Study on the effect of coix seed extract on enzymes of lipometabolism and glycometabolism

Research Institution:	2nd Unit, Laboratory of Pharmacokinetics, Tianjin Institute of Materia Medica
Person in charge:	Liu Changxiao
Experimenter:	Gao Jing, Yu Fei, Zeng Yong
Contact person:	Gao Jing
Tel：	+86-022-23004535

Study on the effect of coix seed extract on enzymes of lipometabolism and glycometabolism

Gao Jing[1], Yu Fei[2,1], Zeng Yong[1], Liu Changxiao[1]

1. *State Key Laboratory of Pharmacokinetics and Pharmacodynamics, Tianjin Institute of Materia Medica*
2. *Department of Pharmaceutical Engineering, School of Chemical Engineering, Tianjin University*

Abstract Objective: To study the in-vitro inhibition effect of coix seed extract on fatty acid synthase (FAS) in animals. An experiment (medication given by gastric perfusion) in rats was applied to evaluate the influence of coix seed extract on activities of some enzymes（FAS、MDH、LPL、HL、TG and G-6-PD）of lipometabolism and glycometabolism. **Methods:** FAS: separated and purified from duck liver and coix seed extract was mixed and reacted at 25°C, then the residual activity was determined and compared with the blank group to study the inhibition effect of coix seed extract on FAS in vitro. The rats were administered coix seed extract (2.5、5.0 and 10.0ml/kg) through gastric perfusion for 10 consecutive days. The activities of FAS、MDH、LPL、HL、TG and G-6-PD in plasma, liver and fatty tissues were separately measured by kit. **Results:** 2 hours after the reaction of coix seed extract 10μl/ml and enzymes, the residual activities of enzymes for the complete reaction of FAS, ketoacyl reduction reaction, enoyl reduction reaction and AcAcCoA reduction reaction were 8.2%、21.6%、39.5% and 8.9%. And the inhibition effect of coix seed extract on FAS was correlated to dosage. After the rats were administered with coix seed extract (2.5, 5.0 and 10.0ml/kg) through gastric perfusion for consecutive 10 days, the activity of FAS in liver was inhibited. There was significant difference at the dosage of 10.0ml/kg of coix seed extract（$P<0.05$）compared with the blank control group while the activity of HL in plasma and liver and PLP in plasma were all significantly elevated（$P<0.05$）. No obvious influence was found on MDH activity and TG content in plasma, liver and fat at the dosage of 2.5, 5.0 and 10.0 ml/kg. The three dosages of coix seed extract mentioned above had slight effect on G-6-PD activity to different extent indicating the reduction percentage of MHb in blood was reduced ($P<0.01$). **Conclusion:** The study demonstrated that coix seed extract had inhibition effect on FAS of animals in vitro. And its inhibition effect on ketoacyl reduction and AcAcCoA reduction reaction was stronger than that on enoyl reduction reaction. In vivo study showed that it had inhibition effect on the activity of FAS in liver. This study provided theoretical support for coix seed extract new target of inhibition effect on FAS and its clinical administration in treating cancer.

Key words: TCM; Coix seed; Coix seed extract; FAS; Enzyme of glycometabolism; Inhibition effect; In vitro study; In vivo study; Target

Preface

Coix seed extract is an oily substance extracted from a Traditional Chinese Medicine -Seeds of *Coix Lacryma-jobi* (family *Cramineae*) (Figure 1) by Zhejiang Kanglaite Pharmaceutical Co., Ltd. The main active ingredient is a compound of triglycerides containing 4 kinds of fatty acid (Figure 2) and is formulated into an emulsion for injection which has been proved to be an effective and safe TCM new product by preclinical animal anticancer studies, pharmacokinetics and safety studies. Through the proof of phase I, II and III clinical trials and clinical application in tens of thousand cases, the product has adjuvant therapeutic effects to cancer patients. It could improve the response rate when combined with chemotherapy, radiotherapy or surgery, regulate energy of advanced patients and improve life quality so as to prolong survival time [1-5]. *Science* magazine in 2003 and *Asian Journal of Pharmacodynamics and Pharmacokinetics* in 2006 reported its academic research [4, 5].

As key materials for synthesis of long-chain fatty acid in organism, FAS extensively exists in fat and liver tissues of human and animals. FAS in animal is a kind of compound enzyme with two subunits. The molecular weight of each subunit is about 2.73 million and it has 6 different active sites and 1 ACP in each subunit covalently linked to a phosphopantetheinyl prosthetic group. Every structural domain of the active site is responsible for one step in catalytic reactions of fatty acid synthesis. The six active sites are MAT, β-KS, β-KR, β-DH, ER and TE. It is reported that there was close correlation between FAS and some obesity-related diseases such as diabetes and cardiovascular disease. FAS is also closely related to the genesis of cancer. The overexpression of FAS has been found in many tumors and FAS inhibitor was proved to have anticancer activity, which made FAS become a new target in search of related drugs at home and abroad [6-10]. Some apoproteins and glycometabolic enzymes are required in the process of FAS synthesis[11]. Therefore the measurement of the activities of some apoproteins and glycometabolic enzymes in blood and tissues can further prove the regulation of FAS in the process of canceration. MDH plays an important role in materials and energy transport; LPL is important for degeneration of chylomicron and VLDL in plasma; HL mainly acts on degeneration of IDL and HDL; The NADPH generated from the catalytic reaction of G-6-PD in red blood cell is the coenzyme of glutathion reductase while reduced glutathione is essential to maintain stability of hemoglobin and integrity of red blood cell membrane. So, lack of G-6-PD can cause damage to the function of red blood cell membrane and eventually induce hemolysis. It is very important to inhibit FAS activity, improve the reduction capacity of methemoglobin and enhance content of G-6-PD in red blood cell in clinical prevention and treatment of cancer.

