Research advance on ethenopharmacology, pharmacodynamics, pharmacokinetics and clinical therapeutics of Coix seed and its preparation, Kanglaite injection

Da-Peng Li
Zhejiang University of Chinese Traditional Medicine
Zhejiang Kanglaite pharmaceutical Co. Ltd.
11th Street Economic& Technical Development Zone, Hangzhou, 310018 China

Abstract
Coix seeds come from the coix plant. This herb is the seed of Coix Lacryma-jobi (family Cramineae). In traditional Chinese medicine, coix seeds serve several functions. The stimulate function of the spleen and lung, remove heat (which helps in the drainage of pus) and induce diuresis. Coix seeds are also used to treat the symptoms of diarrhea and arthritis. In this paper, author summered the ethenopharmacology, pharmacology, pharmacokinetics and clinical application of the coix seed and its preparation according to published literatures by scientists of Chinese and other countries, as well as myself research information. The mechanism of action of Kanglaite injection (KLT) is as follows: (1) the drug inhibits the mitosis of tumor cells during G2/M phases, (2) induces apoptosis of tumor cells, (3) affects the genetic expression of tumor cells by up-regulating FAS/Apo-1 gene expression and down-regulating Bc1-2 gene expression, (4) inhibits the tumor angiogenesis, (5) counteracts the cachexia of cancers, and (6) reverses the multi-resistance of tumor cells to anticancer drugs and the resistance modification in some of chemotherapeutics. Kanglaite injection (KLT) has been successfully applied in the treatment of a variety of malignant tumor such as carcinomas of lung, liver, stomach, esophagus, colon, pancrease, kidney, ovaries, malignant lymphoma, leukemias for more than 200,000 cases. The prospective, randomized and large-scale clinical studies have been carried out in 1408 cases. The clinical studies have shown that KLT could not only inhibit cancer cells, but also enhance body immunoity. Moreover, it has synergistic and toxicity-reducing effects when KLT combined with chemo- or radio-therapy. It could also relieve pains, improve patient life-quality and extend the survival time. In 2001 and 2002, clinical trial of kanglait injection has been started in USA, and Russia, respectively.

Key words Coix seed; Coix Lacryma-jobi; ethenopharmacology; pharmacodynamics; pharmacokinetics and clinical application; anticancer activity; mechanism of action, preparation; Kanglaite injection

Introduction
Coix seeds come from the coix plant (Fig 1 and 2). This herb is the seed of Coix Lacryma-jobi (family Cramineae) produced mainly produced in the Fujian, Hebei and Liaoning provinces in China. The seeds are usually oval-shaped or egg-shaped, with a milky white outer surface and a slightly sweet taste.

Correspodence to Professor Da-Peng Li,
Zhejiang Kanglaita pharmaceutical Co. Ltd.
11th Street Economic& Technical Development Zone, Hangzhou, 310018, China. Tel +86-571-86913770, Fax +86-571-86913769
E-mail lidapeng@mail.hz.zj.cn

Coix seeds are harvested in the fall when the Coix plant ripens and are dried in the sun. They are usually used unprepared, or they are stir-baked until the outer shell has a yellowish color. In traditional Chinese medicine, coix seeds serve several functions. The stimulate function of the spleen and lung, remove heat (which helps in the drainage of pus) and induce diuresis. Coix seeds are also used to treat the symptoms of diarrhea and arthritis.

Epidemiologists had long suspected that the low cancer rates is southeast China might be related to coix, a grasslike relative of maize that is a dietary staple in the region and a key ingredient of many traditional Chinese medicines.
Author in 1975 began trying to coax the anticancer compounds out of the plant’s seed. In 1995, we won government approval to market the fruits of this research, a drug called Kanglaite injection, to help cancer patients fight their disease and reduce the side effects from other treatment. Kanglaite injection has been taken by more 200,000 patients with cancer, and is a best-selling cancer treatment in China. After the phase II clinical trial applied Kanglaite injection with another chemotherapy to treat non-small-cell lung cancer, the results suggested that Kanglaite injection enhances the efficacy of chemotherapy and mitigates side effects such as fatigue, nausea, and hair loss. In 2003, the U.S. Food and Drug Administration approved a phase II trial to test its efficacy in treating non-small-cell lung cancer.

In this paper, author summered the ethenopharmacology, pharmacology, toxicity, pharmacokinetics and clinical application of the coix seed and its preparation according to published literatures by scientists of Chinese and other counties, as well as myself research information.

Ethenopharmacological activities

In traditional Chinese medicine, the herb is sweet and tasteless in flavour, slightly cold in nature. It acts on spleen, stomach and lung channels. Tasteless for removing dampness, sweet for tonifying spleen, slight coldness for eliminating heat, and neutral, not oily and not drastic, coix seed as a herb for eliminating heat and dampness is indicated for edema, diarrhea, damp arthralgia and pulmonary abscess. The major effects are Inducing diuresis, excreting dampness, strengthening spleen, arresting diarrhea, clearing heat and pus. The indications are three aspects: (1) The herb is often used in combination with umbellate pore-fungus, poria, oriental water plantain rhizome and other herbs for inducing diuresis and alleviating edema, to treat water and dampness retention, edema, beriberi and dysuria; with Dangshen, white atractylodes rhizome, poria, Chinese yam rhizome and other herbs for strengthening spleen and arresting diarrhea, such as Shenling Baizhu Powder, to treat diarrhea and anorexia due to spleen deficiency. (2) The herb is often used in combination with phellodendron bark, atractylodes rhizome, achyranthes root for clearing heat and dampness, alleviating arthralgia and pain, to treat arthralgia due to damp-heat and swelling, heat and pain in joints; and with apricot kernel, round cardamon seed and other herbs for eliminating dampness and heat and promoting Qi circulation, such as Sanren Decoction, to treat early damp-heat syndrome, fever, fatigue, chest tightness and greasy tongue. And (3) The herb can be used in combination with common reed root, Chinese waxgourd seed and other herbs for clearing heat from the lung and pus, such as Weijing Decoction, to treat pulmonary abscess, cough and purutemr phlegm; with rhubarb, peach kernel, dandelion and other herbs for clearing heat and suppuration of the intestine to treat acute appendicitis and abdominal pain.
The amount of coix seeds taken depends on the condition being treated. A typical dosage is between 10-30 grams, which can be combined with water for a decoction, or ground into powder.

