

PHARMACEUTICAL COMPOUND COMPRISING 13 GLYCERIDES, FORMULATION AND APPLICATION THEREOF

Technical Field

The present invention relates to the pharmaceutical field, specifically, the present invention relates to a pharmaceutical composition containing 13 glycerides, the pharmaceutical preparations thereof, a process for the preparation of same and the use thereof in the treatment of tumors and in the immuno-enhancement.

Background Art

Coix seeds are dried ripe seeds of *Coix lacryma-jobi* L. var *ma-yuen* (Roman.), Stapf, a genus of plant in the Poaceae family. It is a dampness-eliminating drug and has been used as a medicinal and edible plant for a long time. Modern researches have found that Coix seeds have many pharmacological effects, such as analgesic, anti-inflammatory, immunomodulatory, anti-ulcer, hypolipidemic and anti-obesity effects. In recent years, researchers around the world have studied the chemical composition of the Coix seed by using TLC, HPLC-MS, GC, etc., and found a variety of active ingredients in it, including coixenolide, triglycerides, fatty acids, lactams, coix lactones, saccharides, sterols and triterpenoids. Among them, esters are the first discovered components having anti-tumor activities and the most reported chemical composition attracting the most attention. Kanglaite injection, in which the active ingredient is Coix seed oil, has been widely used in present Chinese clinical applications. However, there is rarely report on the substance basis and active antitumor ingredients. Xiang zhiming, et al, have analyzed qualitatively triglycerides in Coix seed oil and presumed tentatively 12 triglycerides: trilinolein, olein-dilinolein, palmitin-dilinolein, diolein-linolein, palmitin-linolein-olein, dipalmitin-linolein, triolein, olein-linolein-stearin, palmitin-diolein, palmitin-linolein-stearin, dipalmitin-olein and diolein-stearin (China Journal of Chinese Materia Medica, 2005, 30 (18): 1436-1438). However, they have not further studied the specific chemical structures and pharmacological activities of these ingredients. In fact, Coix seed oil also contains monoglycerides, diglycerides and fatty acid esters, etc. It can be seen that Coix seed contains complex components. This will inevitably be a great challenge for the quality control in the practical production process and the safety in clinical applications.

Therefore, the development of a safe, effective and controllable medicine for the treatment of tumors and the immuno-enhancement became a focused issue of the invention. In the present invention, diglyceride and triglyceride ingredients have been isolated from Coix seed oil one by one, their structures have been confirmed and their pharmaceutical activities have been screened. Among them, the inventors have selected 13 glycerides having obvious antitumor and immuno-enhancement. These 13 glycerides have been combined in different ratios and their pharmacodynamic tests have been conducted. Thus, the pharmaceutical composition of the invention and pharmaceutical preparations thereof and uses thereof, in the treatment of tumors and in the immuno-enhancement, have been provided. The use of the pharmaceutical composition of the invention, having confirmed

ingredients and definite composition, in medication can ensure the stability of quality in each batch in the industrial production and avoid toxic and side effects induced by the complication of ingredients in crude Coix seed oil when it is directly adopted.

Disclosure of the Invention

The first aspect of the invention is to provide a pharmaceutical composition containing glycerides, specifically, 13 ingredients in the following mass percentages:

1,3-diolein	0.41-0.59
1-linolein-3-olein	0.93-1.33
1,2-diolein	0.24-0.35
1-olein-2-linolein	0.68-0.97
1,2-dilinolein	0.33-0.48
trilinolein	2.01-2.89
1-olein-2,3-dilinolein	23.46-33.72
1-palmitin-2,3-dilinolein	3.33-4.78
1,3-diolein-2-linolein	21.6-31.05
1-palmitin-2-linolein-3-olein	9.99-14.35
1,3-dipalmitin-2-linolein	0.39-17.80
triolein	12.39-17.80
1-palmitin-2,3-diolein	4.26-6.12

Preferrably, the 13 ingredients are in the following mass percentages:

1,3-diolein	0.46-0.56
1-linolein-3-olein	1.04-1.28
1,2-diolein	0.27-0.34
1-olein-2-linolein	0.76-0.93
1,2-dilinolein	0.38-0.46
trilinolein	2.26-2.57
1-olein-2,3-dilinolein	26.39-32.25
1-palmitin-2,3-dilinolein	3.74-4.58
1,3-diolein-2-linolein	24.30-29.70
1-palmitin-2-linolein-3-olein	11.23-13.73
1,3-dipalmitin-2-linolein	0.44-0.53
triolein	13.93-17.03
1-palmitin-2,3-diolein	13.93-17.03

More preferrably, the 13 ingredients are in the following mass percentages:

1,3-diolein	0.50-0.52
1-linolein-3-olein	1.14-1.18
1,2-diolein	0.30-0.31
1-olein-2-linolein	0.83-0.86

1,2-dilinolein	0.41-0.43
trilinolein	2.47-2.57
1-olein-2,3-dilinolein	28.73-29.91
1-palmitin-2,3-dilinolein	4.08-4.24
1,3-diolein-2-linolein	24.46-27.54
1-palmitin-2-linolein-3-olein	12.23-12.73
1,3-dipalmitin-2-linolein	0.47-0.49
triolein	15.17-15.79
1-palmitin-2,3-diolein	5.22-5.43

The above 13 glyceride ingredients can be isolated by using the method described in the examples of the description, or prepared by using conventional synthesis in the art, or purchased from markets.

The second aspect of the invention is to provide a pharmaceutical preparation containing glycerides, specifically, it comprises a therapeutically effective amount of the pharmaceutical composition of the invention and one or more pharmaceutically acceptable carriers.

Pharmaceutically acceptable carriers can be selected from pharmaceutical conventional dilutions, excipients, fillers, emulsifiers, binders, lubricants, absorption accelerators, surfactants, disintegrants, lubricants and antioxidants, if necessary, flavoring agents, sweeteners, preservative and/or coloring agents.

Pharmaceutically acceptable carriers can be selected from one or more in the group consists of: mannitol, sorbitol, sodium metabisulfite, sodium bisulfite, sodium thiosulfate, cysteine hydrochloride, thioglycolic acid, methionine, soybean lecithin, vitamin C, vitamin E, EDTA disodium, EDTA calcium sodium, monovalent alkali metal carbonate, acetate, phosphate or its aqueous solution, hydrochloric acid, acetic acid, sulfuric acid, phosphoric acid, amino acids, sodium chloride, potassium chloride, sodium lactate, ethylparaben solution, benzoic acid, potassium sorbate, chlorhexidine acetate, xylitol, maltose, glucose, fructose, dextran, glycine, starch, sucrose, lactose, mannitol, silicic derivatives, cellulose and its derivatives, alginates, gelatin, polyvinyl pyrrolidone, glycerin, Tween 80, agar-agar, calcium carbonate, calcium bicarbonate, surfactants, polyethylene glycol, cyclodextrin, β -cyclodextrin, phospholipid material, kaolin, talc, and calcium stearate or magnesium stearate.

The pharmaceutical preparation of the invention can be oral solid preparations, oral liquid preparations or injections.

Preferably, the oral solid preparation is selected from any one of capsules, tablets, dripping pills, granules, and concentrated pills; the oral liquid preparation is selected from any one of aqueous or oily suspensions, solutions, emulsions, syrups or elixirs, and a dry product that may be reconstructed by water or other suitable carrier before use; and the injection is selected from any one of nano suspensions, liposomes, emulsions, lyophilized powder for injection and aqueous injections.

More preferably, the injection comprises the following components: the pharmaceutical composition of the invention 50-350 g, soybean lecithin for injection or soybean lecithin acceptable for injection 10-40 g, glycerin for injection or glycerin acceptable for injection 15-50 g, and Water for

injection added to 1000 mL.

The injection of the invention can be prepared by a method comprising steps of:

adding appropriate amount of water for injection to a formulated amount of soybean lecithin for injection or soybean lecithin acceptable for injection; dispersing the mixture with a high shear dispersing emulsifier to give a dispersion without bulks or granules; adding a formulated amount of glycerin for injection or glycerin acceptable for injection; then adding water for injection to a specified amount, and stirring the mixture to give a water phase;

weighing a formulated amount of the pharmaceutical composition of the invention having 13 active ingredients; heating the weighed oil and the water phase separately to 60-70°C, then mixing them and emulsifying the mixture in a high pressure homogenizer, in which the low pressure is 5-12 MPa and the high pressure is 25-50 MPa; repeating the cycle of homogenization for 3-6 times until the amount of particles below 2 µm is no less than 95% and particles above 5 µm are undetectable; if necessary, using NaOH or HCl to adjust the pH to 4.8 to 8.5, preferably 6.8 to 7.0, most preferably 6.8; and

filtering the resulting homogeneous emulsion by nitrogen pressure through a microporous filter of 3 µm or less; filling the emulsion with nitrogen, sterilizing and cooling to afford the injection.

The capsule of the invention comprises the following components: the pharmaceutical composition of the invention having 13 active ingredients 200-800 g, antioxidant(s) and/or emulsifier(s) 0.20-0.60 g for 1000 capsules.

The capsule of the invention can be prepared by a method comprising steps of:

preparing glue solution: weighing gelatin, purified water, glycerin and a preservative at a weight ratio of 1 : 0.6-1.2 : 0.3-0.8 : 0.0001-0.01; adding glycerin, purified water and preservative (selected from any one of 10% ethylparaben solution, benzoic acid, potassium sorbate and chlorhexidine acetate) sequentially into a glue melting tank; heating to 70°C - 90°C; then adding gelatin and constantly stirring the mixture under vacuum until the gelatin is completely dissolved; filtering the glue solution and storing the filtered glue solution at 56-62°C for use;

preparing drug liquid: adding formulated amount of Coix seed oil, antioxidant (Vitamin E) and/or emulsifier (Tween 80) into an dosing tank, and stirring the mixture constantly until being homogeneously mixed; and

pressing capsules: choosing proper pellet dies according to the capsule size; pressing capsules in a temperature of 15-30°C and a relative humidity of less than 35%; drying the pressed and shaped capsules; after removing capsules of abnormal size, washing the normal capsules with 95% medicinal ethanol, and drying them continuously to a moisture content of less than 12%; visually inspecting and removing unqualified capsules; finally printing and packaging to afford the capsules.

It is demonstrated, in pharmacodynamic experiments, that the pharmaceutical composition of the invention and pharmaceutical preparations thereof have shown different degrees of inhibition on a variety of human tumor cell lines. Thus, they can be used to treat neoplastic diseases.

Therefore, another aspect of the invention is to provide a use of the above pharmaceutical composition and pharmaceutical preparations thereof in the preparation of antitumor drugs and the

drugs enhancing immunity function.

The pharmaceutical composition of the invention or the pharmaceutical preparation thereof can be administered alone or in combination with LAK cells (lymphokines activated killer cells).

Preferably, the tumor refers to lung cancer, liver cancer, pancreatic cancer, prostate cancer, ovarian cancer or breast cancer, in early, middle or late stage.

The following experimental data are used to illustrate beneficial effects of the pharmaceutical composition of the invention and the pharmaceutical preparations thereof in anti-tumor and immuno-enhancement.

I. Inhibition of the pharmaceutical composition of the invention and preparations thereof on 8 human tumor cell lines in MTT method in vitro

A. Experimental materials and the preparation thereof:

(1) Cell lines: PANC-1 (human pancreatic cancer cells), SKOV3 (human ovarian cancer cells), MCF-7 (human breast cancer cells), Bcap-37 (human breast cancer cells), SMMC-7721 (human hepatic cancer cells), HepG-2 (human hepatic cancer cells), A549 (human lung cancer cells) and H460 (human lung cancer cells), stored and passaged maintainably in Research and Evaluation Center for Pharmacology, Shanghai Institute of Pharmaceutical Industry;

(2) DMEM complete medium supplied with 10% newborn calf serum (GIBCO BRL), 1% of penicillin (100 U/mL) + streptomycin (100 µg/mL);

(3) 0.25% trypsin solution, purchased from Invitrogen Corp. and stored at -20°C;

(4) Phosphate buffer (PBS): NaCl 8g, KCl 0.2g, Na₂HPO₄ 1.15g and KH₂PO₄ 0.2g, dissolved in 1L double-distilled water and autoclaved at 121°C for 20 min, then stored at 4°C;

(5) MTT (AMRESCO) solution: 5mg/ml in PBS;

(6) Formazan crystal dissolving solution: SDS 10g, isobutanol 5ml and concentrated hydrochloric acid 0.1ml, dissolved in 100ml of deionized double distilled water.

