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Plants with Liver Protective Activities (II)

Potential Hepatotonic Activities of *Plantago asiatica* Seed

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强肝劑로 使用된 生藥의 調査研究 (Ⅱ)

車前子의 强肝效果에 關하여

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Total methanol extract of *Plantago asiatica* seeds showed significant hepatotonic activities against animal model of hepatitis. The methanol extract was further fractionated with petroleum ether, chloroform, n-butanol and water. Among these fractions, methanol extract and water and chloroform fractions exhibited hepatonic activities, whereas petroleum ether and n-butanol fractions appeared to give rise a rather toxicity. Measurements of serum GOT, GPT and duration of sleeping time induced by hexobarbital after CCl_4 intoxication indicated that water fraction showed most potent activities.

Introduction

According to various medical surveys including W.H.O. report, the exceptionally high occurrence rate of liver diseases like viral hepatitis and cirrhosis to Korean peoples have brought about one of serious problems in area of public health[1,2]. At the present time, it is uncertain whether such high occurrence rate is due to the dietary habit and/or the lack of sanitation. Nonetheless, it is desirable to control or to cure such diseases by means of proper medications. Although many efforts have been made to develope the therapeutic remedies for liver illness, no significant progresses have been achieved yet.

For the purpose of treating liver illness or protecting liver damages from various factors such as virus and toxic chemicals, the present study is aimed to investigate potential hepatotonic substances and/or therapeutic agents originated from natural products like medicinal plants and herbs. Our previous report described the plaussible medicinal plants appeaed in various literatures including old oriental medicinal books and the results of preliminary screening test with respect to hepatotonic activities[3]. In present paper, we attempted to isolate the most active fraction from total

methanol extract of *Plantago asiatica* seed which showed potent hepatotonic activities through the preliminary screening test against animal model of hepatitis. In addition, in order to ascertain the hepatotonic activities of *Plantago asiatica* seed fractions, biochemical studies were carried out by assaying serum glutamate-oxaloacetate transaminase and glutamate-pyruvate transaminase enzymes activities.

Materials and Methods

Animals and plant samples *Plantago asiatica* seeds were purchased from the local herb drug dealers. This sample was identified taxonomically by botanist in the Natural Products Research Institute, Seoul National University, Seoul (110), Korea.

Mice, Swiss albino, were supplied by Animal Care Laboratory of Seoul National University. All mice were housed in air-conditioned room under proper light control (7A.M.-9P.M.).

Preparations of extracts Six kilograms of air-dried *Plantago asiatica* seeds were placed in 10 L. flask and were refluxed with 90% methanol (v/v) for 6 hours. Then it was filtered off and the filtrates were concentrated under reduced pressure into complete dryness.

In order to extract the residual components as much as possible, the extraction procedures were repeated for two times. Total 200g of extract was yielded and this extract was fractionated with various solvents such as methanol, water, petroleum ether, chloroform, and n-butanol.

Fractionations in details were described in Chart 1.

After each fraction was dried under reduced pressure, the residues were dissolved in 0.9% saline and were subjected to oral administation. In case of insoluble plant fraction in saline, few drops of Tween 80 were added and the plant sample were homoginized to form suspension.

Animal model of hepatitis the animal model was produced by CCl_4 intoxication. The proce-

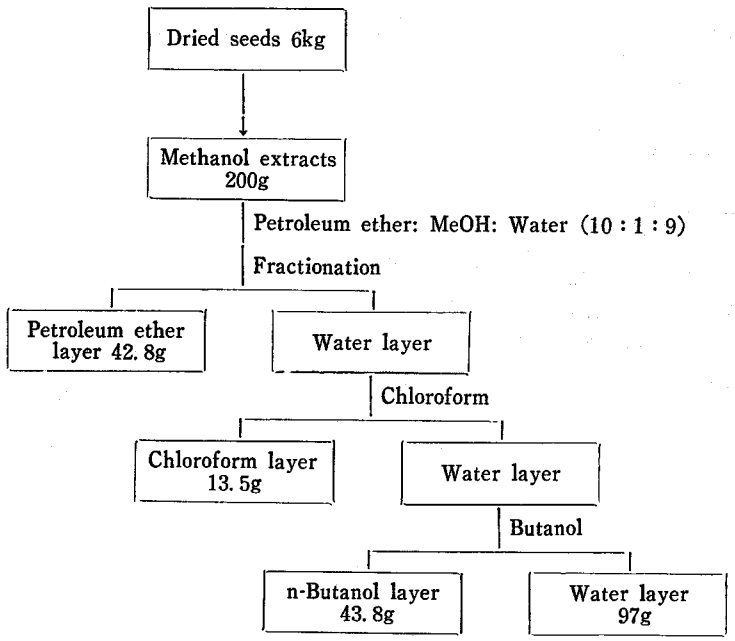

Chart 1. Fractionation of *Plantago aciatica* seeds.

dure in detail was reported previously.[3]~[5]

Pharmacological evaluations of hepatotonic activities Evaluations were undertaken by measuring the duration of sleeping time induced by hexobarbital administrations. Details in procedure were described in Table I.