This herb can be used for the treatment: (1) Cancers of digestive tract, lung and ovary, and chorioepithelioma. For deficient spleen, excessive dampness and disorderly digestion in carcinoma of digestive tract, it is used with oldenlandia, white atractylodes rhizome, poria, greenbrier rhizome, shell of areca nut and others. For phlegm-heat accompanied with dampness in lung cancer, it is used together with luidium seed, trichosanthes fruit, dandelion, hout-tuynia, poria and others. For accumulation of dampness and excess toxin in cancers of uterine cervix and ovary, it is used with phellodendron bark, seven-lobed yam, Hindu lotus stamen and toona. For chorioepithelioma, it is used with houttuynia, adski bean seed, patrinia, astragalus root, rubia rot, waxgourd seed, chinese ange-lica root, danghsen, donkey-hide gelatin, licorice root, etc. It induces antitumour and enhances immunologic function. The extract and decoction of coix seedinhbit various tumourcells. Its active component, coixenolide, promotes cellu-lar immunity and humoral immunity. (2) Diarrhea due to spleen deficiency,arthralgia caused by wind-dampness, edema, jaundice,lung abscess,appendicitis, chy-luria, leukorrhea, etc.

**Studies on active chemical components and functional characterization**

Fatty acids, such as hexadecanoic acid (16-C), octadecanoic acid (18-C-1), octadecadienoic acid, (18-C-2), palmitic acid, stearic acid, oleic acid, and linoleic acid are presented in coix seeds. Its active component, coixenolide, promotes cellu-lar immunity and humoral immunity. These fatty acids were assayed to be bioactivity.

At National Institute of Health, Tokyo, Japan, Numata et al find four free fatty acids from the seeds of the gramineous plant Coix lachryma-jobi L. var. ma-yuen Stapf. An acetone extract of the seeds was fractionated by the aqueous alkali method and by silica gel column chromatography in order to identify the antitumor components. Antitumor activity, as assayed by an in vivo growth inhibition test on a transplantable mouse tumor, was attributed to an acidic fraction. Infrared spectroscopy and gas-liquid chromatography showed that this acidic fraction was composed of four free fatty acids: palmitic, stearic, oleic, and linoleic acids.[10] An activity-directed fractionation and purification process was used to identify the antioxidative components of adlay hulls. Hulls of adlay (Coix lachryma-jobi L. var. ma-yuen Stapf) were extracted with methanol and then separated into water, 1-butanol, ethyl acetate, and hexane fractions. The 1-butanol-soluble fraction exhibited greater capacity to scavenge 2,2′-diphenyl-1-picrylhydrazyl (DPPH) radicals when compared with fractions soluble in water, ethyl acetate, and hexane phases. The 1-butanol fraction was then subjected to separation and purification using Diaion HP-20 chromatography, silica gel chromatography, and HPLC. Six compounds showing strong antioxidant activity were identified by spectroscopic methods ((1)H NMR, (13)C NMR, IR, and MS) and by comparison with authentic samples to be coniferyl alcohol, syringic acid, ferulic acid, syringaresinol, 4-ketopinoresinol, and a new lignan, mayuenolide.[11] The seed storage proteins of Coix, sorghum and maize are codified by homologous genes which are coordinately expressed in the endosperm in a temporal-specific fashion. Opaque2 (O2), a bZIP protein originally isolated from maize, has been described as a transcription activator of alpha- and beta-prolamin genes. The isolation and characterization of cDNA and genomic clones encoding the Opaque2 homologue from Coix are reported here. The coding region of the Coix O2 gene is interrupted by five introns and codifies a polypeptide of 408 amino acids. Comparison of the deduced amino acid sequence with two different sequences of maize O2 protein showed that the Coix O2 protein is similar to the maize O2 isolated from W22 maize inbred line. The Coix O2 protein has the same binding specificity and expression pattern of the maize O2. The O2 proteins together with OHP1, OsBZIPPA, SPA, CPRF2 and RITA1 were assigned to one of the five bZIP plant families in an updated classification of plant bZIP according to bZIP domain similarity.[12]
A protein inhibitor of locust gut alpha-amylase was purified from seeds of Job's Tears (Coix lachryma-jobi) using ammonium sulphate precipitation, affinity chromatography on Red Sepharose and reversed-phase HPLC. It consisted of two major isomers, each a dimer of two closely similar or identical subunits of Mr about 26,400, and associated by inter-chain disulphide bonds. These isomers also had closely similar amino acid compositions. The major isomer showed no inhibitory activity against amylases from other sources: human saliva, porcine pancreas, Bacillus subtilis, Aspergillus oryzae and barley malt. The manual DABITC/PITC method was used to determine about half of the amino acid sequence of the major isoform. This showed a high degree of homology with previously reported sequences of endochitinase enzymes from several species (tobacco, potato, barley, bean), and endochitinase activity was demonstrated by following the release of radioactivity from \(^3\)H-chitin. This novel combination of functions may be relevant to protection of the grain from insect feeding and fungal infection.\[13\]

Seeds of 3 accessions of Job's tears (Coix lacryma), obtained from a germplasm repository, were ground and extracted with hexane. Whole kernel oil yields and levels of four phytonutrients (free phytosterols, fatty acyl phytosterol esters, ferulate phytosterol esters, and gamma-tocopherol) in the oils were measured. Among the seeds tested, oil yields ranged from 2.19 to 4.83 wt %, the levels of ferulate phytosterol esters in the oil ranged from 0.047 to 0.839 wt %, the levels of free phytosterols in the oil ranged from 0.54 to 1.28 wt %, the levels of phytosterol fatty acyl esters in the oil ranged from 0.76 to 3.09 wt %, the levels of total phytosterols in the oil ranged from 1.40 to 4.38 wt %, and the levels of gamma-tocopherol in the oil ranged from 0.023 to 0.127 wt %.\[14\]

At Federal University of Ceara, Brazil, Ary et al studied the major trypsin inhibitor from seeds of Coix lachryma-jobi. The major trypsin inhibitor was purified by heat treatment, fractional precipitation with \((\text{NH}_4)_2\text{SO}_4\), ion-exchange chromatography on DEAE-Sepharose, gel-filtration on Sephadex G-75 and preparative reverse-phase HPLC. The complete amino acid sequence was determined by analysis of peptides derived from the reduced and S-carboxymethylated protein by digestion with trypsin, chymotrypsin and the S. aureus V8 protease. The polypeptide contained 64 amino acids with a high content of cysteine. The sequence exhibited strong homology with a number of Bowman-Birk inhibitors from legume seeds and similar proteins recently isolated from wheat and rice.\[15\]

The expression of Brazil nut storage albumin genes is highly regulated during seed development. Several sequences in the promoter of one of these genes show homologies with the target sites of the maize O2 bZIP regulatory protein. We therefore asked whether the O2 protein would recognize these promoter sequences. We show that the O2 protein binds to three different sequences (F1, F2 and F3). F1 and F3 are hybrid C/G and A/G boxes, respectively, that are homologous to the O2-binding site of a maize alpha-zein gene. F2 is a new O2-binding sequence related to the O2 target sites of the Coix alpha-coxin, the maize b-32 genes and the AP-1 pseudopalindrome. Molecular modelling showed that an Asn and a Ser in the O2 DNA binding domain make different base-specific contacts with each operator. 5' Promoter deletions of the be2S1 gene showed that the domain containing the O2 target sites F1 and F2 is required for detectable reporter gene expression in transgenic tobacco seeds. Moreover, the homologous coix O2 protein was shown to in situ transactivate the promoter region encompassing the three O2-binding sites F1, F2 and F3. Thus, these sites may be in vivo regulatory sequences mediating activation by bZIP regulatory proteins.\[16\]

