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BIOACTIVITY OF NEOLIGNANS FROM FRUCTUS SCHIZANDRAE

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Fructus Schisandrae sinensis *Baill, a traditional Chinese medicine, used as tonic and sedative, has been shown at the beginning of 70's to lower the elevated serum glutamic-pyruvic transaminase (SGPT) levels of patients suffering from chronic viral hepatitis. During past 20 years, a series of neolignans have been isolated and identified as effective principles. Pharmacological studies revealed that they increased liver protein and glycogen synthesis, antagonized liver injuries from CCl_4 and thioacetamide. The mechanism of SGPT lowering was considered as a hepato-protective and membrane stabilize action, although inhibition of the activity of liver GPT may also be existed. It was found that some principles of Schisandrae have an inducing effect on hepatic microsomal drug-metabolizing enzyme system P-450, thus explained their anti-toxic, anti-carcinogenic and anti-mutagenic effects. A synthetic derivative compound of Schisandrin called DDB has most of the above mentioned actions now used widely in China as a hepato-protective drug with high effectiveness in normalizing liver functions and very low side effects. From natural Schisandrin to synthesized DDB, pointed out a successful way in the development of new drugs from natural products.*

Key words: *Fructus Schizandrae* – *Schizandraceae* – dibenzocyclooctene neolignans – hepatoprotective agents – DDB

The fruits of *Schizandrae sinensis* Baill., *Schisandrae sphenanthera* Rehd et Wils. and other species belonging to the *Schizandraceae* family were used in traditional Chinese medicine as tonic and sedative for about two thousands years. At the beginning of 70's, clinical investigations reported that *Fructus Schizandrae* are effective for treatment of viral and chemical hepatitis, especially in lowering the elevated serum glutamic-pyruvate transaminase (SGPT) levels. During past 20 years, near 40 compounds were isolated from this kind of herbs, they were identified as derivatives of dibenzo[a,c] cyclooctene, and some of the neolignans were active hepatoprotective principles. Here is a brief review of the pharmacological studies made by Chinese scientists, including their effects on liver injuries and the mechanism of SGPT lowering, effects on hepatic microsomal cytochrome P-450 induction and on anti-oxidation. The investigations lead to discovery of a synthetic derivative of schizandrin called DDB (dimethyl-4,4'-dimethoxy-5,6,5',6',-dimethylenedioxy-biphenyl-2,2'-dicarboxylate) now used widely in China for the treatment of hepatitis.

The chemical structures and nomenclatures are shown in Fig. 1.

EFFECTS ON LIVER INJURIES INDUCED BY CHEMICAL TOXICANTS

Several neolignans isolated from the dried fruit of Schizandraes were shown to be active in lowering the high SGPT level to various extents in CCl_4 and thioacetamide intoxicated mice or rats (Bao, et al., 1979), see Table I.

Table I showed that Sin C, Sol B and Ser B were more potent than other principles. From another report (Shanghai Institue of Materia Medica, 1976), Ser A, the highest yield lignan in *Schisandrae sphenanthera* was also very effective in lowering SGPT. Histological studies revealed that in CCl_4 intoxicated liver, widely necrosis and acid degeneration of liver cells were seen in the central regions of lobules, which were substituted by neutrophilcyte infiltrations. The liver of treated animals were much better. Ultrastructural modifications of hepatic cells of Ser A on acute and chronic CCl_4 intoxication were shown as Fig. 2. The hepatic cellular injuries were protected and this component has no direct toxic action on liver cells.

