高氏柴胡的資源開發與藥效評估

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摘　要

柴胡爲『神農本草經』上品，爲『傷寒論』少陽病之主藥，含柴胡的方劑，被廣泛地應用於炎症、肝膽疾患、過敏疾患、代謝性、內分泌及免疫性患。其中在肝炎治療中，柴胡爲最要及使用量最多的生藥。

爲了開發台灣產高氏柴胡的生藥資源，評估其品質與藥效而從事生藥組織學、栽培試驗、活性成分之定性與定量、保肝之藥理學與病理學研究及抗炎症與免疫學的研究。

其結果顯示，高氏柴胡具有良好的保肝效果，其保肝作用優於北柴胡。高氏迆胡根內皮層寬，髓線發達，含有多量之柴胡皂甘，經栽培試驗，發現高氏柴胡的種子發芽率優於三島柴胡，且容易栽培及適應本省環境。其所含 saikosaponin a, d, f 具有大白鼠足蹠浮腫抑制之抗炎症效果對 D- 半乳醣胺引起之肝損傷具有良好的保肝及修復改善效果。活性成分 saikosaponin a, c, d, 含量較三島柴胡及北柴胡，高品質良好。在免疫學方面，高氏柴胡所含柴胡皂甘對 BALB/C 品系小鼠體內免疫學方面，高氏柴胡所含柴胡皂甘對BALB/c品系小鼠體內免疫試驗均具有體液性免疫力活化作用，對細胞性免疫力同時有加強及抑制作用，尤其以 saikosaponin d 及 a 之作用較明顯。而體外培養刺激巨噬細胞產生 IL-1 的能力也顯示各成分具誘導產生能力，證實高氏柴胡具有良好之免疫賦活作用。

柴胡爲『神農本草經』上品，『傷寒論』少陽病之主藥，能解熱、鎭痛、解毒、消炎，主治胸脅苦滿、往來寒熱、黃疸、肝炎、胃腸炎、膽囊炎等。含柴胡之柴胡劑則被廣泛地用於炎症疾患，精神神經疾患、過敏疾患、代謝、免疫及內分泌疾患。尤其最具代表性處方小柴胡湯，常被用治肝膽疾患，增強肝機能，改善肝實質細胞障礙，增強免疫功能。而柴胡爲小柴胡湯之主要組成生藥，爲目前肝炎治療生藥中，最重要及用量最多之生藥。

有關本省市售柴胡之基源，迄今未見證實，爲究明本省市售柴胡之基源，從事了生藥學的研究，經由比較組織學的實驗，證實本省市售柴胡之基源爲：

⑴北柴胡 *Bupleurum chinense* DC.

⑵栽培種三島柴胡 *B. falcatum* L. var. *komarowi* Koso-Polj

⑶野生柴胡 *B. kaoi* Liu, Chao et Chuang

爲了評估台灣野生柴胡—高氏柴胡的品質，確認其藥效，進行了以四氯化碳誘生大白鼠急性肝炎之藥理學及病理學研究。由實驗結果顯示，野生高氏柴胡及栽培種三島柴胡及北柴胡根水抽出物均能抑制大白鼠血清轉胺SGOT、SGPT值之上升，並改善肝小葉壞死，氣球樣變性，炎症細胞 Kupffercells、lymphocytes 增殖性炎症浸潤，脂肪變性等病理現象，其中又以高氏柴胡及栽培種三島柴胡之保肝效果優於北柴胡。進一步以薄層層析法分析了上述三種柴胡，發現高氏柴胡含有多種柴胡皂苷。由於野生高氏柴胡量少及濫採，頻於絕種，從組織學的研究中，又發現高氏柴胡根內部皮層寬廣，髓線發達，木部纖維較少等及含多量柴胡皂苷等品質優良特徵，因此繼續進行了高氏柴胡之栽培研究，經栽培試驗，證實了高氏柴胡種子發芽率優於栽培種三島柴胡，其最佳發芽溫度爲約 18 ℃左右，幼苗移植以具四片葉片爲最佳移植植株，田間栽種以密植優於疏植。栽培結果顯示高氏柴胡極能適應本省環境，根肥大，香味濃郁、根質柔軟、病蟲害較少。而栽培種之高氏柴胡其內部組織構造特徵與野生種高氏柴胡一致。

爲了評價栽培種高氏柴胡之品質及藥效，著者首次利用 TLC Scanner 對不同種柴胡所含活性成分柴胡皂苷 Saikosaponin a,c,d 做定性定量之研究，同時評估調入不同柴胡之小柴胡湯，高氏柴胡及栽培三島柴胡莖葉之柴胡皂甘含量，結果顯示，一年生栽培種高氏柴胡根所含 Saikosaponin a,c,d 含量均爲栽培種三島柴胡根的 2 ～ 3 倍，進口北柴胡根的 10 倍。而高氏柴胡地上部分含有少量柴胡皂苷。又含有高氏柴胡根之小柴胡湯所含柴胡皂苷含量最豐富。另一方面，以四氯化碳誘發大白鼠急性肝損傷的動物模式研究中，由血清轉胺及肝病理損傷之改善，證實了調配高氏柴胡根的小柴胡湯具有最優越的肝臟保護效果，且高氏柴胡地上部份（莖葉）之水抽出物亦具類似的肝保護效果，因此著者首次證實了高氏柴胡含有最豐富的柴

胡皂苷及良好之保肝效果，且全株可供作藥物資源。

爲了究明及評估高氏柴胡的藥效，進行了高氏柴胡之活性成分柴胡皂苷的分離研究，並將分離得到的純化物進行了各項藥理學及免疫學的研究。著者經由系統性柱層層析初步分離得到 14 個化合物，經光譜之分析證實了其中五個化合物爲α -Spinasteryl- β -D-Glucoside(1) ， 23-0-Acetyl-Saikosponin a(2) ， Saikosaponin d(3) ， Saikosaponin a(4) ， Saikosaponin f(5) 。由 Carrageenan 誘發 Wistar品系大白鼠足蹠浮腫的抗發炎動物模式實驗中， Saikosaponin a 及 Saikosaponin d 在每公斤大白鼠體重注射一毫克的劑量時即具有足蹠浮腫抑制效果，而 Saikosaponin f 及高氏柴胡含有之 Crude Saponin(皂苷粗抽提物) 在三毫克及十毫克的劑量下具有明顯的足蹠浮腫抑制效果，顯示出柴胡皂苷的藥效與劑量爲呈現 Dose Denpendency 之作用。

在許多致肝毒性的藥物中， D- 半乳糖胺(D-Galactosamine ， D-GalN) 對大白鼠 (Wistar 品系) 所引起的肝病變與人類病毒性肝炎之病變極爲接近。在經由D-GalN 誘發大白鼠急性肝損傷的動物模式實驗中，對第 24 小時急速升高的血清轉胺酶之值， Saikosaponin a(3mg/kg) 、 Saikosaponin d(3mg/kg) 、 Crude Saponin (3mg/kg) 、 H_2O-layer(10mg/kg) 等藥物組均顯示具有明顯的降 SGOT 與 SGPT 值之作用，而 Saikosaponin f(3mg/kg) 組降 SGOT 與 SGPT 值之作用未很明顯。而肝病理病變的變化，由 D-GalN 所導致的大白鼠肝門脈區域之發炎浸潤，肝小葉散布之局部細胞壞死，玻質樣變性等現象，上述五種藥物組均能有效的予以抑制，顯示出良好至中等程度的修護改善效果。