At Universidade de Sao Paulo, Brasil, Garratt et al studied the three-dimensional structure of \(\alpha\)-prolamins. \(\alpha\)-Prolamins are the major seed storage proteins of species of the grass tribe Andropogonea. They are unusually rich in glutamine, proline, alanine, and leucine residues and their sequences show a series of tandem repeats presumed to be the result of multiple intragenic duplication. Two new sequences of \(\alpha\)-prolamin clones from Coix (pBCX25.12 and pBCX25.10) are compared with similar clones from maize and Sorghum in order to investigate evolutionary relationships between the repeat motifs and to propose a schematic model for their three-dimensional structure based on hydrophobic membrane-helix propensities and helical "wheels." A scheme is proposed for the most recent events in the
evolution of the central part of the molecule (repeats 3 to 8) which involves two partial intragenic duplications and in which contemporary odd-numbered and even-numbered repeats arise from common ancestors, respectively. Each pair of repeats is proposed to form an antiparallel alpha-helical hairpin and that the helices of the molecule as a whole are arranged on a hexagonal net. The majority of helices show six faces of alternating hydrophobic and polar residues, which give rise to interstitial holes around each helix which alternate in chemical character. The model is consistent with proteins which contain different numbers of repeats, with oligomerization and with the dense packaging of alpha-prolamins within the protein body of the seed endosperm.\[17\]

Pharmacodynamics studies

**Influence on the cytotoxic activity of peripheral lymphocytes**

At Department of Laboratory of Medicine, Osaka University School of Medicine, Kaneda and his co-researchers studied the influence on the cytotoxic activity of peripheral lymphocytes. Coix seed has been used in patients with verruca vulgaris and verruca planae juveniles, which have been considered to be induced by viral infection. Moreover, coixenolide, component in the seeds of coix, was reported to show anti-tumor activity. Possibly coix seed may have some influence on the cytotoxic activity of peripheral lymphocytes but there has been no data on this. Then we investigated the changes in number of cytotoxic lymphoid cells in seven volunteers before, during (four weeks) and after taking six coix seed tablets. Lymphocyte subsets were analyzed with monoclonal antibodies using a flow cytometer. The level of CD3+CD56+ (MHC-non restricted cytotoxic T cells) markedly increased at four weeks (before 1.9±0.5% vs four weeks 4.2± 0.7%, \(p < 0.01\)). The level of CD16+CD57- (the mature, most active natural killer cells) increased at three weeks (before 4.5±0.8% vs three weeks 5.2±0.8%, \(p < 0.05\)). The level of CD3-CD56+ (natural killer cells) and the level of CD16+CD57+ (the variable active natural killer cells) decreased at one week and returned to normal level thereafter (before 13.7 ± 2.1% vs one week 11.2± 1.5%, \(p < 0.05\); before 8.8±1.5% vs one week 6.9±1.3%, \(P < 0.05\), respectively). These results indicate that Coix seed modulate the peripheral blood lymphocyte subsets and may be effective to virus disease through the enhancement of cytotoxic activity.\[18\]

**Inhibitory effects on nitric oxide and superoxide production**

Overproduction of nitric oxide (NO) or superoxide (O2-) by activated macrophages is known to be involved in acute or chronic inflammation. The seeds of Job's Tears (Coix lacryma-jobi L. var. ma-yuen) have been used as anti-inflammatory medicine and health food. However, it is still unclear how the seeds show anti-inflammatory properties. Using murine macrophage-like RAW 264.7 cells, we tried to know whether the overproduction of NO and O2 by activated macrophages could be prevented by the methanol (MeOH) extract of the seeds of Job's Tears. RAW 264.7 cells were activated with interferon-gamma plus lipopolysaccharide to produce NO and with pholbol ester to produce O2-. The MeOH extract showed marked inhibition of NO production by activated RAW 264.7 cells in a dose-dependent manner via suppression of inducible NO synthase mRNA expression. The MeOH extract also showed inhibition of O2- production by activated RAW 264.7 cells in dose- and time-dependent manners, possibly by interfering with NADPH oxidase machinery of macrophages. Collectively, these results demonstrate that the MeOH extract of the seeds of Job's Tears shows anti-inflammatory properties which may, in part, involve an inhibition of NO and O2- production by activated macrophages.\[19\]

**The effect on fibrinolytic system of blood plasma**

At University of Nairobi, Kenya, Check et al studied the effect on fibrinolytic system of blood plasma in rats Experimental wister rats were fed on coix-mixed diet for 30 days in a view to study the effect of Coix feeding on the state of haemostatic mechanisms. Blood plasma which was obtained after cardiac puncture was analyzed for fibrinogen levels, euglobulin lysis time, fibrinolytic activity by protamin sulfate degradation and inhibitors of
plasmin. These experimental models were set with a view to analyze situations mimicking processes associated with haemostasis and interpolate such situations in changes associated with the development of atherosclerosis. To study the plasma fibrinogen levels in experimental and control animals, spectrophotometric method was applied. Feeding the animals with coix-pellets mixed diet caused a decrease in fibrinogen levels as compared with controls. An overall decrease of this plasma protein was observed in both sexes. It was shown that euglobulin lysis time (ELT) was insignificantly changed in the experimental animals. However, fibrinolytic activity by degradation of protamin sulfate showed an increased fibrinolytic potential in experimental animals. In the same experimental models the analysis for the activity of inhibitors of plasmin showed no significant differences in mean values. It was found that coix has got vital nutritional value in lowering fibrinogen levels while at the same time creating a tendency of reducing fibrinolytic activity. More experiments should be conducted to show the possible mechanisms by which the observations can affect the development of atherosclerosis in man.

