



Antitumor activity of Cassia Tora Leguminosae on Balb Sarcoma 180 Injected Mice

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Abstract

Juemingzi is the seed of the Cassia tora L. plants and has been used as a laxative, tonic, anti-aging, anticancer and anti-oxidant effects as well as a being a popular health drink. The anticancer activity of the seed was tested against the sarcoma 180 tumor bearing mice. The average tumor weight of the sarcoma 180 cell bearing mice receiving a normal diet (control) was 4.2± 0.3 g, where as that of the mice that were treated with 50 and 100 mg/kg body weight. Juemingzi was 3.9± 0.4 and 3.1 ± 0.4g. The extract (50, 100 and 200mg/kg b.w) treated mice demonstrated tumor growth inhibitory rates of 14.2, 31.0, and 39% respectively. The AST levels in the normal mice were 33.6± 1.2U/ml however, that of the sarcoma 180 control mice was significantly increased to 46.9 ± 1.1 karmen U/ml. The levels of AST in the mice that were treated with 50, 100 and 200 mg/kg b.w. Juemingzi were 42.0 ± 1.1, 40.0 ± 0.6 and 37.3 ± 1.3 karmen U/ml. respectively. The ALT levels in the normal group were 22.3 ± 0.7 karmen U/ml, where as those in the control group were 36.3 ± 1.0 Karmen U/ml, reflecting marked increase. The ALT levels in the 50, 100 and 200mg/kg b.w. Juemingzi group decreased to 30.1 ± 2.1, 28.2 ± 1.4 and 25.6 ± 1.2 karmen U/ml respectively. The serum TNF alpha and IL- I beta levels of the mice in the Juemingzi treated group were significantly higher than those in control group. The increase in TNF alpha and IL-I beta levels in the 200mg/kg b.w. Juemingzi treated mice were 50.6 ± 1.8 and 470.2 ± 10.2 mg/ml, respectively compared with those of the control group. The TNF levels in the mice that were treated with 50 and 100 mg/kg b.w. Juemingzi were 36.2± 0.6 and 40.1 ± 0.2, respectively and II-I beta levels were 345.6 ± 18.1 and 408.2 ± 23.0 respectively. The present study provided further data indicating the *in vivo* anticancer effects of Juemingzi.

Key words: Cassia tora Leguminosae, Balb sarcoma 180, Mice, Antitumor activity

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1. Introduction

Juemingzi is the seed of the *Cassia tora* L. (Leguminosae) plants and has been used as a laxative and a tonic, as well as a being a popular health drink. Pharmaceutical research has concentrated on beneficial activities of Juemingzi, including its anti-aging, anticancer and anti oxidant effects. It contains anthraquinones, fatty acids, amino acids and inorganic elements. Types of Juemingzi with high anthraquinones content, including chrysophanol, physcion and obtusin may aid in cancer prevention. The sarcoma 180 mouse cell line is derived from a sarcoma that has been described to grow in multiple inbred mouse strain due to Beta-2 micro globulins deficiency, major histocompatibility complex (MHC) class I destabilization and lack of recognition by host cytotoxic T lymphocytes. An injection of these cells into the mice results in mortality due to the accumulation of ascites fluid. BALB/c mice are distributed globally and are among the most widely used inbred strains that are used for animal experimentation. Balbo/c mice are often used for in vivo cancer research. The sarcoma 180 tumor bearing mouse model was a staple research animal modal that was used for the tumor and metastasis study.

2. Materials and Methods

Preparation of Juemingzi:

It is purchased from Yunnan Baiyao group.Co.Ltd, Chennai, stored at -80°C and freeze dried to produce a powder. A 20 fold volume of methanol was added to the powdered sample and extracted twice by stirring overnight. The methanol extract was evaporated using a rotary evaporator concentrated and dissolved in dimethylsulfoxide to adjust to the stock concentration (205w/v).

Animals:

Female six week old Blab/c mice (=50) were purchased. The mice were maintained in a temperature controlled facility with light/dark cycle and free access to a standard mouse diet and water. The study protocol was approved by animal ethical committee.

Cell preparation:

Mouse sarcoma 180 cells were purchased. The sarcoma 180 cell line was cultured for 7-10 days in the abdominal cavity of the mouse and cultured cells were harvested with the peritoneal fluid and centrifuged at 300 g for 10 min in phosphate buffered saline (PBS). The separated sarcoma cells were suspended in PBS.

Serum aspartate aminotransferase (AST):

Alanine transaminase and blood urea nitrogen levels. The AST, ALT and BUN levels in the serum were determined using enzyme linked immunisorbent assay (ELISA) kit.

