



## Effect of Resveratrol on Serum and Liver Lipid Profile and Antioxidant Activity in Hyperlipidemia Rats\*

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**ABSTRACT :** The antioxidant activity of resveratrol in cholesterol-fed rats, along with its hypolipidemic effects was determined. Thirty two male Sprague-Dawley (SD) rats were randomly divided into three groups (Control, Res30 and Res70) and fed a hyperlipidemic diet for 4 weeks. Resveratrol was suspended in 0.3% carboxymethyl cellulose (CMC) solution and given to rats of the Res30 and Res70 groups once a day for 4 weeks by oral intubation at a dose of 30 and 70 mg/kg body weight, respectively. The control group received 0.3% CMC solution alone. Resveratrol significantly lowered serum lipid, hepatic cholesterol (TC) and triglyceride (TG) levels compared to the control. Excretion of bile acids was significantly enhanced by resveratrol. The overall potential of the antioxidant system was significantly enhanced by the resveratrol as plasma and hepatic thiobarbituric acid reactive substances (TBARS) levels were lowered while serum superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) and catalase (CAT) activities were increased in the cholesterol-fed rats. These findings suggest that resveratrol maintains an antioxidant efficacy as well as its anti-hyperlipidemic effect. (**Key Words :** Resveratrol, Lipid Profile, Antioxidant Enzymes, Rat)

### INTRODUCTION

Cardiovascular diseases such as coronary heart disease and stroke are the leading cause of death in the world. Resveratrol is a phenolic compound found in many families of plants. It was first reported in the peel of grape berries for disease resistance and, later, in wines to benefit health. It was found to be the major polyphenol in the root of *P. cuspidatum*. Its content was much higher in *P. cuspidatum* than in grapes. The root of this plant was traditionally used in China as a folk medicine for the treatment of atherosclerosis and for other therapeutic purposes (Shan et al., 1990). Resveratrol has been reported to have strong antioxidant activity *in vitro* (Fauconneau et al., 1997) and anti-inflammatory activities (Kawada et al., 1998; Rotondo et al., 1998; Jang et al., 1997), to exhibit cancer chemopreventive activity (Jang et al., 1997) and to modulate low-density lipoprotein oxidation (Frankel et al.,

1993). Daiki et al. (2003) reported that dietary resveratrol dose-dependently suppressed both serum triglyceride and very-low-density + low-density lipoprotein (VLDL+LDL)-cholesterol levels in hepatoma-bearing rats. Otherwise, in rats fed cholesterol for 7 days, daily oral administration of resveratrol (50 mg/kg) failed to reduce serum cholesterol and triglyceride concentrations (Arichi et al., 1982). In rabbits fed cholesterol for 60 days, a daily oral dose of resveratrol (0.6-1 mg/kg) exerted no influence on lipoprotein-cholesterol concentrations (Wilson et al., 1996).

Recent interest in plant polyphenols and herbs has focused on their potential benefits to human and animal's health and metabolism (Al-Mamun et al., 2007). Many of the health benefits related to polyphenols would seem to arise from their various antioxidant activities. Phenolic compounds act as a primary antioxidant, chelator and superoxide anion scavenger (Zhang et al., 1999; Noguchi et al., 2000; Hosoda et al., 2006). In association with cardiovascular disease, it was also recently announced that various phenolic compounds improve blood lipid components (O'Brien, 1977; Huff and Telford, 1989; Yugarani et al., 1992). All organisms are exposed to reactive oxygen species (ROS) or reactive oxygen metabolites, such as hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), superoxide anions (O<sub>2</sub><sup>-</sup>) or hydroxyl radicals (·OH), as a by-product of oxidative metabolism or through exposure to radical-generating

\* This work was supported by the Youth Innovation Fund of Shandong Agricultural University and the National Eleventh "Five Plan" Scientific Fund (2007BAD70B01).