柴胡與柴胡劑的代表藥方”小柴胡湯”近年來被積極的研究證實具有抗腫瘍及各種免疫促進的效果，在本研究中，著者同時再進行了高氏柴胡所含活性成分柴胡皂苷的 BALB/c 品系小鼠體內免疫作用研究，各藥物組分別爲 Saikosaponin a(3mg/kg) ， Saikosaponin d(3mg/kg) ， Saikosaponin f(3mg/kg) ， Crude Soponin(3mg/kg) ， H_2O-layer(10mg/kg) 。以隔天腹腔注射給藥，每週每組犧牲十隻小鼠的方式連續觀察四週，獲得下列幾項結果：

(1)胸腺與體重比值，各藥物組呈現逐週遞減的效應。

(2) Th/Ts 之比值，在 Saikosaponin f 及 H_2O-layer 組呈現逐週遞增的效果， Saikosaponin a ， Saikosaponin d 及 Crude Saponin 組，其比值分別在第二及第三週達到高峰後遞減。

(3) IL-2 的濃度，各藥物組雖有增加趨勢，惟單一藥物組間每週增減變異較大，無法做常態性統計分析評估，須再進一步與 IL-2-dependent 細胞株做體外研究。

(4)脾臟與體重比值，各藥物組呈現逐週遞增的效應。

⑸刺激 IgM 產生的能力，依次爲 Saikosaponin d(第一週)，Saikosaponin a(第二週)，Saikosaponin f(第二週)，Crude Saponin(第三週)， H_2O-layer(第四週)。

刺激IgA產生的能力，依次爲 Saikosaponin d(第一週)，Saikosoponin f 及 Crude Saponin(均爲第二週)， Saikosaponina 及 H_2O-layer 均爲第三週)。

刺激IgG產生的能力，依次爲 Saikosaponin d(第二週)，Saikosaponin a(第三週)，Saikosaponin f ， Crude Saponin 及 H_2O-layer(均爲第四週)。

⑹各藥物於BALB/c品系小鼠的體內實驗顯示均有體液性免疫力活化作用，在細胞性免疫力同時有加強及抑制作用，尤其以 Saikosaponin d 及 Saikosaponin a 較明顯，其詳細機轉有待進一步研究。

⑺體外培養刺激巨噬細胞產生 IL-1 的能力，各藥物在濃度爲 50 μ g/ml 時，顯示出最佳的誘導產生效果，其中以 Saikosaponin d 的誘導產生能力最強，Saikosaponin a ， Crude Saponin ， 23-O-Acetyl-Saikosaponin a 次之，Saikosaponin f 及 H_2O-layer 誘導產生能力較弱。由以上結果證實高氏柴胡具有良好之免疫賦活作用。

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TABLE 1.

Effects of *Bupleurum chinense*, cultivated *B. falcatum* var. *komarowi* and *B. kaoi* on CCl_4-induced acute hepatic damage in rats.

Liver function test / Groups	Dose (ml/100g)	sGOT (IU/1)	sGPT (IU/1)	Total bilirubin (mg/dl)	Total protein (g/dl)	Total cholesterol (mg/dl)
(A) Normal	0	93.03 ± 5.3*	47.72 ± 3.4*	0.3 ± 0.03	6.2 ± 0.2	55.9 ± 10.5
(B) Olive oil	0.3	98.12 ± 5.3*	44.12 ± 3.2*	0.3 ± 0.03	5.9 ± 0.2	63.6 ± 2.9
(C) CCl_4/olive oil	0.3	369.0 ± 17.0	237.0 ± 10.2	0.4 ± 0.03	5.9 ± 0.2	49.0 ± 12.8
(D) B. chinense	1.0	250.0 ± 13.0**	178.0 ± 22.2***	0.3 ± 0.03	6.1 ± 0.2	64.5 ± 6.5
(E) B. falcatum var. komarowi	1.0	145.0 ± 3.02*	97.0 ± 12.5*	0.4 ± 0.02	5.9 ± 0.3	60.2 ± 5.6
(F) B. kaoi	1.0	170.0 ± 12.8*	124.9 ± 5.4*	0.3 ± 0.02	6.1 ± 0.1	62.2 ± 3.7

Each value represents mean ± S.E. of 6 rats
Statistical analysis was carried out by means of the Student's t-test, and was significantly different from the CCl_4 group.
*$p < 0.001$, **$p < 0.01$, ***$p < 0.05$

TABLE 2.

The pathological change of liver under different drugs group treatment.

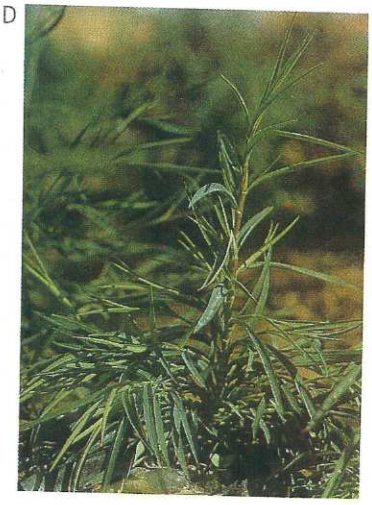
groups / pathological change	CCl_4/olive oil	*B. chinense*	*B. falcatum* var. *komarowi*	*B. kaoi*
lymphocyte increase	+ + + +	+ + +	+ ~ + +	+ ~ + +
kupffer cell increase	+ + + +	+ + +	+ ~ + +	+ ~ + +
cell necrosis	+ + +	+ +	+	+
ballooning degeneration	+ + + +	+ + + +	+	+
fatty change	+ + + +	+ + ~ + + +	+ ~ + +	+ ~ + +
cytoplasm loss	+ + +	+ +	+	+

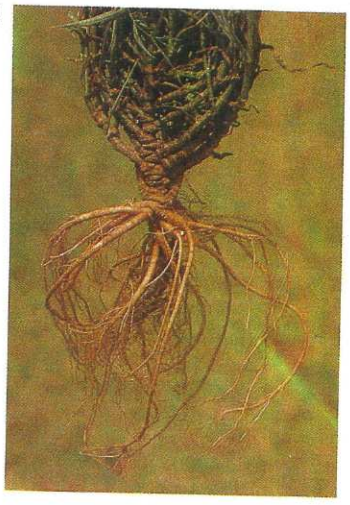
+ + + +: very significant, + + +: significant,
+ +: moderate change, +: slight change.









