performed.

$$\text{Tumor Inhibition Rate} = \frac{T - C}{C} \times 100$$

T = Average weight of tumor in treated group

C = Average weight of tumor in control groups

B. RESULTS

Experiments demonstrated that 9405 can inhibit the growth of mouse sarcoma 180 significantly under the dosage of 50mg /kg. 100mg /kg, and 200mg /kg.

Tab. 1. Effect of 9405 on the Growth of Mouse Sarcoma 180

Jan. 1995

GROUP	DOSAGE (mg /kg)	Mice NO. Beginning	Mice NO. Fin	Body Weights (g) Beginning	Body Weights (g) Fin.	Weights of Tumor (g)	Inhibition Rate (%)	P Value
Control		10	10	20.4+1.17	34.5+1.90	3.01+0.83		
Cytoxan	100x1	10	10	20.1+1.79	29.7+2.36	0.69+0.34	77%	<0.1
9405	50x8	10	10	21.9+1.10	33.4+3.53	2.55+1.02	15%	>0.05
	100x8	10	10	21.4+1.26	31.1+3.18	1.88+0.74	7.5%	<0.05
	200x8	10	10	22.3+1.34	32.6+3.06	1.55+0.42	48.5%	<0.05

Tab. 2. Effect of 9405 one the Growth of Sarcoma 180

Jan. 1995

GROUP	DOSAGE (mg /kg)	Mice NO. Beginning	Mice NO. Fin	Body Weights (g) Beginning	Body Weights (g) Fin.	Weights of Tumor (g)	Inhibition Rate (%)	P Value
Control		10	10	19.2+1.23	29.5+3.14	2.74+0.53		
Cytoxan	100x1	10	10	20.1+1.29	28.0+2.74	0.32+0.11	88.3%	<0.01
9405	50x8	10	10	20.3+0.82	28.3+2.37	1.82+0.61	33.6%	<0.05
	100x8	10	10	20.3+0.95	29.6+2.44	2.03+0.32	25.9%	<0.05
	200x8	10	10	20.5+0.97	29.2+1.53	1.63+0.27	40.5%	<0.05

Tab. 3. Effect of 9405 on the Growth of Mouse Sarcoma 180

March 1995

GROUPS	DOSAGE (mg /kg)	Mice NO. Beginning	Mice NO. Fin	Body Weights (g) Beginning	Body Weights (g) Fin.	Weights of Tumor (g)	Inhibition Rate (%)	P Value
Control		10	10	23.8+2.14	33.7+2.4	2.53+0.61		
Cytoxan	100x1	10	10	23.6+3.17	32.8+2.20	0.18+0.09	92.9%	<0.01
9405	50x8	10	10	23.7+3.16	31.2+2.82	1.35+0.61	46.6%	<0.05
	100x8	10	10	23.0+4.10	32.5+2.01	1.08+0.57	57.3%	<0.05
	200x8	10	10	24.1+3.70	32.3+2.41	1.47+0.64	41.9%	<0.05

EFFECT OF 9405 ON THE GROWTH OF WALKER CARCINOSARCOMA 256 IN RATS

A. MATERIALS AND METHODS

ANIMALS: Wister rats, male 70－90g, provided by the Experimental Animal Center of Chinese Medical Academy Sciences.

TRANSFER OF TUMORS: Under aseptic conditions ascites of Day 7 were taken from a tumor－bearing rat. 0.2ml of the ascites was injected subcutaneously in axillary region of rate.

GROUPING & EXPERIMENTAL SIZE: Rats transplanted with tumor were randomized into five groups, namely th hing, middle and low dosage group, control group and positive control group.

DRUG ADMINISTRATION SCHEDULE: The drug was administered orally for 9 days and also checked. The tumor inhibition rate was calculated and the statitical significance was analyzed.

B. RESULTS

9405 can inhibit the growth of Walker carcinosarcoma 256 under the dosage of 50mg /kg. 100mg / kg and 200mg /kg respectively. Statistically the inhibitory effect is significant different from control goup.

