Antitumor and immunomodulating activities of rapeseed polyphenols

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Abstract

The polyphenol of rapeseed shell was isolated and purified. The effects of the rapeseed polyphenol L-0, I, and II on the cellular immune response of Sarcoma 180-bearing mice were investigated. Mice were treated with three doses of the polyphenol L-0 (50, 100, and 200 mg/kg body weight), rapeseed polyphenol L-0, I (50 mg/kg body weight), and rapeseed polyphenol II (50 mg/kg body weight) for 10 days. It is studied from the aspects of tumor weight, pathology of tumor tissues, relative spleen and thymus weight, delayed-type hypersensitivity (DTH) response, phagocytosis of macrophage, Splenocyte antibody formation, lactic dehydrogenase (LDH) activity, Hemolysin in serum, and catalase activity. For the dose of the polyphenol L-0 (50, 100, and 200 mg/kg body weight), rapeseed polyphenol L-0, I(50 mg/kg body weight), or rapeseed polyphenol II(50 mg/kg body weight), a significant increase ($p < 0.05$＝in relative spleen and thymus weight, DTH, and phagocytosis of macrophage was observed, and a significant decrease in tumor formation, as well as a dosage effect of rapeseed polyphenol L-0 on tumor weight was also obtained. The results showed that rapeseed polyphenol exhibited significant antitumor and immunomodulating activity on mice-transplanted sarcoma 180.

Key words: Rapeseed meal; Polyphenol;Antitumor; Immunomodulation;Sarcoma 180

Introduction

Phyto-polyphenol is a kind of polyphenol with molecular weight ranged from 500 to 3000. Phyto-polyphenol shows a lot of physiological activities, such as anti-inflammatory, bacteriostasis, anti-oxidation, anti-mutation, and many other activities due to its combination with protein and radical cleanup [1-4]. Recently more and more attention was paid to the biological activities of phyto-polyphenol in medicine.

There is a lot of poly-phenol in rapeseed meal. However, rapeseed meal was usually used as feedstuff and fertilizer. Some bioactive compounds, such as rapeseed protein, poly-phenol, polysaccharide and phytin were isolated and purified to increase the value of rapeseed meal [5]. Rapeseed polyphenol exists as the forms of phenolic acid and tannin. It has been reported that rapeseed phenolic acid exhibites as the same anti-oxidative activity as the tertbutyhydroquinone, and higher anti-oxidative activity than the mixture of butylated hydroxyanisol, butylated hydroxytoluene, and monoglyceride citrate [6].

The anti-oxidation and mechanism of rapeseed polyphenol were studied in some papers, in which results showed that rapeseed polyphenol was a good scavenger of reactive oxygen species (ROS), and inhibitor of lipoxygenase in vitro and in vivo. The mechanism of rapeseed polyphenol to inhibit oxidation is probably due to the capabilities of high de-oxidation and inhibiting the enzymes associated with oxidation [7-8].

The main purpose of this paper is to research the antitumor and immunomodulating activities of rapeseed polyphenol from the aspects of tumor weight, pathology of tumor tissues, relative spleen and thymus weight, delayed-type hypersensitivity response, phagocytosis of macrophage, Splenocyte antibody formation, lactic dehydrogenase activity, Hemolysin in serum, and catalase activity.

Materials and methods

Preparation and purification of polyphenol. The cold squeezed Huaza No.4 rapeseed meal was used (Wuhan city, China). Polyphenol was extracted from the rapeseed meal sealed in a container in organic solvent A and water (65%v/v) with $1.249 \times l0^{-2}$g/ml adjunct B at 51°C for 40min. After cooled to room temperature, the extract solution was filtered to remove the meal. Organic solvent A was removed from the filtrate in a vacuum rotary evaporator below 40°C. The aqueous solution was extracted in equal volume petroleum ether. The ether phase was freeze-dried. It was applied to Special No.1 Macro reticular Resin. After washed with distilled water to remove polysaccharide and protein impurity, it was washed with 74% ethanol aqueous solution with 0.1mol/l HCl 0.5%(v/v). The ethanol solution was collected and evaporated to remove ethanol below 40°C. After freezing, its corresponding fraction was white powder(rude polyphenol).It was then applied to Sephadex LH-20 to purify the polyphenol and washed with water, methanol-water(1:2v/v) and methanol-water(1:1v/v) successively, the corresponding polyphenol L-0, polyphenol L -I and polyphenol L -IIwas obtained.

Animals. Male and female Kunming mice (18-20g, 7-9 weeks old) were purchased from the Animal Research Center, Institute of Disease Control and Prevention of Hubei Province. The mice were housed under standard laboratory conditions, and fed with standard mouse-food pellets and water ad libitum.