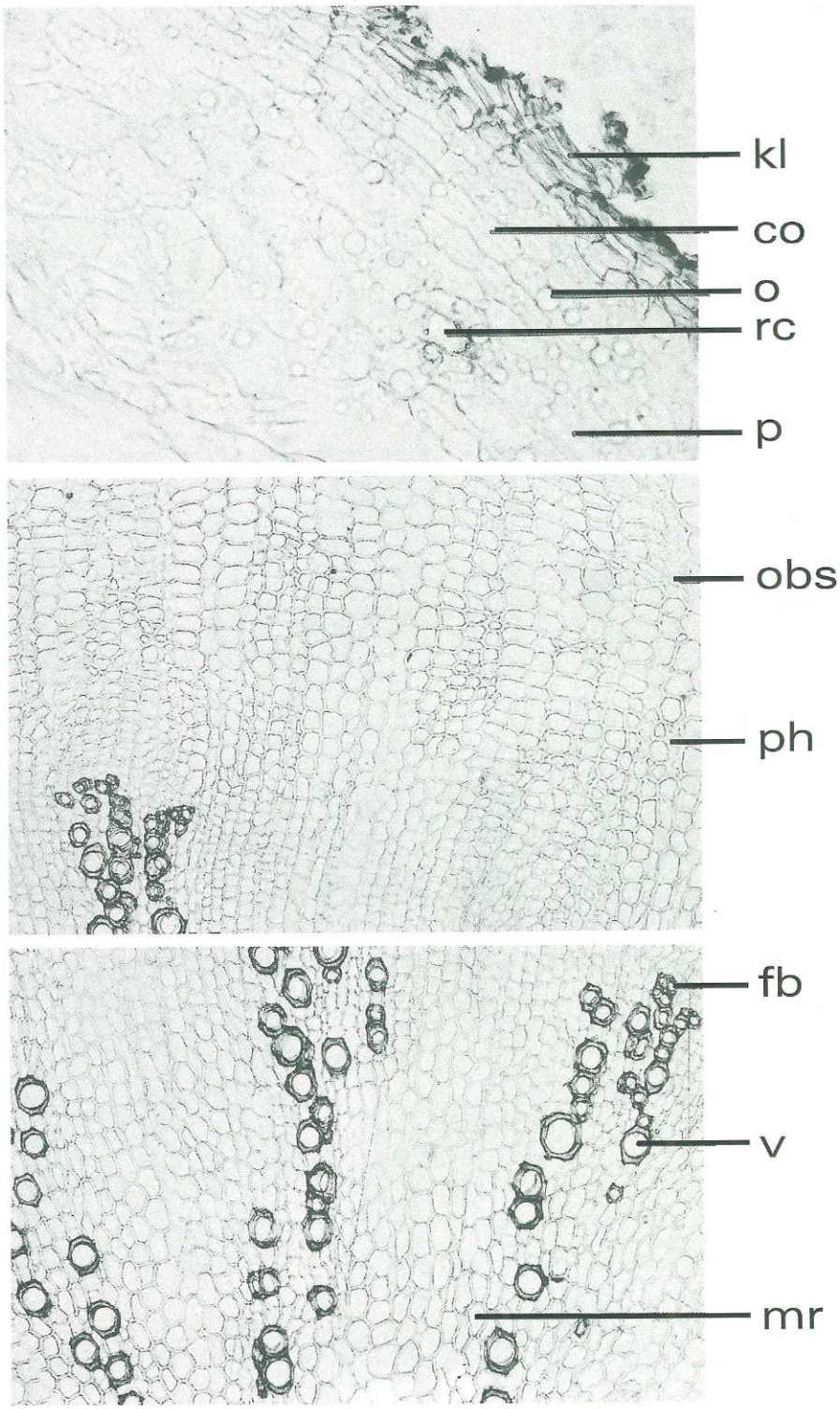




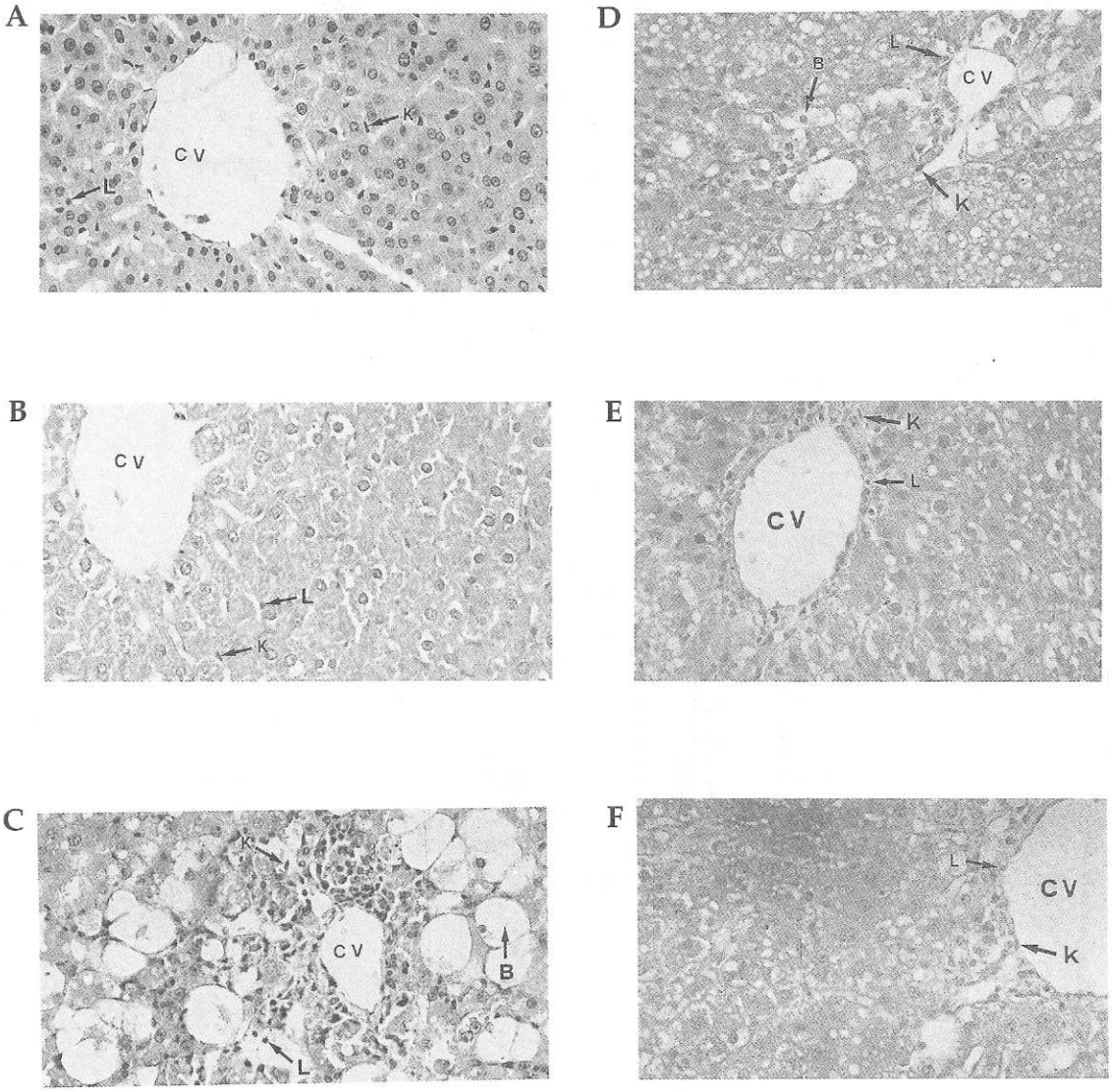


照片一・

A.高氏柴胡（右）與三島柴胡（左）種子發芽情形
B.高氏柴胡幼苗（尚未定植）
C.高氏柴胡幼苗田間定植堆肥
D.高氏柴胡田間定植三個月後之植株
E.高氏柴胡田間定植五個月後根部發育情形
F.栽培近一年的高氏柴胡
G.開花期的栽培種高氏柴胡