Coix seeds have not only applicable value in treating cancer but also therapeutic effect in treating diabetes and obesity based on literature. Extensive researches have been conducted on the mechanism and target related to cancer treatment of Kanglaite Injection produced by Zhejiang Kanglaite Pharmaceutical Co., Ltd. with coix seed extract as starting material. To study whether coix seed extract has influence on FAS we conducted in vitro experiment on its inhibition effect on FAS in animals to find out its capacity of inhibiting FAS and the possible action targets. We also studied

changes of FAS, MDH, LPL, HL, TG and G-6-PD in animals after orally feeding coix seed extract to search for a new theoretical support for coix seed extract in clinical adjuvant treatment of cancer.

Experimental materials

Drug: Coix seed extract (batch no. 060309) provided by Zhejiang Kanglaite Pharmaceutical Co., Ltd. Through GC analysis on quality, the quality uniformity between batches was good (Figure3).
Animals: Beijing duck: body weight 2-2.5kg, purchased from Tianjin farm product market. Wistar rat, body weight 170-210g, provided by the animal house of our institute.
Main reagents for in vitro experiment: AcCoA, MalCoA, NADPH and AcAcCoA were all from SIGMA. Ethyl crotonate, ethyl acetoacetate, dithio threitol (DTT) and other reagents were domestic analytical grade. FAS was extracted and purified from duck liver by our institute.

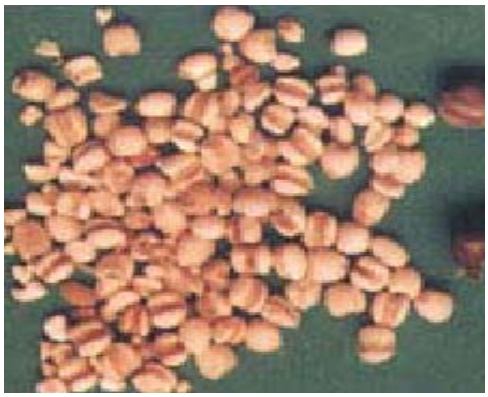

Fig.1 Coix seeds

$$\begin{array}{l} H_2C\text{-}O\text{-}R_1 \\ \quad | \\ H\text{-}C\text{-}O\text{-}R_2 \\ \quad | \\ H_2C\text{-}O\text{-}R_3 \end{array}$$

Hexadecanoic acid (C16) R_1, R_2, R_3 = $-CO(CH_2)_{14}CH_3$
Octadecoic acid (C18) $-CO(CH_2)_{16}CH_3$
Octacenic acid (C18-1) $-CO\text{-}(CH_2)_7CH{=}CH(CH_2)_7CH_3$
Octadedienoic acid (C18-2) $-CO\text{-}(CH_2)_7\text{-}CH{=}CH\text{-}CH_2CH{=}CH(CH_2)_4CH_3$

Fig.2 Chemical structure of the main active ingredient in coix seed

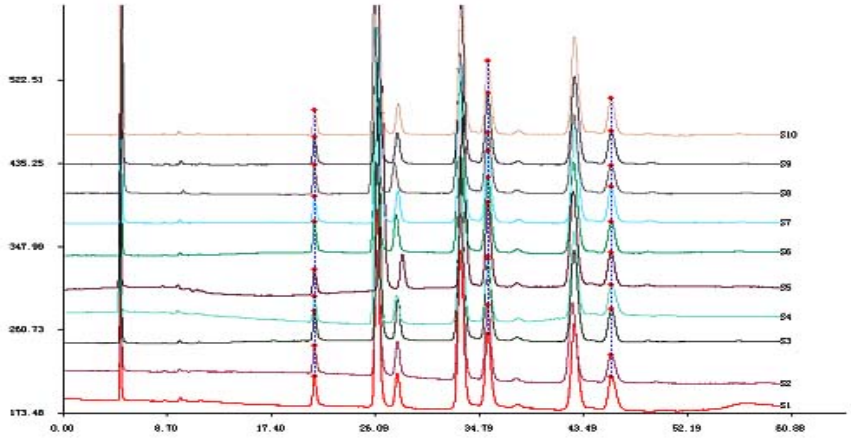

Fig 3　Inter-batch quality control of active ingredient in coix seed by GC method

Major reagent kits for in vivo test: glucose-6-phosphate dehydrogenase (G-6-PD), malate dehydrogenase (MDH plasma, tissue), plasma total lipase (lipid protein lipase LPL and hepatic lipase HL), tissue total lipase (LPL and HL), and protein determination with coomessie brilliant blue were procured from Jiancheng Bioengineering Lab, Nanjing; Triglyceride kit was procured from Zhongshengbeikong Biotech Co., Ltd, Beijing. Acetyl coenzyme A (AcCoA), malonyl coenzyme A (MalCoA) and NADPH were from SIGMA.