Treatment of mice with Sarcoma 180 cells. Sarcoma 180 cells (purchased from Tongji Medical College,

Huazhong University of Science and Technology, Wuhan) were passed into mice ascites. Then, ascites was inoculated subcutaneously 0.2 ml (1 × 106 cells) into the right axilla of each mouse.

Normal control mice were not inoculated Sarcoma180 (group I). The mice inoculated Sarcoma 180 was divided into nine groups (group II–VIII). The mice were treated as following: (Group I) normal control, received normal saline; (Group II) model control, received normal saline; (Group III), the polyphenol L-0 (50 mg/kg body weight); (Group IV), polyphenol L-0 (100 mg/kg body weight); (Group V), polyphenol L-0 (200 mg/kg body weight); (Group VI), polyphenol L -I(50 mg/kg body weight); (Group VII), polyphenol L -II(50 mg./kg body weight); (Group VIII), positive control, received Cyclophosphamide (Cy, 20 mg/kg body weight).

Normal and model control mice received the saline intraperitoneally (i.p.), while positive control mice cyclophosphamide. The rapeseed polyphenols was dissolved in saline and was administered (i.p.) for 10 days. The dose volume was 0.2 ml.

Measurements of tumor weight, relative thymus, spleen, and liver weight. After administration for 10 days, mice were sacrificed by cervical dislocation. Spleen, thymus, and tumor weights in the mice were measured [9].

Lactic dehydrogenase (LDH) activity assay. LDH activity was measured spectrophotometrically. 3.0 ml of reaction mixtures contained 6.5 mmol/l NAD, 100mmol/l Tris HCl and 100mmol/l KCl, were added into 100μl mice serum and kept at 30°C for 30s. The absorbance of the mixture was noted at 340 nm for 2 min at regular intervals, the change of absorbance per minute (ΔA/min) is calculated. The unit activity of LDH was expressed as 1μmol /min of NADH. Total LDH activity is as the following equation [10].

$$LDH(U/mL) = 4.984\Delta A/min \tag{1}$$

Catalase activity assay. The catalase (CAT) activity was measured spectrophotometrically at 230 nm. The blood from mice eyepit, after anticoagulation, centrifugation and wash with physiological saline, the erythrocytes were obtained and diluted 100 times with distilled water. 4.0ml 0.3% hydrogen peroxide was added into 6ml 50mmol/l phosphate buffer (pH 7.0),10μl erythrocyte solution was added into 3.0ml of the buffer at 25°C. The mixtures were used to measure the catalase activity. The CAT activity was measured by H_2O_2 consuming as described [11].

Phagocytosis of macrophage assay. Chicken red blood cells (CRBC) were used to assess the phagocytosis of macrophage [12-13]. Briefly, mice were sacrificed 30 min after intraperitoneally injected 1 ml 1% CRBC, and then injected 2.5 ml Hank's. Activated Macrophages were obtained by lavage of the cavity into microscope slide. After centrifugation at 150 × g for 10 min, the supernatant was removed and the free CRBC were lysed by sterile 0.16 mmol/l NH_4Cl lysing buffer. Macrophages were dyed within 0.5–1.0 min right after the microscope slide was air-dried. Microscope slide were washed by PBS and counted with microscope. The phagocytosis index was measured by counting the number of phagocytosed CRBC per 100 macrophage cells [14].

Delayed-type hypersensitivity reaction to dinitrofluorobenzene. Mice were sensitized to dinitrofluorobenzene (DNFB) by placing 25 μl 1% DNFB in acetone–gingili oil on the shaved abdominal skin of recipients on the third and fourth day. Five days later, 10 μl 1% DNFB solution was placed on the right ear. Twenty four hours later, the antigen challenge was evaluated by measuring weight difference of right and left ear with an analytical balance [15].

Splenocyte antibody assay. Splenocytes taken from mice after administration of 0.2ml 20% sheep red blood cells (SRBC) were suspended in phosphate buffer, 1.0ml 0.4% SRBC and 1.0ml cavy serum (1:10) were mixed with the splenocyte suspension, and kept at 37°C for 1 hour. After centrifugation, the absorbance of the supernatant was measured at 413nm [16].

Hemolysin in serum assay. Hemolytic assays were performed using a modification of Zhang's method [17]. 5ml 20% SRBC was injected into the mice twice at a seven days interval for immunity. The serum of each mouse was divided equally into two parts. One part was added into equal volumes of mercaptoethanol. Then, the solution was incubated at 37°C for 30 min to destroy IgM, and diluted 500 times. 0.5ml IgG (1:25), 0.5ml cavy serum (1:5) and 1.0ml physiological saline were added into a mixture of 0.5ml of the diluted solution treated with 2-mercaptoethanol and 0.5ml 5%SRBC to determine IgG hemolysin. 0.5ml of another part of serum from the treated mice by 20% SRBC, 0.5ml 5%SRBC, 0.5ml cavy serum (1:5) and 1.5ml physiological saline were mixed to determine IgM serum. All samples for assay of IgG and IgM serum were incubated at 37°C for 1 hour, and shaken once at the middle of time. The samples were then centrifuged at 3000 × g for 10min, were determined. All measurements of the supernatants were made at 540 nm. IgG hemolysin (HCIgG) and IgM hemolysin(HCIgM) were calculated as follows,

$$HC_{IgG} = A_m \times n \tag{2}$$

$$HC_{IgM} = A_b \times n \tag{3}$$

where Am was absorbance of the serum treated by mercaptoethanol, Ab was absorbance of the serum without treatment of mercaptoethanol, n was the diluted times of serum.