Tab. 4. Effect of 9405 on the Growth of Walker Carcinosarcoma 256 in Rate

April 1995

GROUPS	DOSAGE (mg /kg)	Rat. NO.		Body Weight (g)		Weights of Tumor (g)	Inhibition Rate (%)	P Value
		Beginning	Fin	Beginning	Fin.			
Control		9	9	100.2＋11.1	141.6＋9.04	7.84＋2.72		
Cytoxan	100x1	10	10	90.6＋8.00	109.2＋11.67	0.12＋0.13	98.5%	<0.01
9405	50x9	10	10	124.8＋9.86	126.0＋13.86	7.11＋2.10	9.31%	<0.05
	100x9	10	10	92.6＋7.06	126.6＋19.00	4.36＋1.34	44.4%	<0.05
	200x9	10	10	90.8＋5.67	124.9＋18.00	4.61＋2.40	41.2%	<0.05

Tab. 5. Effect of 9405 on the Growth of Walker Carcinosarcoma 256 in Rats

April 1995

GROUPS	DOSAGE (mg /kg)	Rat NO.		Body Weight (g)		Weights of Tumor (g)	Inhibition Rate (%)	P Value
		Beginning	Fin	Beginning	Fin.			
Control		10	10	70.3＋7.12	88.9＋20.2	5.47＋1.45		
Cytoxan	100x1	10	10	90.6＋6.01	91.0＋16.6	0.18＋0.08	96.7%	<0.01
9405	50x9	10	10	80.8＋4.85	100.0＋19.7	3.97＋1.89	27.4%	<0.05
	100x9	10	10	71.6＋5.94	87.8＋15.0	3.04＋1.40	44.4%	<0.05
	200x9	10	10	91.4＋4.72	87.3＋16.8	2.84＋0.94	48.1%	<0.05

EFFECT OF 9405 ON THE GROWTH OF HEPATOMA H22 IN MICE

A. MATERIALS AND METHODS

ANIMALS: Male mice, 18－22g, provided by the Experimental Animal Center of Chinese

Acadmy of Medical Sciences.

TUMOR TRANSFER: Under sterile conditions ascites was taken from a mouse bearing H22 tumor seven days after transplantation. The ascites was diluted (1:3) with sterile physiological saline and 0.2ml of the cell suspension was injected subcutaneously in axillary region.

GROUPING & EXPERIMENTAL SIZE: After th tumor transplantation, animals were divided radomly into five groups, namely the control group, high, middle and low dosage group and a positive control group.

DRUG ADMINISTRATION SCHEDULE: Drugs are administered orally for 8 consecutive days. Twenty four hours after the last dosage all animals are sacrificed and weighed. The tumors were also weighed and th tumor inhibition rate was calculated and the data were subjected to statistical analysis.

$$\text{DEGREE OF TUMOR INHIBITION} = \frac{T - C}{C} \times 100$$

T: Average weight of tumor in treated group

C: Average weight of tumor in control group

B. RESURTS

Experiments demonstrated that 9405 can inhibit the growth of hepatoma H22 significantly under the dosage of 25mg /kg. 50mg /kg, and 100mg /kg.

Tab. 6. Effect of 9405 on th Growth of Hepatome H22 in Mice

Jan. 1995

GROUPS	DOSAGE (mg /kg)	Mice NO. Beginning	Mice NO. Fin	Body Weights (g) Beginning	Body Weights (g) Fin.	Weights of Tumor (g)	Inhibition Rate (%)	P Value
Control		10	10	21.7+1.32	30.3+2.94	2.84+0.66		
Cytoxan	100x1	10	10	21.5+0.93	27.4+0.92	0.44+0.24	84.5%	<0.01
9405	25x8	10	10	20.1+1.72	28.6+4.10	1.60+0.87	43.7%	<0.05
	50x8	10	10	21.4+0.92	28.8+2.05	1.39+0.96	51.1%	<0.05
	100x8	10	10	20.3+1.32	27.9+2.47	1.18+0.23	58.5%	<0.01

Tab. 7. Effect of 9405 on the Growth of Hepatome H22 in Mice

Jan. 1995

GROUPS	DOSAGE (mg /kg)	Mice No. Beginning	Mice No. Fin	Body Weight (g) Beginning	Body Weight (g) Fin.	Weights of Tumor (g)	Inhibition Rate (%)	P Value
Control		10	10	21.0+2.27	24.6+4.50	1.43+0.55		
Cytoxan	100x1	10	10	22.2+1.62	21.8+6.38	0.17+0.08	88.1%	<0.01
9405	25x8	10	10	19.1+1.12	24.9+2.59	1.29+0.71	9.8%	<0.05
	50x8	10	10	22.6+0.88	25.8+2.86	0.65+0.31	54.6%	<0.05

