BIOLOGICAL ACTIVITIES OF FRAXINUS RHYNCHOPYLLA

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INTRODUCTION

Fraxini Cortex has been used as decoction for heat regulat ion and visual obstruction from ancient period(1). Recently, it is reported that Fraxinus cortex may posses the anti-inflammatory effect, analgesic effect, and diuretic effects(2). The liver cells may be easily encountered with numerous of chemicals and biological agents, such as plant products, fungal products, bacterial metabolites, medicinal agents, pesticides, and industrial by-products, and taken as food contaminants or as accidental inhalation or ingestion(3, 4). These foreign compounds is mainly converted to more hydrophillic or nontoxic metabolites by microsome enzymes of liver parenchymal cell, but some compound converted into highly reactive and toxic materials in liver. Alteration of xenobiotics to highly reactive intermediates produce radicals, and which binds covalently with cell component, and lead to inhibition of a variety function of intracellular enzymes or disruption of integrity of liver cell membrane(5, 6). In this case, cytoplasmic components release into extracellular fluid, and lipidperoxidation occurs in cell membrane. While, protection mechanisms may bring about rapid removal of radicals and inactivation. In this experiment, author have investigated the liver toxicity, and evaluate activities of potential hepatoprotective agent prepared from natural sources(7, 8). Fraxini cortex were used as water extract for application to disease in oriental medicine, but the information is lack about the effect of Fraxinus rhynchopylla methanol-extracts on liver injury induced by hepatotoxicants.

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EXPERIMENTAL METHODS

EXPERIMENTAL ANIMALS

Spraque-Dawley rats (200-250g) and ICR mice (about 20g) were housed three and five per plastic cage on hard wood chips and acclimatized for at least 7 days prior to use. The animal room temperature was maintained at 20-24°C, relative humidity at 50-60%, and controlled lightning interval. Rats were fed an unrefinded diet and tap water *ad libitum*.

SAMPLE PREPARATION AND TREATMENT

Fraxinus rhynchophylla were purchased from Kyung-dong Korean market in Seoul. The 1 kg of this herb was disintegrated and extracted in hot MeOH for 6 hours, and dryed with evaporator and freeze dryer. After 1 weeks of acclimatization, the rats and mice were divided into four

groups; control group, CCl_4 group, high dose of extracts and CCl_4 group, low dose of extracts and CCl_4 group. The extract was administered orally for 4 days at the dose of 100mg/kg and 20mg/kg, and CCl_4 (0.5ml/kg) was administered orally once at third day in each group with the exception of control.

PREPARATION OF LIVER HOMOGENATE AND MICROSOME FRACTION

Six rats from each group were killed by decapitation at 4 hours after final administeration. Livers were perfused *in situ* with ice cold 1.15% KCL containing 0.1mM EDTA. Whole liver homogenates were prepared by mincing and then homogenizing with Ultra-Turrax. The whole homogenate was centrifuged at 3,000 x g for 10 min. The supernatant was centrifuged again at 10,000 x g for 20 min, and the supernatant fraction centrifuged once more 105,000 x g for 1 hour in ultracentrifuge. Pellet was resuspended in PBS solution. All procedure was done below 4°C.

MDA CONTENTS

MDA contents measured using liver homogenates and microsome fraction according to previously described(9). In briefly, liver homogenates and sodium lauryl sulfate were mixed and incubated for 30 minutes. 0.1 N of HCL and TBA were added then, heated at 95°C for 1ours. After centrifugation, reaction products were measured. Protein was determined by the method of Lowry et al(11).

SERUM BIOCHEMICAL FACTORS

The rats were anesthetized with ethyl ether, and whole blood was withdrawn with heartpuncture using plastic syringe. After standing in dark room, tubes containing blood was centrifuged at 3,000 rpm for 30 minutes in order to get serum. The level of AST and ALT was measured by enzymatic method(10).

STATISTICAL ANALYSIS

Student's t-test was employed to asscess the statistical significance. Values which differ from contrl over $p<0.05$ were considered as significant.