B. Experimental method

The inhibition effects of samples on the above-mentioned cell lines were detected by using MTT method. The specific procedures were as follows:

(1) Cell culture: (a) Stored cells were taken out from the liquid nitrogen, thawed quickly in a 37°C water bath, then aseptically transferred into 6ml of cellular medium in a 10ml centrifugal tube, and centrifuged at 1000rpm for 5 min. The supernatant was discarded, then the precipitated cells were re-suspended in 5-6ml cellular media by pipetting and transferred into a flask in a 37°C incubator for cell culture; (b) Next day, the flask was taken out from the incubator and the used medium was discarded, then the cells were incubated in 5-6ml fresh medium in the 37°C incubator; (c) On the third day, the flask was taken out from the incubator and the used medium was discarded, then 2-3ml of PBS (pH7.4) was added into the flask with rocking for cleaning it and the used PBS was discarded. Such a cellular cleaning step was repeated once again. 3-5 drops of 0.25% trypsin solution were added into the flask with sloshing, thus well-distributed in it. The flask was capped and

placed in a 37°C incubator for about 3 min, and the separation of cells from the flask wall was observed under the microscope. 2ml of cellular medium was added and cells were separated completely from the flask wall by pipetting, then the cell suspension was transferred into 2 separate clean flasks, each containing 5-6ml medium. The cell suspension was well-distributed by pipetting, then the flask was placed in a 37°C incubator. (d) Step(c) was repeated every other day. In the whole cultivation process, adherent cells were not allowed to grow too dense and suspension cells were always maintained at a logarithmic growth stage.

(2) Preparation of the sample and the control: A proper amount of the pharmaceutical composition of the invention was dissolved in DMSO to obtain a solution in a concentration of 10mg/ml. This solution was diluted in a gradient dilution with PBS to obtain a set of sample solutions in the concentration of 10mg/ml, 5000µg/ml, 2500µg/ml, 1250 µg/ml, 625 µg/ml and 312.5 µg/ml, respectively.

(3) Each diluted sample solution was added into duplicated wells of a 96 well flat-bottom microplate (10µl /well). The correspondingly diluted DMSO solutions, as controls, were added into the wells of the microplate.

(4) Cells in a logarithmic growth stage were trypsinized and washed, then re-suspended in the medium containing 10% calf serum. The number of living cells was counted in Trypan blue dye exclusion method and cell suspensions were adjusted into a density of 2×10^5 cell/ml.

(5) The cell-contained 96 well flat-bottom microplate was placed in a 37°C incubator and cells were incubated under 5% CO₂ for 48h.

(6) 20 µl of 5 mg/ml MTT solution was added into each well and cells were incubated continuously in the incubator for 3-4h.

(7) 100 µl of crystal dissolving solution was added into each well and cells were incubated continuously in the incubator overnight, so as to dissolve the resulted formazan crystals sufficiently. Then, the absorbance value was measured at 570nm for each well.

(8) Based on absorbance values, inhibition rates on the cell growth were calculated for sample groups of various concentrations. The calculation formula was as follows:

$$(1 - \text{mean absorbance of experimental wells} / \text{mean absorbance of control wells}) \times 100\%$$

C. Experimental results

Table 1. Inhibition rates of samples in various concentrations
on the growth of 8 cell lines (%)

Cell line	Concentration of Sample					
	1000µg/ml	500µg/ml	250µg/ml	125µg/ml	62.5µg/ml	31.25µg/ml
PANC-1	98.74	72.27	24.27	15.90	15.61	1.48
SKOV3	98.92	60.16	25.08	17.78	9.07	0.33
MCF-7	98.37	64.34	22.14	11.40	6.76	0.33

Bcap-37	98.94	71.16	40.65	18.07	3.39	0.79
SMMC-7721	97.98	64.01	38.32	23.26	12.06	2.74
HepG-2	98.09	67.67	34.48	27.61	16.92	1.26
A549	99.15	75.73	57.01	41.67	19.82	1.07
H460	96.98	72.98	55.32	42.48	16.77	6.89

Table 2. IC₅₀ values of samples in 8 cell lines in vitro (μg/ml)

Cell line \ Sample	Pharmaceutical composition of the invention	Positive control (Taxol)
PANC-1	230.6	0.44
SKOV3	259.8	0.22
MCF-7	283.6	0.18
Bcap-37	240.6	0.28
SMMC-7721	225.9	0.41
HepG-2	224.4	0.45
A549	177.4	0.46
H460	173.2	0.49

D. Conclusion

The pharmaceutical composition of the invention and preparations thereof in various concentrations have shown inhibition effects on 8 human tumor cell lines in different degrees.

II. Effects of the injection of the invention on immunological functions in mice

Animals and materials

Animal:

Kunming mice, male, 18-22g, provided by Shanghai Institute of Pharmaceutical Industry, Certificate of quality No. 107;

C57BL/6 mice, male, 18-20g, provided by Shanghai Experimental Animal Center;

DBA/6 mice, male, 18-20g, provided by Shanghai Experimental Animal Center, Certificate No. 005.

Materials:

Injection of the invention: prepared according to the invention by Zhejiang Hospital of Traditional Chinese Medicine;

Solvents (blank control): provided by Zhejiang Hospital of Traditional Chinese Medicine;

Lentinan (positive control): 4mg/2ml, produced by Fuzhou Meifeng Pharmaceutical Factory, Batch No. 911026;

Medium: RPMI1640 (Difco), containing 15% calf serum, mercaptoethanol and Hepes, etc.;

³H-TdR (1 mci/ml): provided by Shanghai Institute of Atomic Nucleus;

Concanavalin A(ConA): 50 μg/ml, Sigma;

YAC-1 cell strain, L1210 cell strain and CTLL cell strain: reserved in the applicant's laboratory.

Method and Results

1. Influence of the injection of the invention on the splenic lymphopoiesis induced by ConA in mice in vitro

Spleens were picked up from C57BL/6 mice in aseptic condition. Splenocytes were separated and the concentration of cells was adjusted to 1×10^7 cells/ml with RPMI 1640+15% FCS medium.

The experiment groups were as follows: the injection of the invention in four concentrations, lentinan (positive control group) in four concentrations, corresponding solvent (blank control I) and medium (blank control II). 100 μ l Cells, 100 μ l solution of drug/control and 50 μ l ConA were added in each well in triplicate. The 96 well microplate was incubated in a 37°C incubator under 5% CO₂ for 48h. ³H-TdR (0.5 μ ci/well) was added and the incubation was continued for 20h. Cells were collected, and CPM values were determined in a liquid scintillation counter and compared with control groups. It is shown in the results (table 3) that the injection of the invention, as same as lentinan, has obviously facilitated the splenic lymphopoiesis in mice in vitro and the effect is linear dependent to the concentration.

Table 3. Influence of the injection of the invention on the splenic lymphopoiesis in mice in vitro

Group	Concentration (μ l)		CPM ($\bar{x} \pm SD$)
Injection of the invention	100	(1:4)	7765 $\pm$ 1008**
Injection of the invention	100	(1:2)	7165 $\pm$ 1163**
Injection of the invention	100	(1:1)	6540 $\pm$ 946**
Injection of the invention	100	(stock solution)	6230 $\pm$ 1130**
Lentinan	100	(1:4)	11555 $\pm$ 1279**
Lentinan	100	(1:2)	11025 $\pm$ 1133**
Lentinan	100	(1:1)	7700 $\pm$ 747**
Lentinan	100	(stock solution)	7420 $\pm$ 957**
Corresponding solvent	100		4612 $\pm$ 719
Medium	100		4795 $\pm$ 487

**P<0.01, Compared with control groups, corresponding solvent and medium

2. Influence of the injection of the invention on the splenic lymphopoiesis in mice bearing cancer

Each of 60 DBA/2 mice was inoculated subcutaneously with 1×10^4 L1210 leukemia cells of mice. Next day, the mice were grouped randomly into 6 groups (10 mice per group): Lentinan 20mg/kg, the injection of the invention (6.25 ml/kg, 12.5 ml/kg and 25 ml/kg), solvent control and NS control. Animals were administered, i.v., for 7 days, then sacrificed. Spleens were picked up in aseptic condition. Splenocytes were counted and adjusted into 1×10^7 /ml. Cells (100 μ l/well) and medium (100 μ l/well) were added into wells of a 96 well microplate in triplicate. The 96 well microplate was incubated in a 37°C incubator under 5% CO₂ for 48h. Then, ³H-TdR (0.5 μ ci/well) was added and the incubation was continued for 20h. Cells were collected, and CPM values were determined and compared with control groups. It is shown in the results (table 4) that the injection of the invention has obviously enhanced the splenic lymphopoiesis in mice bearing L1210 leukemia cells, and the immuno-enhancement effect has increased with the increasing dose. Lentinan has also shown immuno-enhancement effect.

Table 4. Influence of the injection of the invention on the splenic

lymphopoiesis in mice bearing L1210 leukemia cells

	Dose (ml/kg)	Dose regimen	No.	CPM($\bar{x} \pm SD$)
Injection of the invention	6.25	iv×7	10	8970 ± 415**
Injection of the invention	12.5	iv×7	10	10720 ± 565**
Injection of the invention	25.0	iv×7	10	14330 ± 360**
Lentinan	20mg/kg	iv×7	10	7410 ± 770**
Solvent	10.0	iv×7	10	5690 ± 1180 [△]
NS	10.0	iv×7	10	5230 ± 455

**P<0.01, compared with solventor NS; [△] P>0.1, compared with NS.

3. Influence of the injection of the invention on the activity of natural killer cells (NK cells) in mice in vitro

Method: The activity of NK cells were detected by the inhibition on ³H-TdR incorporation.

Spleens were picked up from C57BL/6 mice in aseptic condition. Splenocytes, as effector cells, were adjusted to 1×10⁶ cells/ml. YAC-1 cells, target cells, which have been cultured for 24h, were adjusted to 1×10⁴ cells/ml. The cells were added into wells of a 96 well microplate (100μl/well). The experiment groups were as follows: the injection of the invention in four concentrations, lentinan (positive control group) in four concentrations, corresponding solvent (blank control I) and medium (blank control II). To each well of a 96 well microplate were added Splenocytes: YAC-1 cells = 100: 1 (100μl), test sample (100μl) and ³H-TdR 0.5 μci/well. After being incubated in a 37°C incubator under 5% CO₂ for 24h, cells were collected, and CPM values were determined. The specific percentage inhibition (Pi), indicating the activity of NK cells, was calculated according to the formula:

$$Pi = (1 - CPM_{Exp.} / CPM_{Cont.}) \times 100\%$$

It is shown in table 5 that the injection of the invention has, as same as lentinan, activated NK cells inordinately in mice in vitro, thus it possesses immune activation.

Table 5. Influence of the injection of the invention on the activity of NK cells in mice in vitro

Group	Concentration (μ l)		CPM ($\bar{x} \pm SD$)	Pi %
Injection of the invention	100	(1:4)	5840 $\pm$ 1045**	52.2
Injection of the invention	100	(1:2)	6830 $\pm$ 1085**	44.1
Injection of the invention	100	(1:1)	8355 $\pm$ 1250**	31.7
Injection of the invention	100	(stock solution)	10925 $\pm$ 790	12.7
Lentinan	100	(1:4)	5544 $\pm$ 85**	54.5
Lentinan	100	(1:2)	7892 $\pm$ 995**	35.5
Lentinan	100	(1:1)	8130 $\pm$ 930**	33.5
Lentinan	100	(stock solution)	8625 $\pm$ 1135	29.5
Solvent (Controller I)	100		12710 $\pm$ 1125	
Medium (Controller II)	100		12235 $\pm$ 725	

**P<0.01, compared with solvent and medium control groups.

4. Influence of the injection of the invention on the activity of NK cells in mice bearing cancer

Each of 60 DBA/2 mice was inoculated subcutaneously with 1×10^4 L1210 leukemia cells of mice. Next day, the mice were grouped randomly into 6 groups (10 mice per group): Lentinan 20mg/kg, the injection of the invention (6.25 ml/kg, 12.5 ml/kg and 25 ml/kg), solvent control and NS control. Animals were administered, i.v., for 7 days, then sacrificed. Spleens were picked up in aseptic condition. Splenocytes were prepared and adjusted into 1×10^6 /ml. The activity of NK cells was detected by using the inhibition on ^3H -TdR incorporation. To wells of a 96 well microplate were added splenocytes cells (effector cells) 100 μ l/well, YAC-1 cells (target cells, 1×10^4 cells/ml) 100 μ l/well and ^3H -TdR 0.5 μ ci/well, in triplicate. After being incubated in a 37°C incubator under 5% CO₂ for 24h, cells were collected, and CPM values were determined. The specific percentage inhibition (Pi), indicating the activity of NK cells, was calculated according to the above formula.

