The Histological and Biological Studies on Cynanchum taiwanianum

Chun- Ching Lin[1] #, Ming- Hong Yen[1], Yueh- Wern Wu[2] and Guo- Jun Xu[3]

1. School of Pharmacy, Kaohsiung Medicinal College, Kaohsiung, Taiwan, ROC

2. Graduate Institute of Natural Products, Kaohsiung Medicinal College, Kaohsiung, Taiwan, ROC

3. China Pharmaceutical University, Nanjing, China

Summary

The botanical origins of Cynanchum species on Taiwan market has been established, they were the roots of C. atratum Bunge (=Pai-wei); C. stauntonii (Decne.) Hand.-Mazz. (=Pai-chein); and C. taiwanianum Yamazaki (=Wan-ling-shu). Furthermore, the water extract of C. taiwanianum root (CTR) significantly reduced carrageenan-induced paw edema in rats. Moreover, CTR also significantly declined the acute increase in sGOT and sGPT levels caused by CCl_4-; β-D-galactosamine-; and acetaminophen-induced hepatotoxicity. The metamorphology of liver damage has been improved by pretreatment with CTR.

Key words : Cynanchum taiwanianum, histology, antiinflammation, hepatoprotection

Introduction

Many substitutes of Cynanchum species were used distinctly as a remedy in ancient China and Asia. Some of them have poisonous (Mitsuhashi and Shimizu, 1960). However, some confusion exists in these crude drugs because similar outward appearance (Shie et al., 1959). In Taiwan, root of C. formosanum has been used as febrifuge, diuretic, and to reduce swellings (Perry, 1980). Recent years, a related Cynanchum species named "Wan-ling-shu" derived from C. taiwanianum has been used in Taiwan market for treatment of skin diseases, diarrhea, constipation, hepatitis, leukemia, hypertension, etc. But all effects have noy been proved yet by pharmacological test. In the present paper, the pharmacognostical and biological studies on C. taiwanianum were made.

Material and Method

Histological examination

Samples were collected locationally from Taiwan market. Their morphological and histological observation were made on the transverse sections of 1-2 cm beneath the proximal end (roots), and the characteristics were compared to the identified original plants.

Pharmacological studies

Animals

Male Wistar albin66o rats (4-6 weeks) were obtained from the Animal Center, College of Medicine, National Cheng Kung University. They were housed in air-conditioned room at 22 ± 3 ℃ under natural lighting condition.

Preparation of crude drug extract

Roots of C. taiwanianum (CTR) were collected from Neman, Kaohsiung Hsien, decocted with boiling water for 1 hr three times. The decoction was filtered, mixed, concentrated and lyophilized to produced dry powder (Yield 8.9%).

Carrageenan-induced rat paw edema

The method was according to Winter et al. (1962). CTR was administered to the rats s.c. 1 hr prior to the injection of carrageenan. The paw volume of each rat was measured with a plethysmomter 7150, Ugo Basile (Italy) before and at 1-5 hrs after carrageenan was injected, and the edema rate and inhibition rate were calculated.

CCl_4-induced hepatic damage

CCl_4 (guaranteed, Merck, 50% in olive oil) was injected s.c. into rats as a dose 3ml/kg. CTR was administered concomitantly with CCl_4 administration. Two additional injections of CTR were administered at 24 and 48 hrs later. After 72 hrs, all rats were sacrificed after collection of blood from carotid artery.

β-D-galactosamine(D-GalN)-induced hepatic damage

The procedure was according to our previous report (Yen et al., 1994), CTR was administered i.p. 2 hrs prior to the injection of D-GalN (Sigma, 400mg/kg). The blood of each rat was collected at 12 and 24 hrs after D-GalN was injected.

Acetaminophen(APAP)-induced hepatic damage

The method was modified from Muriel et al. (1992). Rats were fasted 16 hrs before and throughout experiment. CTR was administered i.p. 2 hrs prior to the injection of APAP (500mg/kg in 25% PEG400). The blood of each rat was collected at 24 hrs after APAP was injected.

Statistical analysis

All data were assessed by one-way analysis of variance (ANOVA) and expressed as mean $\pm$ S.E. (n=6). The difference of data was analyzed by using Student's t-test and/or Newman-Keuls test.

Result and Discussion

The characteristics of Cynanchum species related in Taiwan are shown in Table 1. The origin of "Wan-ling-shu" is derived from the root of C. taiwanianum Yamazaki. On the other hand, some confusion is arisen about the origin of :Pai-wei" and "Pai-chein" not only in the therapy but also in the name. It is recommended that these two crude drugs should be applied to the right gennine origin and should not be further

confused each other in the future.

Carrageenan-induced edema in rat's hind paw was significantly reduced by treated with CTR as shown in Table 2. As carrageenan- induced edema is believed to be biphasic in nature, the result suggests the mechanism of CTR inhibit a phagocytic inflammatory response should to be considerable (Vinegar et al., 1987).