UV-Visible spectrum analysis.UV-Visible spectrum was obtained in a Shimazu UV-265 spectrometer.

Statistical analysis. The data were analyzed statistically using SAS8.2. The level of significance was at a P value less than 0.05.

Results

The effect of rapeseed polyphenol on Tumor inhibition. A significant tumor regression was observed at three doses of polyphenol L –0, 50 mg/kg of polyphenol L -I and 50 mg/kg of polyphenol L –IIwhile compared with model control (Figure 1). Tumor inhibition rates of polyphenol L –0 at the doses of 50-200 mg/kg were ranged from 30.23% to 44.19%, and exhibited a dosage effect. It indicated that rapeseed polyphenol could inhibit significantly the tumor although the tumor inhibition rates at polyphenol L –0(three dosage), L -I and L –II were less than that of Cy at the dosage of 20 mg/kg (Table 1). For a dosage of 50 mg/kg, the tumor inhibition rate of polyphenol L -Iwas stronger than these of polyphenol L –0 and L –II.
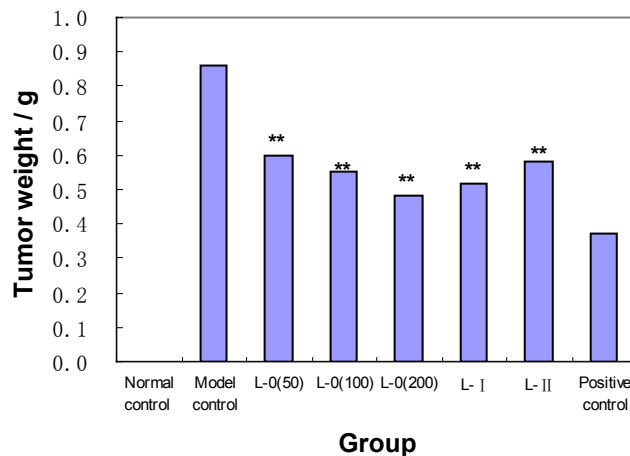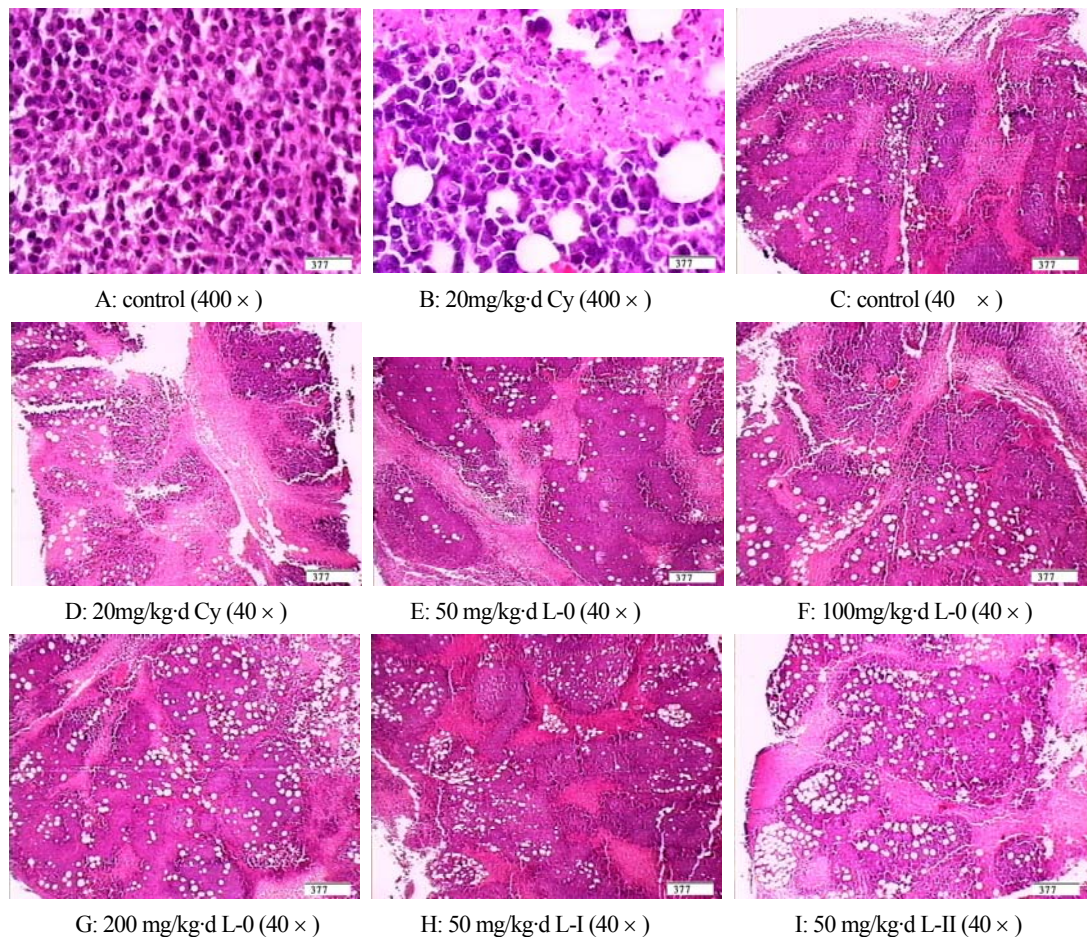