RESULTS AND DISCUSSION

Recently, a lot of efforts have been made to elucidate the biochemical pathway of liver necrosis and protective compounds against hepatotoxins(12, 13). But, the exact mechanism involved in the cytotoxicity of those chemicals to liver cells are not understood entirely, it is generally recognized as the result of any or combination of a variety of biochemical alterations in the cell. Membrane damage, which is the direct cause of cell death, can influence the intracellular or membrane associated protein, such as receptor, transporters, and the other enzymes. Toxic agents may reacteither with the protein or lipid components and significantly alter transport function and thus cellular integrity. These effect may disrupt a variety of transport or permeability mediated physiological and biochemical functions and result in a wide

specrum of toxic events(14). In the case of CCl_4 induced liver toxicity, the basic sequence of events involves initial generation of the trichloromethyl radical at cytochrome locus of the monooxygenase system(15). These initial events are accompanied by covalent binding of CCl_4 cleavage product largely to lipid and protein of liver cell ER(16) and by the initiation of lipid peroxidation(17). Breakdown of the cell membrane by covalent binding with free-radical causes the disturbance of the function of those membrane bound enzymes to the extracelluar fluid. The leakage of cytoplasmic enzyme, AST, ALT, and lipid peroxidation are known as good signs of membrane damages. Therefore, evaluation parameters of hepatic membrane injury in this study were assessed by AST and ALT activity, BUN in the serum, and MDA contents in liver homogenates and microsome. As shown in the results, FRE decreased ALT and AST activities which is increased by CCl_4 toxicities in serum. And, it also showed a protective effect on MDA production induced by CCl_4 intoxication. This results imply the possibility that FRE possess some radical scavenging components as antioxidants. These antioxidants affects the protection system, such as glutathione peroxidase(18, 19), glutathione-S-transferase(20, 21), glutathione reductase(22), superoxide dismutase(23), and catalase(24). Once reactive metabolites are formed in liver, Protection and defense mechanism may bring about their rapid removal and inactivation. Toxicity then depends on the balance between th rate of metabolite formation and the trate of removal. Glutathione is the most important and widely occuring nonprotein thiol in living system that plays a major role in many redox and detoxification reaction in the liver(25). The availability of GSH may be the factor stimulating the excretion of the reactive and radical intermediate through conjugation reaction in Phase II. In cells, GSH(reduced glutathione) converted into GSSG(oxidized glutathione) to detoxify the endogeneous hydrogen peroxide or lipid peroxides. And the redox status of glutathione can be maintained by NADPH/NADP sytem and glutathione reductase(22, 26) The tetrachlorocarbon is converted into reactive compounds, trichloromethyl radical and trichloro -methylperoxy radical, in liver microsome, and those attack membrane and/or deplete GSH. Therefore, Liver injury may be prevented by some compounds which stimulate GSH-production and/or scavenge the radical intermediates.

In this study, FRE showed a protective effect on liver damage induced by tetrachlorocarbon. Though precise mechanism is not clear, it is supposed that FRE may act on, at the least, one of defense system mentioned above.

Table I. Effects of FRE on indics (MDA) of lipid peroxide concentrations in liver homogenates.

GROUP	MDA (nmol/mg protein)
Control	1.31 ± 0.24
CCl_4	4.89 ± 1.36
CCl_4 + FRE(100mg/kg)	3.16 ± 0.89*
CCl_4 + FRE(20mg/kg)	4.53 ± 0.72

FRE: Fraxinus rhynchopylla extracts
*: Significant, $p<0.05$

Table II. Antiperoxidative effect of FRE in liver microsome fraction.

GROUP	MDA(nmol/mg protein)
Control	2.04 ± 0.46
CCl_4	5.96 ± 0.91
CCl + FRE (100mg/kg)	3.47 ± 0.79*

FRE: Fraxinus rhynchopylla extracts
*: significant, p<0.05

Table III. Effects of FRE on AST activities in CCl_4 intoxicated rats.

GROUP	AST activities (IU/L)
Control	133.35 ± 14.42
CCl_4	323.62 ± 17.68
CCl_4 + FRE(100mg/kg)	213.17 ± 18.56*
CCl_4 + FRE(20mg/kg)	321.25 ± 21.73

FRE: Fraxinus rhychopylla
*: significance, P<0.05

Table IV. Effects of FRE on ALT activities in CCl_4 intoxicated rats.

GROUP	ALT activities (IU/L)
Control	67.57 ± 12.02
CCl_4	203.14 ± 21.90
CCl_4 + FRE(100mg/kg)	122.81 ± 17.62*
CCl_4 + FRE(20mg/kg)	179.65 ± 13.14

FRE: Fraxinus rhynchopylla extracts
*: significant, p<0.05

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