Tab. 8. Effect of 9405 on the Growth of Hepatoma H22 in Mice

March 1995

GROUPS	DOSAGE (mg /kg)	Mice No. Beginning	Mice No. Fin	Body Weights(g) Beginning	Body Weights(g) Fin.	Weights of Tumor (g)	Inhibition Rate (%)	P Value
Control		10	10	21.7+0.32	30.3+2.94	2.84+0.66		
Cytoxan	100x1	10	10	21.5+0.93	27.4+0.92	0.44+0.24	84.5%	<0.01
9405	25x8	10	10	19.9+13.6	26.9+2.15	1.47+0.53	48.2%	<0.05
	50x8	10	10	19.2+1.48	26.3+4.58	1.11+0.59	60.9%	<0.01
	100x8	10	10	20.1+2.10	0.84+0.11	27.6+2.62	70.4%	<0.01

EFFECT OF 9405 ON LIPID PEROXIDATION OF RAT LIVER MICROSOME

A. MATERIALS AND METHODS

1. Preparation of Liver Microsome:

Rate fasted for overnight and are sacrificed on the next day. The rat liver are washed and perfused with ice cold physiological saline and then put on a filter paper. The liver is weighed and a 20% homogenite was made with TMS buffer (0.05M Tris-HCL, 0.2 M sucrose, 3mM MgC12, pH7. 4) or 0. 1 M phosphate buffer (pH 7. 4). After a high speed centrifugation to ultracentrifugation (105,000g x 60 min). The pellet was used as microsome fraction.

2. Lipid peroxidation assay of rat liver microsome induced by Fe + + /cystein.

0.1 ml of rat liver microsome fractin was taken. 10 ul of different concentration of the drug and solvent for control were added. 10mM of cystein 20 ul, 1 mM FeSo4 50 ul and 0. 01 M phosphate buffer solution (PBS) were also added to the final volume of 1. 0 ml. The samples were incubate for 30 min, at 37 C. 0. 3 ml of 20% trichloracetic acid (TCA) was used to terminate the reaction, After centrifugation at 2,000 cpm for 15 min., remove the supernatant 1. 0 ml and 0.8 ml of 0.67% thiobarbituric acid was added and heated in boiled water for 10 min. After cooling the optical density (O.D.) of th sample was determined at wavelength of 532 nm.

B. RESULTS

Expertments indicated that 0.3125 ug /ml to 40 ug /ml of 9405 has no significant effect on lipid peroxidationt of rat liver microsome.

Tab.9 Effect of 9405 on Lipid Peroxidation of Rat Liver Microsome

Feb. 1995

GROUPS	CONCENTRATION (ug /ml)	OD	Inhibition Rate (%)	P Value
Control		1.186 0.03		
9405	10	1.006 0.029	15.0	>0.05
	20	1.135 0.027	4.3	>0.05
	30	1.105 0.009	6.8	>0.05
	40	1.061 0.072	10.5	>0.05

Tab. 10. Effect of 9405 on Lipid Peroxidation of Rat Liver Microsome

Feb. 1995

GROUPS	CONCENTRATION (ug /ml)	OD	Inhibition Rate (%)	P Value
Control		1.106 + 0.185		
9405	0.3125	1.006 + 0.071	9.04	>0.05
	0.6250	0.952 + 0.045	13.92	>0.05
	1.250	0.943 + 0.041	14.74	>0.05
	2.500	0.926 + 0.005	16.27	>0.05
	10.000	0.898 + 0.009	18.81	>0.05

PROTECTIVE EFFECT OF 9405 ON LOWERING THE WHITE BLOOD CELL NUMBER INDUCED BY CYCLOPHOSPHAMIDE (CYTOXAN) IN THE PERIPHERAL BLOOD OF MICE

A. MATERIALS AND METHODS

ANIMALS: Kunming Mice. 18 – 22g, provided by the Experimental Animal Center, Chinese Academy of Medical Sciences.

