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Hypolipidemic and Hepatoprotective Effects of Picrorrhiza Rhizoma in High Fat Diet Supplied Mice. A Pevention Sudy.

Hyeung Sik LEE[1], Sung Jung Woo[1] and Sae Kwang Ku[2,3,*]

[1]Department of Herbal Biotechnology, Daegu Haany University, Gyeongsan 712-715, Korea
[2]Department of Anatomy and Histology, College of Oriental Medicine, Daegu Haany University, Gyeongsan 712-715, Republic of Korea
[3]Development Team for The New Drug of Oriental Medicine (BK21 program), Daegu Haany University, Gyeongsan 712-715, Republic of Korea

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Abstract – The preventive hypolipidemic effect of the aqueous extracts of Picrorrhiza Rhizoma (PR) was observed in a high fat diet (HFD) feeding hyperlipidemic mouse with their hepatoprotective effects. PR extracts (50, 100 and 200mg/kg) were orally dosed once a day for 12 weeks initiated with HFD supply, and changes on body weight and gains, liver weight, serum aspartate transferase (AST) and alanine transferase (ALT) levels were monitored with serum low density lipoprotein (LDL), high density lipoprotein (HDL), triglyceride and total cholesterol levels. The efficacy of test articles was compared to that of 10mg/kg of simvastatin (SIMVA). Dramatic decrease of both absolute and relative liver weight was dose-dependently observed in all PR extract dosing groups as compared with HFD control group. The serum AST and ALT levels were dose-dependently decreased in PR extract dosing groups. The serum LDL, triglyceride and total cholesterol levels were dose-dependently decreased in PR extract dosing groups compared to that of HFD control group. The serum HDL levels were slightly but dose-dependently increased in PR extract dosing groups as compared with control group. The efficacy on the serum lipid levels of PR extracts was slighter than that of SIMVA. Based on these results, it is concluded that water extract of PR has a relatively good favorable preventive effects on the HFD inducing hyperlipidemia and hepatopathy.

Keywords □ Picrorrhiza Rhizoma, extracts, high fat diet, hyperlipidemia, effect, liver

INTRODUCTION

High blood cholesterol has been ranked as one of the greatest risk factors contributing to the prevalence and severity of coronary heart disease (Neaton *et al.*, 1984; Grundy, 1986). In general, more than 200 mg/dl of blood cholesterol levels or > 180 mg/dl of blood triglyceride levels are regarded as hyperlipemia. In addition, hyperlipemia is also induced by secondary effect of diabetes (Pari *et al.*, 2004), and liver damages are generally induced in the condition of hyperlipemia as dramatic increase of serum aspartate transferase (AST) and alanine transferase (ALT) levels (el-Saadany *et al.*, 1991). Therefore, the agents having some hepatoprotective and/or anti-diabetic effect also showed favorable effect to the hyperlipemia (Hoyos *et al.*, 2000; Bhandari *et al.*, 2002; Le *et al.*, 2004; Park *et al.*, 2004). HMG Co A reductase inhibitor has been used as a treatment of hyperlipemia (Vickers *et al.*, 1990) and simvastatin (SIMVA) is one of the most prevalently used HMG Co A reductase inhibitors (Steinmetz *et al.*, 2005). The treatment of hyperlipemia has been conducted to reduce the increased low density lipoprotein (LDL) and triglyceride, and to increase the decreased high density lipoprotein (HDL). Simple and sensitive *in vitro* assay systems are essential to screen active agents efficiently. However, although a variety of *in vitro* screening systems for hypolipemic agents have been developed, the results of the *in vitro* experiments do not always match those of *in vivo* experiments. In animal models of high fat diet (HFD), dramatical increase of serum LDL, triglyceride and total cholesterol levels were reported with relatively slight changes on HDL (Morishita *et al.*, 1986). The effect of various hypolipemic agents has been evaluated by using this HFD models, and the effects of a test article would be based on

*Corresponding author
Tel: +82-53-819-1549, Fax: +82-53-819-1269
E-mail: gucci200@hanmail.net

the serum LDL, HDL, triglyceride and total cholesterol levels (Yokode *et al.*, 1990; Han *et al.*, 2005). In addition, liver damages are also induced in HFD models (el-Saadany *et al.*, 1991; Senthil Kumar *et al.*, 2001).