Major instruments: Beckman J2-21 high-speed freezing centrifuge from Beckman, USA; Low-pressure column chromatograph from High-tech Application Lab, Beijing; UV-190 spectrophotometer from Shimadzu Corporation, Japan.

Methods

Separation & purification of FAS from duck liver[12]

Bleed the animal to death, immediately take out the liver and put it in ice bath for cooling and weighing. Add 1.8ml/g pre-cooled buffer solution (0.1mol/L potassium phosphate buffer solution containing 0.07mol/L $KHCO_3$, 1mmol/L DTT and 1mmol/L EDTA, pH7.8-8.0) to make a homogenate in ice bath (centrifugation at 4°C 8,000rpm×30min). Filter the supernatant fluid of the homogenate with gauze and centrifuge again (18,000rpm×60min). Collect the supernatant fluid and store at -75°C for purification. Defreeze 70ml of the supernatant fluid from duck liver homogenate at 4°C. Add

saturated $(NH_4)_2SO_4$ to reach a saturation of 25%. Allow it to stand at 4°C for 2 hours. Centrifuge and collect supernatant fluid, add saturated $(NH_4)_2SO_4$ to reach a saturation of 50%. Allow it to stand at 4 °C overnight, centrifuge it to collect precipitate which is dissolved with 200ml of balanced buffer solution (5mmol/L potassium phosphate buffer solution containing 1mmol/L DTT) and then for centrifugation. Apply the supernatant fluid onto the balanced DEAE-sepharose FF column (2.6cmx50cm) at a flow rate of 1.4ml/min. Elute the column with balanced buffer solution until A280<0.01. Then elute the column with 5mmol/L-0.3mol/L potassium phosphate buffer solutions by a method of linear gradient elution. Collect the eluate stepwise and test FAS activity. Combine the sections with active peak, add same volume saturated $(NH_4)_2SO_4$ and allow to stand at 4°C overnight, centrifuged to collect precipitate, dissolve it with appropriate amount of buffer solution (0.1mol/L potassium phosphate buffer, 1mmol/L DTT and 20% glycerin, pH7.0) and dialyze the buffer solution overnight. The dialyzed solution was sub-packed and stored at -75°C after activity determination.

Determination of FAS activity

Activity is determined according to reference[13] method. It is determined with 0.1mol/L potassium phosphate buffer solution (pH7.0) containing 1mmol/L EDTA and 1mmol/L DTT (volume 2ml, optical diameter 1cm) at constant 37°C water bath for 5 minutes, add diluted enzyme solution (about 20μg) to initiate the reaction. Continuously monitor the absorbance change under 340nm wavelength. The absorbance changes since NADPH was catalyzed and oxidized by FAS into NADP. The determination is conducted in a UV-spectrophotometer. The substrate concentrations in the FAS complete reaction and activity determination of each activity center are listed below.

(1) Determination of activity of complete reaction reactase: 6μmol/L AcCoA, 12μmol/L MalCoA, and 40μmol/L NADPH.
(2) Determination of activity of ketoacyl reductase: 0.8mmol/L ethyl acetoacetate and 40μmol/L NADPH.
(3) Determination of the Activity of enoyl reductase: 45mmol/L ethyl butylene acid and 40μmol/L NADPH.
(4) Determination of the Activity of AcAcCoA reduction: 31μmol/L AcAcCoA and 40μmol/L NADPH. This determination contains reactions at active centers including transacylation, ketoacyl reduction, dehydration and enoyl reduction, etc.
(5) Calculation of enzyme activity: The unit of FAS activity is defined as the enzyme quantity consumed to oxidize 14nmol/L NADPH in a minute. In this way the unit of enzyme activity in one milliliter can be calculated with an equation as follows.

$$\text{Activity (u/ml)} = \triangle A_{340} \times 10^6 \times C / (\varepsilon \times V)/14$$

Where $\triangle A_{340}$ is the absorbance change value monitored at 340nm wavelength/min in the activity

determination, ε is the mmol extinction coefficient of NADPH at 340nm wavelength which is 6.022 at optical diameter 1cm. V is the volume of activity determination system (2ml). C is dilution folds (here is 40). 10^6 is the coefficient in conversion from mmol to nmol.

Determination of in vitro inhibitory effect of coix seed extract on animal FAS

Add certain amount of coix seed extract into the enzyme solution, mix for some time at 25°C, rapidly centrifuge it to separate the oil from water, determine the activity A of the enzyme solution. And A_0 means the activity when coix seed extract is replaced by water. A/A_0 multiplied by 100% is the residual activity at that moment. Determine the residual activities at a series of time points and draw a figure with activity to time.

Effect of coix seed extract on FAS and glucometabolic enzymes in rats

42 rats are divided randomly into 4 groups: control group (10 rats), test group I with 2.5ml/kg coix seed extract (10 rats), test group II with 5.0ml/kg coix seed extract (10 rats) and test group III with 10.0ml/kg coix seed extract (12 rats). The male/female ratio is 1:1 in each group. The animals are given coix seed extract through gastric perfusion once per day for 10 consecutive days. The rats are anaesthetized with ether after the last dose. Blood samples are taken from abdominal aorta of the rats and separate the plasma, dissect the liver and fat to obtain tissue homogenate. Determine the activities of FAS, MDH, LPL, HL, TG and G-6-PD in plasma and tissues. Compare the results with that in control group and conduct bio-statistical evaluation (t test) to study the impact of coix seed extract on fat and glucometabolic enzymes in rats.

