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Antioxidant activity of stilbene glycoside from *Polygonum multiflorum* Thunb *in vivo*

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Abstract

Stilbene glycosides were isolated from the ethanol extract of the roots of *Polygonum multiflorum* Thunb. Two samples were obtained; a fraction separated by macroporous resin and pure crystals of 2,3,5,4′-tetrahydroxystilbene 2-*O*-β-glucopyranoside. The antioxidant activities of these two samples were evaluated using antioxidant tests of rats *in vivo*. The activities of superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) of the serum and the organs (liver, heart and brain of rats) of D-galactose induced senile rats which were fed with stilbene glycoside, were increased; however, the content of 2-thiobarbituric acid-reactive substances (TBARS) was decreased. It is concluded that the stilbene glycoside from *Polygonum multiflorum* Thunb possesses high *in vivo* antioxidant activity.
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Keywords: Stilbene glycoside; *Polygonum multiflorum* Thunb; Antioxidant activity; D-galactose

1. Introduction

Free radical induced oxidative damage has long been thought to be the most important consequence of the aging process (Harman, 1992). Free radicals are capable of causing cellular damage, which leads to cell death and tissue injury (Ho, Magnenat, Gargano, & Cao, 1998). Studies show that these radicals also affect the equilibrium between pro-oxidants and antioxidants in biological systems, leading to modifications in genomes, proteins, carbohydrates, lipids and lipid peroxidation (Romero et al., 1998), thus inactivating antioxidant defense.

The dried roots of *Polygonum multiflorum* Thunb (PM) have been used as a tonic and an antiaging agent in many remedies in traditional Chinese medicine (Chen & Li, 1993; Huang, 1993). Recent pharmacological studies have demonstrated that a crude PM extract could produce a hypolipidaemic action in rats (Zhang, Zhuang, & Mei, 1983) and a vasorelaxant effect on isolated rat aorta (Chang, Huang, Chiu, & Chao, 1990). Given the involvement of free radical-mediated reactions in the development of age-related cardiovascular disorders, including atherosclerosis and hypertension (Halliwell & Gutteridge, 1990, 1999), the medicinal effects of PM in the treatment of these age-related diseases are possibly mediated by the antioxidant capacity of this plant. The antioxidant activity of PM *in vivo* and *in vitro* had been demonstrated (Ip, Tse, Poon, & Ko, 1997). Research indicates that PM enhances the cellular antioxidant activity, increases the function of superoxide dismutase (SOD), significantly inhibits the formation of oxidized lipids (Xiao, Xing, & Wang, 1993), and represses lipid peroxidation in rat heart mitochondria (Hong, Lo, Tan, Wei, & Chen, 1994). The objectives of this study were to investigate the *in vivo* antioxidant activity of the components isolated from PM.

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2. Materials and methods

2.1. Preparation of extracts of stilbene by resin fractionation

The dried roots of PM were crushed and extracted with 60% ethanol, at a ratio of solution to solid of 1:10 (v/w), at room temperature for 2 days. The plant material was filtered off, and the ethanolic extracts were combined and concentrated under reduced pressure using a rotary evaporator. The dry extract obtained was then subjected to open column chromatography (CC) packed with macroporous resin (Chemical Plant of Nankai University, Tianjin, China). The column was eluted stepwise with each of five different concentrations of ethanol (10%, 20%, 30%, 40%, and 50%). The 40% aqueous–ethanol fraction was then concentrated under reduced pressure using a rotary evaporator and designated as "Resin fractionated PM stilbene glycoside" (RF-PM-SG) for the following study.