**Downregulation of progesterone biosynthesis**

*Coix lacryma-jobi* L. var. *ma-yuen* Stapf. (adlay) has long been used as a traditional Chinese medicine for dysfunctions of the endocrine system and inflammation conditions. However, the effect of adlay seed on the endocrine system has not yet been reported. At Institute of Food Science and Technology, National Taiwan University, Hsia et al studied the downregulation of progesterone biosynthesis in rat granulosa cells by adlay. In this study, the effects and the mechanisms of methanolic extract of adlay bran (ABM) on progesterone synthesis in rat granulosa cell were studied. ABM was further partitioned with different solvents including water, 1-butanol, ethyl acetate and n-hexane. Four subfractions named ABM-Wa (water fraction), ABM-Bu (1-butanol fraction), ABM-EA (ethyl acetate fraction) and ABM-Hex (n-hexane fraction) were obtained. ABM-Bu was further fractionated using Diaion HP-20 resin column chromatography with gradient elution. Granulosa cells were prepared from pregnant mare serum gonadotropin-primed immature female rats and challenged with different reagents including human chorionic gonadotropin (hCG 0.5 IU·mL⁻¹), forskolin (10 µM), 8-bromo-adenosine-3',5'-cyclic monophosphate (8-Br-cAMP, 1 mM), A23187 (10 µM), phorbol 12-myristate 13-acetate (PMA, 0.01 µM), 25-OH-cholesterol (0.1-10 µM) and progrenolone (0.1-10 µM) in the presence or absence of ABM-Bu (100 µg·mL⁻¹). The functions of steroidogenic enzyme including protein expression of the steroidogenic acute regulatory protein (StAR) and cytochrome P450 side-chain cleavage enzyme (P450scC) protein were investigated. Expressions of both P450scC and StAR mRNA have also been explored. They found that ABM decreased progesterone production via an inhibition on (1) the cAMP-PKA and PKC signal transduction pathway, (2) P450scC and 3beta-hydroxysteroid dehydrogenase (3beta-HSD) enzyme activity, (3) P450scC and StAR protein and mRNA expressions and (4) the phosphorylation of ERK1/2 in rat granulosa cells.²²

**Biostatic activity**

Water extract of *Coix lacryma* seeds (Co-Ex) was separated into several components; dissolved with Tris-C1 buffer and the supernatant (WC1), ammonium sulfate treatment supernatant (WC2) and the pellet (WC3), QAE column chromatography of WC1 and the peak portions; WC4, WC5 and WC6. Murine peritoneal macrophages in DMEM containing 10% heat-inactivated FCS were infected with tachyzoites of *Toxoplasma gondii*, RH strain, in vitro. By adding modulators such as Co-Ex, WC1, 2, 3, 4, 5, 6 and LPS or IFN-gamma for 24 hrs, toxoplasmastatic activity of macrophages was examined in relation to nitrite production. Nitrite production of macrophages was enhanced especially in the series of WC2, WC1 and the combination sample (WC1+WC2+WC3) by order, than other components or fractions (WC4, WC5, WC6) tested. Toxoplasmastatic actions such as percentage of the macrophages infected by *T. gondii* and fold increase of *T. gondii* in macrophages showed retroverse relations with the amount of nitrite production; i.e., as nitric oxide (NO) increased the phagocytic index of macrophages and the fold increase of tachyzoites in macrophages decreased. Nitrite (NO2) production was increased by adding IFN-gamma in all cases.
together with enhancement of biostatic effects. Through the results obtained, it is speculated that some components other than the non-proteinous and defatted components in Coix lacryma seeds may contribute to activate macrophages through induction of NO for the biostatic activity.\[22\]

**Abortifacient effects**

At institute of Toxicology, College of Medicine, National Taiwan University, Tzeng et al carried out a study to evaluate the abortifacient effects of the extracts of seeds of Coix lachryma-jobi L. var. ma-yuen Stapf (adlay) in pregnant rats. Pregnant rats were treated with oral administration of adlay seed extracts on d 6 of pregnancy and their fetuses were examined for growth and malformations on d 20 of pregnancy. Following oral administration of 1 g·kg\(^{-1}\) body weight of water extracts but not methanolic extracts, fetal resorptions were significantly increased and mortality of postimplantation was increased. There were no significant differences in the uterine and fetal weight compared to control. Fetal malformations were not observed in the adlay seed extracts-treated pregnant rats. The contractile activity of uteri isolated from rats on d 20 of pregnancy was assessed. The spontaneous uterine contractions were significantly enhanced in rats treated with water extracts of adlay seeds (1 g·kg\(^{-1}\) body weight). Immunoblotting of uteri from rats treated with water extracts of adlay seeds demonstrated an induction of cyclooxygenase-2 (COX-2) protein expression. The water extracts of adlay seeds also enhanced extracellular signal-regulated protein kinase (ERK) 1/2 phosphorylation and protein kinase C (PKC)-alpha translocation from cytosolic to particulate fractions in uteri. These results indicate that the water extracts of adlay seeds are capable of inducing embryotoxicity and enhancing uterine contractility during pregnancy. The enhanced activities of PKC-alpha, ERK1/2, and COX-2 may contribute to these responses\[23\]

**Hypolipidemic effects**

In the hypolipidemic effects of crude extract of coix seed to find out whether the expressions of these adipocyte markers are influenced by oriental medicine, obesity rats induced by high fat diet (HFD) for 8 weeks were injected with 50 mg/100 g body weight adlay seed crude extract (ACE), daily for 4 weeks. The results are summarized as follows: HFD + ACE group significantly reduced food intakes and body weights. Weights of epididymal and peritoneal fat were dramatically increased in HFD groups compared with those of normal diet (ND) group but significantly decreased more in HFD+ACE group than those of HFD+saline group (sham). Those of brown adipocytes were increased in HFD+ACE group compared to ND and sham groups but there was no significant difference. The sizes in white adipose tissue (WAT) by microscope were markedly larger in HFD groups than ND group but considerably reduced in HFD + ACE group compared with sham group. The levels of triglyceride, total-cholesterol and leptin in blood serum were significantly decreased in HFD+ACE group compared to those of sham group. Leptin and TNF-alpha mRNA expressions in WAT of rats were remarkably increased more in sham group than in those of ND group. Those of HFD+ACE group were significantly decreased compared with those of sham group, especially. TNF-alpha mRNA expression in HFD+ACE group was declined more than that of ND group. Their study suggested that the treatments of ACE modulated expressions of leptin and TNF-alpha and reduced body weights, food intake, fat size, adipose tissue mass and serum hyperlipidemia in obesity rat fed HFD. Accordingly, the oriental medicine extract, adlay seed crude extract, can be considered for obesity therapies controlling.\[24\]

**Anticancer activity**

Department of Physiology, Graduate Institute of Medicine, and School of Technology for Medical Sciences, Kaohsiung Medical University, Taiwan, Republic of China. This study examined the effects of different extracts of adlay seed on the growth of human lung cancer cells in vitro and in vivo. The data showed that a methanolic extract, but not a water extract, of adlay seed exerted an antiproliferative effect on A549 lung cancer cells by inducing cell cycle arrest and apoptosis. It was also found that tumor growth in vivo was inhibited by the methanolic
extract in a dose-dependent manner. The chemopreventive effect of adlay seed on the tobacco-specific carcinogen 4-(methyl nitrosamino)-1-(3-pyridyl)-1-butanol (NNK)-induced lung tumorigenesis in A/J mice was also investigated. Groups of mice were pre-fed with different diets, followed by feeding with NNK-containing drinking water for 8 months. The results indicated that feeding with diet containing 30% of powdered adlay seed reduced the number of surface lung tumors by approximately 50%. Taken together, these results indicate that the components of adlay seed exert an anticancer effect in vitro and in vivo and may be useful for the prevention of lung tumorigenesis.[25]

