

Studies on the Concurrent Administrations of Sosiho-Tang Extract and Methionine

Effects on the Liver Lesion Induced by Carbon Tetrachloride in Rats

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Abstract—The preventive effect of Sosiho-Tang extract administered concurrently with methionine on hepatotoxicity induced by carbon tetrachloride (20% CCl₄ in olive oil, 5 ml/kg) in rats was studied. S-GOT values were decreased significantly by the concurrent administration of Sosiho-Tang extract 100 mg/kg with methionine 100 mg/kg and also Sosiho-Tang 200 mg/kg with methionine 100 mg/kg. S-GPT values were decreased remarkably by the concurrent administration of Sosiho-Tang extract 200 mg/kg with methionine 100 mg/kg. In histological observations, the pattern of the contrast group indicated the marked necrosis of the parenchymatous cells around the centrilobular area and the fatty changes of the hepatic cells around the midzonal area, but the concurrent administration of Sosiho-Tang extract 200 mg/kg and methionine 100 mg/kg revealed better improvements than their respective single administration in all pathological aspects.

Keywords—Sosiho-Tang extract • methionine • carbon tetrachloride • S-GOT • S-GPT • hepatotoxicity • histological observation

Sosiho-Tang(小柴胡湯) is a representative remedy for chest fevers. It is the basic remedy of *Bupleurum* Prescriptions consisting of *Bupleuri Radix*, *Scutellariae Radix*, *Ginseng Radix*, *Glycyrrhizae Radix*, *Gingiberis Rhizoma*, *Zizyphi Fructus* and *Pinelliae Tuber*. And it is used mainly for the cure of the Soyang disease(少陽病) and related diseases in the chinese herb medicine.

According to the documentary studies, Sosiho-Tang is the prescription recorded in the ancient Chinese medical literatures including *Tong Eui Bo Gam*¹⁾ and *Bang Yak Hap Pyun*²⁾ in Korea and *Sang Han Ron*³⁾ and *Kum Kyaе Yo Ryak*⁴⁾ in China. It is prescribed as a remedy for the Soyang disease or chills and fevers related diseases in *Tong Eui Bo Gam* and *Bang Yak Hap Pyun*. At present it is applied for the cure of

the acute hepatitis, chronic hepatitis, common cold, gall bladder infection, T.B., tonsillitis otitis media, gastritis, jaundice, etc.⁵⁾

As a modern medico-pharmacological study of *Bupleuri Radix*, Arichi, et al⁶⁾ reported in 1977 on "Effects of Drugs including *Bupleuri Radix* and Saiko-saponin to Hepatitis". The same research team reported again in 1978 on "Effects of Saiko-saponin to Hepatic Injury Induced by D-galactosamine"⁷⁾ and in 1980 on "The Study on the Mechanism of Action of Saikosaponin"⁸⁾. Again in 1980 the same research team reported the result of its investigation on "Pharmacological Studies on the Prescriptions Containing *Bupleuri Radix*"⁹⁾.

As described above, a number of pharmacological studies have been conducted on the preve-

ntive effects of the *Bupleurum* prescriptions in the recent years, however, there have been very few studies undertaken on the concurrent administration of *Bupleurum* herbs and other synthetic medicines. The authors have taken a notice of this fact and started a study on the preventive effects of the respective and combined administrations of Soshiho-Tang extract and methionine on the carbon tetrachloride induced hepatic toxicity or hepatic injury in the rats. As a result of studying and comparing influences and effects on the carbon tetrachloride induced hepatotoxicity in rats by respective and concurrent administrations of Soshiho-Tang extract and methionine; the test group concurrently administered with Soshiho-Tang extract 100 mg/kg and 200 mg/kg with methionine 100 mg/kg not only revealed a significant decrease respectively in S-GPT values but also a remarkable decrease in necrosis and fatty changes of the hepatic cells around the central vein in the histological observations.

Materials and Methods

1. Materials

Soshiho-Tang herbs was purchased from a Chinese herb medicine shop (at the Kyung Dong Market, Seoul) and its properties were accurately tested. Then, *Bupleuri Radix* 0.7 kg, *Scutellariae Radix* 0.3 kg, *Ginseng Radix* 0.3 kg, *Glycyrrhizae Radix* 0.2 kg, *Gingiberis Rhizoma* 0.4 kg, *Zizyphi Fructus* 0.3 kg, *Pinelliae Tuber* 0.5 kg (100 portions of one dose based on Sang Han Ron) added to 10 litres of distilled water and boiled for 5 hour twice for extraction and filtered through the gauze. The filtrate was concentrated at the reduced pressure and dried to obtain 360 g of Soshiho-Tang extract. DL-Methionine, a product of Kokusan Chemical Works, was used for this experimentation.