Splenocyte proliferation assay:

Splenocyte were obtained by gentle disruption of the spleen of the Balb/c female mice and filtered via a 40-micro meter nylon cell stainer. Splenocyte were treated with mitogens including lipopolysaccharides and concanavalin at 10 micro g/ml and co cultured with the test sample in 24 well plates for 24, 48 and 72 h at 37°C in humidified atmosphere of 5% CO_2 .

ELISA analysis of serum inflammation related cytokines:

For the serum cytokine assay, blood from the inferior venacava was collected into a tube and centrifuged at 1,800g for 10 min at 4°C . The serum was aspirated and assayed as follows. The serum concentration of the inflammatory related cytokines, tumor necrosis factor and interleukin's were measured by ELISA according to the manufacturer's instructions. The supernatant of the homogenized serum were incubated at 37°C in CO_2 for 2 hrs. Subsequent to be washed with PBS, streptavidin-horseradish peroxidase was added and plates incubated for 30 min at room temperature. Absorbance measured at 450nm using micro plate reader.

Statistical analysis:

Analysis of variance (ANOVA) was performed and results are presented at the mean standard deviation. $P < 0.05$ was considered to indicate a statistically significant difference.

3. Results and Discussion

Tumor Growth Inhibitory Effect of Juemingzi:

The anticancer activity of the seed was tested against the sarcoma 180 tumor bearing mice. The average tumor weight of the sarcoma 180 cell bearing mice receiving a normal diet (control) was 4.2 ± 0.3 g, where as that of the mice that were treated with 50 and 100 mg/kg body weight. Juemingzi was 3.9 ± 0.4 and 3.1 ± 0.4 g. The extract (50, 100 and 200mg/kg b.w) treated mice demonstrated tumor growth inhibitory rates of 14.2, 31.0, and 39% respectively. According this a good antitumor effect was observed in that were treated with high concentration.

Effect on serum AST, ALT and BUN levels:

The AST levels in the normal mice were 33.6 ± 1.2 U/ml however, that of the sarcoma 180 control mice was significantly increased to 46.9 ± 1.1 karmen U/ml. The levels of AST in the mice that were treated with 50, 100 and 200 mg/kg b.w. Juemingzi were 42.0 ± 1.1 , 40.0 ± 0.6 and 37.3 ± 1.3 karmen U/ml. respectively (fig.1).

Table.1

Group	Tumor weight	Inhibitory rate(%)
Control	4.2+- 0.3 ^a	
Juemingzi		
50mg/kg b.w	3.9+- 0.4 ^b	14.2
100 mg/kg b.w	3.1+- 0.4 ^c	31.0
200 mg/kg b.w	2.6+-0.3 ^d	39.0

^{a-d} mean values with different letters in the same column are significantly different (P<0.05)