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Received October 20, 2007; Accepted January 3, 2008

compounds (Yu, 1994; Yuan et al., 2007). Free radicals and ROS are closely associated with various degenerative diseases including atherosclerosis, ischemic heart disease and aging, etc. (Comporti, 1985; Halliwell and Gutteridge, 1990; Steinbrecher et al., 1990; Belch et al., 1991). Superoxide anions are readily generated through the one-electron reduction of oxygen by transition metal ions and then dismutated into hydrogen peroxide by enzymatic and nonenzymatic mechanisms (Fridovich, 1989). Hydrogen peroxide is then further converted into hydroxyl radicals ( $\cdot\text{OH}$ ) which are highly reactive as the initiating species for cellular and plasma lipid peroxidation (Aust and Svingen, 1982; Fridovich, 1989). They promptly react with cellular macromolecules such as lipids, proteins, and nucleic acids eventually leading to cell death. Superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSH-Px) are the primary intracellular antioxidant enzymes which cooperate on the detoxification of the free radicals produced during normal aerobic respiration (Chung et al., 2007; Yuan et al., 2006). SOD resolves  $\text{O}_2^{\cdot-}$  into  $\text{H}_2\text{O}_2$  and  $\text{O}_2$ , whereas CAT and GSH-Px catalyze the reduction of  $\text{H}_2\text{O}_2$  to  $\text{H}_2\text{O}$ .

Although different hypolipidemic effects of resveratrol were reported (Arichi et al., 1982; Wilson et al., 1996; Daiki et al., 2003), little was known about its antioxidant effects in hyperlipidemia animals. Accordingly, in this study we investigated the antioxidant activity of resveratrol in cholesterol-fed rats, along with its hypolipidemic effects.

## MATERIAL AND METHODS

### Animals and experimental design

Thirty two male Sprague-Dawley (SD) rats weighing 180-200 g were obtained from the National Rodent Laboratory Animal Resource Shanghai Branch (Shanghai, China). The rats were housed in individual stainless steel cages in an air-conditioned room with controlled temperature (20-23°C) and automatic lighting (alternating a 12 h period of light and dark) and fed with a pelletized chow diet for 1 week after arrival. Next, the animals were randomly divided into three groups (Control, Res30 and Res70,  $n = 8$ ) and fed with a hyperlipidemic diet for 4 weeks. The rats were provided with feed and water *ad libitum* during the entire experimental period.

Resveratrol (laboratory owned, 99%) was suspended in 0.3% carboxymethyl cellulose (CMC) solution and given to rats of the Res30 and Res70 groups once a day at 8:00 am for 4 weeks by oral intubation at a dose of 30 and 70 mg/kg body weight, respectively. The rats in the control group received 0.3% CMC solution alone.

During the final consecutive 3 days of the experiment, feces were collected to determine the fecal content of bile acids. At the end of the experiment, animals were fasted for

12 h and anesthetized with chloral hydrate (100 g/L). Venous blood samples were taken from the eyepits of rats into a tube, and the serum was obtained by centrifuging the blood at 1,000 g for 15 min at 4°C. The livers were removed and rinsed with physiological saline. All samples were stored at -70°C until analyzed.

### Experimental hyperlipidemic diet

The experimental diet consisted of a well pulverized mixture of cholesterol (1%), cholic acid (0.3%), lard (10%) and normal laboratory diet (88.7%) (Nanjing Animal Science and Technology Co. Ltd.).

### Lipid analyses

Serum TC, TG, HDL-C and LDL-C concentrations were measured enzymatically using commercially available kits (Shanghai Kexin Technology Institute). The assays were performed in accordance with the manufacturer's instructions.

The hepatic lipids were extracted using the procedure developed by Folch et al. (1957). The dried lipid residues were dissolved in 1 ml of ethanol for the cholesterol and triglyceride assays. The hepatic cholesterol and triglycerides were analyzed with the same enzymatic kit as used in the plasma analysis.