2. Methods

Male rats 170~200 g of the Sprague-Dawley breed raised under a fixed condition for 3 weeks are used as test animals. Test animals have been divided into 3 groups-control, contrast, and test-of 6 rats in each group. The test group has been subgrouped into 1) the single administrations subgroup of methionine 100, 200, 400 mg/kg and Soshiho-Tang extract 100, 200, 400 mg/kg respectively and 2) the concurrent administrations subgroup of Soshiho-Tang extract plus methionine 100+100, 200+100, 400+100 mg/kg and the prescribed doses were orally administered twice daily for the duration of 3 days. One hour after the last dose on the third day, 20% CCl₄ (v/v olive oil) has been injected at the volume of 5 ml/kg to the test group animals; olive oil to the control group; 20% CCl₄ (v/v olive oil) to the contrast group animals. The animals have been starved for 24 hours after the injection of CCl₄. Then, the rats were lightly intoxicated by ether for the extraction of blood from inferior vena cava. The blood sample was let alone for 20 min before it was centrifuged for 15 min at 3000 rpm for the isolation of serum. S-GOT and S-GPT values have been measured by the Reitman-Frankel method.

After the taking of blood samples from the rats, the hepatic cells were isolated and placed in 10% formalin. According to the routine method, the samples were dyed with hematoxylin-eosin for the observation of histological changes.

Results and Discussion

1) Effects on the Hepatic Functions

S-GOT values of the methionine 100, 200, and 400 mg/kg unilateral administration group animals were 64.0, 60.7 and 56.2 unit respectively. As compared with those of the contrast group animals, S-GPT values of the test animals decreased significantly. S-GOT values of the

Table I. Effects of Methionine, Soshiho-Tang Extract and Soshiho-Tang Extract + Methionine on S-GOT, S-GPT after Administration of 20% CCl₄ (5 ml/kg s.c.)

Drugs	No. of animal	Dose (mg/kg) p.o.	S-GOT Karmen units mean ± S.E.	S-GPT Karmen units mean ± S.E.
Control	6	—	94.2 ± 6.3	34.3 ± 7.1
Contrast	6	—	334.6 ± 30.6	235 ± 19.5
Methionine	6	100	193.3 ± 17.8**	64.0 ± 10.5**
Methionine	6	200	137.2 ± 15.8**	60.7 ± 8.8**
Methionine	6	400	136.8 ± 14.0**	56.2 ± 7.9**
Soshiho-Tang Extract	6	100	253.0 ± 20.7	118.2 ± 44.2*
Soshiho-Tang Extract	6	200	229.5 ± 38.6	48.2 ± 9.7**
Soshiho-Tang Extract	6	400	205.5 ± 15.7**	47.2 ± 4.5**
Soshiho-Tang Extract + Methionine	6	100 + 100	131.8 ± 13.6**	57.7 ± 12.9**
Soshiho-Tang Extract + Methionine	6	200 + 100	106.2 ± 15.6**	40.8 ± 1.7**
Soshiho-Tang Extract + Methionine	6	400 + 100	157.8 ± 19.7**	56.0 ± 11.6**

Significantly different from CCl₄ positive control, *p < 0.05 **p < 0.01

Soshiho-Tang extract 400 mg/kg unilateral administration subgroup animals indicated a meaningful change. Especially S-GPT values of the Soshiho-Tang extract 100, 200, and 400 mg/kg revealed 118.2, 48.2 and 47.2 units respectively in commensurate with the amount of doses administered.

On the other hand, for the concurrent administration test subgroup of Soshiho-Tang extract and methionine 100+100, 200+100 mg/kg, S-GOT values registered 131.8, 106.2 units respectively; S-GPT values, 57.7, 40.8 units respectively. Both values indicated the significant decreases as compared with the contrast group rats. (Table I)

Therefore, the concurrent administration of Soshiho-Tang extract and methionine more significantly improves the hepatic functions of CCl₄ induced rats than that of the unilateral administration test subgroup.

2) Effect on the Hepatic Histology

24 hours after the injection of 20% CCl₄ (v/v olive oil) 5 ml/kg to the contrast group rats, they indicated necrosis of hepatic cells around the central veins and ballooning caused by the protein change of the hepatic cells around the midzonal area, and the spread of cell inflammation

and severe hepatic blood congestion around the central vein. However, to the Soshiho-Tang extract unilateral administration 100 mg/kg group; there occurred the fatty changes and blood congestion in the hepatic cells around the central vein. Nevertheless, its extent was in a much better condition than that of the contrast group. The 200 mg/kg and 400 mg/kg unilateral administration test group indicated a far better improvement. Also in the methionine unilateral administration test group, the symptom was similar indicating the improvements in the hepatic condition corresponding to the amount of doses.

On the other hand, for the Soshiho-Tang extract and methionine concurrent administration test subgroup especially for the Soshiho-Tang extract 100 mg/kg and methionine 100 mg/kg test subgroup and the Soshiho-Tang extract 200 mg/kg and methionine 100 mg/kg concurrent administration test subgroup, there were remarkable improvements in the hepatic conditions particularly in the last concurrent administration test subgroup. (Fig. 1~7)

Conclusion

The followings are the results of this study by

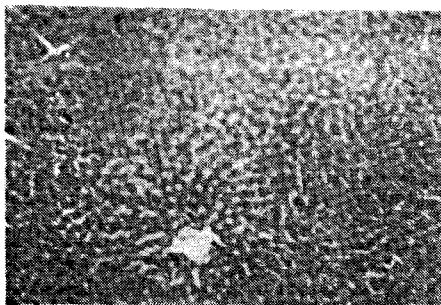


Fig. 1. Liver from normal group (H&E, $\times 60$).