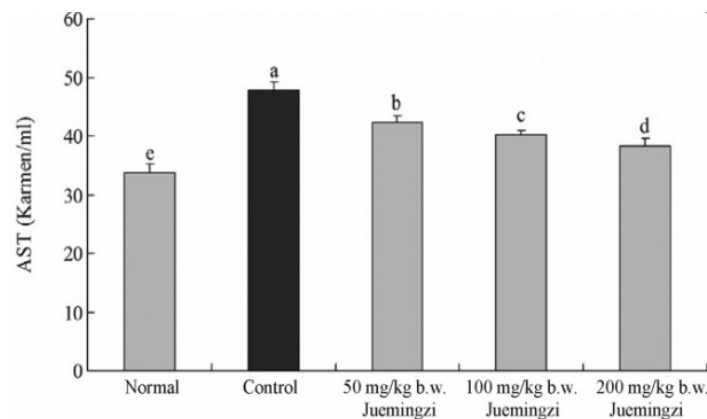


Figure.1

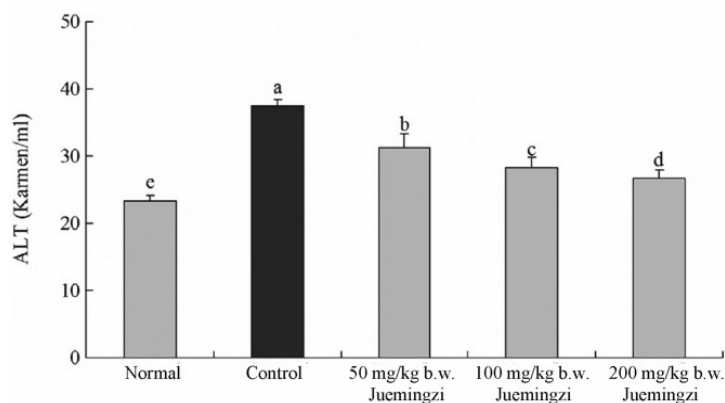


Figure.2

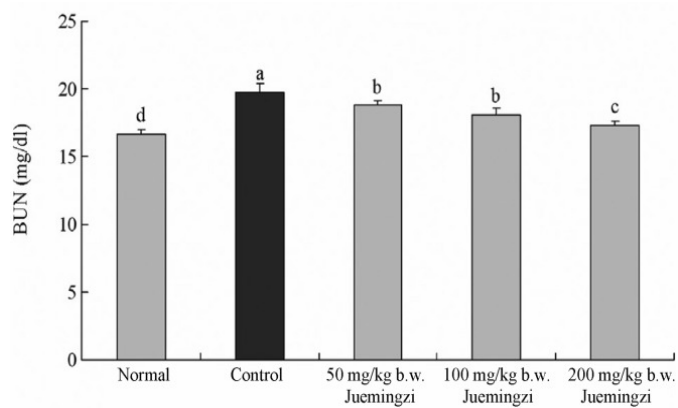


Figure.3

The ALT levels in the normal group were 22.3 ± 0.7 karmen U/ml, where as those in the control group were 36.3 ± 1.0 Karmen U/ml, reflecting marked increase. The ALT levels in the 50, 100 and 200mg/kg b.w. Juemingzi group decreased to 30.1 ± 2.1 , 28.2 ± 1.4 and 25.6 ± 1.2 karmen U/ml respectively. The levels of BUN in the 50,100 and 200mg/kg b.w. Juemingzi group were 18.6 ± 0.2 , 18.0 ± 0.3 and 17.1 ± 0.2 mg/dl, which were marginally lower than those of the control group (19.6 ± 0.04 mg/dl). However, the BUN levels in the 200 mg/kg b.w. normal group were 16.4 ± 0.3 mg/dl (Fig. 1). The AST, ALT and BUN levels in the Juemingzi groups were lower than those in the control group and the mice from the 200-mg/kg b.w.

Effect of Juemingzi on lymphocyte proliferation:

The lymphocytes of the sarcoma 180 tumor bearing mice in each group were irritated by LPS or Con A. The irritated lymphocytes were compared with the non-irritated PBS Lymphocytes. After 24 hrs the proliferation rate of the Con-A treated was greater than that of the LPS-treated lymphocytes. The con-A treated lymphocytes in the 200 mg/kg b.w. Juemingzi group demonstrated a marginally lower lymphocytes proliferation rate than that of the normal group. The lymphocytes proliferation rate in the 50, 100 mg/kg b.w. Juemingzi group was and respectively.

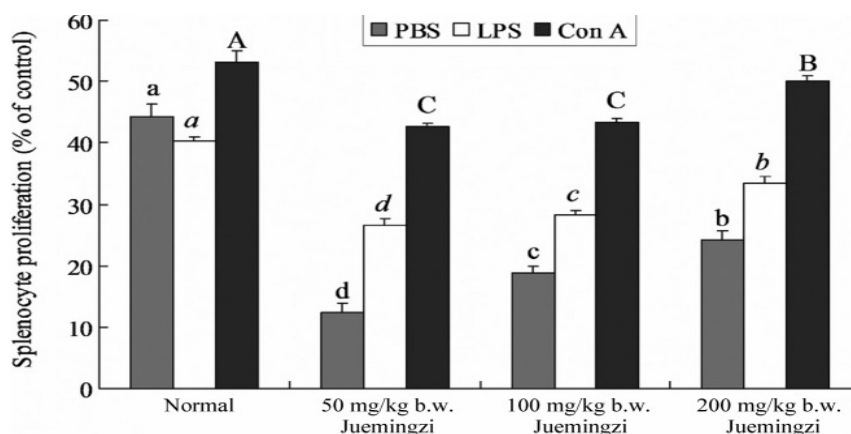


Figure.4

Effect of Juemingzi on serum TNF alpha And IL-I beta levels:

