ADVANCES IN PHARMACOLOGICAL STUDIES OF SILYMARIN

RUI Yao-Cheng

College of Pharmacy, Second Military Medical University, Shanghai 200433, China

Silymarin is the flavonoids extracted from the seeds of Silybum marianum *(L) Gearth as a mixture of three structural isomers: silybin, silydianin and silychristin, the former being the most active component. Silymarin protects liver cell membrane against hepatotoxic agents and improves liver function in experimental animals and humans. It is generally accepted that silymarin exerts a membrane-stabilizing action preventing or inhibiting membrane peroxidation. The experiments with soybean lipoxygenase showed that the three components of silymarin brought about a concentration-dependent non-competitive inhibition of the lipoxygenase. The experiments also showed an analogous interaction with animal lipoxygenase, thus showing that an inhibition of the peroxidation of the fatty acid in vivo was self-evident. Silybin almost completely suppressed the formation of PG at the highest concentration (0.3 mM) and proved to be an inhibitor of PG synthesis in vitro. In our experiments, silybin at lower dose (65 mg/kg) decreased liver lipoperoxide content and microsomal lipoperoxidation to 84.6% and 68.55% of those of the scalded control rats respectively, and prevented the decrease of liver microsomal cytochrome p-450 content and p-nitroanisole-0-demethylase activity 24 h post-scalding. Effects of silymarin on cardiovascular system have been studied in this university since 1980. P. O silymarin 800 mg/kg/d or silybin 600 mg/kg/d reduced plasma total cholesterol, LDL-C and VLDL-C. They however, enhanced HDL-C in hyperlipemic rats. Further studies showed that silymarin enhanced HDL-C but didn't affect HDL-C, a property of this component which is beneficial to treatment of atherosclerosis. The results showed silymarin 80 mg or silybin 60 mg decreased in vitro platelet aggregation (%) in rats. The maximal platelet aggregation induced by ADP declined significantly, and time to reach maximal platelet aggregation and five-minute disaggregation didn't change. In our experiments, iv silybin 22,4 mg/kg lowered the amplitude and duration of diastolic blood pressure (DBP) more than those of systolic (SBP), but the descending aortic blood flow, cardiac contractility and ECG did not change significantly in anesthetized open-chest cats. The results indicated a reduction of peripheral resistance and dilatatory action on the resistant blood vessels. These effects are beneficial to coronary heart disease. We also observed the effects of silybin on morphological change, the release of glutamic oxaloacetate aminotransferase (GOT) and lactate dehydrogenase (LDH) as well as the radioactivity of ³H-TdR incorporated into DNA in normal cardiac cells and cells infected by coxsackie B₅, virus of newborn rats. The results showed that silybin did not affect the morphology of normal cell, and that the pathological change of cells infected by virus was delayed and reduced as compared to control. We have investigated the effect of silybin on synthesis and release of LTs in the cultured porcine cerebral basilar arteries (PCBA). Silybin 100 and 500 μmol/L declined the amounts of LTs released from the PCBA incubsated in the presence of A 23187, AA and indomenthacin. The result suggests that silybin can inhibit the activity of 5-lipoxygenase of cerebral blood vessel and may protect the brain from ischemia.*

Key words: silymarin − hepatoprotective actions − platelet aggregation − cultured cells − 5-lipoxygenase

Silymarin is a flavonoids extracted from the seeds of Silybum marianum (L) Gearth as a mixture of three structural isomers: silybin, silydianin and silychristin (Fig. 1), the former being the most active component. Silymarin protects liver cell membrane against hepatotoxic agents and improves liver function in experimental animals and humans (Wagner et al., 1986). Recently, studies on its mechanism of liver protection and its cardiovascular actions as well as its metabolism of arachidonic acid (AA) have aroused great interests in this university.