It is shown in table 6 that the injection of the invention has, as same as lentinan, activated NK cells in mice bearing cancer, thus it possesses immune activation.

Table 6. Influence of the injection of the invention on the activity of NK cells in mice bearing cancer

Group	Dose (ml/kg)	Dose regimen	No.	CPM ($\bar{x} \pm SD$)	Pi (%)
Injection of the invention	6.25	iv $\times$ 7	10	10350 $\pm$ 1600	16.8
Injection of the invention	12.5	iv $\times$ 7	10	8000 $\pm$ 960**	34.2
Injection of the invention	25.0	iv $\times$ 7	10	6210 $\pm$ 890**	50.1
Lentinan	20mg/kg	iv $\times$ 7	10	5660 $\pm$ 260**	54.5
Solvent	10.0	iv $\times$ 7	10	12510 $\pm$ 430 ^A	
NS	10.0	iv $\times$ 7	10	12450 $\pm$ 340	

**P<0.01, compared with solvent and NS control; ^AP>0.1, compared with NS.

5. Influence of the injection of the invention on the production of IL-2 in mice bearing cancer

Each of 60 DBA/2 mice was inoculated subcutaneously with 1×10^4 L1210 leukemia cells of mice.

Next day, the mice were grouped randomly into 6 groups (10 mice per group): Lentinan 20mg/kg, the injection of the invention (6.25 ml/kg, 12.5 ml/kg and 25 ml/kg), solvent control and NS control. Animals were administered, i.v., for 7 days, then sacrificed. Spleens were picked up in aseptic condition. Splenocytes were prepared and adjusted into 1×10^7 /ml. To each well of a 24 well plate were added splenocytes cells (2ml) and Con A (5 μ g/ml). After the plate was incubated in a 37°C incubator under 5% CO₂ for 24h, the supernate was collected. The activity of IL-2 was determined in IL-2 dependent CTLL cell strain by using the method of ³H-TdR incorporation.

As shown in table 7, the injection of the invention has promoted the production of IL-2 in animals bearing cancer and this effect increased in a dose-dependent way.

Table 7. Influence of the injection of the invention on the production of IL-2 in mice suffering from L1210 leukemia

Group	Dose (ml/kg)	Dose regimen	No.	CPM ($\bar{x} \pm SD$)
Injection of the invention	6.25	iv×7	10	1460 $\pm$ 184
Injection of the invention	12.5	iv×7	10	2080 $\pm$ 386
Injection of the invention	25.0	iv×7	10	6020 $\pm$ 910**
Lentinan	20mg/kg	iv×7	10	2750 $\pm$ 123**
Solvent	10.0	iv×7	10	1750 $\pm$ 487 ^Δ
NS	10.0	iv×7	10	1830 $\pm$ 95

**P<0.01, compared with NS; ^ΔP>0.1, compared with NS.

6. Influence of the injection of the invention on the phagocytosis of macrophages of mice

Healthy Kunming mice, 18-22g, were divided randomly into a treating group and a control group (corresponding solvent), i.p. x 7 day. Each mouse was injected with 2% chicken erythrocytes (1ml), i.p. after the final administration. 30 Min later, the mouse was sacrificed via cervical dislocation and fastened in a supine position. The skin in the middle of abdomen was scissored and 2ml NS was injected into the abdominal cavity. The board was rotated for 1 min. 1 ml washing liquid was aspirated from the abdomen and dripped onto two glass slides. The glass slides were put into an enamel box padded with a wet gauze. After being incubated in a 37°C incubator for 30 min, the glass slides were rinsed in NS to remove non-adherent cells and dried in the air. The slides were fixed with acetone-methanol (1: 1) and dyed with 4% (v/v) Giemsa-phosphate buffer for 3 minutes, then rinsed with distilled water thoroughly and dried.

100 macrophages were counted for one slide under oil immersion lens. Percentage of phagocytosis and index of phagocytosis were calculated according to the formulas:

$$\begin{aligned} \text{Index of phagocytosis} &= \text{Count of swollen chicken erythrocytes} / 100 \text{ macrophages} \\ \text{Percentage of phagocytosis} &= \\ &= (\text{Count of Cells having swollen chicken erythrocyte} / 100 \text{ macrophages}) \times 100\% \end{aligned}$$

As shown in table 8, the injection of the invention (12.5 ml/kg, 6.25 ml/kg, i.p., x 7 days) has obviously promoted the phagocytic activity of peritoneal macrophages in mice.

Table 8. Activation of the injection of the invention on peritoneal macrophages in mice

	Dose (ml/kg)	Dose regimen	No.	Phagocytosis on chicken erythrocyte	
				percentage of phagocytosis (%)	Index of phagocytosis
Injection of the invention	6.25	ip×7	10	31.05 ± 1.96	0.68 ± 0.03**
Injection of the invention	12.5	ip×7	10	51.45 ± 3.28	1.75 ± 0.14**
Solvent	12.5	ip×7	10	14.00 ± 1.08	0.34 ± 0.03

**P<0.01, compared with control.

Conclusion:

The injection of the invention has shown an acceleration on splenic lymphopoiesis in mice, an activation on the activity of NK cells in mice bearing cancer, an enhancement on the production of IL-2 in mice bearing cancer, and an obvious promotion on the phagocytic activity of peritoneal macrophages in mice. Thus, the injection of the invention can enhance immunity and this is in favour of anti-tumor effects.

III. Influence of the injection of the invention on the curative effect of LAK cells

Materials & Methods

Sample: The injection of the invention, provided by Pharmaceutical Lab., Zhejiang Hospital of Traditional Chinese Medicine, Batch 930924, stored in 4°C refrigerator ;

Target cells: K562 cells, Daudi cell, introduced from Japanese National Cancer Research Center ;

Activating fluid: RPMI 1640 medium + 10% inactivated human AB-type serum + 2mM glutamine + 100 U/ml penicillin + 100 µg/ml streptomycin + rIL-2, used for the introduction of LAK cells;

Nutrient solution: RPMI 1640 medium + 10% neonatal bovine serum (NBS) + 2mM glutamine + 100 U/ml penicillin + 100 µg/ml streptomycin, used for the culture of target cells.

Introduction of LAK cells:

To the peripheral blood of healthy donors was added lymphocyte separating medium. Mononuclear cells obtained by centrifuging this suspension were washed with Hanks solution twice and suspended, in a concentration of 1×10^6 /ml, in LAK cells activating fluid containing RPMI 1640 medium, penicillin, streptomycin, glutamine, inactivated human AB-type serum and rIL-2. The cells were cultured for 10 days, centrifuged for 15min, washed with Hanks solution twice and suspended in NS injection containing 5% serum albumin of average persons and rIL-2. NK activity was indicated as killing activity of mononuclear cells separated from the peripheral blood of healthy donors.

Treatment of tumor cells with the injection of the invention:

Tumor cells used were K562 cells sensitive to NK cells and Daudi cells tolerant to NK cells. To

the suspension of tumor cells ($1 \times 10^5/\text{ml}$) was added the injection of the invention (diluted with RPMI 1640 medium in 1:8) in a ratio of 1:1. After being treated for 2h, the cell suspension served as target cells.

Test of cell killing activity:

Effector cells were washed with RPMI 1640 medium for 3 times and suspended. The suspended effector cells and/or target cells were added into each well of 96 well U bottom microplate (in a ratio of 15:1). After adding $0.5 \mu\text{Ci } ^3\text{H-TdR}$ (10 $\mu\text{l/well}$), the microplate was incubated for 18h. Then the reaction was terminated and CPM values were determined. The killing activity was calculated according to the formula of:

$$\text{Killing activity (\%)} = ((B + C - A) / C) \times 100$$

A: CPM of the well having effector cells and target cells;

B: CPM of the well having effector cells alone;

C: CPM of the well having target cells alone.

Results:

1. NK Activity: Killing activity of NK cells on K562 cells treated with the injection of the invention for 2h had no change, but on Daudi cells, increased from 4.9% to 11.0% ($P < 0.01$).

2. LAK Activity: Similarly, LAK Activity on K562 cells treated with the injection of the invention had no change, but on Daudi cells, increased from 31.1% to 43.2% ($P < 0.01$).

Discussion:

Killing activities of LAK cells and NK cells on Daudi cells treated with the injection of the invention have been significantly enhanced. Thus the injection of the invention can be used in combination with LAK therapy in clinic.

In conclusion, the pharmaceutical compositions and preparations thereof have shown inhibition to a certain degree on cell lines of pancreatic cancer, ovarian cancer, breast cancer, hepatic cancer and lung cancer, etc., in vitro; and the injection of the invention can enhance body immunity and have synergistic effects in combination with LAK therapy

It is proved by experiments that all pharmaceutical compositions and pharmaceutical preparations thereof in various contents described in the Specification can obtain effects shown in the above experiment examples.

The following examples further illustrate the invention, but are not construed as a limitation to the invention.

Detailed description of the preferred embodiments

Example 1 Preparation of Coix seed oil

1000g of Coix seeds having moisture content $\leq 10\%$ were crushed into 100 mesh powder and extracted by using a supercritical CO_2 extraction system, in which the extraction temperature was 50°C , the extraction pressure was 22 Mpa, the separation temperature was 40°C , the separation pressure was 8 Mpa, and the flow rate of CO_2 was 500 L/h. A continuous extraction for 3 h afforded a

crude Coix seed oil. To the crude Coix seed oil was added 46% petroleum ether by weight of the crude oil and 1% NaOH aqueous solution to alkali-refine the crude Coix seed oil. After layering, the upper organic phase was added with 5% activated neutral alumina by weight of the crude oil, and filtered. The filtrate was heated to 45°C and added with 4% activated kaolin by weight of crude oil, then filtered. The filtrate was concentrated under a reduced pressure to remove the solvent, and added with 10% activated neutral alumina based on the oil weight. The mixture was filtered, and the filtrated oil was sterilized by dry heating under vacuum at 170°C. After cooling, the oil was filtered to obtain Coix seed oil.

Example 2

8000 mg of Coix seed oil was dissolved in 10 ml n-hexane by using ultrasonic dissolving method, and prepared to be a Coix seed oil solution in acetone (50 mg/mL). This solution was separated in CHEETAH-HP100 preparative high performance liquid chromatography (Column: Venusil XBP silica, 20*250mm, 10µm; Mobile phase: n-hexane/acetone = 94:6 (v/v); Injection volume 15 ml; Flow rate: 18 mL/min; ELSD Detector: temperature of drift tube 45°C, flow rate of carrier gas 2.0 L/min). Peak fraction at retention time of 15.8 min was collected and concentrated under vacuum at 30°C. The concentrated fraction was transferred into a 10 ml sample vial and blow dried with nitrogen at ambient temperature to obtain a colourless oil, 1,3-diolein.

Q-TOF/MS: quasi-molecular ion peaks $[M+Na]^+ = m/z$ 643.5277 (*Calcd.* = 643.5272, $C_{39}H_{72}O_5Na$), Degree of unsaturation = 4.

1H -NMR data and ^{13}C -NMR data are shown in Table 9.