A traditional Korean herbal medicine, Picrorrhiza Rhizoma (PR) is a dried root and stem of *Picrorrhiza kurroa* (Scrophulariaceae), and has been used as hepatoprotective agents such as jaundice. Until now, the nitric oxide scavenging activity (Jagetia and Baliga, 2004), cardioprotective effect (Senthil Kumar *et al.*, 2001), anti-cancer effect (Jeena *et al.*, 1999; Joy *et al.*, 2000), anti-diabetic activity (Joy and Kuttan, 1999) and anti-viral effect (Mehortra *et al.*, 1990) of PR extracts have been evaluated. The agents having some hepatoprotective and/or anti-diabetic effect also have been showed favorable effect to the hyperlipemia (Hoyos *et al.*, 2000; Bhandari *et al.*, 2002; Le *et al.*, 2004; Park *et al.*, 2004). Although, the therapeutic effect of PR on HFD induced hyperlipemic model (Lee *et al.*, 2006a) and preventive effects in PX-407 model (Lee *et al.*, 2006b) are already known, the preventive effects of PR on HFD induced hyperlipemia has not been evaluated yet.

The purpose of this study is to evaluate the hypolipemic potentials of PR extracts in HFD hyperlipemic mice with their hepatoprotective effects. In this study, PR extracts (50, 100 and 200mg/kg) were orally dosed once a day for 12 weeks initiated with HFD supply to detect preventive effects. The efficacy of test articles was compared to that of 10mg/kg of SIMVA.

MATERIALS AND METHODS

Animals and husbandry. Thirty male ICR mice (6-wk old upon receipt, Charles River, Japan) were used after acclimatization for 7 days. Animals were allocated 5 per polycarbonate cage in a temperature (20-25°C) and humidity (30-35%) controlled room. Light : dark cycle was 12hrs : 12hrs, and feed and water were supplied free to access. After acclimatization, twenty-five animals were supplied HFD (Dyets, PA, USA) containing 1% cholesterol and 0.25% sodium cholate free to access listed in Table I to induce the hyperlipemia during experimental periods, and normal mouse pellet diets (Samyang, Korea) were supplied in 10 animals for normal control.

Experimental design. Experimental groups were divided into normal control, HFD control, 10 mg/kg of SIMVA-dosing group, 50, 100 and 200 mg/kg of PR extracts-dosing groups. To observe the preventive effects, PR extracts were orally dosed once a day for 12 weeks initiated with HFD supply. Aqueous PR extracts (absorption rate 25.63%) were prepared by routine methods using rotary vacuum evaporator (Lab. Camp, Korea) and programmable freeze dryer (IIShin Lab., Korea) from root and stem of *Picrorrhiza kurroa*, which were purchased from Cho-Heung Pharmaceutical Ind. Co. (Daegu, Korea). Powders of PR extracts were stored in a desiccator to protect from light and moisture. The PR extract was dissolved in injectable distilled water and administered at a dosage volume of 10 ml/kg by oral gavage.

Table I. Composition of high fat diet used in this study

Ingredient	Kcal/gm	grams/kg	Kcal/kg
Casen	3.72	200	744
DL-Methionine	4	3	12
Cornstarch	3.6	150	540
Sucrose	4	487.5	1950
Cellulose	0	50	0
Corn Oil	9	50	450
Mineral Mix	0.47	35	16.45
Vitamin Mix	3.92	10	39.2
Choline Bitartrate	0	2	0
Sodium Cholate	0	2.5	0
Cholesterol	0	10	0

[Dyets Inc., PA, USA]

Body weight measurement. The body weights were measured at 1 day before dosing, initial dosing, 7, 14, 35, 56, 79, 83 and 84 days (at sacrifice) after dosing. To reduce the erratum originated from feeding, all animals were fasted (water was not restricted) about 18hrs before initial dosing and sacrifice. In addition, the body weight gains during dosing periods were calculated to reduce the individual differences as body weight at sacrifice – body weight at initial dosing.