METHODS: Th mice were divided randomly inot 5 groups with 10 mice in each group. Blood was taken from tail vein of each mouse and counted. The tested drug was administered per day for 7 consecutive days at different dosage. On the fifth day cytoxan was given to each mouse orally at a dosage of 100 mg /kg for single dose except the mice in control group. On the eighth day the white blood cells were counted and the WBC rising rate was calculated according to the following formula:

$$\text{WBC Rising Rate} = \frac{T - C}{C} \times 100\%$$

B. RESULTS

Experiments showed that cytoxan lowered the white blood cells in mice significantly. Under the dosage of 50 mg /kg. 100 mg /kg and 200 mg /kg. Prescription 9405 exhibited protecting activity agaist the lowering of WBC number in mice induced by cytoxan but there is no significant difference between control and treated group statistically.

Tab. 11 Protective Effect of 9405 on the Lowering of WBC in Mice Induced by Cytoxan

17 Feb. 1995

GROUPS	DOSAGE(mg /kg) CYTOXAN	9405	n	WBC NUMBER IN PERIPHERAL BLOOD (1 X 10 /L)	P VALUE
Control			10	9.03 0.923	
Cytoxan	100x1		10	5.49 0.72*	<0.01
9405 +	100x1	50x7	10	7.82 1.40**	<0.05
9405 +	100x1	100x7	10	7.07 2.63**	>0.05
9405 +	100x1	200x7	10	5.95 0.81**	>0.05

* Compared with Control Group

* * Compared with Cytoxan Treated Group

Tab. 12 PROTECTIVE EFFECT OF 9405 ON THE LOWERING OF WBC IN DUCED BY CYTOXAN IN MICE

17 Feb. 1995

GROUPS	DOSAGE(mg /kg) CYTOXAN	9405	n	WBC NUMBER IN BLOOD (1 X 10 /L)	P VALUE
Control			10	10.45+1.94	
Cytoxan	100x1		10	4.35+1.62*	<0.01
9405 +	100x1	50x7	10	4.47+1.16**	>0.05
9405 +	100x1	100x7	10	4.65+1.34**	>0.05
9405 +	100x1	200x7	10	5.10+1.62**	>0.05

* Compared with Control Group

* * Compared with Cytoxan treated Group

PROTECTIVE EFFECT OF 9405 ON THE LOWERING OF NUCLEATED CELLS AND DNA CONTENT OF BONE MARROW CAUSED BY CYTOXAN IN MICE

A. MATERIAES AND METHODS

ANIMALS: Mice 18－22g male, provided by the Experimental Animal Center of Chinese Academy of Medical Sciences.

METHODS: The mice were divided into 5 groups randomly with 10 mice in each group. The drug was administered orally for seven consecutive days. One the 5th day a single oral dose of cytoxan (100 mg /kg) was given to the mice. The animals were sacrificed on th 8th day and both of the femur were removed. One fumur was used for counting the total number of nucleated cells and the other femur was used to measure the DNA content of the nucleated cells of the bone marrow.

B. REUSLTS:

Experiments exhibited that Cytoxan can lower both the number of nucleated cells and the DNA content of nucleated cells of the bone marrow in the mice. The dosages of 50 mg /kg, 100mg /kg, 200mg /kg of 9405 has the significant protecting effect against the lowering of nucleated cells and DNA content of the bone marrow caused by cytoxan in mice.

Tab. 13. Protective Effect of 9405 on DNA Content Change of Nucleated Cells of Bone Marrow caused by Cytoxan in Mice

Feb. 17. 1995

GROUPS	DOSAGE(mg /kg) CYTOXAN	9405	n	OD	P VALUE
Control			10	3.999＋0.02	
Cytoxan	100x1		10	2.760＋0.029＊	<0.01
9405 ＋	100x1	50x7	10	3.810＋0.14＊＊	<0.01
9405 ＋	100x1	100x7	10	3.933＋0.132＊＊	<0.01
9405 ＋	100x1	200x7	10	3.999＋0.003＊＊	<0.01

＊ Compared with the Control group
＊＊ Compared with cytoxan treated group

Tab. 14. Protective Effect of 9405 on Nucleated Cells Number Change of Bone Marrow induced by cytoxan in Mice

Feb. 17. 1995

GROUPS	DOSAGE(mg /kg) CYTOXAN	9405	n	NUMBERS OF NUCLEATED CELL IN BONE MARROW (1 X 10 /mk)	P VALUE
Control			10	2.025	
Cytoxan	100x1		10	1.255＊	<0.01
9405 ＋	100x1	50x7	10	1.485＊＊	<0.01
9405 ＋	100x1	100x7	10	1.475＊＊	<0.01
9405 ＋	100x1	200x7	10	1.555＊＊	<0.01

* Compared with the Control Group

* * Compared with cytoxan treated Group

Tab. 15. Protective Effect of 9405 on the Lowering of DNA Content of Nucleated Cells of Bone Marrow Caused by Cytoxan in Mice.