Figure 1. Effect of the rapeseed polyphenol on tumor regression of tumor-bearing mice. Model and positive control and the polyphenol groups were inoculated sarcoma 180, normal control not. Model control received saline intraperitoneally and positive control cyclophosphamide. The rapeseed polyphenol was dissolved in saline and was administered intraperitoneally. The dose volume was 0.2 ml. Data are expressed with means±S.E. of 10 mice, $P < 0.01$ vs. model control.

Table 1 Characteristics of tumor inhibition of rapeseed polyphenol on the mice inoculated Sarcoma 180.
Values are means±S.E. of 10 mice.

Group	Body weight gain (g)	Tumor inhibition rate (%)
Normal control	11.42±2.02	/
Model control	10.76±1.46	/
L-0 (50 mg/kg)	8.65±2.77	30.23
L-0 (100 mg/kg)	7.89±1.27	36.05
L-0 (200 mg/kg)	9.32±1.64	44.19
L-I (50 mg/kg)	8.80±2.90	39.53
L-II (50 mg/kg)	8.92±2.55	32.56
Positive control (Cyclophosphamide)	7.45±1.57	57.00

Rapeseed polyphenol was an anti-nutrition substance in that rapeseed polyphenol can combine with protein to influence the digestion and absorption of protein [18]. However, there were no significant difference at the index of body weight gain between rapeseed polyphenol group (polyphenol L -0(a), L -I (b), and L –II (c)) and normal control group (Table 1). Possibly because rapeseed polyphenol was injected into the axilla of mice rather than entrance through alimentary canal, rapeseed polyphenol had no chance to contact the nutrition to influence the digestion and absorption of nutrition.

Pathology observation of tumor tissue. The tumor inhibition effect of rapeseed polyphenol was also demonstrated by the micrograph of tumor tissue through HE coloration. Under the high power microscope, karyon showed black color, dyed tumor cells exhibited deeply red color and irregular profile, and some of tumor cells displayed carykinesis phase (Figure 2A), which were the characteristics of tumor during a flourishing growth. The region in the upside of Figure 2B showed lightly red color, there was only a few of purple dots and no full karyon of tumor after the administration of Cyclophosphamide, the purple dots were the residues of necrotic tumor cells. And under the high power microscope, the region of necrotic tumor cell, being lightly red color, exhibited in form of strip and piece (Figure 2E-2I). The dead tumor cells, not owing to necrosis, also showed lightly red color region in control group (Figure 2C), but the area of the region with lightly red color for control group (Fig.4c) was smaller than that of every rapeseed polyphenol group (Figure 2D-2H), it indicated that rapeseed polyphenol inhibited significantly the tumor cell growth, although the inhibition effect of rapeseed polyphenol was lower than that of Cyclophosphamide(Figure 2D). This result of images was identical with that of tumor weight. Simultaneously, the area of lightly red region exhibited a significant dosage effect at three dosages of rapeseed polyphenol L-0.

| A: control (400 ×) | B: 20mg/kg·d Cy (400 ×) | C: control (40 ×) |

| D: 20mg/kg·d Cy (40 ×) | E: 50 mg/kg·d L-0 (40 ×) | F: 100mg/kg·d L-0 (40 ×) |

| G: 200 mg/kg·d L-0 (40 ×) | H: 50 mg/kg·d L-I (40 ×) | I: 50 mg/kg·d L-II (40 ×) |

Figure 2. Microgragh of tumor tissues of various group mice treated with substance at various amplified times. a-i represented control group(no treatment, 400 ×), Cyclophosphamide (20 mg/kg, 400 ×), control group (40 ×), Cyclophosphamide (20 mg/kg, 40 ×), polyphenol L -0 (50 mg/kg, 40 ×), polyphenol L -0 (100 mg/kg, 40 ×), polyphenol L -0 (200 mg/kg, 40×), polyphenol L -I (50 mg/kg, 40 ×), and polyphenol L-II (50 mg/kg, 40 ×), respectively.