### Determination of fecal bile acid contents

Extraction and measurement of fecal bile acids were performed according to the method described by Dvir et al. (2000). Briefly, dried feces (100 mg) were extracted with 10 ml of chloroform-methanol (2:1, v/v) by agitation on a shaker table overnight. Two milliliters of KCl (3.7g/L) was added and the samples were centrifuged for 10 min (1,500 g, 4°C). The supernatant was removed, evaporated to dryness and redissolved in 1 ml of methanol-water (1:1, v/v). Contents of bile acid were measured at 340 nm by colorimetry.

### Serum SOD, GSH-Px, CAT and serum and hepatic lipid peroxidation (TBARS) assays

The assay kits for SOD (20040712), GSH-Px (20040508), CAT (20040428) and TBARS (20040616) were purchased from Jiancheng Biologic Project Company, Nanjing, Jiangsu Province, China. The assays were performed in accordance with the manufacturer's instructions. All other reagents used were of analytical grade.

### Statistical analysis

Results were expressed as mean  $\pm$  standard error of mean (SEM). Statistical analysis was carried out using one-way analysis of variance followed by Duncan's multiple-

**Table 1.** Effects of resveratrol on serum and hepatic lipids in high cholesterol-fed rats\*

Groups	Control	Res30	Res70
<b>Serum</b>			
TC (mmol/L)	4.87±0.21 <sup>A</sup>	4.03±0.14 <sup>B</sup>	3.41±0.18 <sup>C</sup>
TG (mmol/L)	0.99±0.08 <sup>A</sup>	0.67±0.05 <sup>B</sup>	0.59±0.09 <sup>BC</sup>
HDL-C (mmol/L)	0.77±0.07 <sup>B</sup>	0.89±0.12 <sup>A</sup>	0.99±0.05 <sup>A</sup>
LDL-C (mmol/L)	2.24±0.27 <sup>A</sup>	1.96±0.29 <sup>AB</sup>	1.66±0.41 <sup>B</sup>
HDL-C/TC	0.19±0.01 <sup>C</sup>	0.25±0.03 <sup>B</sup>	0.29±0.02 <sup>A</sup>
A.I.	4.34±0.38 <sup>A</sup>	3.30±0.29 <sup>B</sup>	2.98±0.21 <sup>B</sup>
<b>Liver</b>			
Cholesterol (µmol/g)	14.76±2.48 <sup>A</sup>	12.09±2.02 <sup>B</sup>	10.81±2.95 <sup>BC</sup>
TG (µmol/g)	57.64±6.72 <sup>A</sup>	52.62±5.95 <sup>AB</sup>	47.33±3.86 <sup>BC</sup>

\* Mean±SE. TC = Total cholesterol, TG = Triglyceride, HDL-C = High density lipoprotein cholesterol.

LDL-C = Low density lipoprotein cholesterol, A.I. = Atherogenic index ((TC-HDL-C)/HDL-C).

Means in same row not sharing a common superscript are significantly different among groups (p<0.05).

**Table 2.** Effect of resveratrol on SOD, GSH-Px and CAT activities in high cholesterol-fed rats\*

Groups	Control	Res30	Res70
SOD (U/ml)	160.45±4.15 <sup>C</sup>	189.70±17.14 <sup>B</sup>	211.14±15.29 <sup>A</sup>
GSH-Px (U)	1,083.40±100.91 <sup>B</sup>	1,288.44±43.19 <sup>A</sup>	1,307.14±132.20 <sup>A</sup>
CAT (U/ml)	1.85±0.32 <sup>B</sup>	2.30±0.18 <sup>A</sup>	2.60±0.15 <sup>A</sup>

\* Mean±SE. Means in same row not sharing a common superscript are significantly different among groups (p<0.05).

range test (SAS 8.0) and p<0.05 was considered statistically significant.