Liver weight measurement. Liver weights at sacrifice were detected as g levels and regards as absolute weight. In addition, the ratio of liver compared to the body weight was calculated as the relative liver weights to reduce the individual body weight differences as ((Absolute liver weight/Body weight at sacrifice) × 100)

Serum biochemistry. At sacrifice, 1 ml of venous blood was collected from vena cava under anesthesia. All blood samples were centrifuged at 3,000 rpm for 10min

under room temperature using clotting activated serum tube. Serum AST and ALT levels were detected with an automated blood analyzer (Toshiba 200FR, Japan) as IU/l using Kinetic UV methods. Serum LDL, HDL, triglyceride and total cholesterol levels were detected with an automated blood analyzer as mg/dl using enzyme assay.

Statistical analyses. Results are expressed as the mean ± standard deviation. Mann-Whitney U-Wilcoxon Rank Sum W test (MW test) was used to analyze the significance of data with SPSS for Windows (Release 6.1.3, SPSS Inc., USA) and a *P*-value of less than 0.05 was considered to be a significant difference. In addition, the % changes compared to those of normal or HFD controls were calculated as follows to help the understanding of the efficacy of test materials on differences between control and test groups in all data from results except for body weight. Percentage changes compared to that of normal control (%) = [((Data of HFD control – Data of Normal control)/Data of Normal control) × 100]; Percentage changes compared to that of vehicle control (%) = [((Data of test groups – Data of HFD control)/Data of HFD control) × 100].

RESULTS

Changes on the body weight. A significant increase of body weight was detected in HFD control from 14 days after dosing compared to that of normal control. In addition, similar changes on body weight were also detected in SIMVA and all PR extract dosing groups compared to that of HFD control (Table II). The body weight gains during dosing periods were also significantly increased compared to that of normal control in HFD control group. Similar changes on body weight gains were also detected in SIMVA and all PR extract dosing groups compared to that of HFD control, and they showed only -6.03, -5.34, 0.58 and 1.55% changes compared to that of HFD control group, respectively.

Changes on the liver weight. A significant increase of absolute and relative liver weights was detected in HFD control group compared to that of normal control, respectively as 190.43% increased in absolute and 136.98% increased in relative weights compared to that of normal control. However, dramatic decreases of both absolute and relative liver weight were observed in all PR extract dosing groups compared to that of HFD control group with dose dependent patterns. In SIMVA group, similar values on absolute and relative liver weights compared to that of HFD control (Fig. 1A, B) were detected. The absolute liver weight on SIMVA, 50, 100 and 200 mg/kg of PR extract dosing groups were showed 3.50, 11.91, 23.01 and 27.83% decrease compared to that of HFD control group, respectively. The relative liver weight on SIMVA, 50, 100 and 200 mg/kg of PR extract dosing groups showed 1.93, 8.86, 15.83 and 28.82% decrease compared to that of HFD control group, respectively.