GROUPS	DOSAGE(mg /kg) CYTOXAN	9405	n	OD VALUE	P VALUE
Control			10	3.13 + 0.256	
Cytoxan	1 100x1		10	2.04 + 0.027 *	< 0.01
9405 +	100x1	50x7	10	2.185 + 0.053 * *	< 0.01
9405 +	100x1	100x7	10	2.703 + 0.130 * *	< 0.01
9405 +	100x1	200x7	10	2.222 + 0.078 * *	< 0.01

* Compared with the Control Group

* * Compared with cytoxan treated Group

Tab. 16. Protective Effect of 9405 on the Lowering of Nucleated cells of Bone Marrow Induced by Cytoxan in Mice.

Feb. 17. 1995

GROUPS	DOSAGE(mg /kg) CYTOXAN	9405	n	NUMBERS OF NUCLEATED CELL IN BONE MARROW 1 X 10 /L	P VALUE
Control			10	2.71 + 0.21	
Cytoxan	100x1		10	1.04 + 0.014 *	< 0.01
9405 +	100x1	50x7	10	1.41 + 0.014 * *	< 0.01
9405 +	100x1	100x7	10	1.74 + 0.058 * *	< 0.01
9405 +	100x1	200x7	10	1.29 + 0.078 * *	< 0.01

* Compared with the Control Group

* * Compared with cytoxan treated Group

PROTECTIVE EFFECT OF 9405 ON THE PROLIFERATION INHIBITION OF STEM CELLS OF BONE MARROW INDUCED BY CYTOXAN IN MICE.

A. MATERIALS AND METHODS

ANIMALS: Mice 18 - 22g male, provided by the Experimental Anical Center of Chinese Academy of Medical Sciences.

METHONS: Remove th bone marrow cells from both femurs of the mouse under sterille conditions and adjust th concentration of cell suspension to 5 x 10 /ml. Put 100 ul of the cell suspension to the cell suspension to each well of a plate with 96 wells and triplicated and incubated at 37 C in a incubator with 5 % CO2. After 24 hours incubation cytoxan and S9 fraction and the tested drug were added to the well. In addition, a group without S9 fraction and a group in which only 9405 was added were set up and the

incubation was continued for 72 hours. 12 hours before the accomplishment of the experiment H－Yhymidime (H－TdR) 0. 5 uci /well was added. After the termination of experiment the cells were collected and the opm was counted. The opm value represents the proliferation activity of bone marrow cells.

B. BESULTS

Experlments indicated that cytoxan can inhibit the proliferation of bone marrow stem cells of mice significantly. It was found that 9405 has a distinct protective effect against the lowering of proliferation activity of stem cells induced by cytoxan. 9405 alone (10 ug /ml, 50 ug /ml and 100 ug /ml has a stimulating activity on the proliferation of stem cells of bone marrow in mice.

Tab. 17. Protective Effect of 9405 on the Proliferation Inhibition of Stem Cells of Mice Induced by Cytoxan

Feb. 1995

GROUPS	CONCENTRATION(ug /ml) CYTOXAN	9405	cpm. (X SD) (S9+)	RISING RATE OF STEM CELLS(%)
Control			454.17+44.19	
Cytoxan	40		74.00+21.21	
9405 +	10		221.00+143.15	198.6
	50		136.67+7.15	80.6

Tab. 18 Protective Effect of 9405 on the Inhibition of Proliferative Activity of Stem Cells of Bone Marrow Induced by Cytoxan

GROUPS	CONCENTRATION) (ug /ml)	cpm. (X SD) (S9－)	RISING RATE OF STEM CELLS (%)
Control		485.00+205.06	
	10	1814.00+874.99	374.02
9405	10	3891.00+160.93	802.27
	50	2946.33+760.05	607.49