# EVALUATION OF ARALIA ELATA ON BIOLOGICAL RESPONSES

林錫麟*

## INTRODUCTION

The liver helps protect the body from various foreign or dangerous materials by detoxification mechanism. Many noxious or comparatively insoluble compounds are converted to other forms. The conversion to other forms may involve various metabolic reactionesterification, oxidation, reduction, hydroxylation(1). In most case, these hepatic metabolites are less toxic or water soluble and therefore excretable. But some xenobiotics may converted into more toxic metabolites by hepatic function. These transformation of foreign materials into highly reactive intermediates may result in hepatic injury, and loss of normal functions of liver cell. The hepatic cell membrane may be ideal receptor sites for reactive chemicals and biological agents, such as plant products, fungal products, bacterial metabolites, medicinal agents, pesticides, and industrial by-product(2,3). Disruption of the membrane by toxic agents may result in a variety of toxic phenomena, such are dysfunction of protein of ER, release of cytoplasmic and membrane enzymes, and eventual necrosis. Therefore, it may be very important study that evaluate hepatic toxic agent and find out a potent protective agents from natural products(4,5).

Araliae elata has been used for psychotic disorder, constipation,

* 大田大學校 韓醫學大學 藥理學教室

arthritis, and bleeding from ancient period in oriental medicine.(6) In modern medicinal studies, the decoction of this herb has been reported to show the anti hyperglycemic effects(7), inhibitory effect on peptic ulcer(6). It has also been reported that Aralia elata decrease total lipid level andlipidperoxidation(8). But the information is lack about the protective effect of Aralia elata on depletion of intracellular components in liver injured by hepatotoxicants.

# EXPERIMENTAL METHODS

## EXPERIMENTAL ANIMALS

Spraque-Dawley rats (200-250 g) 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 ℃, relative humidity at 50-60%, and controlled lightning interval. Rats were fed an unrefinded diet and tap water ad libitum.

## SAMPLE PREPARATION

The 1 kg of Aralia elata was disintegrated and extracted with hot MeOH in reflux extraction apparatus for 6 hours. An aquous fraction was obtained in each separation steps those were added consequently with hexane, CHCl3, and EtOAc. And finally, BuOH fraction was obtained from aquous fraction, and concentration and drying was done with evaporator and freeze dryer.

## SERUM PROTEIN LEVEL

The level of AST and ALT was measured by enzymatic method(9).

## HEPATOCYTES PREPARATION AND SAMPLE TREATMENT

Hepatocytes were prepared as previously described(10). Viability of cell were estimated by staining with tryptophan blue, about 80% cells were viable initially. The isolated cells were suspended to about 6 x 106 cells/㎖ in DMEM medium buffer(Sigma). Culture dish were incubated at 37C in CO2 incubator. BrCCl3(0.3㎕/㎖) and

various concentration of AEE were added to hepatocytes suspension, and incubated at 37℃ for 30 min

ISOLATION HEPATIC MICROSOME

The hepatocyte suspension was washed three times, and homogenated with polytron pestle. The whole homogenate was centrifuged at 2800 x g, 10 min. The supernatant was centrifuged at 8,000 x g, 10 min to obtain mitochondria fraction in pellet. Supernatant fraction was centrifuge at 10,000 x g, 30 min, pellet was discard. And, centrifugation at 105,000 x g, 60 min result in microsomal pellet. The pellet was resuspended in 0.1M phosphate buffer (PH 7.4). All procedure were done below 4℃.

MDA CONTENTS

MDA contents measured using hepatocyte and microsome according to Stacey et al.(12). In briefly, membrane fraction (or microsome) and sodium lauryl sulfate were mixed and incubated for 30 minutes. 0.1 N of HCL and TBA were added then, heated at 95 ℃ for 1ours. After centrifugation, reaction products were measured. Protein was determined by the method of Lowry et al.(11)

INTRACELLULAR GLUTATHIONE

After the addition of 0.5% picric acid to the washed liver cells, the cells were collected. And then, protein was removed by centrifugation at 12,000 x g, 10 min. The supernatant was withdrawn for the determination of glutathione. Total glutathione and oxidized glutathion were measured as previously described(13).