照片二
栽培種（八個月）高氏柴胡根內部構造詳圖



照片三、

A.正常組大白鼠肝切片
B.注射橄欖油組大白鼠肝切片
C.注射四氯化碳組大白鼠肝切片
D.注射北柴胡根水抽出物組大白鼠肝切片
E.注射三島柴胡根水抽出物組大白鼠肝切片
F.注射高氏柴胡根水抽出物組大白鼠肝切片

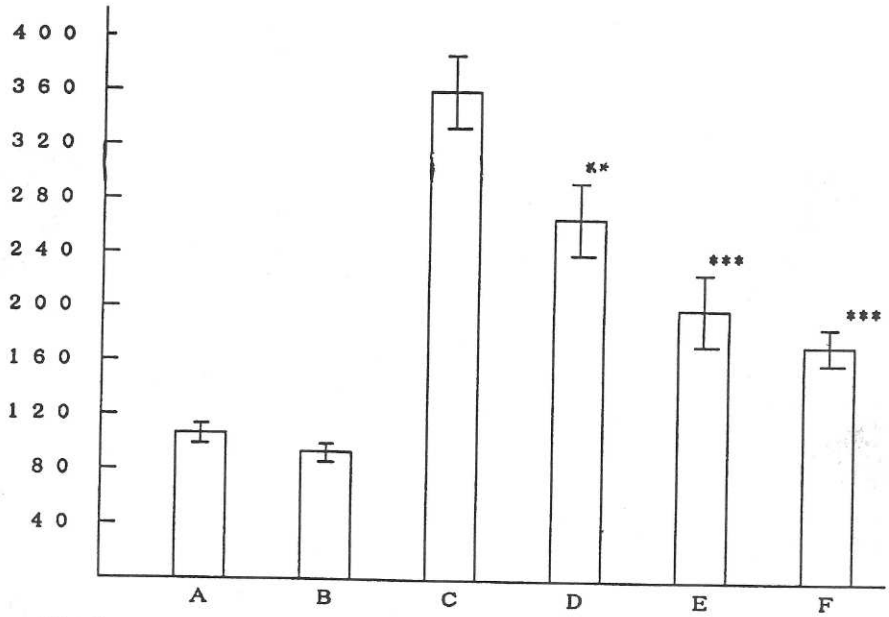


Fig. 1

Hepatic protective effects of XCHT (小柴胡湯) with three different Bupleuri Radix on the CCl_4-induced SGOT level increase. Animal groups of (A) Normal rat group (B) Olive oil group (C) CCl_4 group (D) XCHT with Bupleurum chinense root (E) XCHT with B. falcatum var. komarowi root (F) XCHT with B. kaoi root. Each value represents the mean ± S.D. (N=6). Values significantly different from CCl_4 treated group were evaluated by Student's t-test. (** $P<0.01$; *** $p<0.001$)

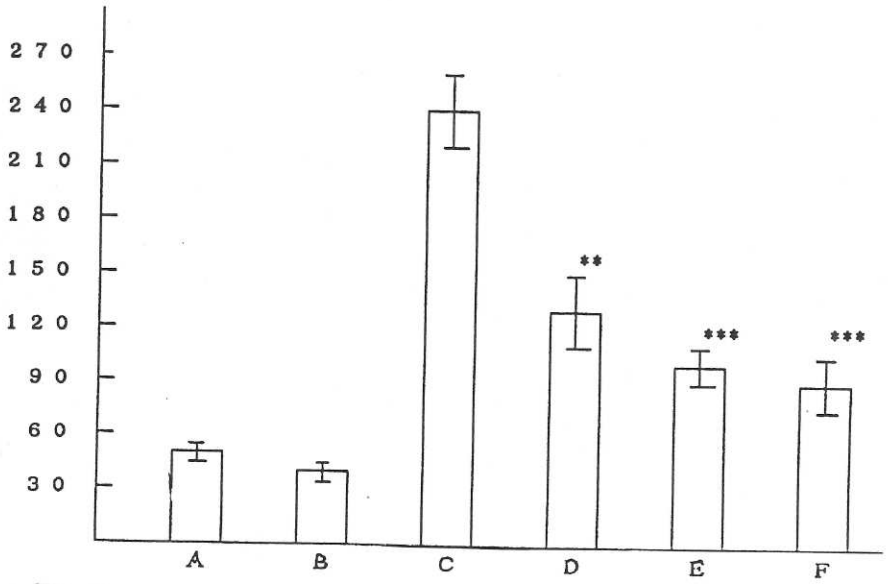


Fig. 2

Hepatic protective effects of XCHT (小柴胡湯) with three different Bupleuri Radix on the CCl_4-induced SGPT level increase. Animal groups of (A) Normal rat group (B) Olive oil group (C) CCl_4 group (D) XCHT with Bupleurum chinense root (E) XCHT with B. falcatum var. komarowi root (F) XCHT with B. kaoi root. Each value represents the mean ± S.D. (N=6). Values significantly different from CCl_4 treated group were evaluated by Student's t-test. (** $P<0.01$; *** $p<0.001$)

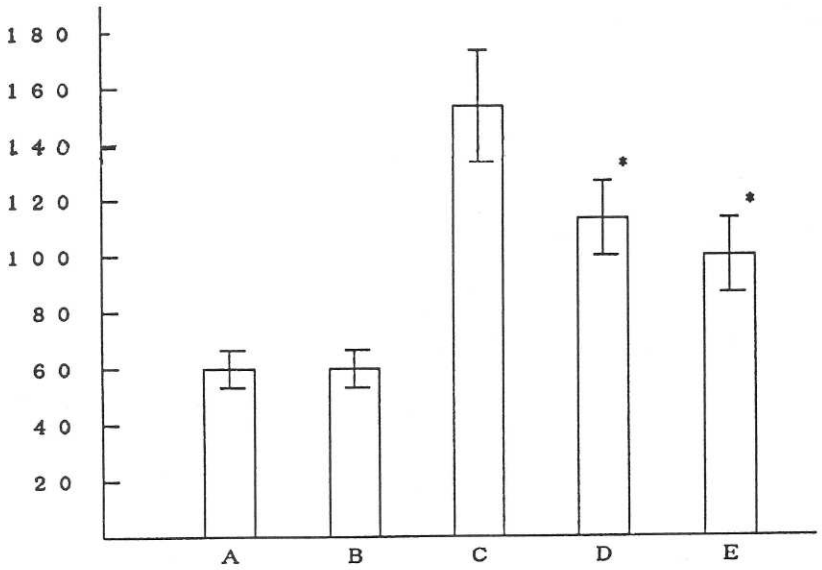


Fig. 3

Hepatic protective effects of Bupleuri aerial parts extracts on the CCl_4-induced SGOT level increase. Animal groups of (A) Normal rat group (B) Olive oil group (C) CCl_4 group (D) B. kaoi group (E) B. falcatum var. komarovi group. Each value represents the mean ± S.D. (N = 6). Values significantly different from CCl_4 treated group were evaluated by Student's t-test. (* $P < 0.05$)