Changes on the serum AST and ALT levels. A significant increase of serum AST and ALT levels was detected in HFD control group compared to that of normal control as 179.02% increase in AST and 246.35% increase in ALT levels compared to that of normal control. However, dramatic decreases of serum AST and ALT levels were observed in all PR extract dosing groups com-

Table II. Changes on the body weight in high fat diet supplied mice after PR extract dosing

Group	Normal control	HFD control	Simvastatin 10 mg/kg	PR extracts		
				50 mg/kg	100 mg/kg	200 mg/kg
Day -1	30.48±1.84	30.62±2.63	30.84±1.63	30.72±0.65	30.48±2.45	30.92±2.06
Day 0[a)]	27.38±2.30	27.68±2.81	27.84±1.71	27.56±1.12	27.50±1.99	28.26±2.04
Day 7	34.46±1.69	37.06±4.15	36.12±2.78	36.14±3.05	37.14±2.30	36.76±2.49
Day 14	34.72±3.79	42.12±3.25*	40.62±2.28*	40.80±2.69**	40.56±2.38**	41.42±3.71**
Day 35	36.82±2.54	44.76±2.60*	44.54±2.63*	43.64±2.72*	43.56±2.83*	44.66±4.24*
Day 56	39.06±1.95	46.42±3.29*	45.68±2.04*	45.34±2.68*	44.86±2.91**	47.16±3.37*
Day 70	40.38±1.88	48.70±3.36*	47.46±2.92*	47.00±3.91*	47.42±2.91**	49.56±3.60*
Day 83	42.28±2.44	50.96±3.51*	49.50±3.81**	50.04±4.63*	50.78±5.21**	51.62±3.52*
Day 84[b)]	39.34±2.47	48.26±3.33*	47.18±3.89**	47.04±4.22**	48.20±5.05*	49.16±3.01*

Mean±S.D., g (n=5); a) At initial dose after fasting; b) At sacrifice after fasting; *P<0.01 compared to that of sham by MW test; **P< 0.05 compared to that of sham by MW test.

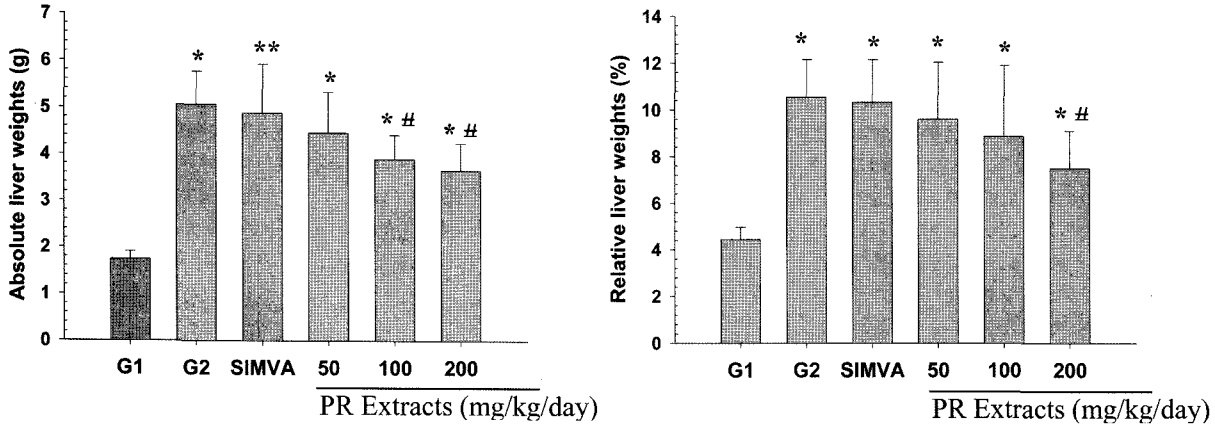

Fig. 1. Effect of aqueous extract of root and stem of *P. kurroa* (PR) on liver weight in HFD supplied mice. **P*<0.01 and ***P*<0.05 compared to G1 by MW test, #*P*<0.01 compared to that of G2 by MW test. *N* = 5 mice (Mean±S.D.). G1=normal control.G2=HFD control. SIMVA = Simvastatin 10 mg/kg/day