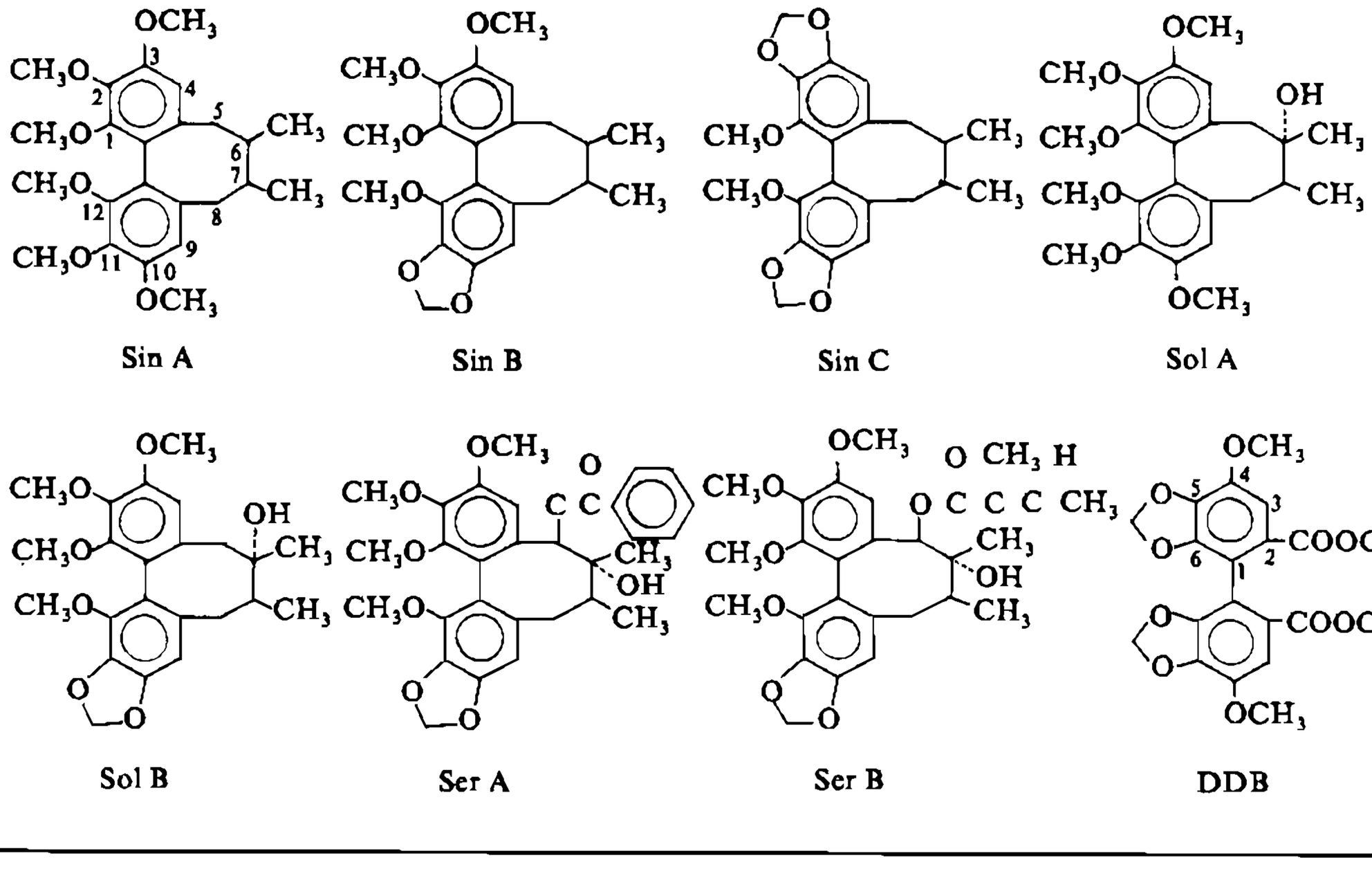

Fig. 1 : the molecular structure of the compounds isolated from *Fructus Schizandrae chinensis* as well as DDB.

TABLE I

Effect of Schizandrae lignans on CCl_4 and thioacetamide (TAA) intoxications of mice SGPT

	Dose mg/kg	Toxicants	SGPT u/100 ml
Control	–		744 ± 100
Sin A	100		797 ± 120
Sin B	100	CCl_4	401 ± 69[a]
Sin C	100	0.01 ml/kg	270 ± 61[b]
Sol A	100	ip	776 ± 127
Sol B	100		200 ± 22[b]
Ser B	100		196 ± 63[b]
Control	–		1112 ± 56
Sin B	50	TAA	1137 ± 36
Sin C	50	100 mg/kg	843 ± 53[a]
Sol B	50	ip	637 ± 90[b]
Ser B	50		438 ± 40[b]

a: $p < 0.05$; *b*: $p < 0.01$ vs Control.