The serum TNF alpha and IL- I beta levels of the mice in the Juemingzi treated group were significantly higher than those in control group. The increase in TNF alpha and IL-I beta levels in the 200mg/kg b.w. Juemingzi treated mice were 50.6 ± 1.8 and 470.2 ± 10.2 mg/ml, respectively compared with those of the control group. The TNF levels in the mice that were treated with 50 and 100 mg/kg b.w. Juemingzi were 36.2 ± 0.6 and 40.1 ± 0.2 , respectively and IL-I beta levels were 345.6 ± 18.1 and 408.2 ± 23.0 respectively. Although Juemingzi has been used in medicine, the scientific data concerning its effects are limited. Juemingzi has previously been demonstrated to have various therapeutic effects on numerous pathological conditions, including inflammation, aging and cancer. Cell injury can release endogenous damage-associated molecular patterns that activate innate immunity. Cell injury can also increase the risk of cancer. AST and ALT are enzymes that are located in cells that leak out into the general circulation when cells are injured. AST is located in a number of body tissues, including the heart, muscle, kidney, brain and lung tissues. A decreased BUN:creatinine ratio indicates malnutrition and syndrome of inappropriate antidiuretic hormone secretion, which is occasionally observed with lung diseases, cancer and diseases of the central nervous system.

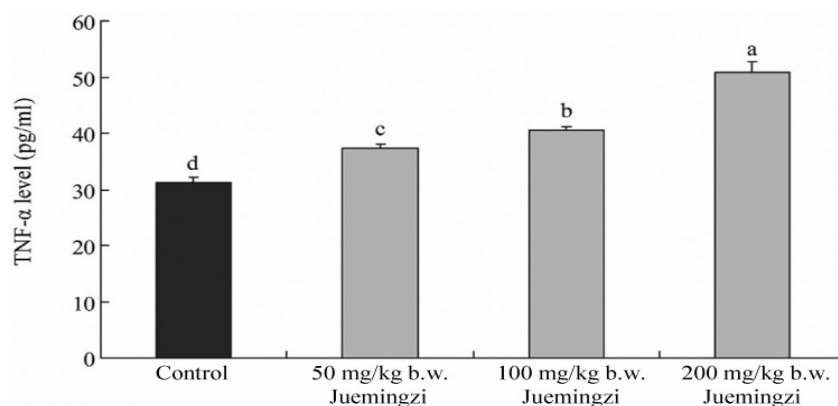


Figure.5

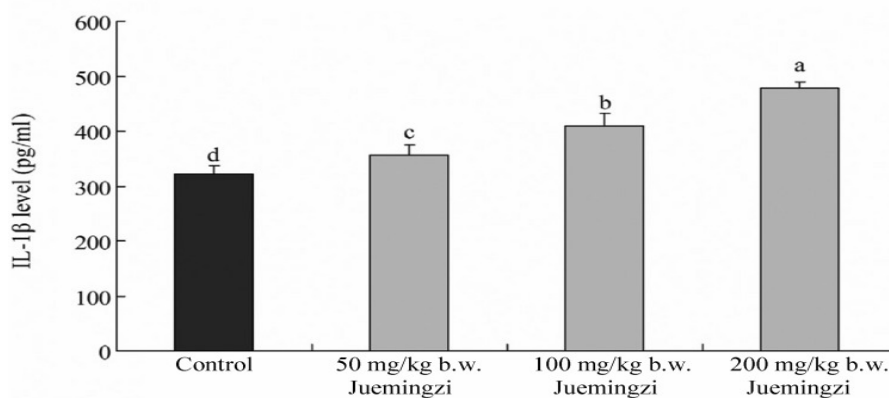


Figure.6

4. Conclusion

TNF- α and IL-1 β are significant regulators of host defense against tumor cells, The observed increase in the production of these cytokines may suggest an enhanced ability of the host to combat the growth of tumors. Macrophages are activated by β -glucans and other cell mediators to kill tumor cells by the production of TNF- α and ILs. The bioactivities of polysaccharides and polysaccharide-protein complexes are dependent on the binding of the surface receptor of immune cells. These receptors, known as pattern recognition molecules, are able to recognize foreign ligands during the initial phases of the immune response. At high levels of IL-1 β , cancer cells that receive genotoxic insults engage the apoptotic pathways. Co treatment with an inhibitor of IL-1 β and TNF- α synthesis has been demonstrated to prevent carcinogen-induced lesions. Oxidation is highly reactive and has the potential to cause damage to cells, including damage that may lead to cancer. Juemingzi demonstrated a preventive effect against atherosclerosis by inhibiting LDL oxidation. Additionally, Juemingzi was found to have *in vitro* anticancer effects in cancer cells. The present study provided further data indicating the *in vivo* anticancer effects of Juemingzi. The results from the present study have demonstrated that Juemingzi is able to decrease sarcoma 180 tumors. Juemingzi exhibited strong activity, as observed by the tumor weight count, serum levels assay and lymphocyte proliferation rate. An increased concentration of Juemingzi is significant for augmenting these antitumor effects on sarcoma 180-treated tumors. The active compounds obtained from Juemingzi (*Cassia tora* L.) need to be identified and evaluated in future studies.

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