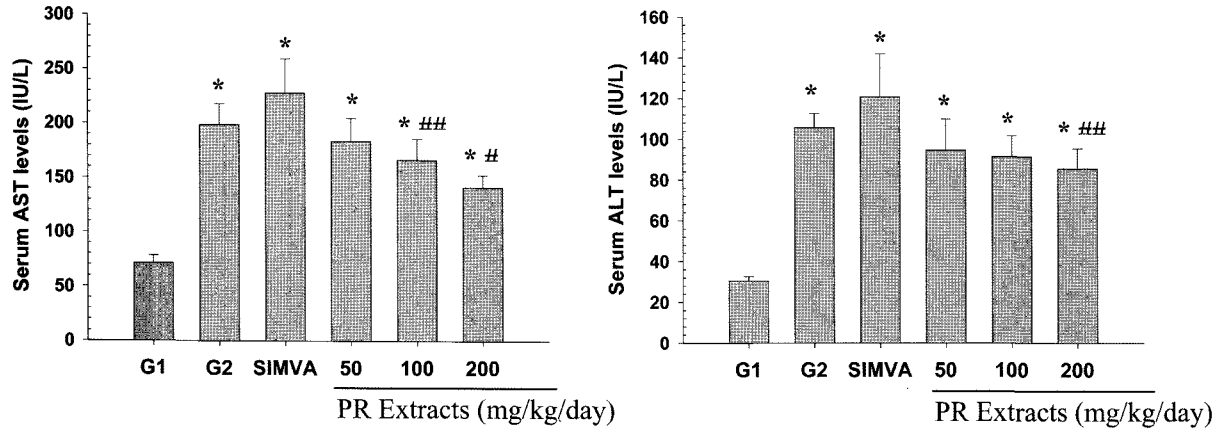

Fig. 2. Effect of aqueous extract of root and stem of *P. kurroa* (PR) on the serum AST and ALT levels in HFD supplied mice. **P*< 0.01 compared to G1 by MW test, #*P*<0.01 and ##*P*<0.05 compared to that of G2 by MW test. *N* = 5 mice (Mean±S.D.). G1=normal control.G2=HFD control. SIMVA = Simvastatin 10 mg/kg/day

pared to that of HFD control group with dose dependent patterns. In SIMVA group, it is detected that some increase but not significant were detected compared to that of HFD control (Fig. 2A, B). The serum AST levels on SIMVA, 50, 100 and 200 mg/kg of PR extract dosing groups showed 14.76, -7.63, -16.24 and -28.84% changes compared to that of HFD control group, respectively. In addition, the serum ALT levels on SIMVA, 50, 100 and 200 mg/kg of PR extract dosing groups showed 14.32, -10.46, -13.47 and -19.05% compared to that of HFD control group, respectively.

Changes on the serum LDL, HDL, Triglyceride and total cholesterols. A significant increase of serum LDL levels as 582.05% was detected in HFD control group compared to that of normal control. However, dramatic decreases of serum LDL levels were observed in all PR extract and SIMVA dosing groups compared to those of HFD control group with dose dependent patterns (Fig. 3A). The serum LDL levels on SIMVA, 50, 100 and 200 mg/kg of PR extract dosing groups were decreased as 33.46, 18.05, 26.32 and 31.95% compared to that of HFD control group, respectively. A significant increase of serum HDL levels as 62.00% was detected in HFD control group compared to that of normal control. Some increases of serum HDL levels were also observed in all PR extract and SIMVA dosing groups compared to those of HFD control group with dose dependent patterns (Fig. 3B). The serum HDL levels on SIMVA, 50, 100 and 200 mg/kg of PR extract dosing groups showed 21.85, 7.48, 9.58 and