Table 9 ^1H NMR and ^{13}C NMR data (CDCl_3)

Position	^1H NMR	^{13}C NMR
C-1', 1"		174.0
C-2', 2"	2.33 (4H, t, $J = 5.0$ Hz)	34.3
C-3', 3"		25.0
C-4', 4"		29.3
C-5', 5"		29.3
C-6', 6"		29.3
C-7', 7"		29.8
C-8', 8"		27.3
C-9', 9"	5.34 (2H, m)	129.9
C-10', 10"	5.34 (2H, m)	130.2
C-11', 11"		27.3
C-12', 12"		29.9
C-13', 13"		29.5
C-14', 14"		29.7
C-15', 15"		29.5
C-16', 16"		32.1
C-17', 17"		22.8
C-18', 18"	0.87 (6H, t, $J = 5$ Hz)	14.3
C-1	4.19 (2H, dd, $J = 11.6, 4.8$ Hz) 4.13 (2H, dd, $J = 11.6, 5.7$ Hz)	65.2
C-2	4.08 (1H, m)	68.6
C-3	4.19 (2H, dd, $J = 11.6, 4.8$ Hz) 4.13 (2H, dd, $J = 11.6, 5.7$ Hz)	65.2

Example 3

8000 mg of Coix seed oil was dissolved in 10 ml n-hexane by using ultrasonic dissolving method, and prepared to be a Coix seed oil solution in acetone (50 mg/mL). This solution was separated in CHEETAH-HP100 preparative high performance liquid chromatography (Column: Venusil XBP silica, 20*250mm, 10 μm ; Mobile phase: n-hexane/acetone = 94:6 (v/v); Injection volume 15 ml; Flow rate: 18 mL/min; ELSD Detector: temperature of drift tube 45 $^{\circ}\text{C}$, flow rate of carrier gas 2.0 L/min). Peak fraction at retention time of 17 min was collected and concentrated under vacuum at 30 $^{\circ}\text{C}$. The concentrated fraction was transferred into a 10 ml sample vial and blow dried with nitrogen at ambient temperature to obtain a colourless oil, 1-linolein-3-olein.

Q-TOF/MS: quasi-molecular ion peaks $[\text{M}+\text{Na}]^+ = m/z$ 641.5121 ($\text{Calcd.}=641.5115$, $\text{C}_{39}\text{H}_{70}\text{O}_5\text{Na}$), Degree of unsaturation = 5.

^1H -NMR data and ^{13}C -NMR data are shown in Table 10.

Table 10 ^1H NMR and ^{13}C NMR data (CDCl_3)

Position	^1H NMR	^{13}C NMR	Position	^1H NMR	^{13}C NMR
C-1'		174.8	C-1''		174.8
C-2'	2.35 (4H, t, $J = 7.6$ Hz)	35.1	C-2''	2.35 (4H, t, $J = 7.6$ Hz)	35.1
C-3'		25.9	C-3''		25.9
C-4'		30.1	C-4''		30.1
C-5'		30.1	C-5''		30.1
C-6'		30.1	C-6''		30.1
C-7'		30.7	C-7''		30.7
C-8'		28.2	C-8''		28.2
C-9'	5.39 (1H, m)	131.0	C-9''	5.39 (1H, m)	130.7
C-10'	5.39 (1H, m)	129.1	C-10''	5.39 (1H, m)	131.0
C-11'	2.80 (2H, t, $J = 6.6$ Hz)	26.6	C-11''		28.2
C-12'	5.39 (1H, m)	128.9	C-12''		30.8
C-13'	5.39 (1H, m)	131.2	C-13''		30.3
C-14'		28.2	C-14''		30.6
C-15'		30.5	C-15''		30.3
C-16'		32.5	C-16''		32.9
C-17'		23.6	C-17''		23.7
C-18'	0.91 (3H, t, $J = 5.0$ Hz)	15.0	C-18''	0.92 (3H, t, $J = 5.0$ Hz)	15.1
C-1	4.21 (2H, dd, $J = 11.5, 4.3$ Hz) 4.16 (2H, dd, $J = 11.5, 5.7$ Hz)	66.0			
C-2	4.11 (1H, m)	69.4			
C-3	4.21 (2H, dd, $J = 11.5, 4.3$ Hz) 4.16 (2H, dd, $J = 11.5, 5.7$ Hz)	66.0			

Example 4

8000 mg of Coix seed oil was dissolved in 10 ml n-hexane by using ultrasonic dissolving method, and prepared to be a Coix seed oil solution in acetone (50 mg/mL). This solution was separated in CHEETAH-HP100 preparative high performance liquid chromatography (Column: Venusil XBP silica, 20*250mm, 10 μm ; Mobile phase: n-hexane/acetone = 94:6 (v/v); Injection volume 15 ml; Flow rate: 18 mL/min; ELSD Detector: temperature of drift tube 45 $^{\circ}\text{C}$, flow rate of carrier gas 2.0 L/min). Peak fraction at retention time of 23 min was collected and concentrated under vacuum at

30°C. The concentrated fraction was transferred into a 10 ml sample vial and blow dried with nitrogen at ambient temperature to obtain a colourless oil, 1,2-diolein.

Q-TOF/MS: quasi-molecular ion peaks $[M+Na]^+ = m/z$ 643.5277 (*Calcd.*=643.5272, $C_{39}H_{72}O_5Na$), Degree of unsaturation = 4.

1H -NMR data and ^{13}C -NMR data are shown in Table 11.

Table 11 ^1H NMR and ^{13}C NMR data (CDCl_3)

Position	^1H NMR	^{13}C NMR
C-1'		173.9
C-1''		173.5
C-2'	2.33 (4H, t, $J = 5.0$ Hz)	34.2
C-2''		34.4
C-3'		25.0
C-3''		25.1
C-4', 4''		29.3
C-5', 5''		29.3
C-6', 6''		29.3
C-7', 7''		29.8
C-8', 8''		27.3
C-9', 9''	5.35 (2H, m)	129.8
C-10', 10''	5.35 (2H, m)	130.2
C-11', 11''		27.3
C-12', 12''		29.9
C-13', 13''		29.5
C-14', 14''		29.7
C-15', 15''		29.5
C-16', 16''		32.1
C-17', 17''		22.7
C-18', 18''	0.88 (6H, t, $J = 5$ Hz)	14.3
C-1	4.32 (2H, dd, $J = 12.0, 4.6$ Hz) 4.24 (2H, dd, $J = 12.0, 5.6$ Hz)	62.1
C-2	5.08 (1H, m)	72.3
C-3	3.73 (2H, d, $J = 3.2$ Hz)	61.8

Example 5

8000 mg of Coix seed oil was dissolved in 10 ml n-hexane by using ultrasonic dissolving method, and prepared to be a Coix seed oil solution in acetone (50 mg/mL). This solution was separated in CHEETAH-HP100 preparative high performance liquid chromatography (Column: Venusil XBP silica, 20*250mm, 10 μm ; Mobile phase: n-hexane/acetone = 94:6 (v/v); Injection volume 15 ml; Flow rate: 18 mL/min; ELSD Detector: temperature of drift tube 45 $^\circ\text{C}$, flow rate of carrier gas 2.0 L/min). Peak fraction at retention time of 24.5 min was collected and concentrated under vacuum at 30 $^\circ\text{C}$. The concentrated fraction was transferred into a 10 ml sample vial and blow dried with nitrogen at ambient temperature to obtain a colourless oil, 1-olein-2-linolein.

Q-TOF/MS: quasi-molecular ion peaks $[\text{M}+\text{Na}]^+ = m/z$ 641.5121 ($\text{Calcd.}=641.5115$,

$C_{39}H_{70}O_5Na$), Degree of unsaturation = 5.

1H -NMR data and ^{13}C -NMR data are shown in Table 12.

Table 12 1H NMR and ^{13}C NMR data ($CDCl_3$)

Position	1H NMR	^{13}C NMR	Position	1H NMR	^{13}C NMR
C-1'		173.9	C-1''		173.5
C-2'	2.33 (2H, t, $J = 5.0$ Hz)	34.2	C-2''	2.33 (2H, t, $J = 5.0$ Hz)	34.4
C-3'		25.0	C-3''		25.1
C-4'		29.3	C-4''		29.3
C-5'		29.3	C-5''		29.5
C-6'		29.3	C-6''		29.3
C-7'		29.8	C-7''		29.9
C-8'		27.3	C-8''		27.4
C-9'	5.37 (1H, m)	129.8	C-9''	5.37 (1H, m)	130.2
C-10'	5.37 (1H, m)	130.2	C-10''	5.37 (1H, m)	128.2
C-11'		25.8	C-11''	2.77 (2H, t, $J = 6.5$ Hz)	25.8
C-12'		29.9	C-12''	5.37 (1H, m)	128.0
C-13'		29.5	C-13''	5.37 (1H, m)	130.4
C-14'		27.4	C-14''		27.4
C-15'		29.5	C-15''		29.8
C-16'		32.1	C-16''		31.7
C-17'		22.8	C-17''		22.7
C-18'	0.89 (3H, t, $J = 6.8$ Hz)	14.3	C-18''	0.88 (3H, t, $J = 6.8$ Hz)	14.2
C-1	4.32 (1H, dd, $J = 11.9, 4.5$ Hz) 4.23 (1H, dd, $J = 11.9, 5.6$ Hz)	62.1			
C-2	5.08 (1H, m)	72.3			
C-3	3.73 (2H, d, $J = 3.2$ Hz)	61.8			

Example 6

8000 mg of Coix seed oil was dissolved in 10 ml n-hexane by using ultrasonic dissolving method, and prepared to be a Coix seed oil solution in acetone (50 mg/mL). This solution was separated in CHEETAH-HP100 preparative high performance liquid chromatography (Column: Venusil XBP silica, 20*250mm, 10 μ m; Mobile phase: n-hexane/acetone = 94:6 (v/v); Injection volume 15 ml; Flow rate: 18 mL/min; ELSD Detector: temperature of drift tube 45 $^{\circ}C$, flow rate of carrier gas 2.0 L/min). Peak fraction at retention time of 27 min was collected and concentrated under vacuum at 30 $^{\circ}C$. The concentrated fraction was transferred into a 10 ml sample vial and blow dried with nitrogen

at ambient temperature to obtain a colourless oil, 1,2-dilinolein.

Q-TOF/MS: quasi-molecular ion peaks $[M+Na]^+ = m/z$ 639.4964 (*Calcd.*=639.4959, $C_{39}H_{68}O_5Na$), Degree of unsaturation = 6.

1H -NMR data and ^{13}C -NMR data are shown in Table 13.

Table 13 ^1H NMR and ^{13}C NMR data (CDCl_3)

Position	^1H NMR	^{13}C NMR	Position	^1H NMR	^{13}C NMR
C-1'		173.9	C-1''		173.5
C-2'	2.32 (4H, t, $J = 5.0$ Hz)	34.2	C-2''	2.35 (2H, t, $J = 5.0$ Hz)	34.4
C-3'		25.0	C-3''		25.1
C-4'		29.3	C-4''		29.3
C-5'		29.5	C-5''		29.5
C-6'		29.3	C-6''		29.3
C-7'		29.9	C-7''		29.9
C-8'		27.4	C-8''		27.4
C-9'	5.37 (1H, m)	130.2	C-9''	5.37 (1H, m)	130.2
C-10'	5.37 (1H, m)	128.2	C-10''	5.37 (1H, m)	128.2
C-11'	2.77 (4H, t, $J = 6.5$ Hz)	25.8	C-11''	2.77 (2H, t, $J = 6.5$ Hz)	25.8
C-12'	5.37 (1H, m)	128.0	C-12''	5.37 (1H, m)	128.0
C-13'	5.37 (1H, m)	130.4	C-13''	5.37 (1H, m)	130.4
C-14'		27.4	C-14'	'	27.4
C-15'		29.8	C-15''		29.8
C-16'		31.7	C-16''		31.7
C-17'		22.7	C-17''		22.7
C-18'	0.89 (3H, t, $J = 6.8$ Hz)	14.2	C-18''	0.89 (3H, t, $J = 6.8$ Hz)	14.2
C-1	4.32 (1H, dd, $J = 11.9, 4.6$ Hz) 4.24 (1H, dd, $J = 12.0, 5.6$ Hz)	62.1			
C-2	5.08 (1H, m)	72.3			
C-3	3.73 (2H, d, $J = 3.2$ Hz)	61.8			

Example 7 Preparation of trilinolein

Isolation was carried out on P3000A preparative high performance liquid chromatography (Column: Superstar BenetnachTM C₁₈, 20 mm×150 mm, 5 μm ; Mobile phase A: acetonitrile, Mobile phase B: acetonitrile/tetrahydrofuran (1:1)). Coix seed oil solution (50 mg/mL) was prepared with mobile phase B, and the injection volume for each separation was 1.5 mL. Gradient conditions were: mobile phase B: 0-27 min: 50%-60%, 27-35 min: 90%, 35-45 min: 100%; and flow rate: 18 mL/min. UV detection was conducted at 208 nm. Peak fractions at retention time of 12.6-14.2 min were collected, and concentrated using a rotary evaporator under vacuum in nitrogen. Residues were

transferred with chloroform to a 10 mL vial, and dried in a vacuum oven at 35°C for 6 h. After filling with nitrogen, the dried samples were frozen in a refrigerator to give the trilinolein.