HEPATOPROTECTIVE ACTIONS AND MECHANISM

It is generally accepted that silymarin exerts a membrane-stabilizing action by preventing or inhibiting membrane peroxidation. Fiebrich
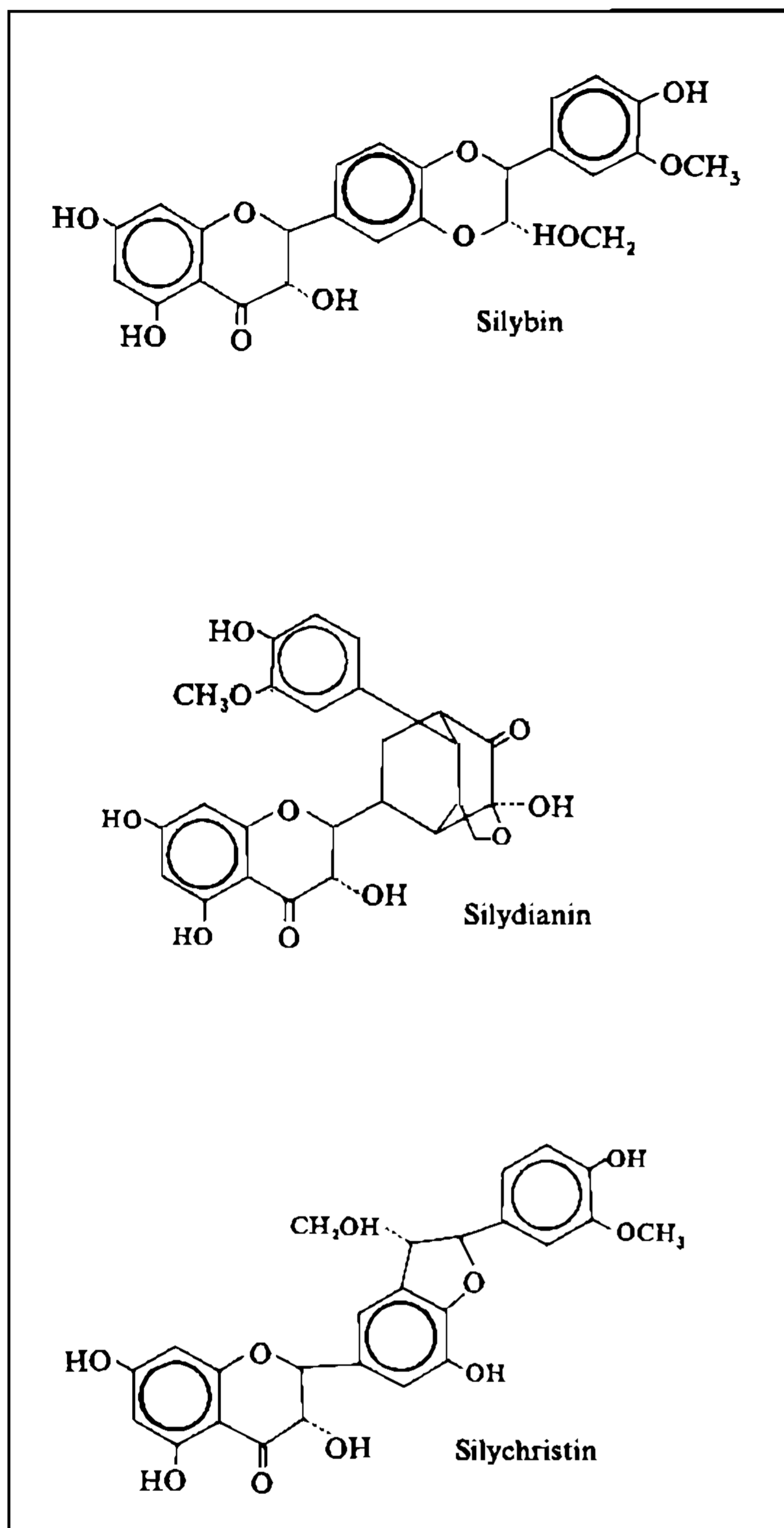
Fig. 1: the structure of silybin, silydianin and sily-christin.

& Kock (1979a) suggested that the effect of silymarin could be due to an inhibition of enzymatic lipoid peroxidation. The experiments were carried out with soybean lipoxygenase and linoleic acid as substrate. The oxidation of linoleic acid in the presence of lipoxygenase and molecular oxygen follows Michaelis-Menten kinetics, the results showed that the three components of silymarin produced a concentration-dependent non-competitive inhibition of the lipoxygenase. The experiments also showed an analogous interaction with animal lipoxygenase, thus showing that an inhibition of the peroxidation of the fatty acid in vivo was evident. Lipoid peroxidation, which is increased by certain hepatic toxins until clinical- pathological symptoms occur, is closely linked to the

formation of prostaglandins (PGs). The primary substrate for the formation of PGs is formed by the polyunsaturated C20 fatty acids, especially arachidonic acid (AA), which are released from the membranes by lipolysis. The effect of AA on lipoid peroxidation and PGs formation in individual organs depends directly on dosage. On the other hand, the inflammatory processes that can occur in tissue and organs as a result of this and similar pathogenic phenomena are linked to the formation of PG. Various liver diseases such as hepatities are also accompanied by inflammatory processes.