## RESULTS

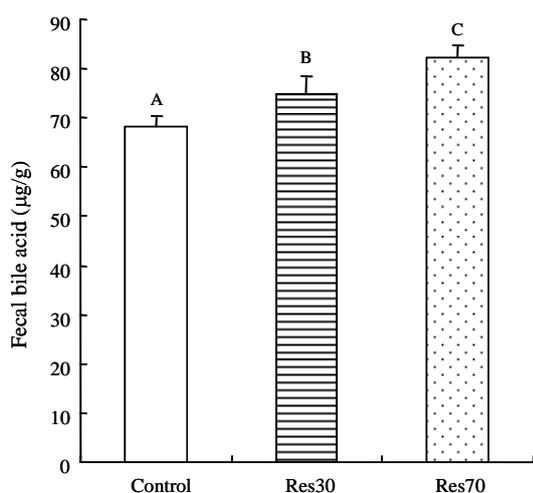
### Effect on serum lipid level

In the Res30 and Res70 groups, the concentration of serum TC, TG, and hepatic TC and serum A.I. were reduced significantly compared to the control group (p<0.05) (Table 1). The serum HDL-C concentration and the HDL-C/TC ratios increased significantly in Res30 and Res70 groups

compared to the control group (p<0.05) (Table 1). The serum LDL-C and hepatic TG concentration did not differ between the Res30 group and the control group (p>0.05), however, in the Res70 group they were reduced significantly compared to the control group (p<0.05) (Table 1). In addition, the serum TC level and HDL-C/TC ratios were both significantly lower in the Res70 than in the Res30 group (p<0.05) (Table 1).

### Effect on fecal bile acids

Figure 1 shows the effect of resveratrol on the fecal excretion of bile acids. After oral intubation of resveratrol, the excretion of bile acids increased significantly (p<0.05). Also, the excretion of bile acids increased significantly in Res70 compared with Res30 (p<0.05).



**Figure 1.** Effect of resveratrol on fecal bile acid excretion in high cholesterol-fed rats. Fecal bile acid of control was 68.1 µg/g feces. Each value represents the mean±SE. A, B, C: Bars with different letters are different at p<0.05.

### Effect on serum antioxidant enzyme activities

The oral intubation of resveratrol enhanced the SOD activity significantly (p<0.05) (Table 2). The SOD activity in Res70 was higher than Res30 (p<0.05).

In the Res30 and Res70 groups, the resveratrol resulted in a significant increase in GSH-Px and CAT activities compared with the control group (Table 2). However, The GSH-Px and CAT activities did not differ significantly between the Res30 and Res70 groups (Table 2).

### Effect on serum and hepatic TBARS level

The levels of serum TBARS were significantly lower in the Res30 and Res70 groups than in the control group (Table 3). In the Res30 and Res70 groups, the resveratrol resulted in decreased the levels of hepatic TBARS

**Table 3.** Effect of resveratrol on serum and hepatic TBARS concentrations in high cholesterol-fed rats\*

Groups	Control	Res30	Res70
Serum TBARS (nmol/ml)	30.00±3.87 <sup>A</sup>	25.82±1.34 <sup>B</sup>	22.46±1.42 <sup>B</sup>
Hepatic TBARS (nmol/mg protein)	2.22±0.33 <sup>A</sup>	1.89±0.16 <sup>AB</sup>	1.67±0.42 <sup>B</sup>

\* Mean±SE. Means in same row not sharing a common superscript are significantly different among groups (p<0.05).

compared to the control group, by 15% and 25%, respectively, and the levels differed significantly between the Res70 and control groups (Table 3).

## DISCUSSION

There is a close relationship between atherosclerosis and an increase or decrease of serum lipids, in particular, very low-density lipoprotein and LDL may be risk factors (Carlson, 1982) and HDL may be a protective factor (Miller and Miller, 1975). Polyphenols are now widely accepted as physiological antioxidants that have a significant potential to protect against the many degenerative diseases linked to free radical-related tissue damage. The health benefits of polyphenols would appear to arise from their antioxidant activity and capacity to protect critical macromolecules, such as chromosomal DNA, structural proteins and enzymes, LDL and membrane lipids, from damage resulting from exposure to reactive oxygen species (ROS) (Rice-Evans, 1996; Dreosti, 2000). The present study investigated the hypolipidemic effects of resveratrol to determine their possible role in a high-cholesterol fed state. The results suggest that the plasma lipid-lowering and antioxidative effects of resveratrol were very potent in high cholesterol-fed rats.