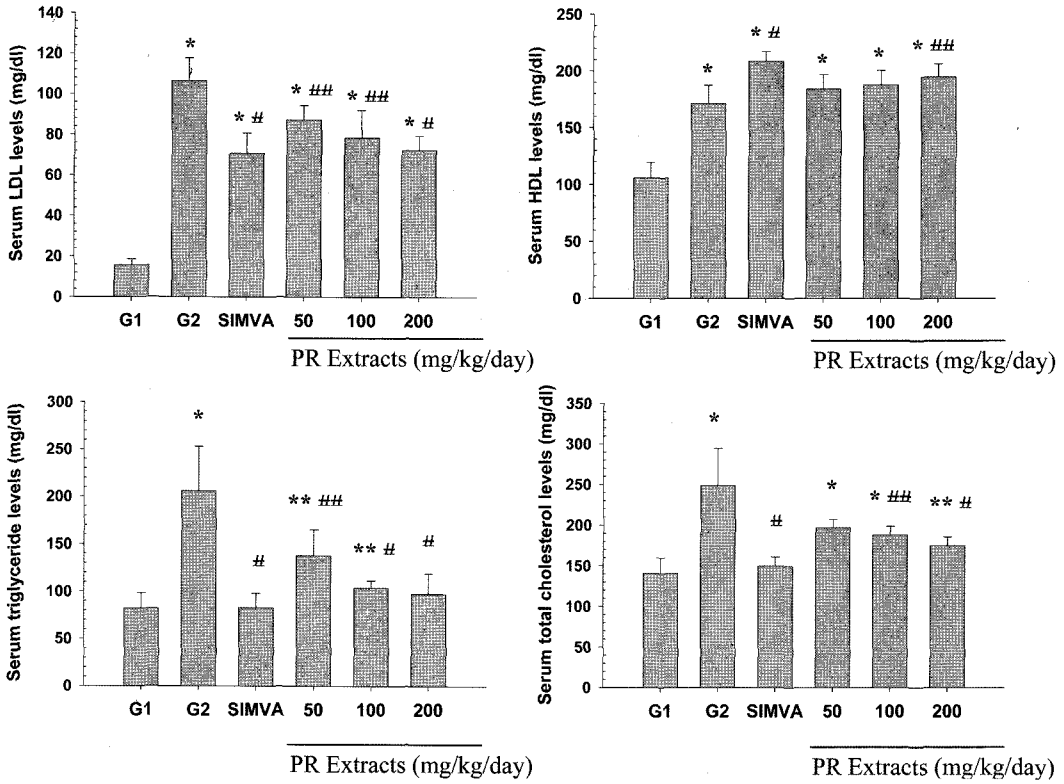

Fig. 3. Effect of aqueous extract of root and stem of *P. kurroa* (PR) on the serum lipid levels in HFD supplied mice. *P<0.01 and ** P <0.05 compared to G1 by MW test, #P<0.01 and ##P<0.05 compared to that of G2 by MW test. N = 5 mice (Mean±S.D.). G1=normal control.G2=HFD control. SIMVA = Simvastatin 10 mg/kg/day

13.67% increase compared to that of HFD control group, respectively. A significant increase of serum triglyceride and total cholesterol levels was detected in HFD control group compared to that of normal control as 150.61% increase in triglyceride and 76.56% increase in total cholesterol levels compared to that of normal control. However, dramatic decreases of serum triglyceride and total cholesterol levels were observed in all PR extract and SIMVA dosing groups compared to those of HFD control group with dose dependent patterns (Fig. 3C, D). The serum triglyceride levels on SIMVA, 50, 100 and 200 mg/kg of PR extract dosing groups were decreased 59.81, 33.11, 49.61 and 52.72% compared to that of HFD control group, respectively. The serum total cholesterol levels on SIMVA, 50, 100 and 200 mg/kg of PR extract dosing groups were also decreased 39.74, 20.84, 24.38 and 29.69 % compared to that of HFD control group, respectively.