Results

Part I: In vitro test

1. Inhibitory effect of coix seed extract on FAS complete reaction

See Tab. 1 and Fig. 4 for the test results. The results showed that over 50% of the FAS activity was inhibited after 0.5h of reaction with 10μl/ml coix seed extract at 25°C. And only 8.2% of FAS complete reaction activity was left after 2h of reaction.

To further evaluate the inhibition of coix seed extract on FAS complete reaction activity we continued our observation on the inhibition of coix seed extract on FAS complete reaction activity with different dosages of coix seed extract (0, 1, 2.5, 5 and 10μl/ml at 25℃ for 2h (See Tab. 2 and Fig. 5). The results showed that there was the dosage dependency in the range 1-10μl/ml concentration.

Tab.1 Results of the inhibition of coix seed extract 10μl/ml on FAS complete reaction

Time (h)	Relative residual activity ($\overline{X}$±*SD*, n=3)
0	100 ± 14.7
0.25	82.8 ± 5.7
0.50	44.3 ± 11.1
0.75	29.9 ± 17.6
1.00	20.5 ± 7.5
1.50	18.9 ± 5.7
2.00	8.2 ± 1.4

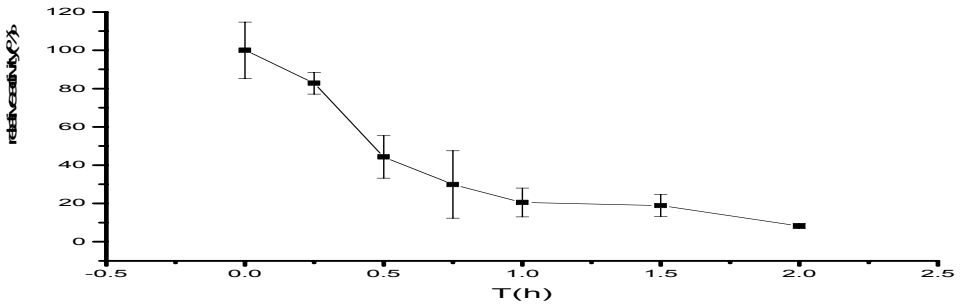

Fig. 4 Inhibition of coix seed extract 10μl/ml on FAS complete reaction

Tab.2 Comparison on inhibition of coix seed extract 1-10μl/ml on complete reaction

Dosage (μl/ml)	Relative residual activity ($\overline{X}$±*SD*, n=3)
0	100 ± 13.2
1.00	53.6 ± 25.9
2.50	29.5 ± 14.9
5.00	20.6 ± 1.5
10.00	8.2 ± 1.4

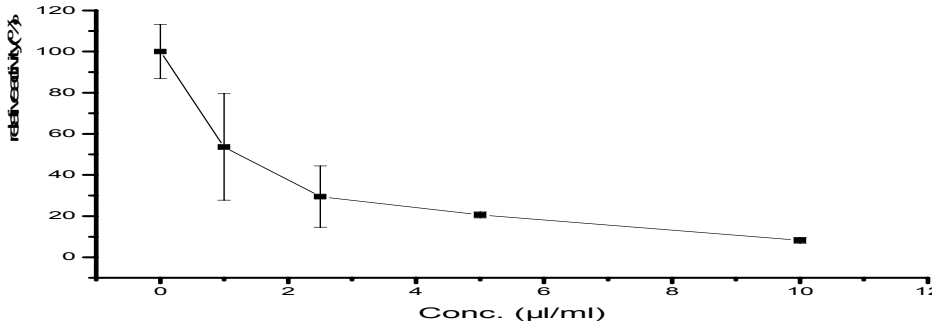

Fig. 5 Inhibition of coix seed extract 1-10μl/ml on FAS complete reaction

2. Inhibition of coix seed extract on FAS ketoacyl reduction

The experimental results were shown in Tab. 3 and Fig. 6. The results showed that over 50% of the FAS activity was inhibited after 0.5h of reaction with 10μl/ml coix seed extract at 25°C. And only 21.6% of FAS complete reaction activity was left after 2h of reaction. The inhibition results of different dosages of coix seed extract on FAS ketoacyl reduction reaction at 25°C for 2h were shown in Tab. 4 and Fig. 4, there was dosage dependency in the range of 1-10μl/ml concentration.

Tab. 3 Results of the inhibition of coix seed extract 10μl/ml on FAS keto-acyl reduction reaction

Time (h)	Relative residual activity ($\overline{X}$±*SD*, n=3)
0	100 ± 8.4
0.25	54.0 ± 8.4
0.50	49.9 ± 10.2
0.75	36.4 ± 8.1
1.00	32.4 ± 16.2
1.50	27.0 ± 4.7
2.00	21.6 ± 8.4