A study of the effect of the interventional therapy y Seed of Job's-stears (SJS) injection combining with lipiodol was to evaluate the effect of SJS as a medicament which can be used in interventional therapy. They repeated the hepatoma-bearing rats, and treated them by interventional therapy with SJS referring to the method which Lindel set up, comparing its effect to which of chemical medicines and lipiodol. SJS or lipiodol alone had an inhibiting effect to liver cancer. The tumor growth rates were 13.89%, 14.05%, and the tumor inhibiting rates reached 38.10%, 37.49%. The curative effect of the SJS/lipiodol group was the best, and its growth rate and inhibiting rate were 3.36% and 85.03%, respectively better than the SJS group and lipiodol group (P<0.01). There was no statistically significant difference between the effect of the SJS/lipiodol group and the mitomycin/lipiodol group. The survival period of SJS/lipiodol group was longer than the rest groups (P<0.01 or P<0.05). The study showed that the interventional therapy by SJS/lipiodol has obvious inhibitory effect on the growth of hepatoma-bearing rats, which is similar to that of MMC/lipiodol. This inhibiting effect is better than that of the SJS or lipiodol group. SJS/lipiodol can prolong the survival period of hepatoma-bearing rats obviously, and this effect is better than that of single lipiodol, SJS or MMC/lipiodol.[26]

**Antiproliferative effect on human lung cancer cells**

Previous results demonstrated that the methanolic extract of adlay seed exerted an antiproliferative effect on human lung cancer cells *in vitro* and *in vivo* and might prevent tobacco carcinogen-induced lung tumorigenesis. In this study, the methanolic extract of adlay seed was tested for its regulation of COX-2 expression of human lung cancer cells. Western blot analysis showed that the methanolic extract of adlay seed inhibited basal and TPA-induced COX-2 expression in a dose-dependent fashion, whereas COX-1 expression was not affected. By using a promoter activity assay, it was found that the methanolic extract inhibited basal and TPA-stimulated COX-2 expression at the transcription level. The effect of the methanolic extract on COX-2 expression in vivo was then investigated. The data demonstrated that treatment of the methanolic extract reduced the PGE(2) level in serum and inhibited COX-2 expression of tumor tissues in nude mice. Taken together, these results suggest that inhibition of COX-2 is one of the mechanisms by which the methanolic extract of adlay seed inhibits cancer growth and prevents lung tumorigenesis.[27]

**Influence on the growth of intestinal bacteria**

Experiments of influence on the growth of intestinal bacteria were conducted to study the effect of a dietary supplement of dehulled adlay (Coix lachryma-jobi L. var. ma-yuen Stapf) on the culture counts of some important groups of intestinal bacteria and their metabolism in the gastrointestinal (GI) tract of Sprague-Dawley rats. Rats were divided into four groups, and each group was fed a diet containing different levels of dehulled adlay for 30 days as follows: 0% (control), 5%, 20%, and 40%. All animals fed with adlay had normal healthy intestinal walls and no pathogenic signs whatsoever. There were no significant differences in body weight gain or the cecal pH between different groups of rats. Both the 20% and 40% groups had lower culture counts of enterics in their feces than the 5% and control groups, whereas the culture counts of fecal lactic acid bacteria were higher in feces of rats fed with adlay than in the control group. Cecal total short-chain fatty acid (SCFA) content and fecal SCFA were significantly higher in the 20% and 40% groups than in the control and 5% groups. All the adlay-fed rats had a higher fecal butyric acid concentration than the
control rats. It is concluded that adlay has a significant influence on the growth of intestinal bacteria, which may ultimately affect the physiology and other functions of GI tracts of rats.

Protein-protein associations with other proteins present in Coix endosperm nuclei

Transient expression and electrophoretic mobility shift assay were used to investigate the cis elements and the DNA-binding proteins involved in the regulation of expression of a 22 kDa zein-like alpha-coixin gene. A set of unidirectional deletions was generated in a 962 bp fragment of the alpha-coixin promoter that had been previously fused to the reporter gene GUS. The constructs were assayed by transient expression in immature maize endosperm. There was no significant decrease in GUS activity as deletions progressed from -1084 to -238. However, deletion from -238 to -158, which partially deleted the O2c box, resulted in a dramatic decrease in GUS activity emphasizing the importance of the O2 box in the quantitative expression of the gene. The -238 promoter fragment interacted with Coix endosperm nuclear proteins to form 5 DNA-protein complexes, C1-C5, as detected by EMSA. The same retarded complexes were observed when the -158 promoter fragment was used in the binding reactions. Reactions with nuclear extracts isolated from Coix endosperm harvested from 6 to 35 days after pollination revealed that the 5 DNA-protein complexes that interact with the alpha-coixin promoter are differentially assembled during seed development. Deletion analysis carried out on the -238/ATG promoter fragment showed that a 35 bp region from -86 to -51 is essential for the formation of the complexes observed. When nuclear extracts were incubated with an antiserum raised against the maize Opaque-2 protein, the formation of 4 complexes, C1, C3, C4 and C5, was prevented indicating that an Opaque-2 like protein participates in the formation of those complexes. Complex C2 was not affected by the addition of the O2 antibody, suggesting the existence of a novel nuclear factor, CBF1, that binds to the promoter and makes protein-protein associations with other proteins present in Coix endosperm nuclei.

Immunopahramcological studies

At Osaka University School of Medicine, Coix seed has been used in patients with verruca vulgaris and verruca planae juveniles, which have been considered to be induced by viral infection. Moreover, coixenolide, component in the seeds of coix, was reported to show anti-tumor activity. Possibly coix seed may have some influence on the cytotoxic activity of peripheral lymphocytes but there has been no data on this. Then we investigated the changes in number of cytotoxic lymphoid cells in seven volunteers before, during (four weeks) and after taking six coix seed tablets. Lymphocyte subsets were analyzed with monoclonal antibodies using a flow cytometer. The level of CD3+CD56+ (MHC-non restricted cytotoxic T cells) markedly increased at four weeks (before 1.9±0.5% vs four weeks 4.2±0.7%, P less than 0.01). The level of CD16+CD57- (the mature, most active natural killer cells) increased at three weeks (before 4.5±0.8% vs three weeks 5.2±0.8%, P less than 0.05). The level of CD3-CD56+ (natural killer cells) and the level of CD16+CD57+ (the variable active natural killer cells) decreased at one week and returned to normal level thereafter (before 13.7±2.1% vs one week 11.2±1.5%, p less than 0.05; before 8.8±1.5% vs one week 6.9±1.3%, P less than 0.05, respectively). These results indicate that coix seed modulate the peripheral blood lymphocyte subsets and may be effective to virus disease through the enhancement of cytotoxic activity.