Hepatic injury from toxicants is judged by assay of serum transaminase levels (sGOT and sGPT) and observed from liver lision under a photomicroscope examination. In the present paper, CTR was significantly reduced the increase of sGOT and sGPT levels which were induced by CCl_4 (Table 3); D-GalN (Table 4) and APAP (Table 5) intoxications, respectively. Furthermore, an acute liver damage from Zone 1 (D-GalN) and Zone 3 (CCl_4 and APAP) was clearly observed. These pathological changes were moderately improved by treated with CTR. As we known, the mechanisms of liver damage on these three hepatotoxicants are quitely different. Benda et al. (1949) indicated the histologic signs of D-GalN hepatitis on rat as compared to human viral hepatitis are very similar. Many reports indicated the reproducible liver cell injury lies in its metabolism (Keppler and Decker, 1969; Bauer et al., 1974). On the other hand, CCl_4 is metabolized by cytochrome p-450 system to produce the trichloromethyl free radical ($CCl_3 \cdot$), which autooxidize cell membrane phospholipids and the cause functional and morphological change in cell membrane (Recknagel, 1967; Packer et al., 1978). APAP mostly excreted by conjugating with glucuronate and sulfate, but few metabolized by cytochrome p-450 system to produce n-acetyl-p-benzoquinon-imine and readily detoxified by react with glutathion (Mitchell et al., 1973; Liu et al., 1993).

In conclusion, we provided evidence that CTR has the antiinflammatory and hepatoprotective activities. The isolation of active principles and determination of the mechanisms requires further investigation.

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Table 1.

The root anatomical characteristics of *Cynanchun* species from Taiwan.

Elements	*C. atratum* (Pai-wei)	*C. stauntonii* (Pai-chein)	*C. formosanum*	*C.lanhsuense*	*C. taiwanianum* (Wan-ling-shu)
existence of cork cell	—	—	+	+	+
existence of stone cell in cortex	—	—	+	+	+
size of parenchyma cell in cortex (μ m)	55-75×45-55	30-45×20-35	25-45×20-30	50-70×25-40	60-105×50-100
diameter of starch grains in cortex (μ m)	5-15	5-10	10-15	5-10	5-15
existence of solitary crystal in cortex	—	—	—	+	—
existence of clustered crystal in cortex	—	—	+	+	+
existence of sclerenchyma cell in cortex	—	—	—	+	—
existence of fiber bundle in cortex	—	—	+	—	—
diameter of vessel (μ m)	50-60	30-40	60-95	35-60	90-160
existence of starch grains in pith	—	—	+	+	+
degree of sclerification in pith	distinct	distinct	indistinct	indistinct	indistinct

Table 2.

Effect of *Cynanchum taiwanianum* root (CTR) on carrageenan-induced paw edema in rats.

Groups	Dose (mg/kg)	Edema rate (E%) after carrageenan administration (hours)				
		1	2	3	4	5
Control (N/S)	—	29.5±2.1	45.3±3.0	50.4±4.3	37.4±5.2	20.9±2.0
Indomethacin	10	9.6±1.4* (66.3)	15.2±2.0* (66.8)	20.2±1.5* (59.9)	21.4±1.8* (42.8)	16.1±1.2
CTR	100	26.6±3.1	33.9±3.3* (26.0)	30.8±2.1* (38.9)	22.2±7.0* (40.6)	14.9±1.8* (28.7)
CTR	300	25.4±2.0	36.9±1.7* (19.4)	28.2±2.0* (44.0)	20.5±2.7* (45.2)	8.9±1.0* (57.4)

*$p < 0.05$ (Student's *t*-test), significantly different from control group.
Values within parentheses indicate percentage of the inhibition rate (I%).

Table 3.

The hepatoprotective effect of *Cynanchum taiwanianum* root (CTR) on carbon tetrachloride (CCl_4)-induced hepatitis in rats.

Groups	Dose (mg/kg)	sGOT (IU/L)	sGPT (IU/L)
Control (N/S)	—	93.8±3.9	23.2±1.8
CCl_4/Olive oil(1:1)	3ml/kg	308.1±20.7	93.2±7.5
Glycyrrhizin	200	172.6±9.8*	54.1±2.6*
CTR	100	229.7±7.8*	66.2±5.3*
CTR	300	206.3±14.9*	58.5±3.2*

Values represent the mean±S.E. of 6 animals each group.
*$p < 0.05$(Student's *t*-test), significantly different from CCl_4-intoxicated group.

Table 4.

The hepatoprotective effect of *Cynanchum taiwanianum* root (CTR) on β-D-galactosamine (D-GalN)-induced hepatitis in rats.

Groups	Dose (mg/kg)	sGOT (IU/L)		sGPT (IU/L)	
		12 hr	24 hr	12 hr	24 hr
Control (N/S)	—	124.6±1.4	102.6±2.3	22.5±1.6	23.9±1.8
D-GalN	400	258.9±15.6	178.7±7.4	80.1±6.5	48.8±1.6
Silymarin	25	185.7±11.4*	129.0±9.3*	54.3±6.4*	31.8±1.3*
CTR	100	192.0±8.2*	142.8±2.8*	54.1±3.5*	30.2±1.7*
CTR	300	195.1±10.0*	134.2±4.7*	54.5±4.3*	31.3±1.1*

Values represent the mean±S.E. of 6 animals each group.
*$p < 0.05$(Student's *t*-test), significantly different from D-GalN-intoxicated group.

Table 5.

The hepatoprotective effect of *Cynanchum taiwanianum* root (CTR) on acetaminophen (APAP)-induced hepatitis in rats.

Groups	Dose (mg/kg)	sGOT (IU/L)	sGPT (IU/L)
Control (N/S)	—	117.1±6.4	27.2±1.7
APAP	500	4567.0±219.7	975.1±31.8
Glycyrrhizin	200	162.2±20.4*	38.1±3.8*
CTR	100	1828.1±268.9*	447.5±87.6*
CTR	300	1848.5±255.8*	666.2±40.9*

Values represent the mean±S.E. of 6 animals each group.
*$p < 0.05$(Student's *t*-test), significantly different from APAP-intoxicated group.