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Systematic analysis of some Chinese medicinal prescriptions I. Antihepatotoxic principles of Ryutan-shakan-to

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Abstract

Six different formulas of Ryutan-shakan-to were described in the original references of well known Chinese medical works. Chern-Shyn-Tzuen-Sheng-Shu (CSTSS) formula, a decoction of the mixture of Gentianae Scrabrae Radix, Bupleuri Radix, Gardeniae Fructus, Hocquartiae Caulis, Forsythiae Fructus, Coptidis Rhizoma, Rhei Rhizoma, Citrus Pericarpium, Paeoniae Radix Alba and Talcum, demonstrated the remarkable antihepatotoxic effect by the oral treatment on the liver damage induced by carbon tetrachloride (CCl_4) in mice. The systematic analysis of this formula was further investigated using CCl_4-induced liver injury. When each composed herbal drug was orally administered, the major antihepatotoxic components in CSTSS formula were Hocquartiae Caulis, Coptidis Rhizoma, Rhei Rhizoma, Bupleuri Radix and Citrus Pericarpium. Among them, Bupleuri Radix and Citrus Pericarpium were essential components. In this experiment, Citrus Pericarpium showed the activity only when it was mixed with other compositions of herbal drugs. When the effect of known ingredients involved in each herbal drug was examined, berberine, hesperidin, saikosaponins, emodin, chrysophanol, oleanolic acid, and geniposide were antihepatotoxic. Furthermore, the combined use of some effective herbal drugs or principle constituents increased the antihepatotoxic activity relative to their single use. These results suggest the importance of the combination of plural herbal drugs in Chinese prescriptions.

Key words Ryutan-shakan-to (Ryûtan-syakan-tô), antihepatotoxic, combined effect, berberine, hesperidine, saikosaponin, oleanolic acid, emodin, chrysophanol, geniposide.

Abbreviations CCl_4, carbon tetrachloride ; CSTSS, Chern-Shyn-Tzuen-Sheng-Shu ; ITJJ, I-Tzong-Jin-Jiann ; LDY, Lii-Dong-Yuang ; IGT, I-Guann-Tarng ; WBHC, Whan-Bing-Hwei-Chong ; GPT, glutamic pyruvic transaminase ; Ryutan-shakan-to (Long-Dan-Xie-Gan-Tang), 竜胆瀉甘湯.

Introduction

Liver disease is a terrible problem in Asian countries. The medicine that can clinically treat hepatitis and cirrhosis is still absent. Historically, there have been many effective prescriptions for the treatment of acute or chronic hepatitis. Twenty six traditional Chinese medicinal prescriptions are commonly used by clinical herbal doctors in China still now. Ryutan-shakan-to (Long-Dang-Xie-Gan-Tang) is one of these remarkable antihepatotoxic prescriptions. Traditionally, Ryutan-shakan-to had been used as a decoction of Gentiana, a main component, to purge the liver in China. It had been applied to eliminate intense fever, dampness from the liver and gallbladder, the manifestations of which were pain in the head, ear and hypochondrium, redness of eyes, bitter taste in the mouth, itching in perineum, yellow and thick leukorrhea, red borders of tongue, yellow fur and rapid taut pulse.

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Ryutan-shakan-to, however, had six different formulas in the long history concerning the original references of the well-known Chinese medical works. The reason for the existence of the six different formulas is unknown, but it is interesting to know the process of the creation of the herbal medicinal mixture. This paper examines which type of formula is most effective on liver injury using an *in vivo* experimental system. Further, the antihepatotoxic herbal drugs, effective principles, and their combined effects were examined to compile scientific proof regarding the medicinal mixture.