Research and development of Kanglaite injection

Since 1975, author and co-workers began the systemic studies on a large number of medicinal herbs, including the extraction and separation of active compounds from herbs, design of recipe and formulation, technical process, quality control and pharmacology of active compounds. Afterwards, many scientists of institutions in China formed a cooperation group to investigate the pharmacology,
pharmacodynamics, pharmacokinetics, toxicology, and mechanism of action of Kanglaite injection, a novel type diphasic anticancer drug prepared by efficacious anticancer components from the Coix seed with the advanced technology and formulated into a lipid emulsion for intravenous and intra-arterial injection. In 1995, the injection was conferred a certificate of new drug by the State Drug Administration of the Ministry of Health and was approved to be manufactured officially and launched into market in 1997. Kanglaite injection (KLT) has been successfully applied in the treatment of a variety of malignant tumors such as carcinomas of lung, liver, stomach, esophagus, colon, pancrease, kidney, ovaries, malignant lymphoma, leukemias for more than 200,000 cases. In 2001 and 2002, clinical trial of kanglaite injection has been started in USA, and Russia, respectively.

Anticancer activity

KLT has marked inhibition effect on many tumor cell strains in in vitro cultivation test of leukemic cells P388, L1210, human cervical carcinoma HeLas3, human colon carcinoma M7609, The IC50 of these strains was 15 µL·mL⁻¹, 28.8µL·mL⁻¹, 12.5µL·mL⁻¹ and 11.9µL·mL⁻¹, respectively.[31] In vitro cultured ovary carcinoma cell 3AO, the IC50 of KLT was 47µg·mL⁻¹.[35]

In the efficacy experiments with mouse rat transplantable tumors, KLT had a prominent inhibitory effect on Lewis lung carcinoma, B16 melanoma, W256 sarcoma and obtained an evident dosae-response relationship.[32] The nude mouse with human liver carcinoma was treated with KLT 25 mL·kg⁻¹ iv for 10 days, an inhibition rate of 83.3% was gained.[33] In nude mice transplanted with human lung carcinoma (SPC), colon carcinoma M7609, breast carcinoma Bcap-37, KLT 25 mL·kg⁻¹ the inhibition rate was 62.4, 57.2 and 50.0%, respectively.[34]

At the Second Affiliated Hospital, The Medical College of Zhejiang University, Hu et al studied the effect of KLT on the proliferation and telomerase activity of rat mesangial cells. They obtained following investigated results: the growth rate of MC was enhanced by IL-1 stimulation, which was accompanied with a reduction of the activity of telomerase. Adversely, the growth rate of MC was reduced by KLT, which was accompanied with an enhancement of activity of telomerase. Moreover, the growth rate of MC and the activity of telomerase were both inhibited by the combinative use of IL-1 and KLT without any influence from the sequence of their administration. It suggested that KLT could inhibit proliferation and telomerase activity of MC with or without pre-stimulation with IL-1. KLT might be useful to prevent and treat glomerular nephritis related to MC proliferation.[36]

When combination of KLT with cancer chemotherapy drugs, KLT can raise the therapeutic response of chemotherapy drugs in vitro system.

In in vivo system, when KLT (20 mL·kg⁻¹) combined with small dose of cyclophosphamide 10 mg·kg⁻¹, the inhibition rate on rat tumor W256 was 72.4%, whereas the inhibition rate of cyclophosphamide alone was only 38.5%. When KLT (25 mL·kg⁻¹) combined with DHAD (2 mg·kg⁻¹) could raise the inhibition rate of DHAD from 50% to 67%.

The combination of KLT with anticancer drugs verified that a combination therapy of KLT can not only remarkably raise the therapeutic efficacy, but also can reduce the toxicity blood system (such as WBC), liver function (such as ALT) and renal function (such as BUN).[37]

Mechanism of action

The investigated results have verified the mechanism of action of KLT as follows: (1) the drug inhibits the mitosis of tumor cells during G2/M phases, (2) induces apoptosis of tumor cells, (3) affects the genetic expression of tumor cells by up-regulating FAS/Apo-1 gene expression and down-regulating Bc1-2 gene expression, (4) inhibits the tumor angiogenesis, (5) counteracts the cachexia of cancers, and (6) reverses the multi-resistance of tumor cells to anticancer drugs and the resistance modification in some of chemotherapeutics.[37]

Effect on cell cycle

The effect on cell cycle was investigated in K562 cell line. When treated with KLT 1µL·mL⁻¹ for 48 h, the percentage of cells at S and G2+M phase increased markedly, while cells at G1 phase decreased evidently. When the dose was increased to 5 µL·mL⁻¹, the percentage of cells at S phase decreased apparently, while cells at G+M phase remarkably rose. When the dose was increased
to 10 µL·mL⁻¹, cells at S phase declined to only 11.6% of the control group, while cells at G2+M increased by 11 times that of control group. At this time, it was not possible to analyze the distribution of the cells in the cell cycle. This result indicated that the key link of KLT’s action was mainly the inhibition of the cells at G2+M phase, thus reduce the number of cells entering G0 and G1 phase and leading to the reduction in percentage of cells at S phase and decreased the mitosis and inhibited the multiplication of tumor cells and resulted in the apoptosis of the cells that were affected. (Fig 3). This study has verified that inhibited mitosis of tumor cells, and then their multiplication. That is probably the key link of the mechanism of action in KLT anticancer. [38]

**Effect on cell apoptosis** Zheng et al study has demonstrated that after being treated with 10 KLT for 6 h, the human erythro-leukemia K562 cells were induced to apoptosis (Fig 4). With a higher dose of KLT, the cells membrane was seen to be damaged and appeared PI overstained, an evidence of necrosis. In respect to solid tumors, the coloncarinoma SW1116 cells could also be induced with 10µL·mL⁻¹ KLT to apoptosis by terminal deoxynucleotidyl transferase labelled Immuno-fluorescence technique (TUNEL) method (Fig 5). The percentage of the positive TUNEL labelled cells decreased with the increase in dose of KLT. This might be related to the increased necrosis rate. The study has also found that after KLT treatment the ultra stucture of tumor had been markedly changed, including large amount of vacuolar degeration, chromatin condensation and apoptosis granules which are morphological characteristics of cell apoptosis. [39]

In Shen’s study, utilized TUNEL for detecting the apoptosis of renal cancer cells. IC₅₀ of KLT for inhibiting renal cancer cells was 19.31µL·mL⁻¹. Both KLT 5µL·mL⁻¹ and 10µL·mL⁻¹ were effective in inducing the apoptoses of cells, the apoptic rates of renal cancer cell lines were 31.1% and 89.76%, respectively. As KLT concentration increased, the number of apoptic cells was conversely decreased. In 15µL·mL⁻¹ and 20µL·mL⁻¹ groups, the apoptotic cells were lower than 10%.These results showed that there is a close relation between the apoptosis and necrosis induced by KLT and the used dose. Different cell line has different apoptotic rate induced by KLT. Therefore, apoptosis and necrosis of human tumor cells induced by KLT are key mechanism for anticancer action. [37]
Effect on expression of tumor cells Shen et al applied MTT method to measure the inhibitory effect of KLT on renal cancer cells, and an immunohistochemical staining method to analyze the effect on the expression of P53 gene and bcl-2 gene. Their test as follows: the Labelled Index (LI) of P53 gene expression in the KLT group was 16.8%, while no expression was observed in the control group. LI of bcl-2 gene expression was 25% in the control group, and 6.6% in the KLT group (10µL·mL⁻¹). The above test results suggested that the apoptosis of tumor cells induced by KLT may be realized by up-regulating P53 gene and down-regulating bcl-2 gene.[40]