Fiebrich & Koch (1979b) also showed that prostaglandin (PG) synthetase from the fundus or rat stomach converted about 30% of AA to defined PG and this formation of PG was significantly inhibited by the three components of silymarin, depending on their concentration. Silybin almost completely suppressed the formation of PG at the highest concentration (0.3 mM). In the case of the partial destruction of membrane structures in the liver disease, increased quantities of C20 fatty acids are released by liposis, which leads to the increased activity of PG synthetase. The suppression of the decomposition of membrane lipoids together with the inhibition of PG formation could provide a plausible explanation for the hepatoprotective functions. Silymarin can also inhibit the hemolysis and lipid peroxidation induced by phenylhydrazine on rat erythrocytes (Valenzuela Guerra, 1985). This effect is ascribed to its antioxidant properties as a free radical scavenger.

We tested the effects of silybin on liver weight, hepatic peroxisome, catalase and carnitine acetyl transferase (CAT) in rats. The results showed that silybin 600 mg/kg/d did not cause hepatomegaly, hepatic peroxisome proliferation and increase of catalase and CAT activities (Wu et al., 1986) (Table I).

In another experiment of ours (Xie & Liao, 1989), silybin at lower dose (65 mg/kg) decreased liver lipoperoxide content and microsomal lipoperoxidation to 84.67% and 68.55% of those of the scalded control rats respectively, and prevented the decrease of liver microsomal cytochrome P-450 content and p-nitroanisole-o-demethylase (PNOD) activity 24 h post-scalding (Table II). Table also shows the effect of 130 mg/kg, but no comments are made. The results prove that silybin can protect liver

TABLE I

Liver weight, catalase and carnitine acetyltransferase activities after-4-wk treatment
$\bar{x} \pm SD$

Drugs (daily dose)	Rats	liver weight (% body WT)	catalase	CAT
			(units/g liver)	
Saline control (5 ml/kg)	9	4.47 ± 0.49	7511 ± 693	153 ± 35
Silybin (600 mg/kg)	8	4.58 ± 0.23^a	7402 ± 817^a	189 ± 69^a
Clofibrate (500 mg/kg)	8	7.86 ± 0.53^b	12768 ± 1611^b	2875 ± 574^b

PFS > 0.05^a; *P* < 0.01 compared to saline control^b.

TABLE II

Effects of silybin on HLPO, MLPO, cyt P-450 and PNOD 24 h postscalding in rats ($\bar{x} \pm SD$).
Number of experiments are given in brackets

	HLPO	MLPO	cyt p-450	PNOD
control	1.723 ± 0.065 (5)	0.337 ± 0.069 (6)	0.169 ± 0.044 (6)	0.143 ± 0.049 (6)
silybin 65 mg/kg	1.49 ± 0.187^a (6)	0.231 ± 0.053 (6)	0.246 ± 0.022^b (6)	0.236 ± 0.028^b (6)
130 mg/kg	1.414 ± 0.218^a (6)	0.177 ± 0.060^b (6)	0.275 ± 0.085^a (6)	0.247 ± 0.056^b (6)

HLPO: nmol MDA/mg protein; MLPO: nmol MDA/mg protein/min; cyt P-450: nmol/mg protein; PNOD: nmol/ mg protein/min; a: P < 0.05; b: P < 0.01.

damage in scalded rats, in not only inhibits liver lipoperoxidation, but also protects liver microsomal drug-metabolizing system from injury after scalding.

EFFECTS ON CARDIOVASCULAR SYSTEM

Effects of silymarin on cardiovascular system have been studied in this university since 1980 and these tests are described as follows.