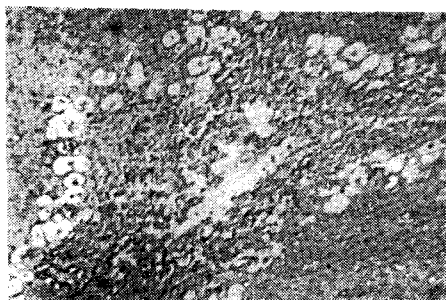


Fig. 2. Liver from CCl₄ group. Marked necrosis of parenchymatous cell around centrilobular area and fatty changes of hepatic cell around midzonal area (H&E, $\times 60$).

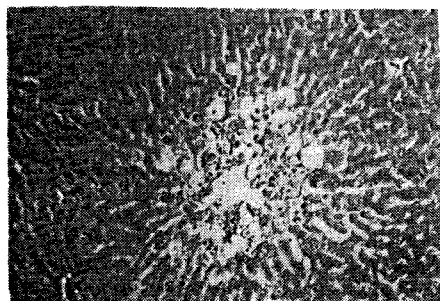


Fig. 3. Liver from CCl₄+Methionine (100 mg/kg). The parenchymatous cell necrosis and fatty changes around centrilobular area are noticed (H&E, $\times 60$).

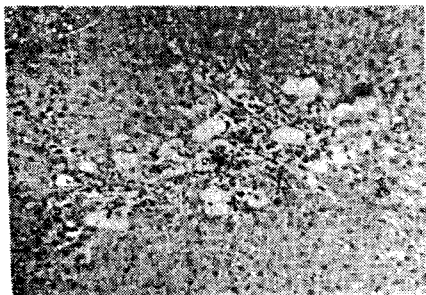


Fig. 4. Liver from CCl₄+Methionine (200 mg/kg). The mild cellular infiltration and fatty changes around centrilobular area were showed. (H&E, $\times 60$).

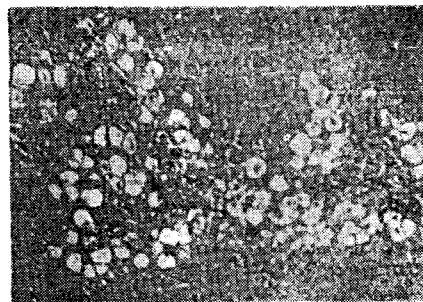


Fig. 5. Liver from CCl₄+Sosiho-Tang (100 mg/kg). The fatty changes and congestion of hepatic parenchymatous cell are remarkable around centrilobular area (H&E, $\times 60$).

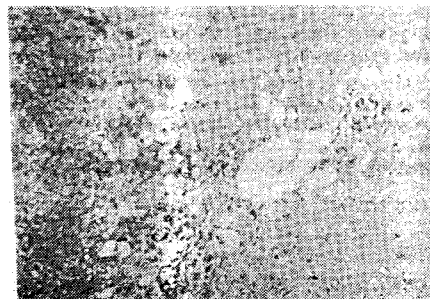


Fig. 6. Liver from CCl₄+Sosiho-Tang+Methionine (100+100 mg/kg). All pathologic patterns are similar to that of Sosiho-Tang (200 mg/kg). But fatty changes around centrilobular area are markedly improved (H&E, $\times 60$).

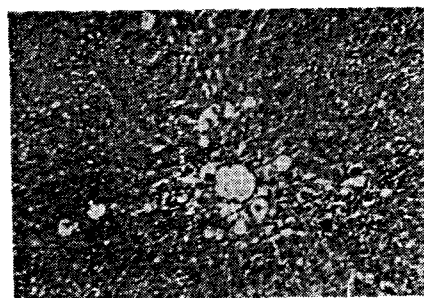


Fig. 7. Liver from CCl₄+Sosiho-Tang+Methionine (200+100 mg/kg). All pathologic patterns are markedly improved (H&E, $\times 60$).

the respective unilateral administrations and concurrent administrations of Sosiho-Tang extract and methionine in various combination doses of varying amounts.

When Sosiho-Tang extract and methionine 100, 200 and 400 mg/kg were administered unilaterally, the hepatic conditions revealed sig-

nificant improvements according to the varying amounts of doses applied. In the case of the concurrent administration of Sosiho-Tang extract 100 mg/kg and methionine 100 mg/kg and the other of Sosiho-Tang extract 200 mg/kg and methionine 100 mg/kg resulted in the most significant improvement in the hepatic conditions of all other test groups and subgroups in this study.

Also in the histological observations, the concurrent administration revealed better improvements in the hepatic condition than those of the unilateral administrations. Especially, the concurrent administration of Sosiho-Tang extract 200 mg/kg and methionine 100 mg/kg resulted in the best improvement of the hepatic condition.

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