At Zhejiang University, Yu conducted a study of effect of KLT on the Fas gene expression on surface of colo 205 cells by RT-PCR method. The cells were pre-treated with KLT for 4 h and then incubated 24 h, the Fas gene expression was apparent. No PCR product was observed in the A FCM method was adopted to measure the Fas gene expression on the surface of Colo 205 cell before and after pre-treatment with KLT. The expression rate of Fas gene expression on the surface of Colo 205 cell before the pre-treatment was 21.1%, and after the expression rate increased to 60.4%. Based on the characteristics of Fas gene which is liable to be activated by its corresponding antibody. They observed the killing effect of the anti-Fas antibody on Colo 205 cells with MTT method. The results have shown that Colo 205 cells were more liable to be injured by anti-Fas antibody after they had been pre-treated with KLT suggesting through up-regulating the functional expression of Fas gene.[41]

Effect on angiogenesis In the study carried out by Zhang et al, a non-serum culture medium was used, in which aortic arches were cultivated in a three dimensional collagen gel for a total of 28 days (0.1 mg·mL⁻¹ Vitamin E as positive control group, 10µL·mL⁻¹ KLT as tested group) the situation of angiogenesis was observed with an Invert-Microscope. As shown in Fig 6, the results revealed that KLT can significantly inhibit the formation of new blood vessels, accelerance the speed of blood vessels entering into regression phase. The inhibitory effect was markedly superior to vitaminine E. It indicated that the inhibition of angiogenesis in tumor is one of the anticancer mechanisms possessed by KLT.[37]

A Anti-cachexia effect At An-Hui Tumor Hospital, Li studied the anti-cachexia effect of JLT in cachectic model by inoculating T739 with tumor LA795 of mice. The experiment result has verified that beginning from the 14th day following the inoculation of tumor cells, the mice were evidently given medication for one week, the food intake and
body weight were evidently restored, there was no significant difference as compared with the normal mice. A significant difference was observed as compared with the cachetic group. Pathological examination has also demonstrated that after the medication with KLT, the growth of tumor has basically stopped. Moreover, part of mice had the occurrence of liquefaction and tumor necrosis and the life span prolonged. The test results of cytokines that were associated with cachexia, have revealed that the serum TNF-α and IL-1 levels were decreased by KLT.\textsuperscript{[37]}

**Effect on multidrug resistance of tumor cells**

The multidrug resistance (MDR) human leukemia K562/VCR was a typical MDR cells which were agents, such as VCR, HAR, ADM, MMC. At Zhejiang University, Zheng et al studied the effect of KLT on MDR tumor cells. The IC\textsubscript{50} of ADM was 0.9 for K562 cells, but 30 for K562/VCR MDR cells. Which was 33 times higher than that of K562 cells. When KLT was used in combination with ADM and 2, 4 and 8µL·mL\textsuperscript{-1} of KLT, the IC\textsubscript{50} of ADM was only 0.705-0.555µg mL\textsuperscript{-1} for K562/VCR MDR cells. Under different concentrations of KLT, the ADM concentration required would be greatly reduced, the resistance modification index of ADM was 42.6\%, 45.2\% and 54.1\%, respectively (Fig 7). This study demonstrated that KLT can markedly reverse the ADM resistance.\textsuperscript{[37,42]}
Pharmacokinetics

Elimination and bioavailability
Using intervenous injection and oral administration of $^3$H-KLT detected the elimination and bioavailability in mice. The concentration-time curves are showed in Fig 8. Its elimination half-life was 15.84 h, the bioavailability was 62%, and the protein binding rate was 80.5%.\(^{[43]}\)

![Fig 8: The concentration-time curves of $^3$H-KLT](image)

Tissue distribution
After intervenous injection of $^3$H-KLT in mice, the content of $^3$H-KLT in liver, spleen, stomach, kidney, lung, brain, intestine pancreas and blood at 0.5, 8, and 24h are showed in Fig 9. This result exhibited that KLT can be widely distributed in various tissues, the highest amount was found in liver, spleen, and lung.\(^{[43]}\)

![Fig 9: The tissue distribution of $^3$H-KLT](image)

Excretion
After intervenous injection of $^3$H-KLT in mice, the determination of excreting amounts of $^3$H-KLT in urine and feces. The test found that excretion amounts in urine and feces were 38.29% to be original drug during 24 h after injection. Of them, 59.4% in urine and 40.6% in feces. The study
Fig 10 Excretion of KLT in mice after injection of $^3$H-KLT

indicated that the drug is majorly excreted by original drug from urine.$^{[43]}$

**Progress of Clinical trials of KLT**

**Clinical Phase I trial**

In 2001 and 2002, clinical trial of kanglaite injection has been started in USA, and Russia, respectively. At Huntsman Cancer Institute, University of Utah, USA, Researchers selected 15 patients in phase I clinical trial. The research result construed that KLT has been well tolerated, no DLT’s have occurred and the MTD has not been defined. One case had reversible, asymptomatic increase in liver enzymes. One case had an asymptomatic decrease in cardiac ejection fraction. There have been no objective response, however, the durations of stable disease of over six months in some patients in encouraging.$^{[44-46]}$

Clinical trials in the treatment in 12 patients with non-small-cell lung cancer were have been verified for therapeuetic effect and safety of KLT at two Russian’s cancer centers. 11 cases were evaluable for response and all cases were evaluable for safety of KLT. KLT injection showed activity in advanced non-small-cell lung cancer. The results suggested that KLT has a good therapeutic effect as well as improved patient’s quality of life. Absence of typical toxicity for cytotoxic agents permits to recommend standard chemotherapy in these patients and receive effect in next clinical phase.