HR-EI-MS: $m/z = 878.7344$ (*Calcd.*=878.7363, C₅₇H₉₈O₆), Degree of unsaturation =9.

IR (KBr film): 1746, 1170, 1098; 2928, 2856, 724; 3008, 1655 cm⁻¹ (weak).

¹H-NMR data are shown in Table 14.

¹³C-NMR data are shown in Table 15.

Table 14 ^1H -NMR spectral data of the compounds of Examples 7-14

No.	G-H	H	2-H	3-H	4-H	5-H	6-H	7-H	8-H	9-H	10-H	11-H	12-H	13-H	14-H	15-H	16-H	17-H	18-H
A LLL	α	4.30																	
	β	5.27	2.32	1.61					2.05	5.36	2.77		5.36	2.05			1.32		0.89
	α'	4.15																	
B OLL	α	4.29																	
	β	5.27	2.32	1.61					2.04	5.37	2.77		5.37	2.04			1.33		0.88
	α'	4.14									2.04				1.33				
C PLL	α	4.30																	
	β	5.27	2.31	1.61					2.05	5.36	2.77		5.36	2.05			1.31		0.88
	α'	4.15							1.31					1.31			0.88		
D OLO	α	4.30																	
	β	5.27	2.32	1.61					2.05	5.36	2.05				1.32		1.32		0.89
	α'	4.15									2.77		5.36	2.05					
E PLO	α	4.15																	
	β	5.27	2.31	1.61					2.04	5.35	2.04		1.28				1.28	1.28	0.88
	α'	4.30							1.28								0.88		
F PLP	α	4.15																	
	β	5.27	2.31	1.61					2.05	5.36	2.77		5.36	2.05			1.28		0.88
	α'	4.30									1.28						0.88		
G OOO	α	4.15																	
	β	5.27	2.31	1.61					2.00		5.34	2.00		1.28					0.88
	α'	4.30																	
H POO	α	4.15																	
	β	5.27	2.31	1.61					2.04	5.34	2.04			1.27					0.88
	α'	4.30								1.27							0.88		

A: trilinolein, B: 1-olein-2,3-dilinolein, C: 1-palmitin-2,3-dilinolein, D: 1,3-diolein-2-linolein, E: 1-palmitin-2-linolein-3-olein, F: 1,3-dipalmitin-2-linolein, G: triolein, H: 1-palmitin-2,3-diolein.

Table 15 ^{13}C -NMR spectral data of the compounds of Examples 7-14

No.	Abb.	Gl-C	C-1	C-2	C-3	C-4	C-5	C-6	C-7	C-8	C-9	C-10	C-11	C-12	C-13	C-14	C-15	C-16	C-17	C-18	
A	α	62.12	173.28	34.05	24.86						130.01	128.08		127.91							
LLL	β	68.91	172.87	34.21	24.90		29.05~29.62			27.21	129.98	128.09	25.64	127.90	130.22	27.21	29.05~29.62	31.53	22.58	14.07	
B	α	62.12	173.28	34.04	24.86						27.22	130.03	128.08		127.91						
OLL	β	68.89	172.87	34.21	24.90		29.07~29.79			27.19	130.00	128.10	25.65	127.90	130.24	27.24	29.07~29.79	31.55	22.60	14.10	
	α'		173.29								129.73	130.03	27.22		29.07~29.79			31.93	22.71	14.14	
C	α	62.12	173.30	34.04	24.89		29.06~29.72			27.21	130.02	128.08		127.909	130.236		29.06~29.72		31.54	22.59	14.09
PLL	β	68.90	172.88	34.20	24.85						129.99	128.09	25.64	127.898	130.236						
	α'		173.34	34.07	24.88						29.06~29.72					31.95	22.71	14.14			
D	α	62.12	173.29	34.05	24.86						27.20	129.73	130.03	27.24		29.07~29.79		31.93	22.71	14.14	
OLO	β	68.89	172.87	34.21	24.90		29.07~29.79			27.22	130.00	128.10	25.65	127.90	130.24	27.24	29.07~29.79	31.55	22.60	14.10	
E	α	62.11	173.28	34.04	24.85						27.18	129.71	130.01	27.21		29.06~29.78		31.92	22.69	14.12	
PLO	β	68.91	172.86	34.20	24.87		29.06~29.78			27.21	129.98	128.09	25.64	127.90	130.22	27.23	29.06~29.78	31.54	22.58	14.07	
	α'		173.32	34.06	24.88						29.06~29.78					31.94	22.71	14.12			
F	α	62.09	173.32	34.05	24.86							29.05~29.70				31.93	22.69	14.12			
PLP	β	68.89	172.86	34.19	24.88		29.05~29.70			27.20	129.97	128.08	25.63	127.89	130.22	27.20	29.05~29.70	31.53	22.58	14.07	
G	α	62.12	173.29	34.04	24.86						27.19	129.72	130.02	27.24							
OOO	β	68.90	172.87	34.21	24.90		29.07~29.78				129.69	130.03				29.07~29.78		31.92	22.70	14.12	
H	α	62.12	173.31	34.04	24.88						129.72	130.02									
POO	β	68.90	172.90	34.21	24.86		29.06~29.78			27.19	129.69	130.03	27.24			29.06~29.78		31.92	22.70	14.12	
	α'		173.35	34.06	24.90							29.06~29.78				31.94	22.70	14.12			

A: trilinolein, B: 1-olein-2,3-dilinolein, C: 1-palmitin-2,3-dilinolein, D: 1,3-diolein-2-linolein, E: 1-palmitin-2-linolein-3-olein, F: 1,3-dipalmitin-2-linolein, G: triolein, H: 1-palmitin-2,3-diolein.

Example 8 Preparation of 1-olein-2,3-dilinolein

Isolation was carried out on P3000A preparative high performance liquid chromatography (Column: Superstar BenetnackTM C₁₈, 20 mm×150 mm, 5 μm ; Mobile phase A: acetonitrile, Mobile phase B: acetonitrile/tetrahydrofuran (1:1)). Coix seed oil solution (50 mg/mL) was prepared with

mobile phase B, and the injection volume for each separation was 1.5 mL. Gradient conditions were: mobile phase B: 0-27 min: 50%-60%, 27-35 min: 90%, 35-45 min: 100%; and flow rate: 18 mL/min. UV detection was conducted at 208 nm. Peak fractions at retention time of 15.4-17.3 min were collected, and concentrated using a rotary evaporator under vacuum in nitrogen. Residues were transferred with chloroform to a 10 mL vial, and dried in a vacuum oven at 35°C for 6 h. After filling with nitrogen, the dried samples were frozen in a refrigerator to give 1-olein-2,3-dilinolein.

HR-EI-MS: $m/z=880.7518$ (*Calcd.* = 880.7520, $C_{55}H_{98}O_6$), Degree of unsaturation = 7.

IR (KBr film): 1747, 1164, 1098; 2925, 2854, 723; 3008, 1655 cm^{-1} (weak).

1H -NMR data are shown in Table 14.

^{13}C -NMR data are shown in Table 15.

Example 9 Preparation of 1-palmitin-2,3-dilinolein

Preliminary isolation was carried out on P3000A preparative high performance liquid chromatography (Column: Superstar BenetnatchTM C_{18} , 20 mm×150 mm, 5 μm ; Mobile phase A: acetonitrile, Mobile phase B: acetonitrile/tetrahydrofuran (1:1)). Coix seed oil solution (50 mg/mL) was prepared with mobile phase B, and the injection volume for each separation was 1.5 mL. Gradient conditions were: mobile phase B: 0-27 min: 50%-60%, 27-35 min: 90%, 35-45 min: 100%; and flow rate: 18 mL/min. UV detection was conducted at 208 nm. Peak fractions at retention time of 17.4-18.1 min were collected, and concentrated using a rotary evaporator under vacuum in nitrogen to give a crude product.

The secondary purification was proceeded on Superstar BenetnatchTM C_{18} Column (10 mm×250 mm, 5 μm) with mobile phase A: acetonitrile and mobile phase B: acetonitrile/ tetrahydrofuran (1:1). A solution of the above crude product (20 mg/mL) was prepared with mobile phase B, and the injection volume for each separation was 1.5 mL. Gradient conditions were: mobile phase B: 0-23 min: 50%-60%, 32-43 min: 60%-90%, 43-60 min: 100%; and flow rate: 3 mL/min. UV detection was conducted at 208 nm. Peak fractions at retention time of 31.2-34.7 min were collected, and concentrated using a rotary evaporator under vacuum in nitrogen. Residues were transferred with chloroform to a 10 mL vial, and dried in a vacuum oven at 35°C for 6 h. After filling with nitrogen, the dried samples were frozen in a refrigerator, to give 1-palmitin-2,3-dilinolein.

HR-EI-MS: $m/z=854.7370$ (*Calcd.* = 854.7363, $C_{55}H_{98}O_6$), Degree of unsaturation = 7.

IR (KBr Flim): 1746, 1165, 1095; 2926, 2854, 722; 3009, 1648 cm^{-1} (weak).

1H -NMR data are shown in Table 14.

^{13}C -NMR data are shown in Table 15.

Example 10 Preparation of 1,3-diolein-2-linolein

Isolation was carried out on P3000A preparative high performance liquid chromatography (Column: Superstar BenetnatchTM C_{18} , 20 mm×150 mm, 5 μm ; Mobile phase A: acetonitrile, Mobile phase B: acetonitrile/tetrahydrofuran (1:1)). Coix seed oil solution (50 mg/mL) was prepared with mobile phase B, and the injection volume for each separation was 1.5 mL. Gradient conditions were:

mobile phase B: 0-27 min: 50%-60%, 27-35 min: 90%, 35-45 min: 100%; and flow rate: 18 mL/min. UV detection was conducted at 208 nm. Peak fractions at retention time of 18.4-20.2 min were collected, and concentrated using a rotary evaporator under vacuum in nitrogen. Residues were transferred with chloroform to a 10 mL vial, and dried in a vacuum oven at 35°C for 6 h. After filling with nitrogen, the dried samples were frozen in a refrigerator, to give 1-olein-2,3-dilinolein.

HR-EI-MS: $m/z=882.7678$ (*Calcd.*=882.7672, $C_{57}H_{102}O_6$), Degree of unsaturation= 7.

IR (KBr film): 1747, 1163, 1097; 2925, 2855, 723; 3007, 1655 cm^{-1} (weak).

1H -NMR data are shown in Table 14.

^{13}C -NMR data are shown in Table 15.

Example 11 Preparation of 1-palmitin-2-linolein-3-olein

Isolation was carried out on P3000A preparative high performance liquid chromatography (Column: Superstar Benetnatch™ C_{18} , 20 mm×150 mm, 5 μm ; Mobile phase A: acetonitrile, Mobile phase B: acetonitrile/tetrahydrofuran (1:1)). Coix seed oil solution (50 mg/mL) was prepared with mobile phase B, and the injection volume for each separation was 1.5 mL. Gradient conditions were: mobile phase B: 0-27 min: 50%-60%, 27-35 min: 90%, 35-45 min: 100%; and flow rate: 18 mL/min. UV detection was conducted at 208 nm. Peak fractions at retention time of 20.3-21.4 min were collected, and concentrated using a rotary evaporator under vacuum in nitrogen. Residues were transferred with chloroform to a 10 mL vial, and dried in a vacuum oven at 35°C for 6 h. After filling with nitrogen, the dried samples were frozen in a refrigerator, to give 1-palmitin-2-linolein- 3-olein.

HR-EI-MS: $m/z=856.7519$ (*Calcd.*=856.7513, $C_{55}H_{100}O_6$), Degree of unsaturation= 6.

IR (KBr film): 1747, 1164, 1098; 2925, 2854, 723; 3008, 1655 cm^{-1} (weak).

1H -NMR data are shown in Table 14.

^{13}C -NMR data are shown in Table 15.