Thymus and spleen weight. Thymus and spleen is immunity organ. Their weights reflect non-specific immunity function. At the dosage of 50, 100, and 200 mg/kg polyphenol L -0, a significant increase ($P<0.05$) in relative organ weight of thymus and spleen was observed (Table 2).

Table 2. Effect of the rapeseed polyphenol on relative spleen and thymus weight of tumor-bearing mice. Relative thymus weight was measured in the ratio of the thymus weight (mg) to body weight (g). Relative spleen weight was measured in the ratio of the spleen weight (mg) to body weight (g). Values are means±S.E. of 10 mice.

Group	Relative thymus index (mg/g)	Relative spleen index (mg/g)
Normal control	3.32±0.43	6.63±1.19
Model control	2.99±0.52	8.64±1.31
L-0(50 mg/kg)	3.21±0.90	9.21±1.83
L-0(100 mg/kg)	3.27±0.52	9.01±1.50
L-0(200 mg/kg)	3.34±0.98	9.13±1.46
L-I(50 mg/kg)	3.19±1.00	9.93±1.53
L-II(50 mg/kg)	3.13±0.96	9.17±1.72
Positive control (Cyclophosphamide)	1.84±0.39	5.44±0.84

Phagocytosis of macrophage. Phagocytosis ability of macrophage increased significantly for the tumor-bearing mice treated with rapeseed polyphenol L-0, IorIIas compared with model group (Figure 3).
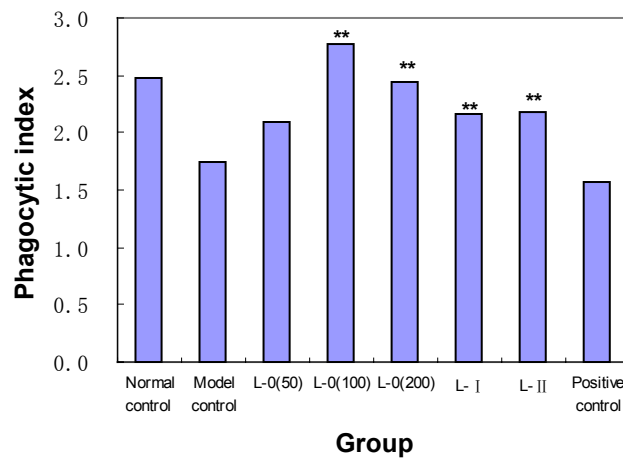
Figure 3. Effect of rapeseed polyphenol on phagocytosis of macrophage. The phagocytic index was measured by counting the number of phagocytosed CRBC per 100 macrophage cells. Values are means±S.E. of eight mice; **$P < 0.01$ vs. model control.

Delayed-type hypersensitivity reaction. Administration of five group of rapeseed polyphenol, a significant increase of weight difference is found while comparing to model control group(Figure 4).
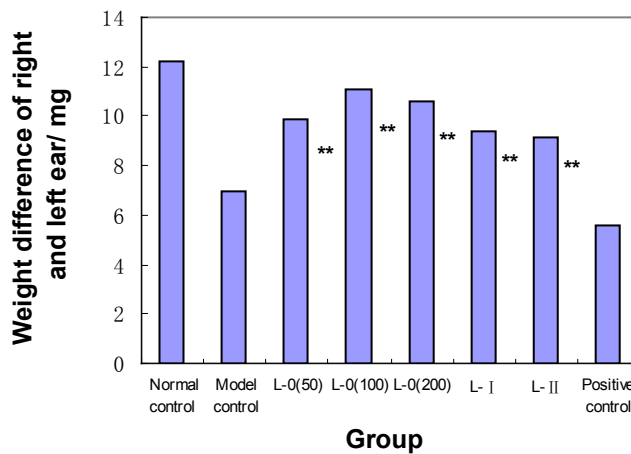
Figure 4. Effect of rapeseed polyphenol on cell immunity (evaluated by DTH to DNFB). Mice were sensitized to dinitrofluorobenzene (DNFB). The antigen challenge was evaluated by measuring weight difference of right and left ear with an analytical balance. Values are means±S.E. of 10 mice; **$P < 0.01$ vs. model control.

Splenocyte antibody formation. To confirm the effect of the rapeseed polyphenol on the cellular immune response, the antibody formation of splenocytes is evaluated from mice. The formation of splenocyte antibody shows the ability of B cell to secrete antibody and the whole immune function of boby fluid [19]. The results indicated that the antibody index of polyphenol groups increased significantly comparing to model group, whereas for polyphenol L-0, their responses were almost restored to normal level (Figure 5).