DISCUSSION

Because of today's increasing demands on the herbal agents that have been regarded as relatively safe in use, numerous types of herbal extracts were tested in various *in vivo* or *in vitro* systems. The hypolipemic effects have been also evaluated in various animal methods such as *Averrhoa bilimbi* (Tan *et al.*, 2005), *Boerhavia diffusa* (Pari *et al.*, 2004), *Cercipia obtusifolia* (Herrera-Arellano, 2004), Coptidis Rhizoma (Yokozawa *et al.*, 2004), *Aleurites moluccana* (Pedrosa *et al.*, 2004), *Nigella sative* (Le *et al.*, 2004), *Allium porrum* (Movahedian *et al.*, 2004), Radix Paeoniae Rubra (Zhu and Zhu, 2004), *Ficus bengalensis* (Shukla *et al.*, 2004), barley leaf (Yu *et al.*, 2004), *Glycine tomenrella* (Ko *et al.*, 2004), *Scutellaria baicalensis* (Regulska-Ilow *et al.*, 2004), *Momordica charantia* (Senanayake *et al.*, 2004), *Cassia tora* (Patil *et al.*, 2004), *Embelia ribes* (Bhandari *et al.*, 2002) and *Bidens pilosa* (Dimo *et al.*, 2002) extracts. In the present study, the preventive hypolipemic potentials of PR extracts in HFD hyperlipemic mice were observed with their hepatoprotective effects.

The increase of body weights after hyperlipemia is generally known (Han *et al.*, 2005) and it can be used as one

of animal models to develop anti-obesity agents (Olsson *et al.*, 2005). Similar to those of previous study (Yamashita and Hayashi, 1990; Han *et al.*, 2005), a significant increase of body weight was detected in HFD supplied groups compared to that of normal control in the present study. However, no favorable changes on the body weight were detected after PR extracts-dosing. Therefore, it is considered that PR extracts has no prevention activity to obesity induced by HFD in mouse model.

In general, the liver damages are induced by hyperlipemia (Mukai *et al.*, 2002), and the changes on the liver weight, serum AST and ALT levels – serum marks of liver damages are generally monitored to observe the hepatoprotective effects with hypolipemic effects. Although it is reported that some materials having hypolipemic effects also showed hepatoprotective effects especially in herbal extracts (Lal *et al.*, 1998; Hoyos *et al.*, 2000; Mukai *et al.*, 2002), SIMVA showed some increase of serum AST and ALT levels (Ble-Castillo *et al.*, 2002). In the present study, some increase of serum AST and ALT levels is detected in SIMVA group but a significant decrease of serum AST and ALT levels was detected in PR extracts-dosing groups with decrease of liver weight compared to that of vehicle control. It is considered of clear evidence of hepatoprotective effects of PR extracts.

Generally, the most critical problem in hyperlipemia is increases of serum LDL, triglyceride and total cholesterol levels with decrease of HDL levels (Milionis *et al.*, 2004; Forrester *et al.*, 2005; Kamada *et al.*, 2005). The efficacy of hypolipemic agents is generally evaluated based on the decrease of serum LDL triglyceride and total cholesterol with increase of HDL levels (Cheng, 2004; Pirat *et al.*, 2004; Zdrenghea *et al.*, 2004). In the present study, the serum HDL levels are increased compared to that of normal control in vehicle control, somewhat different from previous reports (Pirat *et al.*, 2004; Forrester *et al.*, 2005; Tan *et al.*, 2005). These differences are considered as the results of used animal models because some different results were observed with used animals (Morishita *et al.*, 1986). In the present study, the dose dependent decrease of serum LDL, triglyceride and total cholesterol levels - clear evidence of hypolipemic effects of PR extracts is detected with increase trends of serum HDL levels compared to that of vehicle control.

It is concluded that PR water extracts have relatively good favorable prevention effect on the HFD hyperlipemic mouse with good hepatoprotective effects. Therefore, it is expected that PR extract has favorable potency to develop hypolipemic and/or liver protection drugs. In addition, about 200mg/kg of PR extracts have similar effect compared to that of Simvastatin 10mg/kg. Although, somewhat slight effect on prevent of hyperlipemia was also demonstrated, the efficacy dosage of PR water extracts was considered as 50mg/kg because they showed some hypolipemic effects with slight hepatoprotective effects.

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