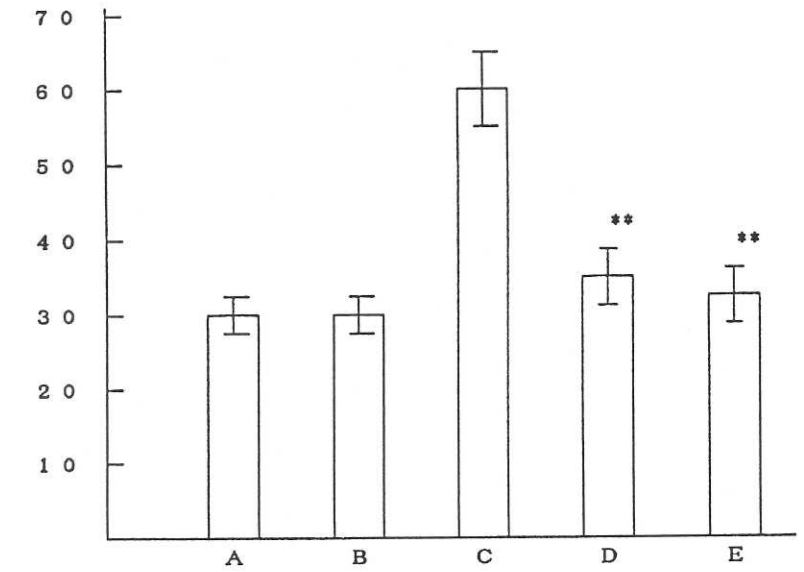


Fig. 4

Hepatic protective effects of Bupleuri aerial parts extracts on the CCl_4-induced SGPT level increase. Animal groups of (A) Normal rat group (B) Olive oil group (C) CCl_4 group (D) B. kaoi group (E) B. falcatum var. komarovi group. Each value represents the mean ± S.D. (N = 6). Values significantly different from CCl_4 treated group were evaluated by Student's t-test. (* $P < 0.05$)

TABLE 3.

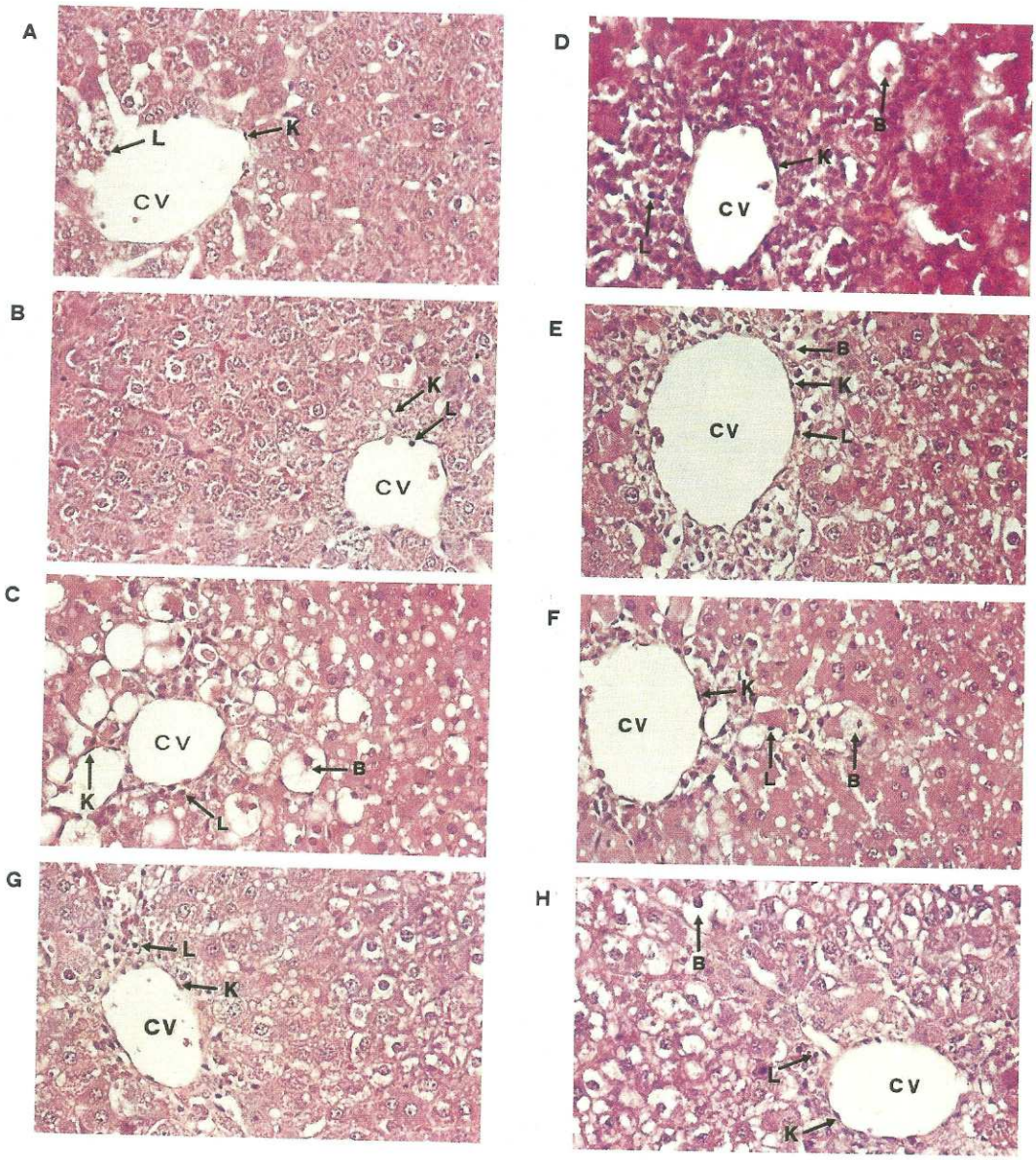
Effects of saikosaponins and the fractions of MeOH ext. from Bupleurum kaoi root on SGOT activities in D-galactosamine treated rats.

Treatment groups	Time after D-galactosamine injection (h)		
	0	12	24
		Serum GOT	
D-Galactosamine	189.8±23.5	749.3±138.3	669.3±84.6
Saikosaponin-a (3mg/kg)	198.0±7.1	556.1±110.7*	362.9±84.0*
Saikosaponin-d (3mg/kg)	189.5±11.3	285.4±71.8**	286.0±75.4**
Saikosaponin-f (3mg/kg)	178.0±18.3	627.9±71.4	498.9±90.2
Crude saponin (3mg/kg)	177.8±6.7	576.2±115.1*	409.3±107.4*
H_2O-layer (10mg/kg)	190.4±11.8	572.7±140*	351.5±93.6*

Effects of saikosaponins and the fractions of MeOH ext. from Bupleurum kaoi root on SGPT activities in D-galactosamine treated rats.