Example 12 Preparation of 1,3-dipalmitin-2-linolein

Isolation was carried out on P3000A preparative high performance liquid chromatography (Column: Superstar Benetnatch™ C_{18} , 20 mm×150 mm, 5 μm ; Mobile phase A: acetonitrile, Mobile phase B: acetonitrile/tetrahydrofuran (1:1)). Coix seed oil solution (50 mg/mL) was prepared with mobile phase B, and the injection volume for each separation was 1.5 mL. Gradient conditions were: mobile phase B: 0-27 min: 50%-60%, 27-35 min: 90%, 35-45 min: 100%; and flow rate: 18 mL/min. UV detection was conducted at 208 nm. Peak fractions at retention time of 25.7-26.2 min were collected, and concentrated using a rotary evaporator under vacuum in nitrogen. Residues were transferred with chloroform to a 10 mL vial, and dried in a vacuum oven at 35°C for 6 h. After filling with nitrogen, the dried samples were frozen in a refrigerator, to give 1,3-dipalmitin-2- linolein.

HR-EI-MS: $m/z=830.7371$ (*Calcd.*= 830.7363, $C_{53}H_{98}O_6$), Degree of unsaturation= 5.

IR (KBr film): 1747, 1164, 1098; 2925, 2854, 723; 3008, 1655 cm^{-1} (weak).

1H -NMR data are shown in Table 14.

^{13}C -NMR data are shown in Table 15.

Example 13 Preparation of triolein

Isolation was carried out on P3000A preparative high performance liquid chromatography (Column: Superstar Benetnatch™ C₁₈, 20 mm×150 mm, 5 μm; Mobile phase A: acetonitrile, Mobile phase B: acetonitrile/tetrahydrofuran (1:1)). Coix seed oil solution (50 mg/mL) was prepared with mobile phase B, and the injection volume for each separation was 1.5 mL. Gradient conditions were: mobile phase B: 0-27 min: 50%-60%, 27-35 min: 90%, 35-45 min: 100%; and flow rate: 18 mL/min. UV detection was conducted at 208 nm. Peak fractions at retention time of 26.6-27.7 min were collected, and concentrated using a rotary evaporator under vacuum in nitrogen. Residues were transferred with chloroform to a 10 mL vial, and dried in a vacuum oven at 35°C for 6 h. After filling with nitrogen, the dried samples were frozen in a refrigerator, to give triolein.

HR-EI-MS: $m/z=884.7851$ (*Calcd.*=884.7833, C₅₇H₁₀₄O₆), Degree of unsaturation= 6.

IR (KBr film): 1749, 1165, 1095; 2925, 2854, 723; 3004, 1654 cm⁻¹ (weak).

¹H-NMR data are shown in Table 14.

¹³C-NMR data are shown in Table 15.

Example 14 Preparation of 1-palmitin-2,3-diolein

Primary isolation was carried out on P3000A preparative high performance liquid chromatography (Column: Superstar Benetnatch™ C₁₈, 20 mm×150 mm, 5 μm; Mobile phase A: acetonitrile, Mobile phase B: acetonitrile/tetrahydrofuran (1:1)). Coix seed oil solution (50 mg/mL) was prepared with mobile phase B, and the injection volume for each separation was 1.5 mL. Gradient conditions were: mobile phase B: 0-27 min: 50%-60%, 27-35 min: 90%, 35-45 min: 100%; and flow rate: 18 mL/min. UV detection was conducted at 208 nm. Peak fractions at retention time of 28.2-29.3 min were collected, and concentrated using a rotary evaporator under vacuum in nitrogen, to give a crude product.

The secondary purification was proceeded on Superstar Benetnatch™ C₁₈ Column (10 mm×250 mm, 5 μm) with mobile phase A: acetonitrile and mobile phase B: acetonitrile/ tetrahydrofuran (1:1). A solution of the above crude product (20 mg/mL) was prepared with mobile phase B, and the injection volume for each separation was 1.5 mL. Gradient conditions were: mobile phase B: 0-23 min: 50%-60%, 32-43 min: 60%-90%, 43-60 min: 100%; and flow rate: 3 mL/min. UV detection was conducted at 208 nm. Peak fractions at retention time of 32.9-35.1 min were collected, and concentrated using a rotary evaporator under vacuum in nitrogen. Residues were transferred with chloroform to a 10 mL vial, and dried in a vacuum oven at 35°C for 6 h. After filling with nitrogen, the dried samples were frozen in a refrigerator, to give 1-palmitin-2,3-diolein.

HR-EI-MS: $m/z=858.7672$ (*Calcd.*= 858.7676, C₅₅H₁₀₂O₆), Degree of unsaturation= 5.

IR (KBr film): 1747, 1166, 1095; 2926, 2854, 722; 3003, 1654 cm⁻¹ (weak).

¹H-NMR data are shown in Table 14.

¹³C-NMR data are shown in Table 15.

Example 15 Preparation of the injection of the pharmaceutical composition

The composition contains following ingredients (in the mass percentage):

1,3-diolein	0.51
1-linolein-3-olein	1.16
1,2-diolein	0.31
1-olein-2-linolein	0.85
1,2-dilinolein	0.42
Trilinolein	2.52
1-Olein-2,3-dilinolein	29.32
1-Palmitin-2,3-dilinolein	4.16
1,3-Diolein-2-linolein	27.00
1-Palmitin-2-linolein-3-olein	12.48
1,3-Dipalmitin-2-linolein	0.48
Triolein	15.48
1-Palmitin-2,3-diolein	5.32

Formulation:

The above composition	100 g
Soybean lecithin for injection	10 g
Glycerin for injection	15 g
Water for injection added to	1000 mL

Process:

To a formulated amount of soybean lecithin for injection was added an appropriate amount of water for injection. The mixture was dispersed with a high shear dispersing emulsifier into a dispersion without bulks or granules. Formulated amount of glycerin for injection was added. Then water for injection was added to a specified amount, and the mixture was stirred to give a water phase.

A formulated amount of the above composition was weighed. The weighed oil and the water phase prepared above were heated separately to 60°C, then mixed and emulsified in a high pressure homogenizer, in which the low pressure was 5 MPa and the high pressure was 25 MPa. The homogenization was repeated for 6 times until the amount of particles below 2 µm was no less than 95% and particles above 5 µm were undetectable. If necessary, NaOH or HCl was used to adjust the pH to 8.5.

The resulting homogeneous emulsion was filtered by nitrogen pressure through a microporous filter of 3 µm or less, then filled under nitrogen, and finally sterilized and cooled to afford the injection.

Example 16 Preparation of the injection of the pharmaceutical composition

The composition contains following ingredients (in the mass percentage):

1,3-diolein	0.59
1-linolein-3-olein	0.95

1,2-diolein	0.24
1-olein-2-linolein	0.97
1,2-dilinolein	0.48
Trilinolein	2.01
1-Olein-2,3-dilinolein	27.94
1-Palmitin-2,3-dilinolein	3.33
1,3-Diolein-2-linolein	31.05
1-Palmitin-2-linolein-3-olein	9.99
1,3-Dipalmitin-2-linolein	0.39
Triolein	17.80
1-Palmitin-2,3-diolein	4.26

Formulation:

The above composition	300 g
Soybean lecithin acceptable for injection	40 g
Glycerin acceptable for injection	50 g
Water for injection added to	1000 mL

Process:

To a formulated amount of soybean lecithin acceptable for injection was added an appropriate amount of water for injection. The mixture was dispersed with a high shear dispersing emulsifier into a dispersion without bulks or granules. Formulated amount of glycerin acceptable for injection was added. Then water for injection was added to a specified amount, and the mixture was stirred to give a water phase.

A formulated amount of the above composition was weighed. The weighed oil and the water phase prepared above were heated separately to 70°C, then mixed and emulsified in a high pressure homogenizer, in which the low pressure was 10 MPa and the high pressure was 50 MPa. The homogenization was repeated for 3 times until the amount of particles below 2 µm was no less than 95% and particles above 5 µm were undetectable. If necessary, NaOH or HCl was used to adjust the pH to 7.1.

The resulting homogeneous emulsion was filtered by nitrogen pressure through a microporous filter of 3 µm or less, then filled under nitrogen, and finally sterilized and cooled to afford the injection.

Example 17 Preparation of the injection of the pharmaceutical composition

The composition contains following ingredients (in the mass percentage):

1,3-diolein	0.58
1-linolein-3-olein	1.08
1,2-diolein	0.27
1-olein-2-linolein	0.93
1,2-dilinolein	0.38
Trilinolein	2.26

30

1-Olein-2,3-dilinolein	32.25
1-Palmitin-2,3-dilinolein	3.74
1,3-Diolein-2-linolein	28.11
1-Palmitin-2-linolein-3-olein	11.23
1,3-Dipalmitin-2-linolein	0.44
Triolein	13.93
1-Palmitin-2,3-diolein	4.79

Formulation:

The above composition	200 g
Soybean lecithin for injection	25 g
Glycerin acceptable for injection	30 g
Water for injection added to	1000 mL

Process:

To a formulated amount of soybean lecithin for injection was added an appropriate amount of water for injection. The mixture was dispersed with a high shear dispersing emulsifier into a dispersion without bulks or granules. Formulated amount of glycerin acceptable for injection was added. Then water for injection was added to a specified amount, and the mixture was stirred to give a water phase.

A formulated amount of the above composition was weighed. The weighed oil and the water phase prepared above were heated separately to 65°C, then mixed and emulsified in a high pressure homogenizer, in which the low pressure was 9 MPa and the high pressure was 35 MPa. The homogenization was repeated for 4 times until the amount of particles below 2 µm was no less than 95% and particles above 5 µm were undetectable. If necessary, NaOH or HCl was used to adjust the pH to 4.8.

The resulting homogeneous emulsion was filtered by nitrogen pressure through a microporous filter of 3 µm or less, then filled under nitrogen, and finally sterilized and cooled to afford the injection.

Example 18 Preparation of the injection of the pharmaceutical composition

The composition contains following ingredients (in the mass percentage):

1,3-diolein	0.50
1-linolein-3-olein	1.20
1,2-diolein	0.30
1-olein-2-linolein	0.83
1,2-dilinolein	0.41
Trilinolein	2.47
1-Olein-2,3-dilinolein	28.73
1-Palmitin-2,3-dilinolein	4.08
1,3-Diolein-2-linolein	28.39
1-Palmitin-2-linolein-3-olein	12.23

1,3-Dipalmitin-2-linolein	0.47
Triolein	15.17
1-Palmitin-2,3-diolein	5.22

Formulation:

The above composition	150 g
Soybean lecithin acceptable for injection	35 g
Glycerin for injection	30 g
Water for injection added to	1000 mL

Process:

To a formulated amount of soybean lecithin acceptable for injection was added an appropriate amount of water for injection. The mixture was dispersed with a high shear dispersing emulsifier into a dispersion without bulks or granules. Formulated amount of glycerin for injection was added. Then water for injection was added to a specified amount, and the mixture was stirred to give a water phase.

A formulated amount of the above composition was weighed. The weighed oil and the water phase prepared above were heated separately to 68°C, then mixed and emulsified in a high pressure homogenizer, in which the low pressure was 8 MPa and the high pressure was 40 MPa. The homogenization was repeated for 5 times until the amount of particles below 2 µm was no less than 95% and particles above 5 µm were undetectable. If necessary, NaOH or HCl was used to adjust the pH to 6.8.

The resulting homogeneous emulsion was filtered by nitrogen pressure through a microporous filter of 3 µm or less, then filled under nitrogen, and finally sterilized and cooled to afford the injection.

Example 19 Preparation of the capsule of the pharmaceutical composition

The composition contains following ingredients (in the mass percentage):

1,3-diolein	0.52
1-linolein-3-olein	1.18
1,2-diolein	1.28
1-olein-2-linolein	0.86
1,2-dilinolein	0.39
Trilinolein	2.57
1-Olein-2,3-dilinolein	28.44
1-Palmitin-2,3-dilinolein	4.24
1,3-Diolein-2-linolein	27.54
1-Palmitin-2-linolein-3-olein	12.73
1,3-Dipalmitin-2-linolein	0.49
Triolein	14.32
1-Palmitin-2,3-diolein	5.43

Formulation:

The above composition	200 g
Vitamine E	0.20 g
to give	1000 capsules

Process:

Glue formulation: Gelatin, purified water, glycerin and 10% ethylparaben solution were weighed at a weight ratio of 1:1.2:0.8:0.01. Glycerin, purified water and 10% ethylparaben solution were sequentially added into a glue melting tank and heated to 70°C. Then gelatin was added and constantly stirred under vacuum until the gelatin was completely dissolved. The glue was filtered and stored at 58°C for use.