Biochemical evaluations of hepatotonic activities Serum glutamate-oxaloacetate transaminase(EC 2.6.1.1.) and glutamate-pyr uvate transaminase(EC 2.6.1.2) activities were determined in order to ascertain pharmacalogical data aforementioned.

Details in procedure by using Auto-Analyzer were described in elsewhere[6]. The blood samples were collected from femoral artery on right thigh, then each blood sample of a group (8 mice/group) was pooled and it was centrifuged in 1490×g for 20 min. at room temperature. The supernatant serum portions were used for assay of enzyme activity.

Results and Discussion

Plantago species have long been used for treatment of asthma and lung diseases and others as a folkloric agents[7]~[9]. And Plantago seeds also are descrised to be a mucilaginous agents in National Formulary. In addition, our previous report showed that mice received methanol extract of Plantago seed exerted marked reduction of sleeping time induced with hexobarbital after CCl_4 intoxication[3].

With these connections, present study aimed to fractionate total methanol extracts of Plantago seed with various solvents in order to attempt isolating active ingredients. As the Chart 1 shows, about 200g of methanol extract was fractionated firstly with petroleum ether-methanol-water (10 : 1 : 9) system. From the petroleum ether layer, 42.8g of dried fraction was obtained. The methanol-water layer was

Table I. Pharmacological Evaluation of Hepatotonic activities.

	Days					Duration of sleep(min)
	1	2	3	4	5	
Control	Saline*				Hexobarbital*	13
Water	Fraction*					11
CCl_4	Saline	CCl_4*		Saline		21
Methanol	Fractions	Fractions+CCl_4		Fraction		19
Water						14
Chloroform						18
Petroleum ether						73
n-Butanol						27

* each group consists of 8 mice, (male, 20±1g)

* saline, 0.9% physiological saline
CCl_4 dissolved in vegetable oil was given orally (0.13ml/kg/day/mouse). Hexobarbital sodium salt was dissolved in physiological saline and the dose of 50mg/kg of mouse was each i.p. injected.

* Each fraction was suspended in physiological saline and the dose of 670mg/kg of mouse was each administered orally.

* The measurement of duration of sleep: as soon as mouse get sleep, they were layed on side, and then they stood up and moved forward, the duration was considered as sleeping time.

further fractionated with sufficient amount of chloroform. From the chloroform layer, about 13.5g of dried fraction was obtained. Remaining water layer was mixed with n-butanol thoroughly. From butanol and water layer, about 43.8g and 97g of dried fraction were obtained, respectively. Many reports indicated that rat liver injury with halogenated hydrocarbon like expecially CCl_4 caused altered structural and metabolic pattens of liver tissue and cells [10-12]. Distinctive changes of the endoplasmic reticulum, depression of microsomal enzymes activities and marked depression of hepatic protein synthesis are well demonstrable with CCl_4 administration[13~15]. Following such disturbances, liver necrosis is usually accompanied[16]. The animal model of hepatitis in present experiment, the chosen dose-schedule of CCl_4 could induce histological changes in appearane of liver closely equivalent to diffused hepatitis in man[4,17]. As the data shown in table I. equal amount of each fraction(670mg/kg/mouse/day) was employed to demonstrate its hepatotonic activities regardless of what amount of fraction obtainable after fractionation. As the data showed, CCl_4 group animals exhibited increased duration of sleep in comparison with those of the control group. In addition, it should be pointed out that the water fraction alone without CCl_4 administration showed slightly less than that of control group. This imply that water fraction exhibited virtually no significant liver injury in terms of sleeping time measurement.