Effects of silymarin on serum lipoprotein-cholesterol in hyperlipemic rats — In Mai & Chou's (1987) experiments, Male wistar rats weighing 100 ± SD 10 g were divided into 4 groups according to plasma lipemic level, the hyperlipemic (feeding of diet containing 1% cholesterol, 5% porcine oil and 0.2% bile salt), hyperlipemic + silymarin (p.o 800 mg/kg), hyperlipemic + silybin (PO 600 mg/kg) and control (normal diet) group. The total choles-

terol (TC), low-density lipoproteins cholesterol (LDL-c), high-density lipoproteins cholesterol (HDL-c) and very low density lipoproteins cholesterol (VLDL-C) were estimated at the 10th and 20th day after dosing. HDL and HDL + LDL were isolated and estimated by Noma's method and cholesterol by enzymatic method.

The results showed that silymarin 800 mg/kg PO at the 10 and 20th day after dosing reduced plasma TC (from 387.0 + 105.9 to 137.4 ± 52.7 mg/dl at 10th day and 407.2 ± 112.6 to 149.8 ± 37.5 mg/dl at 20th day, *P* < 0.01), LDL-C (from 278.9 ± 90 to 170.7 ± 50.5 and 243.1 ± 75.9 to 88.1 ± 38.5 mg/dl, *P* < 0.01) and VLDL (82.5 ± 23.1 to 28.3 ± 13.2 and 141.2 ± 96.6 to 30.3 ± 12.5 mg/dl, *P* < 0.01), They, however enhanced HDL-C (26.4 ± 6.1 to 38.4 ± 9.2 and 22.9 ± 7.1 to 31.4 ± 8.6 mg/dl, *P* < 0.01). The action of silybin was similar to

that of silymarin. Futher studies showed that silymarin enhanced HDL_2-C but didn't affect HDL_3-C (Table III). A property which is beneficial for the treatment of atherosclesosis.

Effects of silymarin and silybin on platelet function in rats — We used the method of retention *in vitro* in Glass Bead Column to measure platelet adhesiveness and platelet aggregometer with a recorder to measure turbidimetrically platelet aggregation. The results showed that silymarin 80 mg or silybin 60 mg decreased platelet aggregation (%) in rats. The maximal platelet aggregation induced by ADP declined significantly, while the time to reach maximal platelet aggregation and five-minute disaggregation didn't change (Tables IV, V).

TABLE III

Effects of simymarin and silybin on serum HDL_2-C and HDL_3-C in hyperlipemic rats

	n	HDL_2-C (mg/dl)	HDL_3-C (mg/dl)	HDL_2-C/ HDL_3-C
silymarin	11	22.2 ± 5.2^b	18.9 ± 3.1	1.2 ± 0.4^b
silybin	11	23.7 ± 6.9^b	17.2 ± 3.2	1.4 ± 0.3^b
hyperlipemic control	11	12.3 ± 5.1	18.1 ± 2.7	0.7 ± 0.3
normal control	7	25.5 ± 3.6	20.3 ± 3.5	1.3 ± 0.2

a: $P < 0.05$; *b:* $P < 0.01$ compared with hyperlipemic control.

TABLE IV

Effects of silymarin and silybin on platelet function in ratws (%, $\bar{x} \pm$ SD)

	n	Adhesiveness rate	
		before	60 min
silymarin (80 mg/kg)	4	82.4 ± 2.5	69.8 ± 4.9^a
silybin (60 mg/kg)	5	83.2 ± 6.3	63.9 ± 9.1^a
control	4	81.3 ± 9.3	84.7 ± 6.3

a: $P < 0.01$ compared with control.

TABLE V

Effects of silymarin and silybin on platelet aggregation in rats ($\bar{x} \pm$ SD)

Maximal aggregation	Silymarin		Silybin	
	control	80 mg	control	60 mg
MAR (%)	73.7 ± 6.6	49.7 ± 12.7^a	76.7 ± 16.1	24.3 ± 13.1^a
Time to reach MAR (min)	2.6 ± 0.8	2.1 ± 0.9	1.1 ± 0.7	0.7 ± 0.3
FMDR	59.8 ± 30.8	64.3 ± 19.3	63.3 ± 46.1	63.2 ± 37.7

a: $P < 0.01$ compared with control; MAR: maximal aggregation rate; FMDR: five-minute disaggregation rate.

Kock & Loffler (1985) reported that 16 natural phenolics and semisynthetic derivatives, including silymarin, flavonoids, catechines and phenolic acids, together with 6 standard drugs with anti-inflammatory, antioxidant and peroxide radical scavenging properties had been tested in an *in vitro* model using human platelets for their inhibitory action against N-ethyl maleimida-induced lipid peroxidation. The malondialdehyde formed during the reaction was estimated by means of thiobarbituric acid method. In all compounds tested, the inhibitory action was clearly dose-dependent.