STUDIES ON THE MECHANISM OF SGPT LOWERING

Effects on tissue enzyme activities – Shanghai Institute of Materia Medica (1976) reported that all the components (e.g. Ser A, Ser B, Sol B) which lowering SGPT could also very significantly lower the activity of liver GPT (LGPT) (Table II). Mice po Ser A 200 mg/kg for 15 days, GPT, GOT and LDH activitities of liver, heart and kidney were assayed. The results (Table III) showed that the lowering of LGPT was very strong and more potent than that of heart and kidney GPT. The effects on GOT activities in these tissues were less potent and almost no effect of Ser A on tissues LDH activities were detected. It is concluded that the LGPT lowering effect of Ser A is very selective.

Effects on LGPT protein content – In order to test if Ser A is a LGPT enzyme inhibitor, GPT protein was purified from rat livers. The purified enzyme was identified by SDS-PAGE electrophoresis as a single bond. The enzyme was used together with Freund complete adjavant as antigen for the immunization to rabbits, then anti-GPT serum was obtained. By means of rocket-electrophoresis of antigen-antibody precipitations, it was found that GPT protein content of Ser A treated animals were not decreased, but the difference of LGPT activities between control (302 ± 170 u) and medicated (93 ± 49 u) groups were very significant, thus concluded that Ser A inhibited the activity of liver GPT (Shanghai Inst. Materia Medica, 1976).