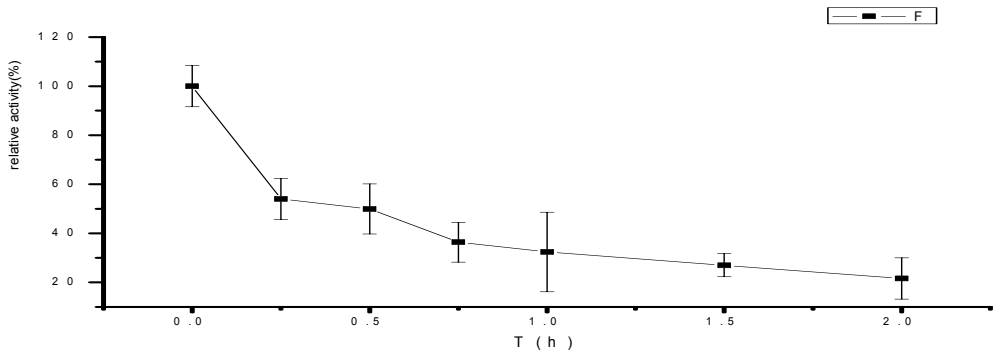

Fig. 6 Inhibition of coix seed extract 10μl/ml on FAS keto-acyl reduction reaction

Tab.4 Comparison on inhibition of coix seed extract 1-10μl/ml on keto-acyl reduction reaction

Dosage (μl/ml)	Relative residual activity ($\overline{X}$±*SD*, n=3)
0	100 ± 31.2
1.00	83.0 ± 23.8
2.50	51.3 ± 30.0
5.00	39.1 ± 12.9
10.00	21.6 ± 8.4

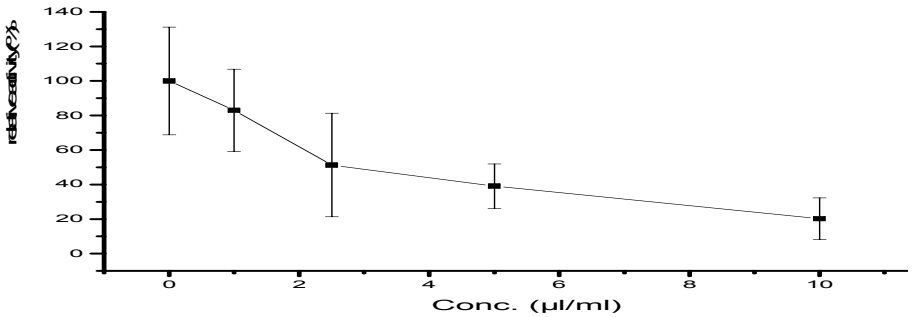

Fig.7 Inhibition of coix seed extract 1-10μl/ml on FAS keto-acyl reduction reaction

3. Inhibition of coix seed extract on FAS enoyl-acyl reduction reaction

See Tab. 3 and Fig. 6 for the experimental results of the Inhibition of coix seed extracts on FAS enoyl-acyl reduction reaction. The results shown that the inhibition of coix seed extracts 10μl/ml on FAS enoyl-acyl reduction reaction was comparatively weak and relative residue after reaction with FAS at 25°C for 2 hours was 39.5%.

Tab.5 Results of the inhibition of coix seed extract 10μl/ml on FAS enoyl-acyl reduction reaction

Time (h)	Relative residue activity ($\overline{X}$±*SD*, n=3)
0	100 ± 16.5
0.25	84.8 ± 15.7
0.50	82.5 ± 19.2
0.75	80.1 ± 22.8
1.00	53.4 ± 4.0
1.50	52.3 ± 12.6
2.00	39.5 ± 24.7

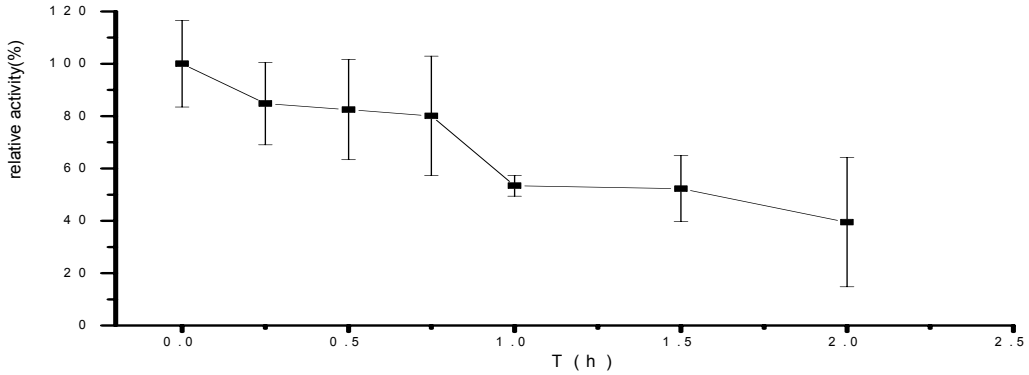

Fig. 8 Inhibition of coix seed extract 10μl/ml on FAS enoyl-acyl reduction reaction

4. Inhibition of coix seed extract on FAS AcAcCoA reduction reaction

See Tab.6 and Fig. 9 for the experimental result of the Inhibition of coix seed extracts on AcAcCoA reduction reaction catalyzed by FAS was comparatively strong. Over 50% of enzyme activity could be inhibited at 25°C for 0.74 hour and the residue activity after 2 hours reaction was only 8.9%. See Tab.7 and Fig.10 for the results of the Inhibition of coix seed extracts at different concentrations on AcAcCoA reduction reaction catalyzed by FAS at 25°C for 2 hours. And the Inhibition of coix seed extracts 1-10μl/ml on AcAcCoA reduction was also dose-dependent.