Effects of silybin on hemodynamics in anesthetized open-chest cats — In our experiments (Rui et al., 1986), iv silybin 22,44 mg/kg lowered the amplitude and duration of diastolic (DBP) more than those of systolic blood pressure (SBP), but the descending aortic blood flow, cardiac contractility and ECG did not change significantly in anesthetized open-chest cats. The results indicated a reduction of peripheral resistance and dilatatory action on the resistant blood vessels. These effects are beneficial to coronary heart disease. Silybin 88 mg/kg iv produced a marked depression of cardiac contractility, maximal rate of change of intraventricular pressure (dp/dtmax) and Vce at common peak isovolumetric intraventricular pressure (Vce-cpIp). Toxic dose (369 ± 26 mg/kg) of silybin caused an elevation of T wave and a depression of AV conduction. The lethal dose was 484 ± 38 mg/kg, and heart rate decreased significantly only at a dose of 318 ± 53 mg/kg. The results suggested that silybin was safe in clinical uses.

Effects of silybin on cultured cardiac cells of rats infected by coxsackie B_5 virus — Dr Cheng in this university observed the effets of silybin on morphological change, the release of glutamic oxaloacetate aminotransferase (GOT) and lactate dehydrogenase (LDH) as well as the radioactivity of ^{3}H-TdR incorporated into DNA in normal cardiac cells and cells infected by coxsackie B_5 virus of newborn rats (Tables VI, VII). The results showed that silybin did not affect the morphology of normal cells, and that the pathological change of cells infected by virus was delayed and reduced as compared to control. Silybin 1.5 μg/pore reduced significantly the elevated levels of GOT and LDH and increased synthesis of DNA in cells 72-96 h after they were infected by coxsackie B_5 virus, suggesting that silybin may protect cardiac cells from viral damege, thus providing theoretical basis for clinical treatment of viral myocarditis.

Effect of silybin on the activity of 5-lipoxygenase of the porcine cerebral basilar artery — The brain tissue and cerebral blood vessels have abundant lipoxygenase and the ability to synthesize leukotrenes (LTs) from AA by way of the lipoxygenase pathway (Wolfe et al., 1982). During cerebral ischemia, the release of LTs increases. We have investigated the effect of silybin on synthesis and release of LTs in the cultured porcine cerebral basilar arteries (PCBA) (Lin & Rui, 1989). The chopped PCBA were incubated in the modified Tyrode solution with calcium ionophore A23187 10 μmol/L in the presence of AA 30.6 μmol/L and indomethacin 2.8 μmol/L (Piper et al.,

TABLE VI

Effects of silybin on cultured cardiac cells of newborn rats infected by coxsackie B_5 virus. n = 10, $\bar{x}$ ± SD

Group	72 h		96 h	
	LDH	GOT	LDH	GOT
Normal cells	43.4 ± 1.2	< 24	49.3 ± 6.6	< 24
Virus	310.7 ± 6.8	46 ± 3.4	252.7 ± 5.4	41.3 ± 6.1
Cells + silybin	35.3 ± 1.9	< 24	46 ± 2.8	< 24
Cells + solvent	66.7 ± 8.4	< 24	107.3 ± 8.2	< 24
Virus + silybin	80 ± 4.3	< 24	137 ± 1.4	28 ± 2
Virus + solvent	292 ± 8.6	54 ± 10.4	268 ± 9.1	54.7 ± 12.2

a: P > 0.05: cells + silybin, cells + solvent vs normal cells. virus + silybin vs normal.
b: P < 0.05: virus vs normal cells
      virus + silybin vs virus
      virus + silybin vs virus + solvent

TABLE VII

Effects of silybin on ^{3}H-TdR incorporation rate in cultured cardiac cells of newborn rats infected by coxsackie B_5 virus 72 h. $\bar{x}$ ± SD

	Cpm
Cells control	11777.5 ± 137.5
Virus	1285.0 ± 153.5
Cell + silybin	11875.0 ± 162.1
Virus + silybin	12122.2 ± 125.8
Cell + solvent	11762.0 ± 133.4
Virus + solvent	1529.5 ± 233.5

a: P > 0.05; b: P < 0.05 as Table VIII.