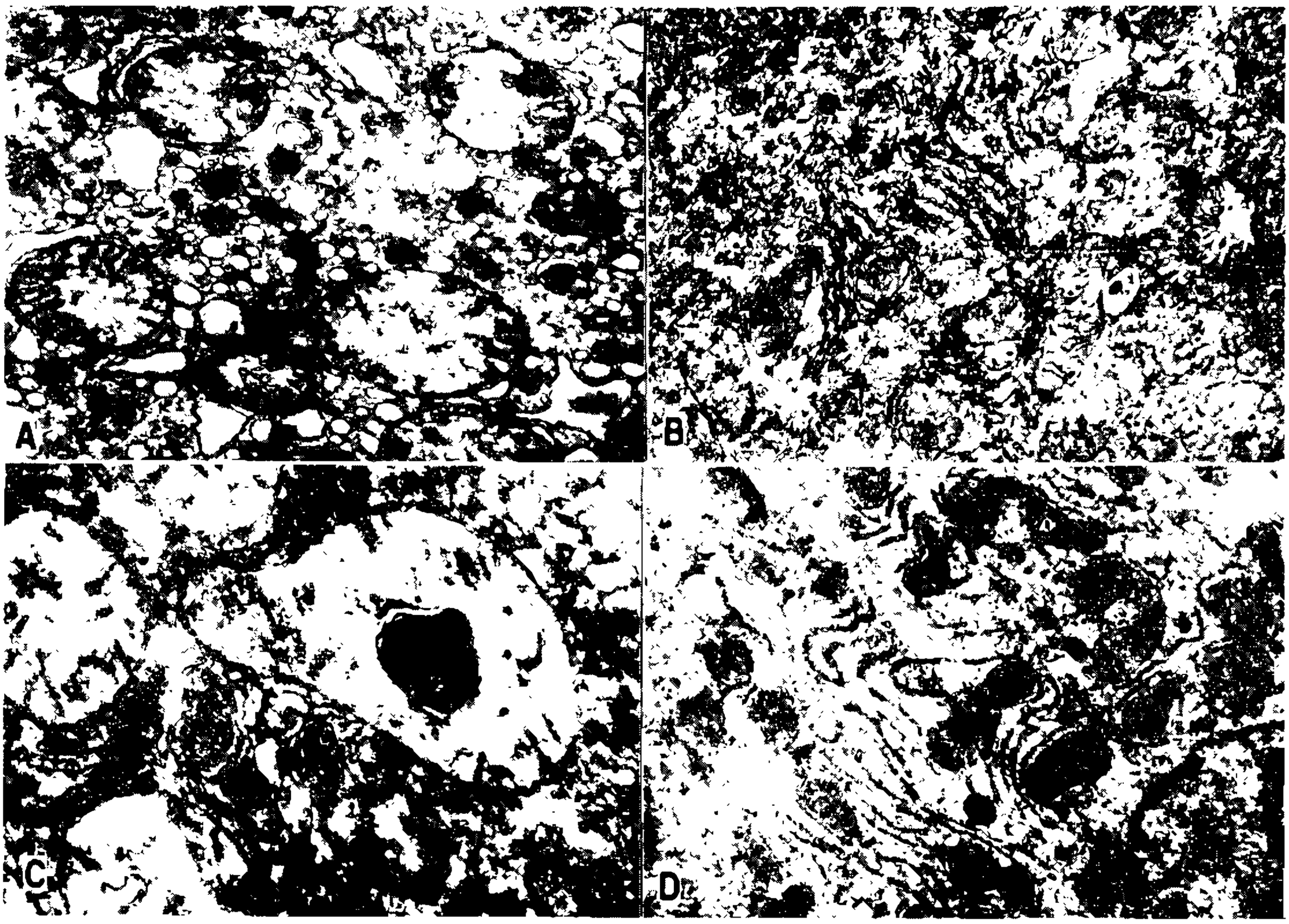

Fig. 2: A – CCl_4 intoxication 19 h after x 16000 – Showing mitochondria swelling, rupture and lysis of inner cristae, dilation and increases of smooth endoplasmic reticulum and formed vesicles. Several small round microbodies and deep electrodensity particles were seen in the plasma. Ribosome granular of rough endoplasmic reticular were decreased. B – CCl_4 acute intoxication with Schizandrer A (Ser A) therapy x 24000 – Showing normal mitochondria. RER and SER were not increased. C – CCl_4 chronic intoxication x 75000 – Mitochondria swelling, cristae rupture and lysis, one of which showing myelinic degeneration. D – CCl_4 chronic intoxication with Ser A therapy x 24000 – Normal mitochondria RER, SER and nuclear.

TABLE II

Effect of Schizandrae lignans on serum and liver GPT activities in CCl_4 intoxicated mice (unit/100 ml or mg)

	Dose mg/kg	SGPT	LGPT
Control	–	343 ± 31	206 ± 5
Sin B	200	202 ± 17[a]	194 ± 6
Sin B	400	177 ± 11[a]	173 ± 6[a]
Sin C	200	201 ± 13[a]	179 ± 6[a]
Sin C	400	196 ± 26[a]	165 ± 3[a]
Sol B	100	208 ± 26[a]	157 ± 7[a]
Ser B	100	228 ± 21[a]	115 ± 6[a]

a: $p < 0.01$ vs Control.

Effect on LGPT protein synthesis – By using of ip ^{14}C-leucine into rats, the radioactivity incorporated into LGPT was 370 ± 80 cpm/mg protein in control group and 490 ± 160 cpm/mg protein in treated group. It is therefore indicated that the synthesis of GPT was not inhibited. The GPT lowering mechanism of Ser A seems to be related to the inhibition of the activity of LGPT, so that after stopping medication, the activity of SGPT soon arised.

TABLE III

Effect of Ser A 200 mg/kg x 15d on tissue enzyme activity (μg pyruvic acid/mg tissue)

		Normal	Ser A
GPT	Liver	188	53
	Heart	11	8
	Kidney	10	8
GOT	Liver	43	33
	Heart	59	47
	Kidney	37	36
LDH	Liver	1.6	1.5
	Heart	1.4	1.5
	Kidney	1.5	1.6

EFFECTS ON LIVER GLYCOGEN CONTENT OF MICE

Glycogenesis was found to be promoted in fasted mice by the administration of Sin A, B, C and Sol B. Among which Sol B was the most effective one with a potency comparable to that of cortisone. Since such an effect can also be shown in adrenalectomized mice, it is resonable to presume that the effect of these agents on glycogenesis is not mediated by the pituitary-adrenal system. No effect on glycogenesis was demonstrated for Ser A, B and Sol A (Table IV).