Tab.6 Experimental results of the inhibition of coix seed extract 10μl/ml on AcAcCoA reduction

Time (h)	Relative residual activity ($\overline{X}$±*SD*, n=3)
0	100 ± 17.2
0.25	71.3 ± 6.2
0.50	56.1 ± 3.8
0.75	42.8 ± 17.5
1.00	34.8 ± 3.8
1.50	23.2 ± 3.1
2.00	8.9 ± 3.1

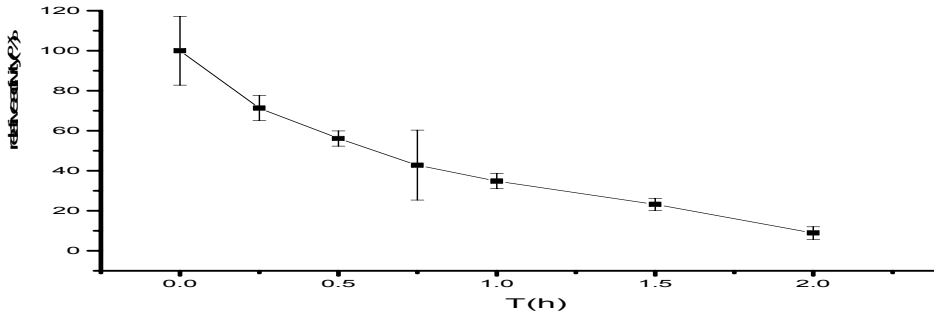

Fig. 9 Inhibition of coix seed extract 10μl/ml on AcAcCoA reduction of FAS

Tab. 7 Comparison on inhibition result of coix seed extract 1-10μl/ml on AcAcCoA reduction

Dosage (μl/ml)	Relative residual activity ($\overline{X}$±*SD*, n=3)
0	100 ± 31.2
1.00	83.0 ± 23.8
2.50	51.3 ± 30.0
5.00	39.1 ± 12.9
10.00	21.6 ± 8.4

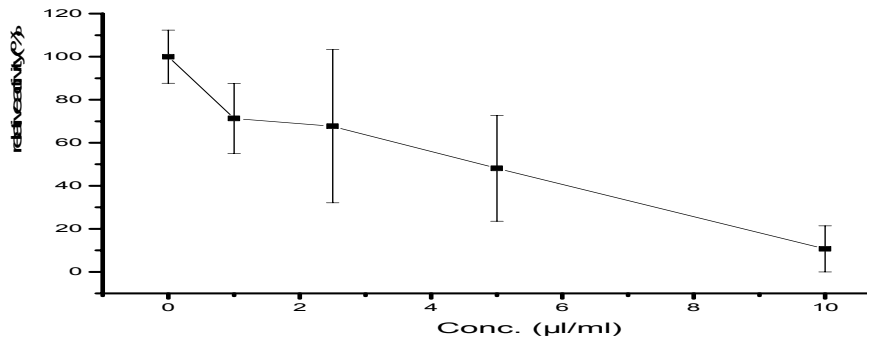

Fig.10 Inhibition of coix seed extracts 1-10μl/ml on AcAcCoA reduction

Part 2 In vivo trial

1. In vivo effect on FAS in rats

Rats were fed with coix seed extract 2.5, 5.0 and 10.0ml/kg through gastric perfusion for 10 consecutive days. Dosage of 10.0ml/kg had apparent inhibitive effect on FAS activity in liver and presented significant difference compared with that in control group P<0.05. No notable effect on FAS activity in fat tissue. See Tab. 8.

Tab. 8 Effect of coix seed extract on tissue FAS activity in rats (n=10)

Dosage（ml/kg）	FAS activity (U/g tissue)（$\overline{X}\pm SD$）	
	In liver	In Fat
Control group	13047.40±3268.21	3510.94±2693.94
2.5	9752.60±4681.41	3004.86±1717.95
5.0	11007.26±2490.75	2894.15±1729.63
10.0	10082.08±2621.14 *	2846.70±1355.86

Note: Compared with control group *P<0.05

2. Effect on MDH activity in rats

Rats were fed with coix seed extracts 2.5, 5.0 and 10.0ml/kg through gastric perfusion for 10 consecutive days. There was no notable effect on MDH activity in plasma, liver and fat tissue. See Tab. 9.

Tab. 9 Influence of coix seed extract on MDH activity in rats (n=10)

Dosage（ml/kg）	MDH activity (U/ml)（$\overline{X}\pm SD$）		
	In plasma	In liver	In fat
Control group	0.570±0.249	12.209±2.950	8.040±2.380
2.5	0.419±0.177	11.924±2.269	10.310±4.127
5.0	0.394±0.141	13.372±1.961	10.833±5.491
10.0	0.452±0.112	12.949±2.227	9.786±2.321

3. Effect on PLP and HL activities in rats

Rats were fed with coix seed extracts for 10 days with dosage of 2.5, 5.0 and 10.0ml/kg through gastric perfusion. Plasma HL activity was notably elevated and compared with control group, P<0.05; Dosage of 10.0ml/kg had effect on plasma PLP activity and liver HL activity, compared with control group, P<0.05. See Tab.10-11.