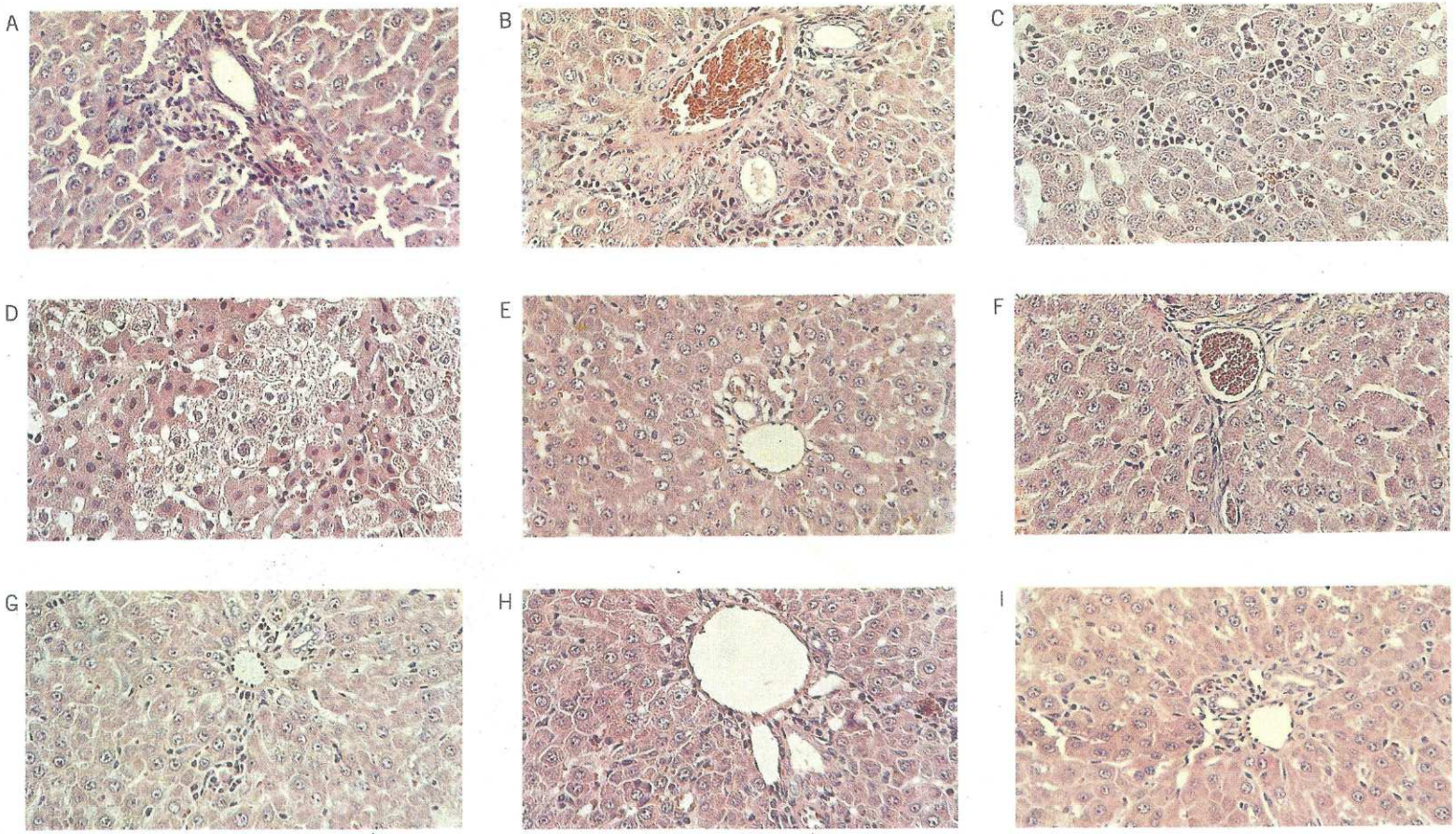
Treatment groups	Time after D-galactosamine injection (h)		
	0	12	24
		Serum GPT	
D-Galactosamine	32.9±4.5	432.7±35.4	298.1±48.7
Saikosaponin-a (3mg/kg)	36.4±4.2	232.3±52.8*	109.5±16.1*
Saikosaponin-d (3mg/kg)	33.4±4.4	133.9±27.9**	130.0±47.0*
Saikosaponin-f (3mg/kg)	34.4±4.3	398.4±125.0	233.2±84.2
Crude saponin (3mg/kg)	41.8±3.4	315.0±72.3*	154.3±42.4*
H_2O-layer (10mg/kg)	34.4±3.9	241.6±85.9*	140.8±52.7*

Rats were i.p. administered drugs, and 2 hr after the administration, D-galactosamine (400 mg/kg) was i.p. injected and blood was obtained at the indicated time. Each value represents the mean±S.E. of 6 animals. Significantly different from D-galactosamine treated control as * $p<0.05$; ** $p<0.01$.



照片四、

A.正常組大白鼠(Wistar Albino Rat)肝病理切片
B.注射橄欖油對照組大白鼠(Wistar Albino Rat)肝病理切片
C.注射四氯化碳控制組大白鼠(Wistar Albino Rat)肝病理切片
D.注射調配北柴胡根之小柴胡湯組大白鼠(Wistar Albino Rat)肝病理切片
E.注射調配三島柴胡根之小柴胡湯組大白鼠(Wistar Albino Rat)肝病理切片
F.注射調配高氏柴胡根之小柴胡湯組大白鼠(Wistar Albino Rat)肝病理切片
G.注射三島柴胡地上部份（莖和葉）水抽出物組大白鼠(Wistar Albino Rat)肝病理切片
H.注射高氏柴胡地上部份（莖和葉）水抽出物組大白鼠(Wistar Albino Rat)肝病理切片



照片五、

A.D－GalN　誘發大白鼠肝門脈週邊炎症性浸潤
B.D－GalN　誘發大白鼠肝門脈週邊炎症性浸潤
C.D－GalN　誘發大白鼠肝小葉局部壞死
D.D－GalN　誘發大白鼠肝小葉玻質樣病變(Hyaline Degeneration)
E.Saikosaponin a (3mg/kg)　組肝臟修復情形
F.Saikosaponin d (3mg /kg)　組肝臟修復情形
G.Saikosaponin f (3mg/kg)　組肝臟修復情形
H.Crude Saponin (3mg/kg)　組肝臟修復情形
I.H_2O—layer (10mg/kg)　組肝臟修復情形

TABLE 4. The mean ratio of thymus weight to its body weight of BALB/c mice from drug treatment

Groups	Time after drug administration (week)			
	一	二	三	四
Control (N/S)	2.92±0.72	2.86±0.68	2.81±0.47	2.82±0.53
Saikosaponin-a (1mg/Kg)	3.08±0.86	2.63±0.41	2.35±0.47	2.54±0.45
Saikosaponin-d (1mg/Kg)	3.53±0.43	2.82±0.55	2.44±0.51	2.34±0.64
Saikosaponin-f (3mg/Kg)	3.52±0.81	2.81±0.84	2.36±0.52	2.32±0.47*
Crude saponin (3mg/Kg)	3.28±0.32	2.88±0.78	2.70±0.72	2.61±0.64
H_2O-layer (10mg/Kg)	3.09±0.65	3.04±0.65	2.71±0.77	2.20±0.68*

Each value represents mean±S.D. of 10 animals.
Statistical values significantly as : *$p<0.05$.