Hemolysin content in serum. Hemolysin content in serum reflects the special immune function of body fluid. From table 3, it is shown that only polyphenol L-0 at high concentration enhanced significantly the content of hemolysin. It implied that rapeseed polyphenol did not increase the immune function through hemolysin effect. However, cyclophosphamide signidicantly reduced the immune function through decreasing hemolysin content.
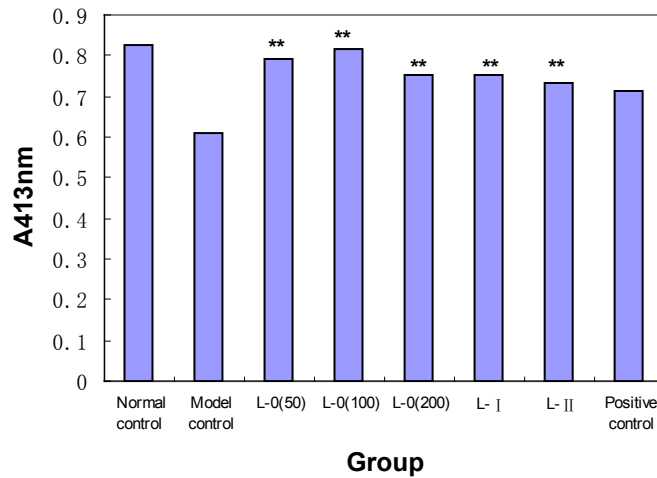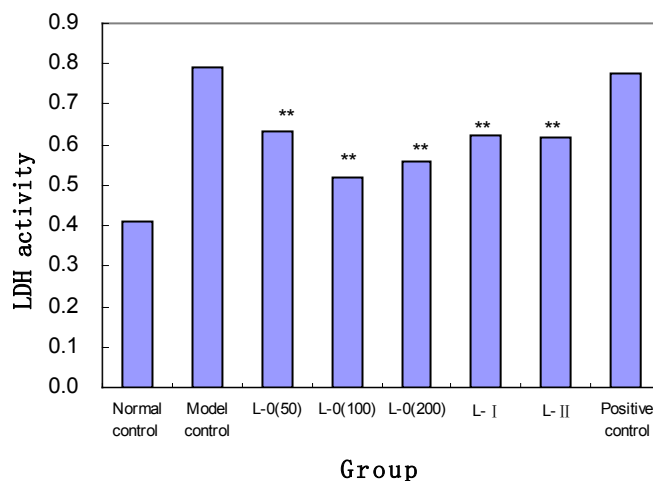
Figure 5. Effect of rapeseed polyphenol on the formation of splenocyte antibody. The concentration of Splenocyte antibody was expressed as the absorption at 413 nm. Values are means±S.E. of 10 mice; **P＜0.01 vs. model control.

Table 3 **Effect of rapeseed polyphenol on content of serum hemolysin in Sarcoma180 bearing mice.** *Values are means±S.E. of 10 mice. P＜0.05 vs. model control..*

Treatment	HC_{LgG}	HC_{LgM}	$HC_{LgG}+HC_{LgM}$
Normal control	229.6±33.7	525.7±79.2	755.3±118.7
Model control	198.4±21.0	414.3±63.0	612.7±79.8
L-0(50 mg/kg)	213.3±21.9	425.5±53.6	638.8±72.4
L-0(100 mg/kg)	214.6±18.4	434.7±50.9	649.3±65.9
L-0(200 mg/kg)	229.1±22.3	478.3±53.6	707.4±73.6
L-I(50 mg/kg)	214.1±23.4	426.4±45.9	640.5±68.1
L-II(50 mg/kg)	213.1±18.9	426.4±46.2	639.5±63.7
Positive control (Cyclophosphamide)	178.7±18.7	215.2±57.6	393.9±73.1

Lactic dehydrogenase activity. In this study, lactic dehydrogenase activity in the serum of sarcoma 180 mice decreased significantly after administration of rapeseed polyphenols (polyphenol L-0, L-I, L-II). Rapeseed polyphenol inhibited lactic dehydrogenase activity(Figure 6).