Hepatic cholesteryl ester is synthesized from cholesterol and acyl-CoA by ACAT enzyme. The cholesteryl ester is stored in the liver cells or packed into the cores of lipoproteins and transported to other tissues (Einarsson et al., 1989; Miller, 1996). The secretion of VLDL cholesteryl ester was reported to be increased in rat liver cells having a high ACAT activity and a large cholesteryl ester mass. In contrast, the secretion of VLDL is suppressed when the hepatic cholesterol pool is reduced. Thus, hepatic ACAT activity has a predominant role in maintaining cholesterol homeostasis and is one of the important determinants of the serum cholesterol level (Krause et al., 1994; Wu et al., 1994). Resveratrol decreased ACAT activity in a dose-dependent manner from the level of  $10^{-3}$  M concentration in HepG2 cells *in vitro* (Park et al., 2004). Resveratrol reduced cholesterol synthesis by inhibiting squalene monooxygenase *in vitro*, a rate-limiting enzyme in cholesterol biosynthesis (Brian et al., 2001). Inhibition of squalene monooxygenase has been shown to be effective in lowering serum cholesterol levels in dogs (Shen et al., 1989). Dietary resveratrol dose-dependently suppressed the serum triglyceride and very-low-density lipoprotein+low-

density lipoprotein (VLDL+LDL)-cholesterol levels in hepatoma-bearing rats (Daiki et al., 2003). In the present study, resveratrol significantly reduced the serum TC, LDL-C and TG level and AI and hepatic TC and TG concentration, but tended to increase the HDL-C level in high cholesterol-fed rats when compared with the control rats (Table 1). These results were consistent with the above reports. In this study, resveratrol stimulated the excretion of bile acids into the feces (Figure 1). Thus, the inhibition of hypercholesterolemia may also have resulted from the increased excretion of fecal bile acids in treated resveratrol rats. Daiki et al. (2003) reported that dietary resveratrol reduced the serum triglyceride levels by increased excretion of bile acids into feces in hepatoma-bearing rats. Dietary rutin and tannic acid also reduced hypercholesterolemia by stimulating sterol excretion into the feces (Park et al., 2002).

Antioxidative enzymes contribute to the antioxidant defense mechanism. Oxidative stress is one of the causative factors that link hypercholesterolemia with the pathogenesis of atherosclerosis (Lee et al., 2002). This stress results from an imbalance between the production of free radicals and the effectiveness of the antioxidant defense system. Resveratrol has been linked to a variety of beneficial effects, including protection from cancer, from free radical damage and from cardiovascular diseases (Fremont, 2000). Several reports in the literature exist, which show the radical scavenger as well as antioxidant properties of resveratrol *in vitro* (Belguendouz et al., 1997; Fauconneau et al., 1997). The results of this current study demonstrated that resveratrol increased the activities of SOD, GSH-Px and CAT (Table 2) in high cholesterol-fed rats compared with the control group. Resveratrol was demonstrated to protect human LDL against copper-catalyzed oxidation *in vitro* at a concentration of 10  $\mu$ M by Frankel et al. (1993). Its antioxidant effectiveness was also reported by Belguendouz et al. (1998) who assessed its ability to protect phospholipid unilamellar liposomes from oxidation by 2,2'-azobis (2-amidinopropane) dihydrochloride (AAPH), and used the reduced formation of thiobarbituric acid reactive substances as a measure of such protection *in vitro*. They reported the dose-response curves to be linear up to 30  $\mu$ M when resveratrol was added to the preparation after formation of liposomes and up to 200  $\mu$ M when added prior to liposome formation. Resveratrol was found to protect against ethanol-induced lipid peroxidation and cell death (Sun et al., 1997). Resveratrol reduced the plasma and hepatic TBARS levels (Table 3) in high cholesterol-fed rats compared to the

control group. That is, resveratrol treatment may reduce the TBARS content by inhibiting the oxidation of LDL, which would seem to imply that resveratrol maintains an antioxidant efficacy as well as an anti-hyperlipidemic effect.

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