EFFECT ON PROTEIN AND NUCLEAR ACID SYNTHESIS IN MICE

Liu et al. (1980) reported that Sin B was found to have protective action on liver injury and to increase the weight of liver in mice. In partially hepatectomized mice, Sin B was shown to increase the protein, RNA and DNA contents as well as mitosis of liver cells. In addition, Sin B was found to enhance the incorporation of ^{14}C-phenylalanine into liver protein and to increase hepatic microsomal cytochrome P-450 and protein content significantly (Table V). It is concluded that Sin B is an inducing agent of drug metabolizing enzyme but different from phenobarbital while the later is not able to antagonize CCl_4 intoxication in mice.

INTERACTION WITH LIVER CYTOCHROME P-450

Seven compounds isolated from *Schizandrae Fructus* and DDB were incubated *in vitro* with ÑADPH-reduced microsomes, Sin B, Sin C, Sol B, Ser A and Ser B generated dual Soret peaks at 455-460 nm and 425-430 nm (Fig. 3). All these compounds more or less inhibit liver microsomal hydroxylation of benzopyrene (BP) demethylation of aminopyrine. Sin B, Sol B and DDB decreased mutagenicity of BP in Ames test. It is quite possible that these compounds are able to protect against mutagenicity of some mutagens mediated by liver microsomal activation (Liu & Lesca, 1982a).

ANTI-OXIDANT ACTIVITY

Liu & Lesca (1982b) reported the anti-oxidant properties of dibenzo[a,c]cyclooctene derivatives isolated from *Fructus Schizandrae* as well as DDB. It is shown that the mechanism of protection against CCl_4-hepatotoxicity of these compounds is to inhibit CCl_4-induced lipid peroxidation and $[^{14}C]Cl_4$ covalent binding to lipids of liver microsomes from phenobarbital-treated mice. The compounds also decreased carbon monoxide (CO) production and cofactor (NADPH, oxygen) utilization during CCl_4 metabolization by liver microsomes (Table VI). It may be postulated that the hepatoprotective effect of these compounds is due to their inhibitory effect

TABLE IV

Effect of p.o. Schizandra lignans on liver glycogen

	Liver glycogen (% of control)							
	Sin A	Sin B	Sin C	Sol A	Sol B	Ser A	Ser B	Cortisone
Normal mice	159	150	89	149	192	97	98	225
Adrenalectomized	177	170	114	151	329	83	105	337

TABLE V

Effect of Sin B on liver protein, RNA and microsomal P-450 content in CCl_4 intoxicated mice

	Normal	CCl_4	Sin B + CCl_4
Protein mg/g	10.1 ± 0.1	10.3 ± 0.4	12.7 ± 0.6[a]
RNA mg/g	0.81 ± 0.06	0.88 ± 0.07	0.88 ± 0.04
P-450 nM/mg pr.	0.173 ± 0.012	0.116 ± 0.015[a]	0.268 ± 0.008[b]

a: $p < 0.05$; *b*: $p < 0.01$ vs normal mice.