Drug liquid formulation: Formulated amount of the above composition and vitamin E were added into an ingredient tank and stirred constantly until thoroughly mixed.

Capsule pressing: Proper pellet dies were chosen according to the capsule size. Capsules were pressed under a temperature of 25°C and a relative humidity of less than 35%, then shaped and dried. After excluding capsules of abnormal size, normal capsules were washed with 95% medicinal ethanol and dried continuously till the moisture content was less than 12%. Unqualified capsules were removed by visual inspection, and the final products were printed and packaged.

Example 20 Preparation of the capsule of the pharmaceutical composition

The composition contains following ingredients (in the mass percentage):

1,3-diolein	0.46
1-linolein-3-olein	1.22
1,2-diolein	0.34
1-olein-2-linolein	0.76
1,2-dilinolein	0.46
Trilinolein	2.77
1-Olein-2,3-dilinolein	26.39
1-Palmitin-2,3-dilinolein	4.23
1,3-Diolein-2-linolein	26.23
1-Palmitin-2-linolein-3-olein	13.73
1,3-Dipalmitin-2-linolein	0.53
Triolein	17.03
1-Palmitin-2,3-diolein	5.85

Formulation:

The above composition	800 g
Tween 80	0.60 g
to give	1000 capsules

Process:

Glue formulation: Gelatin, purified water, glycerin and benzoic acid were weighed at a weight ratio of 1:1.2:0.8:0.01. Glycerin, purified water and benzoic acid were sequentially added into a glue melting tank and heated to 90°C. Then gelatin was added and constantly stirred under vacuum until the gelatin was completely dissolved. The glue was filtered and stored at 56°C for use.

Drug liquid formulation: Formulated amount of the above composition and Tween 80 were added into an ingredient tank and stirred constantly until thoroughly mixed.

Capsule pressing: Proper pellet dies were chosen according to the capsule size. Capsules were pressed under a temperature of 20°C and a relative humidity of less than 35%, then shaped and dried. After excluding capsules of abnormal size, normal capsules were washed with 95% medicinal ethanol and dried continuously till the moisture content was less than 12%. Unqualified capsules were removed by visual inspection, and the final products were printed and packaged.

Example 21 Preparation of the capsule of the pharmaceutical composition

The composition contains following ingredients (in the mass percentage):

1,3-diolein	0.55
1-linolein-3-olein	1.33
1,2-diolein	0.35
1-olein-2-linolein	0.68
1,2-dilinolein	0.33

Trilinolein	2.89
1-Olein-2,3-dilinolein	33.72
1-Palmitin-2,3-dilinolein	4.78
1,3-Diolein-2-linolein	21.60
1-Palmitin-2-linolein-3-olein	14.35
1,3-Dipalmitin-2-linolein	0.56
Triolein	12.73
1-Palmitin-2,3-diolein	6.12

Formulation:

The above composition	500 g
Vitamine E	0.40 g
to give	1000 capsules

Process:

Glue formulation: Gelatin, purified water, glycerin and potassium sorbate were weighed at a weight ratio of 1:0.9:0.6:0.005. Glycerin, purified water and potassium sorbate were sequentially added into a glue melting tank and heated to 80°C. Then gelatin was added and constantly stirred under vacuum until the gelatin was completely dissolved. The glue was filtered and stored at 62°C for use.

Drug liquid formulation: Formulated amount of the above composition and Vitamine E were added into an ingredient tank and stirred constantly until thoroughly mixed.

Capsule pressing: Proper pellet dies were chosen according to the capsule size. Capsules were pressed under a temperature of 30°C and a relative humidity of less than 35%, then shaped and dried. After excluding capsules of abnormal size, normal capsules were washed with 95% medicinal ethanol and dried continuously till the moisture content was less than 12%. Unqualified capsules were removed by visual inspection, and the final products were printed and packaged.

Example 22 Preparation of the capsule of the pharmaceutical composition

The composition contains following ingredients (in the mass percentage):

1,3-diolein	0.51
1-linolein-3-olein	1.16
1,2-diolein	0.31
1-olein-2-linolein	0.85
1,2-dilinolein	0.42
Trilinolein	2.64
1-Olein-2,3-dilinolein	29.37
1-Palmitin-2,3-dilinolein	4.17
1,3-Diolein-2-linolein	27.17
1-Palmitin-2-linolein-3-olein	12.24
1,3-Dipalmitin-2-linolein	0.47

	35	
Triolein		15.46
1-Palmitin-2,3-diolein		5.23

Formulation:

The above composition	600 g
Tween 80	0.3g
to give	1000 capsules

Process:

Glue formulation: Gelatin, purified water, glycerin and chlorhexidine acetate were weighed at a weight ratio of 1:1.0:0.5:0.008. Glycerin, purified water and chlorhexidine acetate were sequentially added into a glue melting tank and heated to 85°C. Then gelatin was added and constantly stirred under vacuum until the gelatin was completely dissolved. The glue was filtered and stored at 56°C for use.

Drug liquid formulation: Formulated amount of the above composition and Tween 80 were added into an ingredient tank and stirred constantly until thoroughly mixed.

Capsule pressing: Proper pellet dies were chosen according to the capsule size. Capsules were pressed under a temperature of 18°C and a relative humidity of less than 35%, then shaped and dried. After excluding capsules of abnormal size, normal capsules were washed with 95% medicinal ethanol and dried continuously till the moisture content was less than 12%. Unqualified capsules were removed by visual inspection, and the final products were printed and packaged.

Example 23 Preparation of the injection of the pharmaceutical composition

The composition contains following ingredients (in the mass percentage):

1,3-diolein	0.57
1-linolein-3-olein	1.21
1,2-diolein	0.34
1-olein-2-linolein	0.78
1,2-dilinolein	0.33
Trilinolein	2.11
1-Olein-2,3-dilinolein	29.12
1-Palmitin-2,3-dilinolein	3.53
1,3-Diolein-2-linolein	29.64
1-Palmitin-2-linolein-3-olein	10.52
1,3-Dipalmitin-2-linolein	0.41
Triolein	17.18
1-Palmitin-2,3-diolein	4.26

Formulation:

The above composition	100 g
Soybean lecithin for injection	10 g

	36
Glycerin for injection	15 g
Water for injection added to	1000 mL

Process:

To a formulated amount of soybean lecithin for injection was added an appropriate amount of water for injection. The mixture was dispersed with a high shear dispersing emulsifier into a dispersion without bulks or granules. Formulated amount of glycerin for injection was added. Then water for injection was added to a specified amount, and the mixture was stirred to give a water phase.

A formulated amount of the above composition was weighed. The weighed oil and the water phase prepared above were heated separately to 60°C, then mixed and emulsified in a high pressure homogenizer, in which the low pressure was 6 MPa and the high pressure was 28 MPa. The homogenization was repeated for 4 times until the amount of particles below 2 µm was no less than 95% and particles above 5 µm were undetectable. If necessary, NaOH or HCl was used to adjust the pH to 6.8.

The resulting homogeneous emulsion was filtered by nitrogen pressure through a microporous filter of 3 µm or less, then filled under nitrogen, and finally sterilized and cooled to afford the injection.

Example 24 Preparation of the injection of the pharmaceutical composition

The composition contains following ingredients (in the mass percentage):

1,3-diolein	0.55
1-linolein-3-olein	1.19
1,2-diolein	0.34
1-olein-2-linolein	0.80
1,2-dilinolein	0.36
Trilinolein	2.38
1-Olein-2,3-dilinolein	30.74
1-Palmitin-2,3-dilinolein	3.96
1,3-Diolein-2-linolein	28.40
1-Palmitin-2-linolein-3-olein	11.62
1,3-Dipalmitin-2-linolein	0.46
Triolein	14.34
1-Palmitin-2,3-diolein	4.86

Formulation:

The above composition	300 g
Soybean lecithin acceptable for injection	40 g
Glycerin acceptable for injection	50 g
Water for injection added to	1000 mL

Process:

To a formulated amount of soybean lecithin acceptable for injection was added an appropriate

amount of water for injection. The mixture was dispersed with a high shear dispersing emulsifier into a dispersion without bulks or granules. Formulated amount of glycerin acceptable for injection was added. Then water for injection was added to a specified amount, and the mixture was stirred to give a water phase.

A formulated amount of the above composition was weighed. The weighed oil and the water phase prepared above were heated separately to 70°C, then mixed and emulsified in a high pressure homogenizer, in which the low pressure was 11 MPa and the high pressure was 46 MPa. The homogenization was repeated for 5-6 times until the amount of particles below 2 µm was no less than 95% and particles above 5 µm were undetectable. If necessary, NaOH or HCl was used to adjust the pH to 7.5.

The resulting homogeneous emulsion was filtered by nitrogen pressure through a microporous filter of 3 µm or less, then filled under nitrogen, and finally sterilized and cooled to afford the injection.

Example 25 Preparation of the injection of the pharmaceutical composition

The composition contains following ingredients (in the mass percentage):

1,3-diolein	0.52
1-linolein-3-olein	1.24
1,2-diolein	0.30
1-olein-2-linolein	0.77
1,2-dilinolein	0.41
Trilinolein	2.52
1-Olein-2,3-dilinolein	28.87
1-Palmitin-2,3-dilinolein	4.16
1,3-Diolein-2-linolein	24.93
1-Palmitin-2-linolein-3-olein	13.45
1,3-Dipalmitin-2-linolein	0.48
Triolein	16.67
1-Palmitin-2,3-diolein	5.68

Formulation:

The above composition	200 g
Soybean lecithin for injection	25 g
Glycerin acceptable for injection	30 g
Water for injection added to	1000 mL

Process:

To a formulated amount of soybean lecithin for injection was added an appropriate amount of water for injection. The mixture was dispersed with a high shear dispersing emulsifier into a dispersion without bulks or granules. Formulated amount of glycerin acceptable for injection was added. Then water for injection was added to a specified amount, and the mixture was stirred to give a water phase.

A formulated amount of the above composition was weighed. The weighed oil and the water phase prepared above were heated separately to 65°C, then mixed and emulsified in a high pressure homogenizer, in which the low pressure was 9 MPa and the high pressure was 36 MPa. The homogenization was repeated for 3 times until the amount of particles below 2 µm was no less than 95% and particles above 5 µm were undetectable. If necessary, NaOH or HCl was used to adjust the pH to 6.5.

The resulting homogeneous emulsion was filtered by nitrogen pressure through a microporous filter of 3 µm or less, then filled under nitrogen, and finally sterilized and cooled to afford the injection.

Example 26 Preparation of the capsule of the pharmaceutical composition

The composition contains following ingredients (in the mass percentage):

1,3-diolein	0.50
1-linolein-3-olein	1.01
1,2-diolein	0.31
1-olein-2-linolein	0.97
1,2-dilinolein	0.45
Trilinolein	2.62
1-Olein-2,3-dilinolein	30.21
1-Palmitin-2,3-dilinolein	1.46
1,3-Diolein-2-linolein	28.36
1-Palmitin-2-linolein-3-olein	13.42
1,3-Dipalmitin-2-linolein	0.51
Triolein	14.47
1-Palmitin-2,3-diolein	5.71

Formulation:

The above composition	200 g
Vitamine E	0.20 g
to give	1000 capsules

Process:

Glue formulation: Gelatin, purified water, glycerin and 10% ethylparaben solution were weighed at a weight ratio of 1:1.2:0.8:0.01. Glycerin, purified water and 10% ethylparaben solution were sequentially added into a glue melting tank and heated to 70°C. Then gelatin was added and constantly stirred under vacuum until the gelatin was completely dissolved. The glue was filtered and stored at 60°C for use.

Drug liquid formulation: Formulated amount of the above composition and Vitamine E were added into an ingredient tank and stirred constantly until thoroughly mixed.

Capsule pressing: Proper pellet dies were chosen according to the capsule size. Capsules were pressed under a temperature of 28°C and a relative humidity of less than 35%, then shaped and dried. After excluding capsules of abnormal size, normal capsules were washed with 95% medicinal

ethanol and dried continuously till the moisture content was less than 12%. Unqualified capsules were removed by visual inspection, and the final products were printed and packaged.