Materials and Methods

Crude drugs : Gardeniae Fructus, *Gardenia jasminoides* ELLIS. ; Gentianae Scabrae Radix, *Gentiana scabra* BUNG. ; Rhei Rhizoma, *Rheum palmatum* L. ; Coptidis Rhizoma, *Coptis chinensis* FRANCH. ; Forsythiae Fructus, *Forsythia suspensa* VAHL. ; Citrus Pericarpium, *Citrus tankan* HAYATA ; Bupleuri Radix, *Bupleurum chinense* DC. ; Paeoniae Radix, *Paeonia lactiflora* PALLS ; Hocquartiae Caulis, *Hocquartia manshuriensis* NAKAI ; Talcum, *Talc* ; Scutellariae Radix, *Scutellaria baicalensis* GEORGI ; Rehmanniae Radix, *Rehmannia glutinosa* LIBOSCH. ; Alismatis Rhizoma, *Alisma orientale* JUZEP. ; Angelicae Radix, *Angelica sinensis* DIELS ; Plantaginis Semen, *Plantago asiatica* L. ; Glycyrrhizae Radix, *Glycyrrhiza uralensis* FISHER ; Ligustici Rhizoma, *Ligusticum chuanxiong* HORT. ; Menthae Folium, *Mentha arvensis* L. ; Phellodendri Cortex, *Phellodendron wilsonii* HAYATA ; Ledebouriellae Radix, *Ledebouriella seseloides* WOLL. ; Schisandrae Fructus, *Schisandra chinensis* BAILL. They were collected from Taipei's Chinese drug market and six formulas of Ryutan-shakan-to based on Table I were prepared.

Animals : ICR male mice, 18－20 g, were supplied from the Animal Center of National Taiwan University. They were kept in an air-conditioned room (24℃) with a 12-hr dark-light cycle. The animals were supplied with standard chow and water *ad libitum*.

Preparation of formula and test solution : Each formula (Table I) and composed crude drug were milled and refluxed twice with 10 volume of water for 6 hr. The filtrates were concentrated under 70℃ *in vacuo*, then lyophilized to make powdered extracts. Each powdered

Table I Crude drug's composition of six original references of Ryutan-shakan-to.

Crude drugs	ITJJ (醫宋金鑑)	LDY (李東垣方)	CSTSS (沈氏尊生方)	IGT (一貫堂)	WBHC (萬病回春)	HJJF (和劑局方)
Gentianae Scabrae Radix (竜胆)	2	2	1	2	1	1
Bupleuri Radix (北柴胡)	0	2	1	0	0	1
Gardeniae Fructus (山梔子)	1	1	1	1.5	1	1
Scutellariae Radix (黄芩)	1	1	0	1.5	1	1
Rehmanniae Radix (地黄)	2	1	0	1.5	1	1
Alismatis Rhizoma (沢瀉)	2	2	0	2	1	1
Angelicae Radix (当帰)	1	1	0	1.5	1	1
Plantaginis Semen (車前子)	1	1	0	1.5	1	1
Hocquartiae Caulis (関木通)	1	1	1	1.5	1	1
Glycyrrhizae Radix (甘草)	1	1	0	0	1	1
Forsythiae Fructus (連翹)	2	0	1	1.5	0	0
Coptidis Rhizoma (黄連)	1	0	1	1.5	0	0
Rhei Rhizoma (大黄)	0	0	1	0	0	0
Citrus Pericapium (陳皮)	0	0	1	0	0	0
Paeoniae Radix (芍薬)	0	0	1	1.5	0	0
Talcum (滑石)	0	0	1	0	0	0
Ligustici Rhizoma (川芎)	0	0	0	1.5	0	0
Phellodendri Cortex (黄柏)	0	0	0	1.5	0	0
Manthae Folium (薄荷)	0	0	0	1.5	0	0
Ledebouriellae Radix (防風)	0	0	0	1.5	0	0

extract was dissolved in saline and orally administered using a stomach tube. Each dose was expressed as the weight of the dried crude drugs.

Ingredients of each crude drug : Berberine HCl, oleanolic acid, hesperidine, geniposide, emodin, chrysophanol and total saikosaponins (saikosaponin fraction) were isolated in our laboratory.

Determination of antihepatotoxic activity : One-half of drugs was orally administered to mice immediately after oral administration of CCl_4 and remaining half was administered 10 hr later (b.i.d.). Serum glutamic pyruvic transaminase (GPT) activity was determined 24 hr after CCl_4-treatment by Karmen method.[1)]

Statistical analysis : The data are indicated as mean±S.E.M. and statistical significance was evaluated by Student's t-test.

Results

Comparative study of different formulas of Ryutan-shakan-to

The assay of six different formulas of Ryutan-shakan-to was shown in Table II. In the control group, CCl_4 elevated the serum GPT to 3910±113 IU/l serum. Oral CSTSS and LDY showed the remarkable antihepatotoxic activity on CCl_4-induced liver injury in a significant manner. Therefore, the systematic analysis of most effective CSTSS formula was examined focusing on the composed crude drugs.