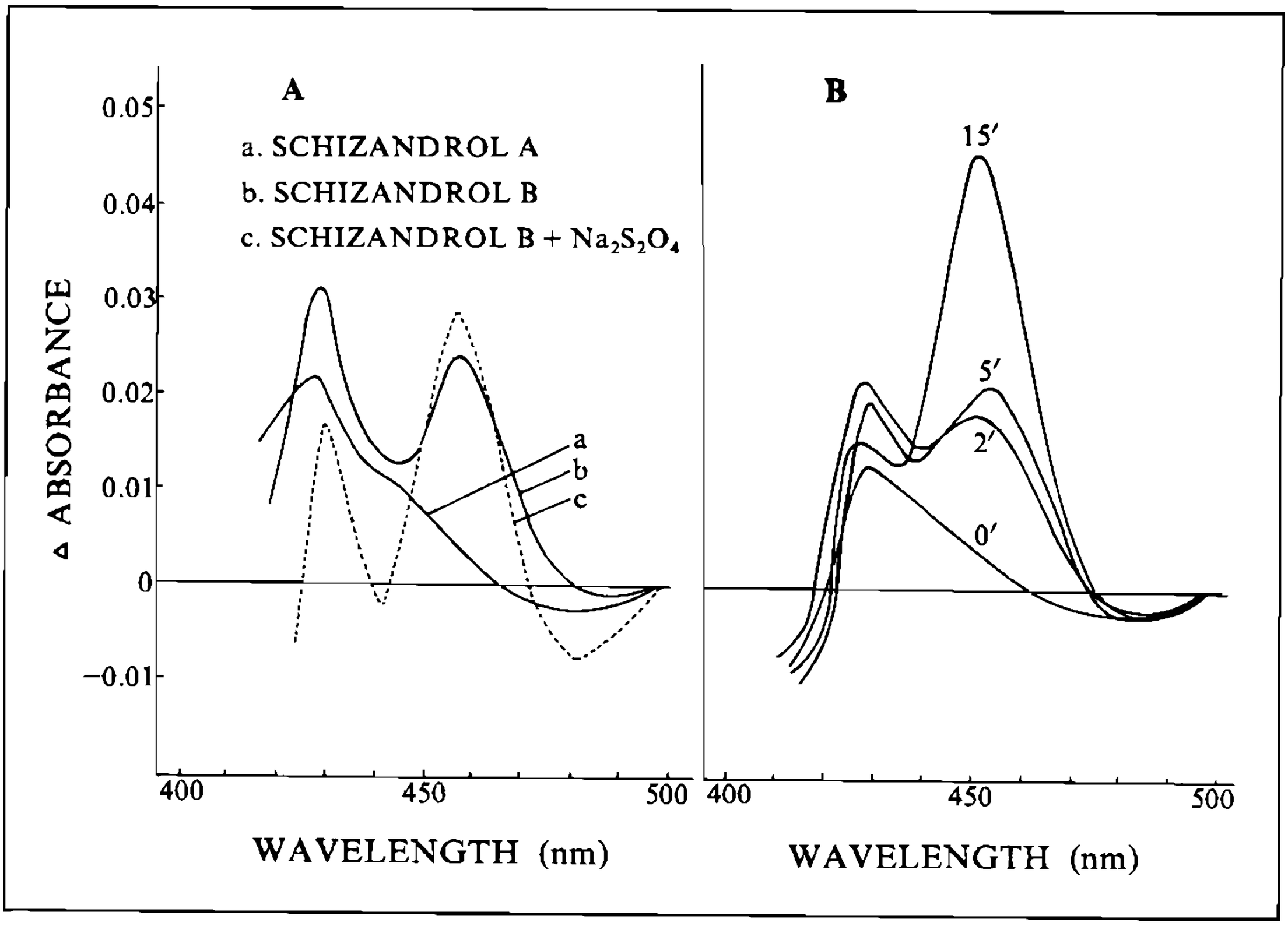

Fig. 3: optical difference spectra formed on incubation of Sol A and Sol B with NADPH-reduced liver microsomes from PB-treated rats. PB-induced liver microsomal suspensions containing 1 mg/ml and cytochrome P-450 1.7 nmol/mg protein; A: solid lines were obtained after addition of Sol A and Sin B to the sample cuvette, respectively; the dotted line was initiated by the addition of $Na_2S_2O_4$ to both cuvettes after 15-min incubation. B: the timecourse of spectral changes after 0-, 2-, 5- and 15-min incubation of Sol B under the same conditions as that in Fig. 3A.

TABLE VI

Summary of the inhibitory effects of schizandrins and DDB on CCl_4-induced MDA formation, lipid binding of $[^{14}C]Cl_4$, CO production, NADPH and oxygen consumption (+) and (−) mean effective and ineffective, respectiviely. The number of plus signs (+) represents the degree of inhibition

Compound	MDA formation	$[^{14}C]Cl_4$ binding to lipids	CO production	NADPH oxidation	Oxygen uptake
Sin A	+ +	−	−	−	−
Sin B	+ + +	+ + +	+ + +	+ +	+ + +
Sin C	+ + +	+ + +	+ + +	+ + +	+ + +
Sol A	+ +	+	−	−	+
Sol B	+	+	+	+	+ +
Ser A	+	+ +	+ +	+ +	+
Ser B	+	+ +	+ +	+ +	+
DDB	+	+ +	+	+ + +	+ +

on CCl_4-induced lipid peroxidation and the binding of CCl_4-metabolites to lipids of liver microsomes.