**Effect on immune function** At Russian Cancer Research Centre of Russian Academy of Medical Sciences, An experiment of Effect on immune function was carried out at 11 patients with lung cancer, treated by KLT injection. The state of cell immunity was estimated by surface antigens expression on peripheral blood lymphocytes. A method of flow cytofluorimetry was used to determine antibodies CD3, CD4, CD5, CD7, CD8, CD11b, CD16, CD20, CD25, CD38, CD50, CD71, CD95, and HLA-DR. Before treatment changes of cell immunity main indexes were revealed in 54 cases and were displayed in the decrease of mature T-lymphocytes relative quantity (CD3+ cells) and their subpopulations CD4+ and CD8+. These parameters were 48.11±3.04, 26.6±2.18, 14.24±1.21, accordingly. Besides, that, in 5of 11 cases, HLA-DR+ lymphocytes quantity was decreased (4.52±0.79), B-lymphocytes was decreased (3.86±0.23) in 3 cases, quinity of lymphocytes, expressing adhesion molecules CD50 was decreased (81.5±21.42) in 4 cases. NK-cell functional activity was depressed in 6 cases. After KLT treatment, the increase of CD3+ was determined in 7 cases of 11 patients to 63.7±4.55, CD4+ to 39.78±2.77and CD8+ to 26.63±3.18. The decrease of CD3+, CD4+ and CD20+ were be determined in 3 cases, NK-cell was increased in 5 cases, and CD16+ decrease in 4 cases. The quanity of cells, expressing activating antigens HLA-DR, CD38, CD25, Cd71, and CD95 were increased in the
half of cases. After the treatment end, increase of IgG, IgM, IgA synthesis was observed in 72%. Increase of antigens CD95 and CD50 level was favorable factor and later on, it will cause improved response on chemotherapy in these patients.\(^{[47]}\)

**Clinical studies of KLT injection in China**

Since 1995, Kanglaite injection has been taken by more 200,000 patients with cancer, and is a best-selling cancer treatment in China. The prospective, randomized and large-scale clinical studies have been carried out in 1408 cases. The clinical studies have shown that KLT could not only inhibit cancer cells, but also enhance body immunoity. Moreover, it has synergistic and chemo- or radio-therapy. It could also relieve pains, improve patient life-quality and extend the survival time.

Clinical pharmacokinetics Tumor patients received the treatment of KLT injection by intravenous infusion 200 mL for 2 h. At different time after stopping injection, blood samples were taken and determined the concentrations of 16-C, 18-C-2 and 18-C-1 in serum by GC-MS method. The concentration-time curves are showed in Fig 11. Calculated pharmacokinetic parameters are listed in Table 1. It is found that the three fatty acids possess different elimination rate Yang H. Collection of the studies of Kanglaite injection against tumors Zhejiang University Press, 1998; 107-109.\(^{[43]}\)

![Fig 11 The concetration-time corves of 16-C, 18-C-2 and 18-C-1 after infussion 200 mL of KLT injection](image)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>16-C</th>
<th>18-C-2</th>
<th>18-C-1</th>
</tr>
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<tbody>
<tr>
<td>( t_{1/2} ) (h)</td>
<td>9.95</td>
<td>8.91</td>
<td>38.31</td>
</tr>
<tr>
<td>AUC(μg.h/mL)</td>
<td>7.93</td>
<td>65.06</td>
<td>12.15</td>
</tr>
</tbody>
</table>

**Clinical phase II trial**

Table 1 The main pharmacokinetic parameters of 16-C, 18-C-2 and 18-C-1 after infusion 200 mL of KLT

Theratment of Primary lung carcinoma At Beijing Guang-An-Men Hospitol, a Multiple-Center Cooperation Group for treating cancer with KLT carried out clinical study in 214 patients treated with KLT and 91 cases treated with chemotherapeutic drugs. The results have demonstrated that the effective rate (CR+PR) was 12.15% (26/214) in the
KLT group, and 14.29%(13/91) in the chemotherapy control group (Table 2). No significant difference between the two groups was observed. In this study, researchers discovered the following findings: symptoms, such as cough, hemoptysis, chest pain, fever, weakness and anorexia in patients with primary bronchogenic lung carcinoma were improved after the treatment with KLT, the therapeutic results were superior to the chemotherapy group. It has also been found that KLT increased the activity of NK-cells and IL-2 level, improved the ratio of T-lymphocyte sub-groups and protected the peripheral blood picture. The study has also verified that no adverse reactions have been observed with regard to liver, kidney and heart functions.\(^{[48]}\)

### Non-small-cell lung carcinoma treated with combined KLT and chemotherapy

A combination of KLT with chemotherapy (PVM scheme) was applied in the treatment of non-small-cell lung carcinoma (NSCLC) was carried out in 72 cases at Tumor Hospital, Chinese Academy of Medical Sciences. The effective rate of KLT+PVM group was 45% (18/40), and that of PVM group was 22% (7/32). A significant difference was found between two groups. This result proved a synergism between KLT and chemotherapy in the treatment of NSCLC and found KLT can improve patient’s general conditions, protect and increase to some extent.\(^{[49]}\)

### Treatment of Primary liver carcinoma

At Beijing Sino-Japanese Friendship Hospital, Dr. Li et al carried out a clinical therapeutic observation of KLT in the treatment of primary liver carcinoma. The trial results have shown that the effective rate of KLT was 11.4% and chemotherapy group (PAF regimen) was 9.8%, no significantly difference can be observed between the two groups (Fig 12). The trial also confirmed that KLT can markedly improve symptoms and life-quality, as well as immune functions. KLT did not inhibit bone marrow, not impair hepatic and renal functions.\(^{[50]}\)
Clinical phase III trial
Effective rate of combined KLT with chemotherapy

A phase III randomized, controlled clinical studies on combined KLT with intervention therapy (KLT+IT) in the treatment of enrolled 198 cases with primary liver carcinoma and primary lung carcinoma. It has demonstrated that the effective rate (CR+PR) of KLT+IT could reach 69.23% (90/130), but the effective rate of IT alone was only 38.23% (26/68), the therapeutic result of KLT+IT was significantly superior to IT alone. Moreover, improving symptoms or Karnofsky score, body weight, and immune functions and protecting peripheral WBC in KLT+IT group were better than that in the IT group. Through another randomized observation on 218 cases of primary lung carcinoma, it has been found that the effective rate of KLT+IT was 52.11% (74/142), and IT alone therapy was only 28.95% (22/76).

Shen et al used combined radiotherapy method for treating 190 cases of carcinomas of lung, esophagus, nasopharynx, etc. The patients were divided into two groups: the first group (KLT with radiotherapy, 104 cases) and the second group (radiotherapy group, 86 cases). In first group, KLT was intravenously injected, 100 mL with 200cGy each time a day for 5 times a week (total dosage of over 6000 cGy), the second group was radiotherapy alone with some radio-dose. Effective rate of (CR+PR)combined group was 82.2%, and that of radiotherapy group was 60.4%, a significant difference between the two groups.

Control of carcinoma pain and improvement of life quality

Dr Li et al performed a phase III clinical study for evaluating the effect of KLT in controlling carcinoma pain and improving life quality in advanced cancer patients (328 cases). They found that the pain would be gradually alleviated 1-3 days after the medication. Among these patients, 96 of them had used morphine type analgesics, 32 of them the dose of morphine could be reduced in the course of the therapy until being totally withdrawn. The withdrawn rate was 32.29%. The remittance could be sustained for 1-7 days after the termination of the medication. The study also shown that the patients used KLT of them had a higher score of Karnofsky score system, of which, patients whose score had been increased by above 20 accounted for 76.6%, the elevated rate of life quality was 91.22%. The study has also found that the body weight of was increased in 42.29% of patients used KLT.

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