Table II Antihepatotoxic effects of six different formulas of Ryutan-shakan-to.

Formulas	GPT[IU/L]	(% of control)
Control	3910±113	(100)
ITJJ	3754±317	(96)
LDY	2792±285*	(71)
CSTSS	2733±274**	(70)
IGT	3789±285	(97)
WBHC	3136±321	(80)
HJJ	3675±371	(94)

Drugs were orally administered to ICR mice (N=5). The dose was the dried crude drugs equivalent 10 g/kg/day (b.i.d.). GPT activity was determined 24 hr after CCl_4 treatment. * and ** : Statistical significance from the control at $p<0.05$ and $p<0.01$, respectively.

Antihepatotoxic action of composed crude drugs

The antihepatotoxic activity of nine crude drugs which composed the CSTSS formula was summarized in Table III. Hocquartiae Caulis, Coptidis Rhizoma, Rhei Rhizoma and Bupleuri Radix showed the antihepatotoxic action. In order to further know which crude drug is important for the antihepatotoxic activity of CSTSS formula, the effect of the formula was examined by the reduction of a certain crude drug as shown in Table IV. When Citrus Pericarpium was reduced from the formula, Ryutan-shakan-to lost

Table III Antihepatotoxic effects of the composed drugs of CSTSS formula.

Crude drugs	Dose (g/kg) p.o.	GPT [IU/L]	(% of control)
Treated control	0.0	3870±159	(100)
Hocquartiae Caulis	10.0	2895±174*	(75)
Coptidis Rhizoma	10.0	2844±356*	(74)
Rhei Rhizoma	10.0	3026±147*	(78)
Bupleuri Radix	10.0	3077±116*	(80)
Gentianae Scabrae Radix	10.0	3320±352	(86)
Gardeniae Fructus	10.0	3541±511	(92)
Citrus Pericarpium	10.0	3901±313	(101)
Paeoniae Radix	10.0	4153±341	(107)
Forsythia Fructus	10.0	3460±310	(89)

Drugs were orally administered to ICR mice (N=5) (b.i.d.). The dose indicated was the dried crude drugs equivalent per day. GPT activity was determined 24 hr after CCl_4 treatment. * : Statistical significance from the control at $p<0.05$.

Table IV Antihepatotoxic effects of the addition and reduction of crude drug from CSTSS formula.

Prescriptions	GPT [IU/L]	(% of control)
Control	4250±132	(100)
Ryutan-shakan-to	2006±132**	(47)
Ryutan-shakan-to (Gentianae Scabrae Radix, Forsythiae Radix, Coptidis Rhizoma)	2282± 89**	(54)
Ryutan-shakan-to (Bupleuri Radix)	3740±570	(88)
Ryutan-shakan-to (Rhei Rhizoma)	3171±247*	(75)
Ryutan-shakan-to (Citrus Pericapium)	4305± 30	(101)
Ryutan-shakan-to (Paeoniae Radix)	3077± 98*	(72)
Ryutan-shakan-to (Hocquartiae Caulis)	2984±221*	(70)
Ryutan-shakan-to (Schisandrae Fructus)	3532±217	(83)

Drugs were orally administered to ICR mice (N=5) (b.i.d.). The dose was 10 g/kg/day of dried crude drugs. GPT activity was determined 24 hr after CCl_4 treatment. * and ** : Statistical significance from the control at $p<0.05$ and $p<0.01$, respectively.

Table V Antihepatotoxic effects of combined components.

Combined components	GPT [IU/L]	(% of control)
Treated control	3917±278	(100)
Berberine+saikosaponins	2017±180**	(52)
Chrysophanol+emodin	2891±200*	(74)
Chrysophanol+emodin+berberine	2440±106**	(62)
Chrysophanol+emodin+berberine Hesperidin	2123±188**	(54)
Chrysophanol+emodin+berberine Hesperidin+saikosaponins	1226±165***	(31)
Chrysophanol+emodin+berberine Hesperidin+saikosaponins+ geniposide+oleanolic acid	823±149***	(21)

Drugs (total 100 mg/kg/day, and the mixed components are the same ratio) were orally administered to ICR mice (N=5) (b.i.d.). GPT activity was determined at 24 hr after CCl_4 treatment. *, ** and *** : Statistical difference from the control at $p<0.05$, $p<0.01$ and $p<0.001$, respectively.

antihepatotoxic activity. The reduction of Bupleuri Radix also reduced the antihepatotoxic activity. On the other hand, Schisandrae Fructus is frequently added to the Ryutan-shakan-to formula in the case of a certain patient's physical condition. Therefore, the effect of the addition of Schisandrae Fructus to the formula was examined. As shown in Table IV, the addition of Schisandrae Fructus showed no significant effect on CCl_4-induced liver injury.