Example 27 Preparation of the capsule of the pharmaceutical composition

The composition contains following ingredients (in the mass percentage):

1,3-diolein	0.42
1-linolein-3-olein	1.14
1,2-diolein	0.31
1-olein-2-linolein	0.91
1,2-dilinolein	0.46
Trilinolein	2.83
1-Olein-2,3-dilinolein	24.85
1-Palmitin-2,3-dilinolein	4.65
1,3-Diolein-2-linolein	28.22
1-Palmitin-2-linolein-3-olein	14.36
1,3-Dipalmitin-2-linolein	0.55
Triolein	15.43
1-Palmitin-2,3-diolein	5.87

Formulation:

The above composition	800 g
Tween 80	0.60g
to give	1000 capsules

Process:

Glue formulation: Gelatin, purified water, glycerin and benzoic acid were weighed at a weight ratio of 1:1.2:0.8:0.01. Glycerin, purified water and benzoic acid were sequentially added into a glue melting tank and heated to 90°C. Then gelatin was added and constantly stirred under vacuum until the gelatin was completely dissolved. The glue was filtered and stored at 56°C for use.

Drug liquid formulation: Formulated amount of the above composition and Tween 80 were added into an ingredient tank and stirred constantly until thoroughly mixed.

Capsule pressing: Proper pellet dies were chosen according to the capsule size. Capsules were pressed under a temperature of 16°C and a relative humidity of less than 35%, then shaped and dried. After excluding capsules of abnormal size, normal capsules were washed with 95% medicinal ethanol and dried continuously till the moisture content was less than 12%. Unqualified capsules were removed by visual inspection, and the final products were printed and packaged.

Example 28 Preparation of the capsule of the pharmaceutical composition

The composition contains following ingredients (in the mass percentage):

1,3-diolein	0.59
1-linolein-3-olein	0.95

	40	
1,2-diolein		0.26
1-olein-2-linolein		0.96
1,2-dilinolein		0.48
Trilinolein		2.89
1-Olein-2,3-dilinolein		23.60
1-Palmitin-2,3-dilinolein		4.78
1,3-Diolein-2-linolein		30.24
1-Palmitin-2-linolein-3-olein		14.22
1,3-Dipalmitin-2-linolein		0.56
Triolein		14.49
1-Palmitin-2,3-diolein		5.97

Formulation:

The above composition	500 g
Vitamine E	0.40 g
to give	1000 capsules

Process:

Glue formulation: Gelatin, purified water, glycerin and potassium sorbate were weighed at a weight ratio of 1:0.9:0.6:0.005. Glycerin, purified water and potassium sorbate were sequentially added into a glue melting tank and heated to 80°C. Then gelatin was added and constantly stirred under vacuum until the gelatin was completely dissolved. The glue was filtered and stored at 61°C for use.

Drug liquid formulation: Formulated amount of the above composition and Vitamine E were added into an ingredient tank and stirred constantly until thoroughly mixed.

Capsule pressing: Proper pellet dies were chosen according to the capsule size. Capsules were pressed under a temperature of 22°C and a relative humidity of less than 35%, then shaped and dried. After excluding capsules of abnormal size, normal capsules were washed with 95% medicinal ethanol and dried continuously till the moisture content was less than 12%. Unqualified capsules were removed by visual inspection, and the final products were printed and packaged.

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CLAIMS

1. A pharmaceutical composition, comprising 13 ingredients in the following mass percentages:

1,3-diolein	0.41-0.59
1-linolein-3-olein	0.93-1.33
1,2-diolein	0.24-0.35
1-olein-2-linolein	0.68-0.97
1,2-dilinolein	0.33-0.48
trilinolein	2.01-2.89
1-olein-2,3-dilinolein	23.46-33.72
1-palmitin-2,3-dilinolein	3.33-4.78
1,3-diolein-2-linolein	21.6-31.05
1-palmitin-2-linolein-3-olein	9.99-14.35
1,3-dipalmitin-2-linolein	0.39-17.80
triolein	12.39-17.80
1-palmitin-2,3-diolein	4.26-6.12

2. The pharmaceutical composition of Claim 1, wherein said ingredients are in the following mass percentages:

1,3-diolein	0.46-0.56
1-linolein-3-olein	1.04-1.28
1,2-diolein	0.27-0.34
1-olein-2-linolein	0.76-0.93
1,2-dilinolein	0.38-0.46
trilinolein	2.26-2.57
1-olein-2,3-dilinolein	26.39-32.25
1-palmitin-2,3-dilinolein	3.74-4.58
1,3-diolein-2-linolein	24.30-29.70
1-palmitin-2-linolein-3-olein	11.23-13.73
1,3-dipalmitin-2-linolein	0.44-0.53
triolein	13.93-17.03
1-palmitin-2,3-diolein	13.93-17.03

3. The pharmaceutical composition of Claim 1, wherein said ingredients are in the following mass percentages:

1,3-diolein	0.50-0.52
1-linolein-3-olein	1.14-1.18
1,2-diolein	0.30-0.31
1-olein-2-linolein	0.83-0.86

1,2-dilinolein	0.41-0.43
trilinolein	2.47-2.57
1-olein-2,3-dilinolein	28.73-29.91
1-palmitin-2,3-dilinolein	4.08-4.24
1,3-diolein-2-linolein	24.46-27.54
1-palmitin-2-linolein-3-olein	12.23-12.73
1,3-dipalmitin-2-linolein	0.47-0.49
triolein	15.17-15.79
1-palmitin-2,3-diolein	5.22-5.43

4. A pharmaceutical preparation, comprising a therapeutically effective amount of the pharmaceutical composition of Claim 1 and one or more pharmaceutically acceptable carriers, wherein said pharmaceutically acceptable carriers are selected from pharmaceutical conventional dilutions, excipients, fillers, emulsifiers, binders, lubricants, absorption accelerators, surfactants, disintegrants, lubricants and antioxidants, if necessary, flavoring agents, sweeteners, preservatives and/or coloring agents.

5. The pharmaceutical preparation of claim 4, wherein said pharmaceutically acceptable carriers are selected from one or more in the group consisting of: mannitol, sorbitol, sodium metabisulfite, sodium bisulfite, sodium thiosulfate, cysteine hydrochloride, thioglycolic acid, methionine, soybean lecithin, vitamin C, vitamin E, EDTA disodium, EDTA calcium sodium, a monovalent alkali metal carbonate, acetate, phosphate or its aqueous solution, hydrochloric acid, acetic acid, sulfuric acid, phosphoric acid, amino acids, sodium chloride, potassium chloride, sodium lactate, ethylparaben solution, benzoic acid, potassium sorbate, chlorhexidine acetate, xylitol, maltose, glucose, fructose, dextran, glycine, starch, sucrose, lactose, mannitol, and silicic derivatives, cellulose and its derivatives, alginates, gelatin, polyvinyl pyrrolidone, glycerin, Tween 80, agar-agar, calcium carbonate, calcium bicarbonate, surfactant, polyethylene glycol, cyclodextrin, β -cyclodextrin, phospholipid material, kaolin, talc, and calcium stearate or magnesium stearate.

6. The pharmaceutical preparation of claim 4, wherein said pharmaceutical preparation is an oral solid preparation, an oral liquid preparation, or an injection.

7. The pharmaceutical preparation of claim 6, wherein:

said oral solid preparation is selected from any one of capsules, tablets, dripping pills, granules, and concentrated pills;

said oral liquid preparation is selected from any one of aqueous or oily suspensions, solutions, emulsions, syrups or elixirs, and a dry product that can be reconstructed by water or other suitable carrier before use; and

said injection is selected from any one of nano suspensions, liposomes, emulsions, lyophilized

powder for injection and aqueous injection.

8. The pharmaceutical preparation of claim 6, wherein said injection comprises the following components:

Composition of Claim 1	50-350 g
Soybean lecithin for Injection or soybean lecithin acceptable for injection	10-40 g
Glycerin for Injection or glycerin acceptable for injection	15-50 g
Water for injection added to	1000 mL

9. A process for preparing the pharmaceutical preparation of claim 8, comprising steps of:
adding appropriate amount of water for injection to a formulated amount of soybean lecithin for injection or soybean lecithin acceptable for injection; dispersing the mixture with a high shear dispersing emulsifier to give a dispersion without bulks or granules; adding a formulated amount of glycerin for injection or glycerin acceptable for injection; then adding water for injection to a specified amount, and stirring the mixture to give a water phase;

weighing a formulated amount of the pharmaceutical composition of Claim 1; heating the weighed oil and the water phase separately to 60-70°C, then mixing them and emulsifying the mixture in a high pressure homogenizer, in which the low pressure is 5-12 MPa and the high pressure is 25-50 MPa; repeating the cycle of homogenization for 3-6 times until the amount of particles below 2 µm is no less than 95% and particles above 5 µm are undetectable; if necessary, using NaOH or HCl to adjust the pH to 4.8 to 8.5, preferably 6.8 to 7.0, most preferably 6.8; and

filtering the resulting homogeneous emulsion by nitrogen pressure through a microporous filter of 3 µm or less; filling the emulsion with nitrogen, sterilizing and cooling to afford the injection.

10. The pharmaceutical preparation of claim 6, wherein said capsule comprises the following components:

Composition of Claim 1	200-800 g
Antioxidant(s) and/or emulsifier(s)	0.20-0.60 g
to give	1000 capsules

11. A process for preparing the pharmaceutical preparation of claim 10, comprising steps of:
preparing glue solution: weighing gelatin, purified water, glycerin and a preservative at a weight ratio of 1 : 0.6-1.2 : 0.3-0.8 : 0.0001-0.01; adding glycerin, purified water and preservative sequentially into a glue melting tank; heating to 70°C - 90°C; then adding gelatin and constantly stirring the mixture under vacuum until the gelatin is completely dissolved; filtering the glue solution and storing the filtered glue solution at 56-62°C for use;

preparing drug liquid: adding formulated amount of the composition of Claim 1, antioxidant(s)

and/or emulsifier(s) into an dosing tank, and stirring the mixture constantly until being homogeneously mixed; and

pressing capsules: choosing proper pellet dies according to the capsule size; pressing capsules in a temperture of 15-30°C and a relative humidity of less than 35%; drying the pressed and shaped capsules; after removing capsules of abnormal size, washing the normal capsules with 95% medicinal ethanol, and drying them continuously to a moisture content of less than 12%; visually inspecting and removing unqualified capsules; finally printing and packaging to afford the pharmaceutical preparation.

12. The process of claim 11, wherein:

said preservative is selected from any one of 10% ethylparaben solution, benzoic acid, potassium sorbate and chlorhexidine acetate;

said antioxidant is vitamin E; and

said emulsifier is Tween 80.

13. Use of the pharmaceutical composition of claim 1 in the preparation of a drug for the treatment of a tumor or for the enhancement of immunity.

14. Use of the pharmaceutical composition of claim 1 in the preparation of a drug for the treatment of a tumor, wherein said drug is used in combination with LAK cells.

15. The use of claim 13, wherein said tumor is selected from a group consisting of lung cancer, liver cancer, pancreatic cancer, prostate cancer, ovarian cancer and breast cancer, in early, middle or late stage.

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ABSTRACT

A pharmaceutical compound comprising glycerides, formulation, and preparation method and application thereof. The pharmaceutical compound specifically consists of the following 13 compounds: 1,3-dioleate diglyceride (0.41-0.59%), 1-linoleate-3-oleate diglyceride (0.93-1.33%), 1,2-dilinoleate diglyceride (0.24-0.35%), 1-oleate-2-linoleate diglyceride (0.68-0.97%), 1,2-dilinoleate glyceride (0.33-0.48%), glyceryl trilinoleate (2.01-2.89%), glyceryl 1-oleate-2,3-dilinoleate (23.46-33.72%), glyceryl 1-palmitate-2,3-dilinoleate (3.33-4.78%), glyceryl 1,3-dioleate-2-linoleate (21.6-31.05%), glyceryl 1-palmitate-2-linoleate-3-oleate (9.99-14.35%), glyceryl 1,3-dipalmitate-2-linoleate (0.39-17.80%), glyceryl trioleate (12.39-17.80%), and glyceryl 1-palmitate-2,3-dioleate (4.26-6.12%).