In case of CCl_4 plus water fraction treated group the duration of sleeping time appeared to be slightly longer than that of control group, but it was significantly shorter than that of CCl_4 alone administered group. The methanol and chloroform administered groups exhibited slightly shorter than that of CCl_4 group, respectively. These data indicated that there is a possibility in which some active components of Plantago seed fractions could be soluble in both solvent systems, however, another possibility in which chemically different components possessing hepatotonic activitity dissolved in both solvents will not be eliminated. Prolongation of duration of sleep with petroleum ether and n-butanol treatment in comparison with that of CCl_4 group is of interest to us. This results implied that certain substances affecting toxicity on injuring liver could exist in both solvent systems.

Balazs et. al.[18] and Zimmerman et.al.[19] have

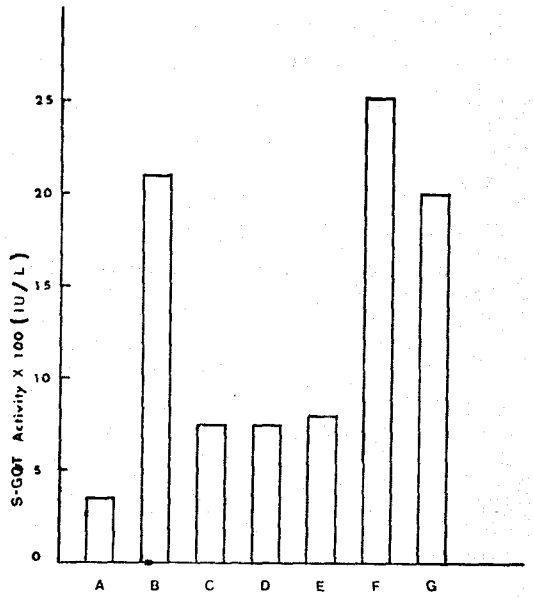

Fig. 1. S-GOT activities: same dose-schedule as in table I were employed.

The blood samples were collected fromfemoral artery of right thigh. All blood in each group were pooled and serum was seperated by centrifugation.

A: Control
B: CCl_4
C: Methanol fraction+CCl_4
D: Water fraction+CCl_4
E: Chloroform fraction+CCl_4
F: Petroleum ether fraction+CCl_4
G: n-Butanol fraction+CCl_4

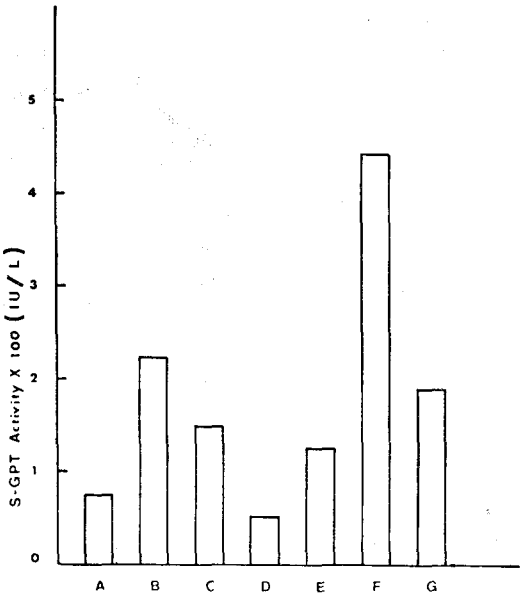

Fig. 2. S-GPT activities: all procedures and dose-schedule were same as in Fig. 1.

shown a relation between S-GTP and S-GOT enzyme activities and CCl_4 liver injury. There fore, we attempted to ascertain the correlations between durations of sleeping time caused by CCl_4 intoxication and the activities of serum GOT and GPT enzymes. As the figures 2 and 3 showed, S-GOT activities of CCl_4 alone treated group exhibited marked increase in comparison with those of control methanol fraction plus CCl_4 and water fraction plus CCl_4 groups, whereas the groups of n-butanol and petroleum ether fractions plus CCl_4 showed still profound elevation of enzymes activities. Therefore,these data appeared to be correlated with the experiments of the measurement of sleeping time aforementioned.

With respect to the evaluation systems employed so far, the most active fraction appeared to be water fraction. In this context, however, it should be pointed out that certain chemicals are able to cause the increase of liver microsomal enzymes responsible for the oxidative metabolism of hexobarbital and disturbance of CCl_4 absorption into gastrointestinal tract[17,20]. They are not hepatotonic agents, though.

With respect to such facts, the results obtained from the present experiments can only be used as an indication for the purpose of preliminary test of hepatotonic activities. Nonetheless, it is believed that present works warrant for further studies.So further fractionation and purification of water fraction and histological studies with liver biopsy are being undergone in authors' laboratory.

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