Antihepatotoxic action of ingredients

The main ingredients involved in each effective crude drug were also studied. Hesperidin, total saikosaponins, berberine, emodin, chrysophanol, oleanolic acid and geniposide inhibited the increase of serum GPT activity in a dose dependent manner as shown in Figs. 1−6. Table V showed the combined effects of the ingredients mentioned above. When the number of principle compounds increased, the inhibitory effect on

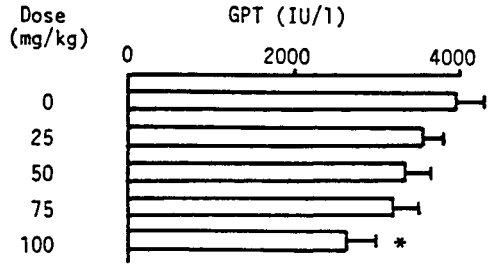

Fig. 1 Antihepatotoxic effect of berberine. Each column indicates the mean±S.E.M. Significant difference from control, $*p<0.05$.

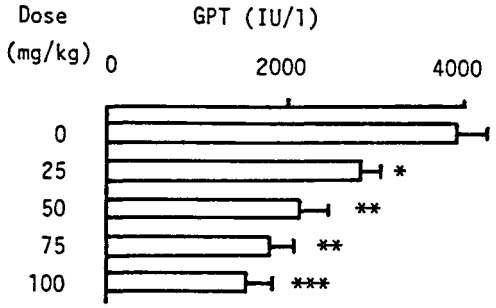

Fig. 4 Antihepatotoxic effect of oleanolic acid. Each column indicates the mean±S.E.M. Significant difference from control, $*p<0.05$, $**p<0.01$, $***p<0.001$.

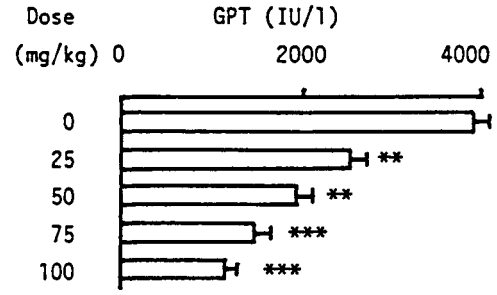

Fig. 2 Antihepatotoxic effect of hesperidin. Each column indicates the mean±S.E.M. Significant difference from control, $*p<0.05$, $**p<0.01$, $***p<0.001$.

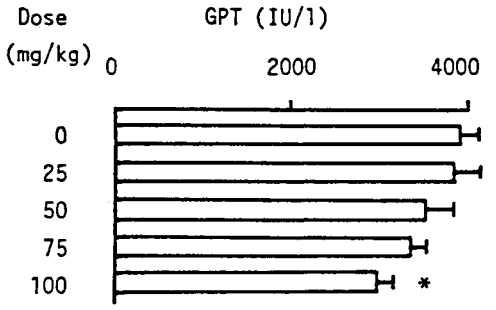

Fig. 5 Antihepatotoxic effect of emodin. Each column indicates the mean±S.E.M. Significant difference from control, $*p<0.05$.

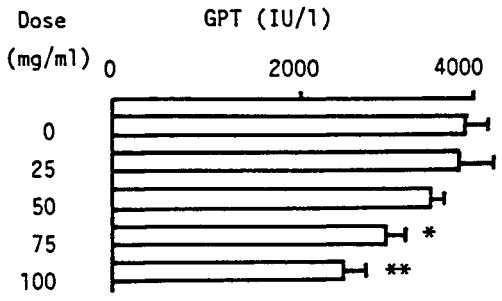

Fig. 3 Antihepatotoxic effect of saikosaponins. Each column indicates the mean±S.E.M. Significant difference from control, $*p<0.05$, $**p<0.01$.