Figure 6. Effect of rapeseed polyphenol on the activity of serum lactic dehydrogenase in S180 bearing mice. *Values are means±S.E. of 10 mice; ** P <0.01 vs. model control.*

Catalase activity. The CAT activity of Erythrocyte was assessed by H_2O_2 consuming. The rapeseed polyphenol showed a significant effect on CAT activity (Figure 7).
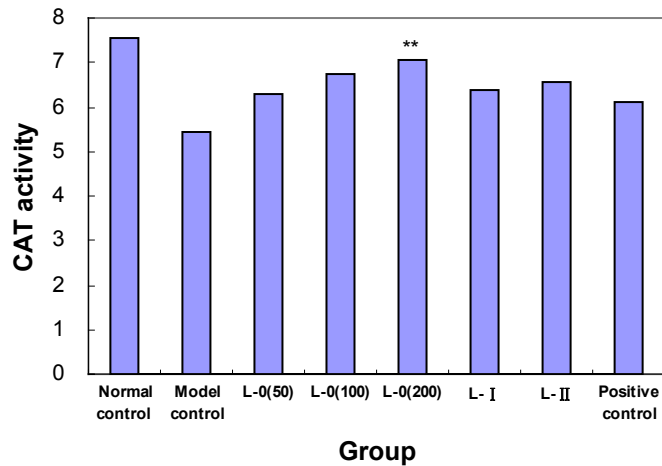
Figure 7. Effect of rapeseed polyphenol on the activity of catalase in erythrocyte of S180 bearing mice. *Values are means±S.E. of 10 mice; **P <0.01 vs. model control.*

Discussion

A tumor-bearing animal model is made to research on the antitumor and immunomodulating activities according to pharmacology. A significant tumor regression of the rapeseed polyphenol L-0 (50, 100, and 200 mg/kg), L-I(50mg/kg), and L-II(50mg/kg) group mice was observed comparing with model control group (Figure 1). The tumor inhibition effect of rapeseed polyphenol was also demonstrated by the micrograph of tumor tissue through HE coloration. It indicated that rapeseed polyphenol inhibited significantly the tumor cell growth, although the inhibition effect of rapeseed polyphenol was lower than that of cyclophosphamide (Figure 2D). This result of images was identical with that of tumor weight. Simultaneously, the area of lightly red region exhibited a significant dosage effect for rapeseed polyphenol L-0.

A significant increase of relative thymus and spleen weight in the polyphenol groups' mice was observed comparing with model control group (Table 1). The relative spleen and thymus weight were important index for nonspecific immunity. Immunopotentiator could increase spleen and thymus weight. Immunosuppressive agent could induce weight decrease of spleen and thymus or decline for immune function. The polyphenol could restore the immunity of mice, which was of the inhibition of tumor retain unclear.

It is confirmed that the polyphenol could augment phagocytosis of macrophage of tumorbearing mice (Figure 3), and Delayed-type hypersensitivity reaction enhanced significantly through administration of the polyphenol L-0, I, or II (Figure 4). The formation of splenocyte antibody shows the ability of B cell to secrete antibody and the whole immune function of boby fluid (19).Our research indicated that the antibody index of tumor-bearing mice treated with rapeseed polyphenol also increased significantly. For polyphenol L-0, the index was almost restored to normal level (Figure 5). Moreover, Hemolysin content in serum reflects the special immune function of body fluid. Table 3 showed that only polyphenol L-0 at high concentration enhanced significantly the content of hemolysin. It implied that rapeseed polyphenol did not increase the immune function through hemolysin effect. However, cyclophosphamide significantly reduced the immune function through decreasing hemolysin content.

Due to relative thymus and spleen weight, and phagocytosis index of macrophage reflect non-specific immune function of body, delayed-type hypersensitivity reaction reflects the cellular specific immune function, and hemolysin in serum reflect reflects the immune function of body fluid. All of these indexes show the whole immune function of body. The results of these indexes demonstrated that rapeseed polyphenols could enhance the immune function.

Lactic dehydrogenase is a key glycolytic enzyme. The energy for tumor cell's growth depends mainly on glycolysis since tumor cells will gangrene without nutrition [20]. Therefore, lactic dehydrogenase activity usually enhanced when tumor cells appeared. In our research, lactic dehydrogenase activity in the serum of sarcoma 180 mice decreased significantly after administration of rapeseed polyphenols (polyphenol L-0, L-I, or L-II). Rapeseed polyphenol inhibited lactic dehydrogenase activity, which availed to inhibit sarcoma 180 growth, but rapeseed polyphenol inhibited sarcoma 180 growth not only through the inhibition of lactic dehydrogenase activity because the lactic dehydrogenase activity did not exhibit a dosage effect for polyphenol L-0. As above experiments, cyclophosphamide inhibited efficiently tumor growth, but the lactic dehydrogenase activity did not change significantly after the administration of cyclophosphamide. It implied that the inhibition of sarcoma 180 growth treated with cyclophosphamide did not depend on mainly lactic dehydrogenase activity effect.

Catalase, an antioxidase, can catalyze hydrogen peroxide to be degraded as water so that the body is avoided to be oxidized. The rapeseed polyphenol L-0, Ior II could increase the catalase activity of in erythrocyte of sarcoma

180 bearing mice.(Figure 7). It demonstrated the antitumor activity of rapeseed polyphenol attributed to antioxide ability of macrophage partly.