TABLE 5. The mean ratio of spleen weight to its body weight of BALB/c mice from drug treatment

Groups	Time after drug administration (week)			
	一	二	三	四
Control (N/S)	3.57±0.64	3.66±0.59	3.61±0.62	3.70±0.68
Saikosaponin-a (1mg/Kg)	3.64±0.89	4.50±0.34**	4.96±0.68**	5.36±0.74***
Saikosaponin-d (1mg/Kg)	5.14±0.82**	5.35±0.76***	5.80±0.95***	6.28±0.92***
Saikosaponin-f (3mg/Kg)	3.79±0.84	4.18±0.81	4.82±0.65**	5.32±0.88***
Crude saponin (3mg/Kg)	3.86±0.76	4.53±0.98*	5.01±0.67***	5.67±0.70***
H_2O-layer (10mg/Kg)	3.67±0.63	4.08±0.47	4.81±0.51**	5.42±0.67***

Each value represents mean±S.D. of 10 animals.
Statistical values significantly as : *$p<0.05$; **$p<0.01$; ***$p<0.001$.

TABLE 6. T helper／T suppressor (Th/Ts) values of BALB/c mice after treated with drugs

Groups	Time after drug administration (week)			
	一	二	三	四
Control (N/S)	1.54±0.14	1.56±0.16	1.57±0.12	1.57±0.14
Saikosaponin-a (1mg/Kg)	2.48±0.16***	2.99±0.42***	3.14±0.87**	3.07±0.48***
Saikosaponin-d (1mg/Kg)	2.64±0.39**	3.21±0.65**	3.14±0.75**	2.96±0.64**
Saikosaponin-f (3mg/Kg)	2.43±0.46**	2.58±0.46**	2.99±0.49***	3.02±0.64**
Crude saponin (3mg/Kg)	2.46±0.50**	2.81±0.50**	3.03±0.58**	2.98±0.74**
H_2O-layer (10mg/Kg)	2.28±0.16***	2.50±0.59*	2.56±0.35**	2.73±0.19***

Each value represents mean±S.D. of 6 animals.
Statistical values significantly as : *$p<0.05$; **$p<0.01$; ***$p<0.001$.

TABLE 7. The serum IL-2 concentration (U/ml) of BALB/c mice after treated with drugs

Groups	Time after drug administration (week)			
	一	二	三	四
Control (N/S)	5.1±1.3	8.7±1.8	6.2±1.3	6.4±1.5
Saikosaponin-a (1mg/Kg)	9.5±2.1**	14.3±2.3**	24.3±3.8***	9.5±1.7*
Saikosaponin-d (1mg/Kg)	7.3±1.6*	16.0±3.1**	11.3±2.6**	10.2±2.3*
Saikosaponin-f (3mg/Kg)	5.5±1.2	21.8±2.9***	8.3±1.6*	13.3±2.4**
Crude saponin (3mg/Kg)	6.6±1.9	13.1±2.0*	16.7±2.5***	11.7±2.1**
H_2O-layer (10mg/Kg)	8.5±1.9*	11.5±2.2*	13.5±2.6**	11.0±2.1**

Each value represents mean±S.D. of 6 animals.
Statistical values significantly as : *$p<0.05$; **$p<0.01$; ***$p<0.001$.

TABLE 8. The serum IgA concentration (mg/l) of BALB/c mice after treated with drugs

Groups	Time after drug administration (week)			
	一	二	三	四
Control (N/S)	444	ND	ND	ND
	400	ND	ND	ND
	444	ND	ND	ND
	444	ND	ND	
	504	ND	ND	ND
	566	ND	ND	ND
Saikosaponin-a (1mg/Kg)	444	566	1040	966
	693	504	566	1110
	400	444	629	1110
	444	759	896	1460
	1040	566	629	966
	400	629	966	1110
Saikosaponin-d (1mg/Kg)	566	1110	1190	1110
	629	759	896	1670
	896	759	896	1566
	566	1040	693	1566
	693	566	896	966
	693	693	896	566
Saikosaponin-f (3mg/Kg)	444	566	566	1420
	566	566	566	1110
	444	444	444	1110
	444	693	1190	759
	444	566	693	1110
	400	566	896	1110
Crude saponin (3mg/Kg)	566	504	759	896
	400	629	759	1260
	400	896	693	1260
	1190	566	966	1040
	400	1190	1040	1750
	444	896	629	1260
H_2O-layer (10mg/Kg)	504	693	629	960
	444	504	966	960
	504	444	444	1110
	400	693	566	966
	444	504	629	1190
	566	504	896	1190

TABLE 9. The statistical values of IgA concentration differ from each drug treated and control groups.

Groups	Time after drug administration (week)			
	一	二	三	四
Saikosaponin-a (1mg/Kg)	—	—	$p < 0.006$	$p < 0.004$
Saikosaponin-d (1mg/Kg)	$p < 0.007$	$p < 0.006$	$p < 0.004$	$p < 0.006$
Saikosaponin-f (3mg/Kg)	—	$p < 0.04$	$p < 0.03$	$p < 0.004$
Crude saponin (3mg/Kg)	—	$p < 0.01$	$p < 0.005$	$p < 0.005$
H_2O-layer (10mg/Kg)	—	—	$p < 0.03$	$p < 0.005$

Statistical significance differ from drug treated and control groups (6 animals) were evaluated by Wilcoxon 2-sample test. $p < 0.05$ was confirmed significant.

TABLE 10. The serum IgG concentration (mg/l) of BALB/c mice after treated with drugs

Groups	Time after drug administration (week)			
	一	二	三	四
Control (N/S)	1730	ND	ND	ND
	980	ND	ND	ND
	1430	ND	ND	ND
	1980	ND	ND	ND
	1150	ND	ND	ND
	1580	ND	ND	ND
Saikosaponin-a (1mg/Kg)	2540	1980	4000	9130
	1150	2710	2150	9130
	980	1580	2290	4600
	2370	2580	5230	7820
	1730	2010	1430	5670
	1290	1290	3240	4600
Saikosaponin-d (1mg/Kg)	2540	2540	5230	9130
	1150	2370	2540	9130
	2370	2540	4810	6590
	4810	6360	6360	4810
	1150	4600	2880	6590
	1150	1580	7070	6060
Saikosaponin-f (3mg/Kg)	1730	2370	1290	3060
	1150	2710	1890	3420
	1150	2880	4000	6590
	2370	1730	4390	5670
	1150	1290	1150	7070
	1730	1010	3590	8860
Crude saponin (3mg/Kg)	2540	1010	980	5450
	1010	1290	1190	5670
	990	1010	6360	8860
	4000	3240	4600	9130
	2880	2710	1290	7320
	2710	3880	4600	5450
H_2O-layer (10mg/Kg)	1890	1290	2880	6590
	980	1730	4190	4810
	980	1580	990	5450
	2710	1150	1010	5450
	1010	2040	3420	2200
	990	1010	2200	3240

TABLE 11. The statistical values of IgG concentration differ from each drug treated and control groups.