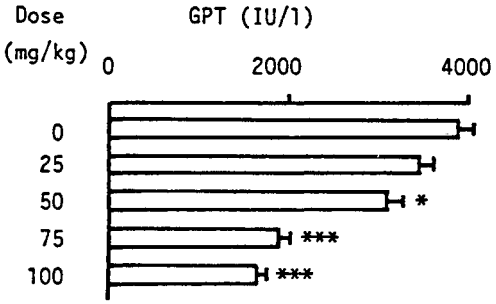

Fig. 6 Antihepatotoxic effect of geniposide. Each column indicates the mean±S.E.M. Significant difference from control, $*p<0.05$, $**p<0.01$, $***p<0.001$.

liver injury became remarkable.

Discussion

Chinese medicines are applied by the differentiation of symptom-complexes on the basis of an overall analysis of symptoms and signs including the cause, nature and location of the illness and patient's physical condition for diagnosis and treatment. They have been used over five thou-

sand years through many dynasties, and many medicinal mixtures were applied for clinical therapy. In such a long history, some prescriptions, each with a certain name, showed the variety of composed crude drugs. In this paper, the six different references of Ryutan-shakan-to formula which had been varied over five thousand years were examined for their antihepatotoxic activity. Among the six formulas, CSTSS[2] showed the most antihepatotoxic activity by oral administration. It is notable that CSTSS showed the remarkable effect on CCl_4-induced liver injury, although all six formulas had been used clinically for the treatment of liver injury. CSTSS, further, inhibited the galactosamine-induced hepatotoxicity and complement-mediated cytotoxicity[3] in primary cultured mouse or rat hepatocytes. Although it is difficult to evaluate the clinical effect of the formula from the *in vitro* experimental results because the metabolism of the formula and it's ingredients are unknown, our results suggest that CSTSS inhibits hepatocellular inflammation as Sho-saiko-to.[4-6] CSTSS is suggested to be effective for acute or chronic hepatitis. Immunoregulatory action of CSTSS may be also influenced on the antihepatitic action. The difference of the action mechanisms of the 6 formulas should be further examined using different hepatic injury models. It is interesting to discover which composed crude drugs are most effective for liver injuries. Therapeutic classification of the composed drugs in original references of Chinese medical works are as follows : Gentianae Scabrae Radix, Coptidis Rhizoma, Gardeniae Fructus and Forsythiae Fructus are febrifugal and antipyretic, Bupleuri Radix is diaphoretic, Rhei Rhizoma is purgative, Citrus Pericapium is carminative, Hocquartiae Caulis and Talcum are diuretic, and Paeoniae Radix is hypotensive. From our results, major antihepatotoxic drugs in the CSTSS formula were Bupleuri Radix and Citrus Pericarpium. Hocquartiae Caulis, Coptidis Rhizoma and Rhei Rhizoma were also effective. Citrus Pericarpium was effective only in the mixture of other composed crude drugs and its single use showed no effect. A certain ingredient involved in Citrus Pericarpium will become an active component when it reacts with other drug's components in the decoction or in the body. When the effect of active ingredients isolated from those effective crude drugs were examined, the result was also noteworthy. Namely, the effects of single use were not so remarkable, but the effects of the mixture of the drugs were definitely remarkable. These results suggest that the combination of plural harbal drugs is essential for the greatest effect of Ryutan-shakan-to.

和文抄録

中国において用いられてきた構成生薬の異なる6種類の竜胆瀉甘湯の肝障害改善作用を検討したところ，沈氏尊生方に記載されているCSTSS (Chern-Shyn-Tzuen-Sheng-Shu) に強い効果がみとめられた。この処方の効果はCCl_4肝障害で強く認められたことから，肝細胞壊死を抑制することが考えられた。この竜胆瀉甘湯の構成生薬について，単味あるいは一味抜き処方を作成して効果を検討してみると，柴胡，陳皮に強い効果が認められた。陳皮の効果は単味では認められず，処方から陳皮を除いた時にのみ処方の効果が消失したことから，陳皮の効果は，他の生薬成分との相互作用により発現することが考えられた。次に，肝障害改善作用を示した生薬の成分についても検討を加えてみるとberberine, hesper idinsaikosaponin, emodin, chrysophahol, oleanolic acid, geniposideに効果が認められ，それらを足し合わせると処方に匹敵する効果が再現された。以上の結果は，古来より行われてきた漢方処方における生薬配合の重要性を示している。

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