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

Liver injury induced by chemicals has been recognized as a toxicologic problem for close to a century. A numerous toxicants, such as halogenated hydrocarbon, pesticides, medicinal compounds, industrial pollutants, have been reported to produce liver necrosis. The membrane is uniquely vulnerable to toxic agents, and which is

an ideal receptor site for reactive chemicals. Toxic agents may react either 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 biochemical functions and result in eventual cell necrosis. Carbon tetrachloride and bromotrichloromethane have been regarded as useful tools for hepatotoxicity studies to improve our understanding of the mechanism of cell damage and cell death induced by hepatotoxic drugs. They are transformed to trichloromethyl radical by cytochrome P450 of mixed function oxidases(14). This free radical is generally considered to be highly reactive. And its action mechanism is that elicit cell membrane damage directly(15, 16). 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. Once reactive metabolites are formed, protection mechanisms within the cell may bring about their rapid removal and inactivation. With some compounds, reduced glutathione play an important protective role by trapping metabolites and preventing their binding to hepatic proteins and enzymes(17, 18). The conjugation of glutathione usually results in the formation of a nontoxic, water soluble metabolites that is then easily excreted.

The leakage of the cytoplasmic proteins, AST and ALT from liver cell is increased in serum of CCl4 intoxicated rats (Table I, II). And, when AEE solution was added to hepatocytes culture, MDA production was reduced (Table III). This results imply the possibility that AEE 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). 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 glutathione may be the factor stimulating the excretion of the reactive and radical intermediate through conjugation reaction in Phase II. In cells, the reduced glutathione converted into the 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(26,27). Consideration that toxicity depends on the balance between the rate of metabolite formation and the rate of removal, and liver injury may be prevented by some compounds which stimulate GSH-production and/or scavenge the radical intermediates, the level of glutathione is very important parameter in estimation of liver toxicity or evaluation of hepatoprotective agents.

In this study, AEE protects the glutathione depletion during liver cell damage(Table V). Though precise mechanism is not clear, it is supposed that AEE may act on, at the least, one of defense system mentioned above.

Table I. Hepatoprotective activities of AEE in CCl4 intoxicated rats.

| GROUP | AST activities (IU/L) |
|---|---|
| Control | 106.63 ±11.75 |
| $CCl_4$(2㎖/kg) | 244.18 ±14.47 |
| $CCl_4$ + AEE(100㎎/kg) | 152.82 ±18.23* |
| $CCl_4$ + AEE(20㎎/kg) | 202.62 ±13.54* |

AAE: Aralia elata Extracts
*: significance, P<0.05

Table II. Effects of AEE on ALT activities in CCl4 intoxicated rats.

| GROUP | ALT activities (IU/L) |
|---|---|
| Control | 67.34 ±9.81 |
| $CCl_4$(2㎖/kg) | 162.13 ±28.29 |
| $CCl_4$ + AEE(100㎎/kg) | 112.02 ±12.68* |
| $CCl_4$ + AEE(20㎎/kg) | 143.165 ±19.13 |

AEE: Aralia elata extracts
*: significant, $p<0.05$

Table III. Effects of AEE on indics (MDA) of lipid peroxide concentrations in hepatocytes.

| GROUP | MDA (㎚ol/$10^6$cells) |
|---|---|
| Control | 1.22 ±0.11 |
| $CCl_3$ | 3.18 ±0.46 |
| $CCl_3$ + AEE(0.5㎎/㎖) | 2.06 ±0.27* |
| $CCl_3$ + AEE(0.05㎎/㎖) | 2.96 ±0.36 |

AEE: Aralia elata Extracts
*: Significant, $p<0.05$

Table IV. Antiperoxidative effects of AEE on microsome prepared from hepatocytes

| GROUP | MDA (㎚ol/㎎ protein) |
|---|---|
| Control | 2.09 ±0.38 |
| $CCl_3$ | 4.62 ±0.63 |
| $CCl_3$ + AEE(0.5㎎/㎖) | 3.21 ±0.56* |
| $CCl_3$ + AEE(0.05㎎/㎖) | 4.48 ±0.86 |

AEE: Aralia elata extracts
*: Significant, $p<0.05$

Table V. Effect of AEE on intracellular glutathione level in rat hepatocytes

| GROUP | Glutathione(nmol/mg protein) |
|---|---|
| Control | 18.66 ±2.46 |
| $CCl_3$ | 5.28 ±1.12 |
| $CCl_3$ + AEE(0.5mg/mℓ) | 11.45 ±3.21* |

AEE: Aralia elata extracts
*: Significant, $p<0.05$

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