Groups	Time after drug administration (week)			
	一	二	三	四
Saikosaponin-a (1mg/Kg)	–	–	$p < 0.03$	$p < 0.005$
Saikosaponin-d (1mg/Kg)	–	$p < 0.02$	$p < 0.005$	$p < 0.005$
Saikosaponin-f (3mg/Kg)	–	–	–	$p < 0.005$
Crude saponin (3mg/Kg)	–	–	–	$p < 0.005$
H_2O-layer (10mg/Kg)	–	–	–	$p < 0.005$

Statistical significance differ from drug treated and control groups (6 animals) were evaluated by Wilcoxon 2-sample test. $p < 0.05$ was confirmed significant.

TABLE 12. The serum IgM concentration (mg/l) of BALB/c mice after treated with drugs

Groups	Time after drug administration (week)			
	一	二	三	四
Control (N/S)	177	ND	ND	ND
	188	ND	ND	ND
	241	ND	ND	ND
	223	ND	ND	ND
	241	ND	ND	ND
	263	ND	ND	ND
Saikosaponin-a (1mg/Kg)	275	348	336	361
	275	361	263	361
	177	241	311	374
	275	263	414	361
	336	374	348	361
	299	299	275	375
Saikosaponin-d (1mg/Kg)	263	374	387	361
	263	374	348	471
	361	361	361	336
	336	299	336	374
	336	515	336	471
	299	471	311	361
Saikosaponin-f (3mg/Kg)	241	241	263	361
	241	361	241	361
	241	252	361	361
	241	252	361	361
	252	287	230	361
	252	252	299	361
Crude saponin (3mg/Kg)	241	252	336	361
	241	252	252	361
	177	230	241	361
	374	252	387	387
	287	374	361	361
	299	471	263	374
H_2O-layer (10mg/Kg)	299	374	263	361
	230	361	252	430
	230	336	208	428
	374	263	241	311
	230	219	299	374
	241	230	336	328

TABLE 13. The statistical values of IgM concentration differ from each drug treated and control groups.

Groups	Time after drug administration (week)			
	一	二	三	四
Saikosaponin-a (1mg/Kg)	–	$p < 0.02$	$p < 0.006$	$p < 0.004$
Saikosaponin-d (1mg/Kg)	$p < 0.008$	$p < 0.005$	$p < 0.005$	$p < 0.005$
Saikosaponin-f (3mg/Kg)	–	$p < 0.04$	$p < 0.05$	$p < 0.003$
Crude saponin (3mg/Kg)	–	–	$p < 0.02$	$p < 0.007$
H_2O-layer (10mg/Kg)	–	–	–	$p < 0.005$

Statistical significance differ from drug treated and control groups (6 animals) were evaluated by Wilcoxon 2-sample test. $p < 0.05$ was confirmed significant.

TABLE 14. The productive activity of IL-1 by macrophage stimulated with different concentrations of saikosaponins, crude saponin and H_2O-layer extracted from Bupleurum kaoi

Groups	Concentration of drugs administration (μg/ml)				
	5	10	50	100	200
Saikosaponin-a	22.5 *	24.0	37.0	35.0	31.0
Saikosaponin-d	24.0	31.0	40.0	34.5	36.0
Saikosaponin-f	24.0	29.0	32.0	30.5	28.0
Crude saponin	23.5	32.0	34.5	32.0	26.0
H_2O-layer	22.0	25.0	31.0	30.5	25.5
23-O-Acetyl-Saikosaponin a	21.5	27.5	35.0	30.5	30.0

* All values were assigned the Intensity from β-counter absorbance as c.p.m.

TABLE 15. Comparison of IL-1 productive activity by saikosaponins, crude saponin and H_2O-layer at the concentration of 50μg/ml in different BALB/c mouse

Groups	Intensity from β-counter absorbance (c.p.m.)
Control	21.3±3.1
Saikosaponin-a	38.2±3.4 **
Saikosaponin-d	42.8±4.1 **
Saikosaponin-f	30.0±2.4 **
Crude saponin	34.6±3.2 **
H_2O-layer	30.1±3.5 *
23-O-Acetyl-Saikosaponin a	35.2±2.7 **

Each value represents mean±S.D. of 8 animals.
Statistical values significantly as : *$p<0.01$; **$p<0.001$.

Development of Crude Drug Resources and Evaluation of *Bupleurum kaoi*

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Abstract

Bupleuri Radix is well known as a valuable crude drug of Chinese medicine. To develop new crude drug resources of Bupleuri Radix, the pharmacognostical、pharmacological、pathological and immunological studies of *B. kaoi* were made .

The results indicate that liver protective effects of *B. kaoi* and *B. falcatum* var. *komarowi* are better than those of *B. chinense*; the gemmate percentage of *B. kaoi* is better than the cultivated *B. falcatum* var. *komarowi* in cultivated study.

The three major bioactive principles:saikosaponin a, c, d, contained in b. kaoi are greater than those of *B. gslvstum* var. *komarowi* aand *B. chinense* in TLC scanner quantitative analysis .

A mixture of *B. kaoi* saonins, containing saikosaponin -a, -d and -f, showed anti-inflammatory effect against carrageenan-induced plantar edema in the rat. The crude saponin mixture and saikosaponin -f exhibited a dose- dependent inhibiting effect. Antihepatotoxic activity was investigated in rats intoxicated with s-D-galactosamine. Except for saikosaponin f、administration of the other compounds significantly reduced the sGOT and sGPT levels.

Pathological changes in rat hepatocytes such as inflammatory infiltration around the portal area, focal necrosis in the liver parenchyma, and hyaline degeneration, have been improved in all drugs treated groups.

The saikosaponin a, d, f, crude saponin mixture and H_2O-layer components which isolated from the root of *B. kaoi*, demonstrated an immunomodulatory effect. Mice of the BALB/c strain were injected peritoneally with drugs to assess immune responses weekly over a 4 week period. The results showed that the mean ratio

of helper T cells to suppressor T cells (Th/Ts) from mice treatd with SSf and HLC gradually increased: however, in mice treated with saikosaponin a, d and CSM, the ratio rose to a maximum at week 3 and then decreased ; the IL-2 level of treated mice was significantly higher than that of the control group throughout the test period ;the ratio of spleen weight to body weight in all drug treated groups increased weekly ; the activity of B-cell stimulation in mice was promoted week by week ; levels of Ig-A 、 IgG and IgM in serum of saikosaponin d and a treated animals increased. An in vitro study, involving stimulation of IL-1 demonstrated the folowing oder of potency : saikosaponin d>a>23-O-Ac a and CSM>f and HLC.