graphing with standard LTB$_4$, C$_4$ and D^4 (Fig. 3). The retention times of LTB$_4$, C$_4$ and D$_4$ in our system were 2.2, 3.2, 5.5 min respectively. The eluates of peaks co-chromatographing with LTB, C and D were collected and tested for contractile activity on the guinea pig iliem. Only the substances which had the similar LTC$_4$ and LTD$_4$ retention time exhibited contractile activities. When PCBA was pre-incubated with 100 and 500 μmol/L silybin, the amounts of LTs released were 29 ± 12 and 14 ± 6 pg/100 mg tissue (control: 70 ± 15 pg/ 100 mg tissue), respectively (Table VIII). The result suggest that silybin can inhibit the activity of 5-lipoxygenase of cerebral blood vessel and may protect the brain from ischemia.
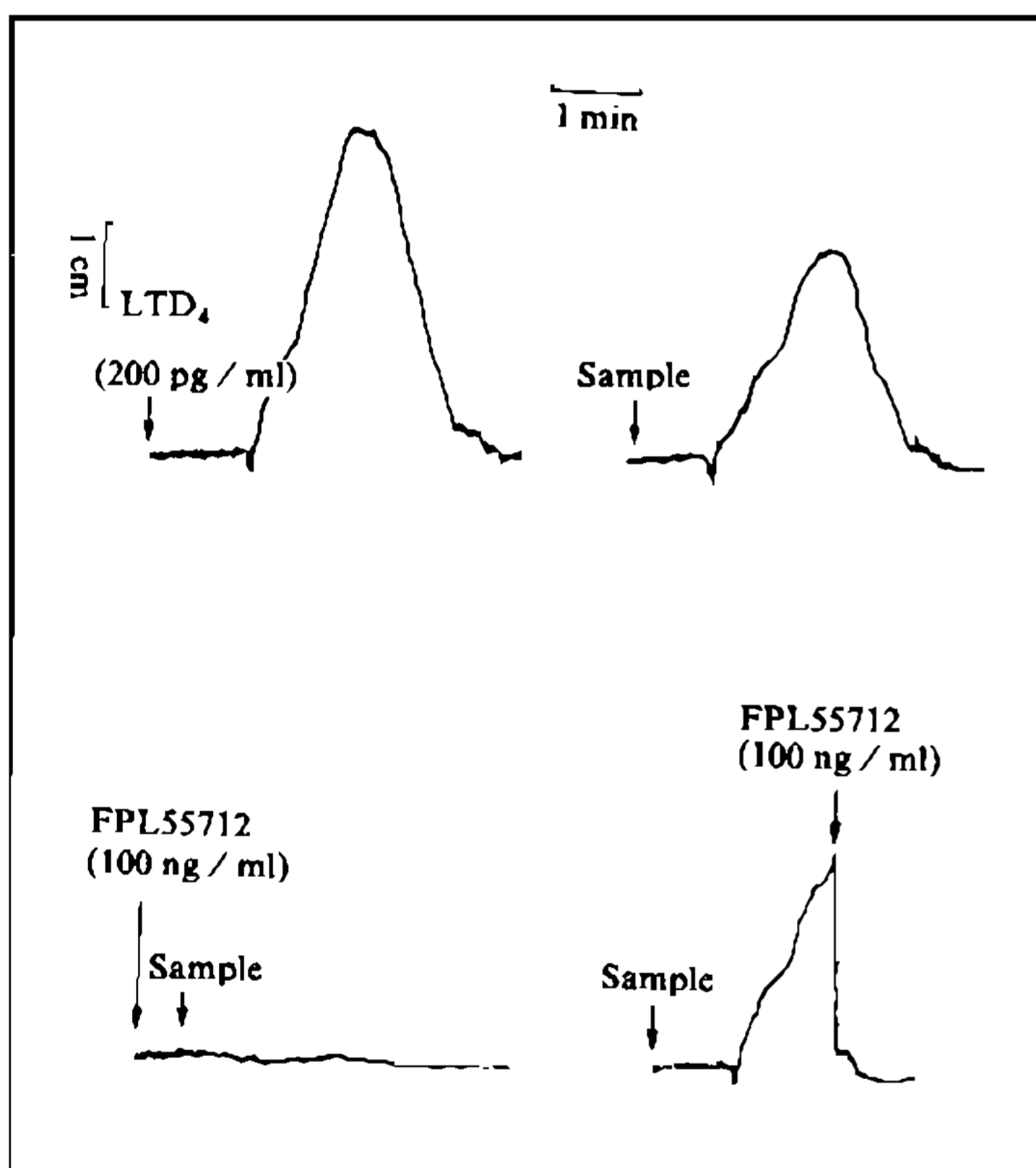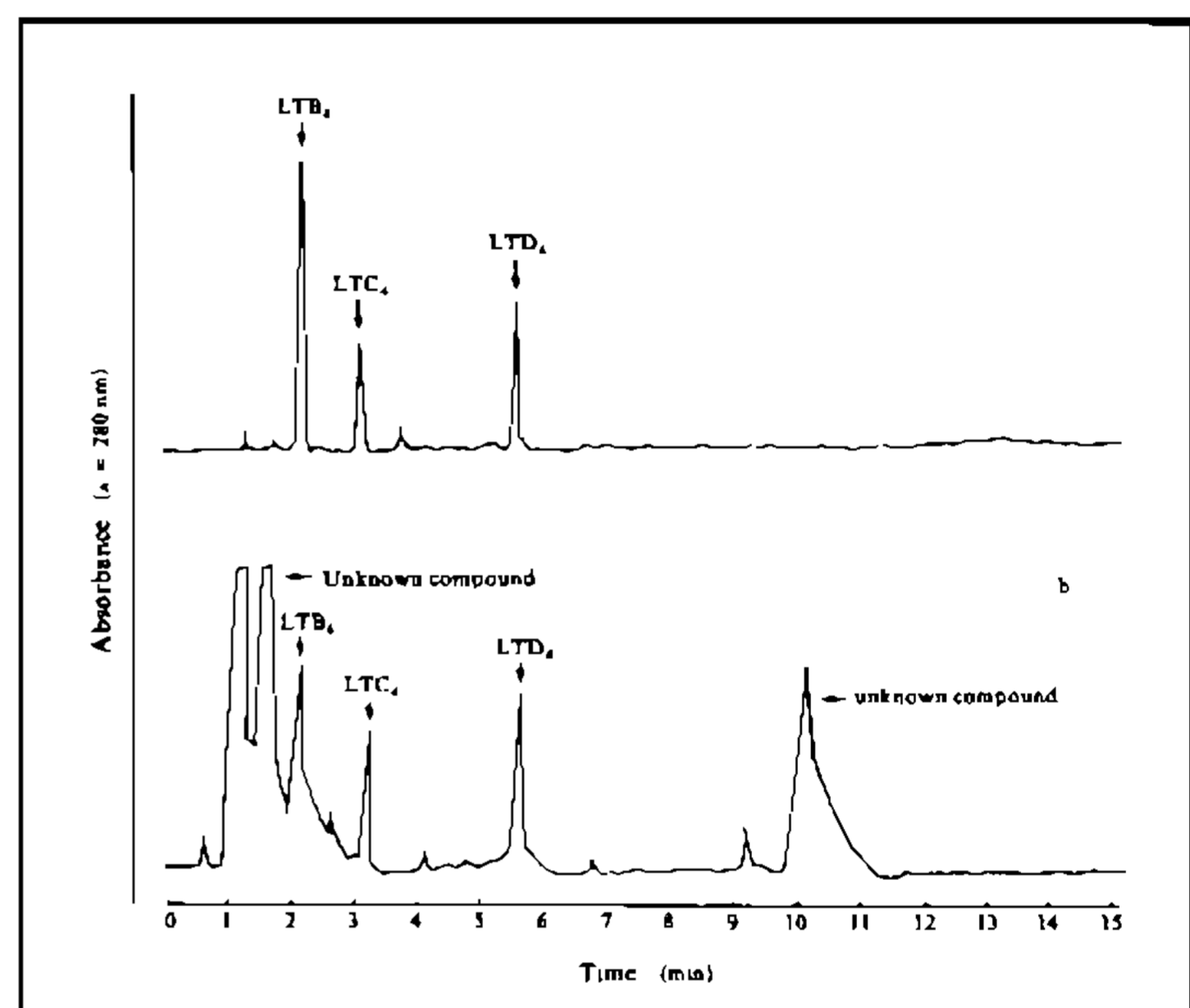