Moreover, Lu & Liu (1989) recently reported that seven of the nine dibenzocyclooctene lignans at a final concentration of 1 mM were

TABLE VII

Effect of Sin B, Sol B and DDB on enzymes of liver smooth and rough endoplasmic reticula (SER, RER)

	Cytochrome P-450 nmol/mg protein		NADPH-cytochrome C reductase nmol/mg protein		Aminopyrine demethylase HCHO nmol/mg protein	
	SER	RER	SER	RER	SER	RER
2% Tween-80	1.06 ± 0.22	1.25 ± 0.30	29.4 ± 7.4	21.1 ± 4.4	12.1 ± 4.3	8.2 ± 6.5
Sin B	1.58 ± 0.44^a	1.40 ± 0.50	36.6 ± 6.1	23.4 ± 2.5	11.7 ± 1.4	15.9 ± 5.8
Sol B	2.33 ± 0.33^b	1.25 ± 0.31	46.6 ± 3.2^b	24 ± 3.7	25.0 ± 8.9^b	14.4 ± 3.3
DDB	2.58 ± 0.53^b	1.02 ± 0.23	42.7 ± 4.3^b	24.4 ± 5.4	24.6 ± 3.0^b	7.1 ± 1.5

	Protein mg/g liver		Glucose-6-phosphotase activity OD/mg protein	
	SER	RER	SER	RER
2% Tween-80	11.4 ± 1.7	10.7 ± 0.4	0.75 ± 0.08	0.85 ± 0.03
Sin B	9.9 ± 4.2	13.7 ± 3.2	0.82 ± 0.10	0.86 ± 0.08
Sol B	14.4 ± 0.8^b	9.4 ± 0.6	0.95 ± 0.07^b	0.70 ± 0.07
DDB	15.6 ± 1.9^b	10.6 ± 1.4	0.79 ± 0.07	0.83 ± 0.06

a: $p < 0.05$; *b*: $p < 0.01$ vs 2% Tween-80.

shown to inhibit Fe^{+++}/cysteine induced lipid peroxidation (MDA formation) of rat liver microsomes as well as superoxide anion (O_2^-) production in xanthine/xanthine oxidase system to different degrees. The action of these lignans were much more potent than vitamin E at the same concentration. Among them, schisanhenol was the most active one. It is therefore suggested that these schisandrae neolignans are anti-oxidants.

EFFECTS ON ENZYMES OF LIVER SMOOTH AND ROUGH ENDOPLASMIC RETICULA (SER, RER)

Oral administration of Sin B, Sol B (150 mg/kg) or DDB (200 mg/kg) to mice once daily for 3 days induced a significant increase of cytochrome P-450 in SER but not in RER. Sol B and DDB also increased NADPH-cytochrome C reductase and aminopyrine demethylase activities as well as protein concentration in SER. In addition, Sol B markedly enhanced the activity of glucose-6-phosphatase in SER, whereas Sin B was without these actions. The results indicated that Sin B, Sol B and DDB selectively induced different effects on drug metabolism enzymes in SER (Li & Liu, 1987) (see Table VII).

CONCLUSION

From the Chinese traditional medicine *Fructus Schizandrae*, several neolignans were isolated and their chemical structures were identified. Animal studies revealed that some of them are effective in protecting liver injuries from chemical intoxicants and enhanced liver protein and glycogen synthesis. It was also found that some neolignans of *Schizandraceae* can induce liver microsomal P-450, thus inhibited xenobiotic metabolism and decreased the mutagenicity of some chemical mutagens.

Different preparations from *Fructus Schizandrae* have been used widely in China in recent years for the treatment of hepatitis, the total effective rate in lowering the elevated SGPT is over 84%, among them 75% reached normal SGPT levels. It is not only effective in viral hepatitis but also for alcohol and drugs induced hepatic injuries. The synthetic derivate DDB has all the above activities and low toxicity, now producted in several Chinese pharmaceutical factories as the first choice drug in lowering elevated SGPT levels on patients suffering from chronic hepatitis. DDB winned a Brussels Eureka Prize of the World

Fair Invention in 1987. From natural *Schizandraceae* lignans to the discovery of DDB pointed out a success way of developing new drugs from natural products.

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