Tab.10 Effect of coix seed extract on LPL activity in rats (n=10)

Dosage（ml/kg）	LPL activity (U/ml)（$\overline{X}\pm SD$）		
	In plasma	In liver	In Fat
Control group	3.318±0.872	0.537±0.183	3.713±1.815
2.5	3.505±0.985	0.418±0.063	4.574±1.910
5.0	2.832±1.057	0.510±0.183	5.714±2.604
10.0	5.620±3.051*	0.525±0.205	5.645±2.414

Note: Compared with control group, *P<0.05.

Tab. 11 Effect of coix seed extracts on HL activity in rats (n=10)

Dosage（ml/kg）	HL activity (U/ml)（$\overline{X}\pm SD$）		
	In plasma	In liver	In fat
Control group	3.980±1.329	0.548±0.106	4.405±2.005
2.5	5.555±1.581*	0.749±0.325	6.946±4.851
5.0	5.376±1.188*	0.677±0.266	6.233±0.935
10.0	6.286±2.787*	0.812±0.294*	6.469±2.901

Note: compared with control group, *P<0.05.

4. Effect on TG content in rat plasma

Rats were fed with coix seed extracts through gastric perfusion for 10 days. The plasma TG content of high, medium and low dosages compared with the blank group, no significant effect was observed.

Tab.12 Effect of Coix seed extract on plasma TG level in rats (n=10)

Dosage (ml/kg)	Plasma TG level (mmol/L) ($\overline{X}\pm SD$)
Blank group	1.119±0.538
2.5	1.027±0.315
5.0	1.061±0.330
10.0	0.942±0.353

5. Effect of Coix seed extract on blood G-6-PD (methemoglobin reduction percentage) in rats

Ten days after gastric perfusion of coix seed extract the blood G-6-PD (methemoglobin reduction percentage) of rats in high, medium and low-dosage groups were reduced to different extent, compared with the blank group, P<0.01. See Tab. 13.

Tab. 13 Effect of coix seed extract on blood G-6-PD in rats (n=10)

Dosage (ml/kg)	Methemoglobin reduction percentage ($\overline{X} \pm SD$)
Blank group	76.542±7.477
2.5	64.746±6.089 **
5.0	60.475±3.308 **
10.0	69.266±2.254 **

Note: Compared to the blank group, ** P<0.01

Discussion

Fatty acid synthase (FAS) is highly expressed in cancer cells while in normal tissues its content is rather low. The products from the action of FAS are the sources of substance and energy for proliferation of tumor cells. Search of drugs that can arrest the activity of FAS to cut off or inhibit the source of substance and energy for the growth of tumor is the new target of anticancer drugs investigated by drug researchers [10]. Currently most of the researches on inhibition of FAS were focused on the formation of fat in body of animals especially on inhibition of the activity of FAS in poultry. Some researchers have studied the inhibitory effect of such plants as *radix polygoni multiflori*, *folium ginkgo* and *green tea* on activity of FAS in human breast cancer cells [14-16] and further proved beneficial effect through the inhibition of FAS.

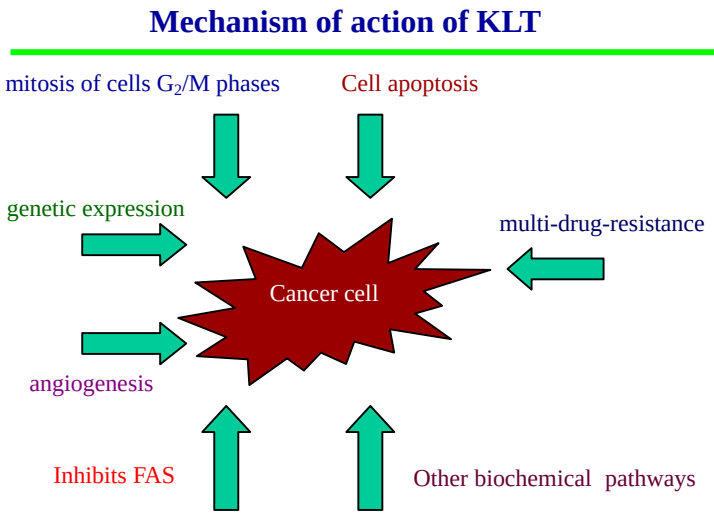

Fig. 11 Inhibition of FAS - a new target by action of the active ingredient of coix seed

This paper has, for the first time, described a new mechanism of action of coix seed extract from the angle of inhibition of FAS. From the results of our experiment we can see that coix seed extract has the action of inhibiting activity of FAS and this inhibition has also a dose-dependent relationship. Furthermore the action of coix seed extract is different at various activity locations of FAS. Its inhibition on ketoacyl reduction and AcAcCoA reduction is much stronger than that on enoyl reduction. These experiments have provided experimental evidences that reflect multiple targets of Traditional

Chinese Medicine (Fig. 11).

Johns Hopkins University School of Medicine has conducted an in vitro study on the mechanism of anticancer action of coix seed extract and reached the conclusion as follows.

Coix seed directly acts on the activity of protein kinase C (PKC), inhibits NFKB regulated COX-2 expression, inhibits expression of MMP-9 probably through inhibiting NFKB signaling and matrigel invasion and Inhibits tumor growth of human cancer xenografts. It has apparent synergy in vitro with agents that inhibit fatty acid synthase. The in vitro study on inhibition of FAS activity has verified that both of 50ml extract/ml and 100ml extract/ml can inhibit the activity of FAS, the same conclusion as from our experiment. This has verified the target of action of coix seed extract and provided a reliable rationale for coix seed extract to be extensively applied in the treatment of cancers. The results of our experiment also support the scientific value of the experimental results by researchers at Johns Hopkins University School of Medicine.