Fig. 2: typical responses of strips of guinea pig ileum to LTD$_4$ and to extract eluted from SEP-PAKs. Reversal by FPL-55712 (100 ng/ml) as indicated. The ileum was preblocked by exposed to atropine 1 μmol/ L, diphenhydramine 1 μmol/L and cyproheptadine 3 nmol/L.

Fig. 3: reversed-phase HPLC of the standard leukotrienes B$_4$, C$_4$, D$_4$ (a) and the sample (b) using a gradient mobile phase. The gradient between 0.0008% (0.1 mmol/L) trifluoroacetic acid (TFA) and 0.02% (2.5 mmol/L) TFA over 20 min (70% acetonitrile in water) was used to separate leukotrienes B$_4$, C$_4$, and D$_4$. The flow rate was 2 ml/min.

TABLE VIII

Inhibitory effects of quercetin and silybin on the activity of 5-lipoxygenase of the isolated porcine cerebral basilar arteries. n = number of experiments, $\bar{x}$ ± SD

Drug (μmol/l)	n	Release of leukotrienes (pg/100 mg tissue)		
		Quercetin	n	silybin
Control	7	70 ± 19	6	70 ± 15
20	5	14 ± 17^b	6	65 ± 20^a
100	5	8 ± 18^c	6	27 ± 12^c
500	6	0^c	6	14 ± 6^c

P < 0.05^b; P < 0.05^b; P < 0.01^c.

1983). The culture was extracted and purified with a SEP-PAK column. The bioassay of the extract was made on the isolated guinea pig ileum and the contraction produced by the extract on the ileum showed the same characteristic as LTD$_4$, and was blocked by the specific LTs antagonist FPL55712 100 ng/ml when given either before or after the incubation with the sample (Fig. 2). Reversed phase high pressure liquid chromatography analysis indicated the presence of peaks co-chromato-

REFERENCES

FIEBRICH, F. & KOCH, H., 1979. Silymarin, an inhibitor of lipoxygenase. *Experientia., 35:* 1548-1550.

FIEBRICH, F. & KOCH, H., 1979. Silymarin, an inhibitor of prostaglandin synthetase. *ibid., 35:* 1550-1552.

KOCH, H. P. & LOFFLER, E., 1985. Influence of silymarin and some flavonoids on lipid peroxidation in human platelets. *Methods Find. Exp. Clin. Pharmacol., 7:* 13-18.

LIN, A. Y. & RUI, Y. C., 1989. Inhibitory effect of silybin on the activity of 5-lipoxygenase of the porcine cerebral basiler artary. *Acta Pharmacol. Sin., 10:* 414-418.

MAI, K. & CHOU, S. J., 1987. The effect of silymarin on serum lipoprotein in hyperlipemic rats. *Chin. J. Cardiol., 15:* 49-51.

PIPER, P. J.; LETTS, L. G. & GALTON, S. A., 1983. Generation of a leukotriene-like substance from porcine vascular and other tissues. *Prostaglandins, 25:* 591-599.

RUI, Y. C.; CHEN, X. S.; GUAN, A. H. & HAN, J. S., 1986. Effects of silybin on hemodynamics in anesthetized open-chest cats. *Acta Pharmacol. Sin., 7:* 34-36.

VALENZUELA, A. & GUERRA, R., 1985. Protective effect of the flavonoid silybin dihemisuccinate on the toxicity of phenylhydrazine on rat liver. *FEBS-Lett, 181:* 291-294.

WAGNER, V. H.; HORHAMMER, L. & MUNSTER, R., 1986. Zur chemie des silymarins (silybin), des wirkprinzips der fruchte von silybun marianum(L) Gaertn. (carduus marianus L.) *Arzneim Forsch., 18:* 688-696.

WOLFE, L. S., 1982. Eicosanoids: Prostaglandins, thromboxanes, leukotrienes, and other derivatives of carbon-20 unsaturated fatty acids. *J. Neurochem., 38:* 1.

WU, S. G.; SHEN, Y. A.; ZHENG, Z. & LONG, K., 1986. Effects of clofibrate, silybin, safflower oil and lamindran on liver peroxisome proliferation and enzyme activities in rats. *Acta Pharmacol. Sin., 7:* 283-284.

XIE, J. E. & LIAO, X. L., 1989. Changes of liver microsomal drug-metabolizing system and lipoperoxidation activity in scalded rats and the effects of silybin. *J. Med. Colleges PLA, 4* (in press).