Acknowledgement

References

[1] Nergarda, C. S.; Diallo, D.; Inngjerdingen, K.; Michaelsen, T. E.; Matsumoto, T.; Kiyohara, H.; Yamada, H.; Paulsen, B. S. Medicinal use of Cochlospermum tinctorium in Mali Anti-ulcer-, radical scavenging- and immunomodulating activities of polymers in the aqueous extract of the roots. *Journal of Ethnopharmacology*, **2005**,96: 255–269

[2] Lambert, J. D.; Yang, C. S. Cancer chemopreventive activity and bioavailability of tea and tea polyphenols. Mutation Research, **2003**, 523–524:201–208

[3] Zdunczyk, Z.; Frejnagel, S.; Wroblewska, M.; Juskiewicz, J.; Oszmianski, J.; Estrella, I. Biological activity of polyphenol extracts from different plant sources. *Food Research International*, **2002**, 35: 183-186

[4] Sakihama, Y.; Cohen, M. F.; Grace, S. C.; Yamasaki, H. Plant phenolic antioxidant and prooxidant activities: phenolics-induced oxidative damage mediated by metals implants. *Toxicology*, **2002**, 177: 67–80

[5] Yan, F.-W.; Wu, M.-C.; Jiang, H.; Wu, J.-Q. Compositive extraction of rapeseed cake. *Transaction of Chinese Society of Agricultural Engineering*, **2004**, *20*(2): 209-212

[6] Udaya, N. W.; Ryszard, A.; Fereidoon S. Partial characterization of natural antioxidants in canola meal. *Food research international*, **1996**, *28*(6):525-530

[7] Yan, F.-W.; Luo Z.-Y.;Xue Z.-H.;Wu, M.-C. Antioxidation of polyphenol from rapeseed. *China Oils and Fats*, **2005**, *30*(7): 54-57

[8]Yan, F.-W.; Luo Z.-Y.; Xue, Z.-H.; Wu, M.-C. Antioxidation effect of polyphenol-1 from rapeseed and the antioxidation mechanism. *Journal of the Chinese Cereals and Oils Association*, **2005**,*20*(5):115-119

[9] Li, W.-D.; Ren, L.-S.; Lin, Z.-B. Antitumor activity and immunopharmacological effects of Linglong liquidum extractum (LLE). *Chin J New Drug*, **2002**; 11: 928– 31.

[10] Mihich, E.; Ehrke, M. J. Anticancer drugs plus cytokines: immunodulation based therapies of mouse tumors. *International Journal of Immunopharmacology*, **2000**, 22:1077-1081

[11] Pang, Z.-J.; Zhou M.; Cheng Y. In *Method of ROS research*, People's Medical Publishing House, Beijing, China, 2000, pp 61– 150.

[12] Li, X.-D.; Rong J.-H.; Wu, M.-C.; Zeng X.-B. Anti-tumor Effect of Polysaccharide from Grifola Frondosa and Its influence on Immune Function. *Journal of Chinese Medicinal Materials*, **2003**, *26*(1):31-32

[13] Zeng, X.-B.; Wu, M.-C.; Li, X.-D. Effect of rapeseed peptides on tumor growth and immune function in mice. *Acta Nutrimenta Sinica*, **2002**, *24*(4): 405-407

[14] Zhang, J.-T.. In *Modern experimental methods in pharmacology*. Beijing Medical University and Peking Union Medical College Press; Beijing, China, 1997.

[15] Fan, M.-Z.; Li, C.-R.; Li, Z.-Z.; Lu, Z.-M.; Shi, G.-Y.; Chen, X.-.X. Evolution on immunity function of strong cordyceps mecylium capsules. *Microbiology*, **2002**, 7:19– 22.

[16] Xu, S. In *Experimental methods for Pharmacology*, People's Medical Publishing House, Beijing, China, 1982

[17] Zhang, C.-J.; Zhou, G.Y.; Li, H.; Guo, C.H.; Li, L. Associating antimutor effect of Grape polyphenol and amycin in mice with liver cancer(H22). *Journal of Shangdong University (Health Sciences)*, **2002**, *40*(5): 474-475

[18] Augustin, S.; Christine, M.; Claudine, M.; Christian,. R. Absorption and metabolism of polyphenols in the gut and impacton health. *Biomed Pharmacother*, **2002**, 56: 276–282

[19] Yan, M.-X.; Chen, Z.-Y.; Xian, B.-K.;Huang, X.-M. Effect of Flammulina Velutipes polysaccharide on serum hemolysin and cell of antibody formation in mice. *Information on Traditional Chinese Medicine*, **2003**, *20*(5): 56-57

[20] Jin, L.-Q.; Lu, J.-X.; Yang, J.Z.; Li, D.; Li, A.-L. Experimental study on the regulative effect of total polysaccharides of paecilomyces cicadidae on immunologic function in old rats. *Chinese Journal of Gerontology*, **2005**, *25*(1): 82-84