Conclusion

Two hours after coix seed extract 10μl/ml reacted with enzyme the remained enzyme activities of FAS complete reaction, ketoacyl reduction, enoyl reduction and AcAcCoA reduction were 8.2%, 21.6%, 39.5% and 8.9% respectively and the inhibition of coix seed extract on enzyme was dose-dependent. The rats were orally administered with coix seed extract 2.5, 5.0 and 10.0ml/kg for 10 consecutive days and the experiment revealed inhibition of FAS activity in liver. Compared with the blank control group, there was a significant difference at dose of 10.0ml/kg ($P<0.05$). HL activities in plasma and liver were elevated and the plasma PLP was also markedly increased ($P<0.05$). At doses of 2.5, 5.0 and 10.0ml/kg the MDH activity and TG content in plasma, liver and fatty tissue were not apparently affected. These three doses of coix seed extract had slight effect on G-6-PD activity to the different extent, indicating a decrease of methemoglobin reduction percentage ($P<0.01$). The in vitro study has verified that coix seed extract had inhibitory action on FAS activity in animal. Its inhibition on ketoacyl reduction and AcAcCoA reduction is stronger than that on enoyl reduction. The in vivo study has demonstrated that coix seed extract had inhibitory action on FAS activity in liver. Our study provided support for the new target of action of coix seed extract in inhibiting FAS, revealing the rationale for coix seed extract to be applied in the treatment of cancer.

References

1. Li Dapeng. Progress of clinical studies of Kanglaite Injection in foreign countries (USA, RUS). Hangzhou: *Zhejing Kanglaite Pharmaceutical Co., LTD*. 2004; 1-28.
2. Yang H. Collection of the studies of Kanglaite injection against tumors. *Zhejiang University Press,* 1998; 107-118.

3. Garin AM, Tjulandin SA, Kadagidze ZG, Bulanov AA, Chitya LV. Clinical trial of Kanglaite Injection in patients with non-small cell lung cancer in Russia, *Progress of Clinical Studies of Kanglaite Injection in Foreign Countries (USA, RUS)*, 2004; 67-70.
4. Normile D. The new face of traditional Chinese medicine. *Science,* 2003; 299: 188-190.
5. Li Dapeng. Research advance on ethenopharmacology, pharmacodynamics, pharmacokinetics and clinical therapeutics of coix seed and its preparation, Kanglaite injection. *Asian Journal of Pharmacodynamics and Pharmacokinetics,* 2006, 6(2): 83-102.
6. Loftus TM, Jaworsky DE, Frehywot GL, Townsend CA, Ronnett GV, Lane MD, Kuhajda FP. Reduced food intake and body weight in mice treated with fatty acid synthase inhibitors. *Science*，2000, (288): 2379-2381.
7. Kuhajda FP. Fatty acid synthase and human cancer: new perspectives on its role in tumor biology. *Nutrition,* 2000, 16(3): 202-208.
8. Maier T, Jenni S, Ban N. Architecture of mammalian fatty acid synthase at 4.5 A resolution. *Science*, 2006; 311:1258-1562.
9. Jenni M, Leibungut, Maier T, Ban N. Architecture of mammalian fatty acid synthase at 5 A resolution. *Science*, 2006; 311:1263-1567.
10. Li Xianghong, Hou Yongsheng. Fatty acid synthase-specific tumor treatment target. *Acta Biochem Biophysic*, 2003, 35(1): 1-5.
11. Zhou Changhai, Keiichi Tanaka. Studies on the activity of fatty acid synthetase and synthesis pathway of fatty acids in meat-duck liver. *Academic Journal of Northeast China Normal University, Natural Science Edition,* 2004, 36 (3): 79-84.
12. Tian WX, Hsu RY, Wang YS. Studies on the reactivity of the essential sulfhydryl groups as a conformational probe for the fatty acid synthetase of chicken liver. *J Biol Chem*. 1985, 260(20): 11375-11387.
13. Li Lichun, Wu Xiaodong, Tian Weixi. The inhibitory effect of radix polygori multiflori extract on fatty acid synthetase. *Chinese Journal of Biochemistry & Molecular Biology,* 2003, 19 (3): 297-304.
14. Zhang Yuanying, Zhang Fengzhen et al. The inhibitory effect of radix polygori multiflori extract on fatty acid synthetase in human breast cancer cells. *Academic Journal of Qiqihar Medical College,* 2004. 25 (10): 1102-1104.

15. Zhang Yuanying, Zhang Fengzhen et al. The inhibitory effect of folium ginkgo extract on fatty acid synthetase in human MCF7 breast cancer cells. *Academic Journal of Taishan Medical College*，2004, 25(5): 433-435.
16. Brusselmans K, De Schrijver E, Heyns W, Verhoeven G, Swinnen JV. Epigallocatechin-3-gallate is a potent natural inhibitor of fatty acid synthase in intact cells and selectively induces apoptosis in prostate cancer cells. *Int J Cancer,* 2003, 106: 856-862.

☑