2.2. Purification of PM stilbene glycoside by chromatography

The dry extract obtained from PM was subjected to an open column CC packed with polycaprolactam (Huangyan Resin Chemical Corp., Huang Yan, China), The column was eluted stepwise with 5 times the volume of the column, for each of the four different concentrations of aqueous ethanol solutions (10%, 20%, 30% and 40%), and collected into four fractions. Fractions collected with 20% and 30% aqueous–ethanol solutions were combined and again subjected to an open CC packed with polycaprolactam. The new fraction obtained by 30% aqueous-ethanolic solution was then put into the refrigerator. An acicular crystal was obtained. The crystals were re-crystallized to obtain pure "stilbene glycoside" (PM-SG) for the antioxidant tests. The structure of obtained pure stilbene glycoside was determined as being 2,3,5,4′-tetrahydroxystilbene 2-*O*-β-D-glucopyranoside as described in a previous publication (Li-Shuang, Gu, Ho, & Tang, 2006).

2.3. Animal preparation and experimental design

Rats (18–22 g) were provided by the animal center of PuKou (Nanjing, China). The rats were housed in stainless steel wire-bottomed cages and acclimatized under laboratory conditions (19–23 °C, humidity 60%, 12 h light/dark cycle). The normal rats were divided into six groups and each group contained ten rats. Group I, Normal control rats received only water; Group II, Model rats received D-galactose (100 mg/kg/day body weight; single hypodermic injection); Group III, Normal rats received PM-SG (20 mg/kg/day body weight) by oral administration; Group IV, Normal rats received PM-SG (40 mg/kg/day body weight) by oral administration; Group V, Normal rats received RF-PM-SG (20 mg/kg/day body weight) by oral administration; Group VI, Normal rats received RF-PM-SG (40 mg/kg/day body weight) by oral administration.

Except Group I, each group was induced by a single hypodermic injection of D-galactose (100 mg/kg/day body weight, dissolved in 20 mg/mL saline). At the same time, PM-SG and RF-PM-SG were freshly prepared and given daily to rats of Group III–VI for a period of 42 days. After being fed normally for another 7 days, all rats were sacrificed. The liver, heart and brain tissues were removed and washed thoroughly with ice-cold saline. The tissues were immediately frozen in liquid nitrogen and kept at −80 °C until analysis. Serum was separated from whole blood by centrifugation (*G* value of 5000 rpm, 10 min, 4 °C) and stored at −80 °C until use.

2.4. Determination of 2-thiobarbituic acid-reactive substances (TBARS)

Serum, heart, liver and brain tissue samples were analyzed for their TBARS values according to the method described by Shahidi and Hong (1991). TBARS values were calculated using 1,1,3,3-tetramethoxypropane as a standard precursor of malondialdehyde (MDA).

2.5. Measurements of antioxidant enzyme activities

Superoxide dismutase (SOD) activity was determined by the epinephrine method (Misra & Fridovich, 1972). This method is based on the measurement of the inhibitory rate of epinephrine autoxidation by SOD contained in the examined samples in 50 mM sodium carbonate buffer, pH 10.2, within the linear range of autoxidative curve. SOD activity was expressed as units/mg protein.

The activity of glutathione peroxidase (GSH-Px) was determined using *t*-butyl hydroperoxide as a substrate by the method of Tamura, Oschino, and Chance (1982) and the activity was expressed as nmol NADPH oxidized min/mg protein. For determination of glutathione-*S*-transferase (GST) activity, 1-chloro-2,4-dinitrobenzene (CDNB) was used as a substrate (Habig, Pabst, & Jakoby, 1974) and the activity was expressed as nmol GSH used min/mg protein. Glutathione reductase (GR) activity was assayed by the method of Glatzle, Vulliemuier, Weber, and Decker (1974) by measuring NADPH oxidation in the presence of oxidized glutathione and the activity was expressed as nmol NADPH oxidized min/mg protein.

All reagents that were used were purchased from Nanjing Jiancheng Institute (Nanjing, China).

2.6. Protein concentration

The concentration of total protein was determined by the biuret method (Lowry, Rosebrough, Farr, & Randal, 1951) using bovine serum albumin as standard.

2.7. Statistical analysis

Results are expressed as mean (SD) and *t*-test was performed to assess the statistical significance (SPSS 10.0 for

Windows). The significance of difference among mean values was determined at level $P < 0.05$.

3. Results

The present study was undertaken to assess the effect of a 42 days dietary supplementation with SG-PM and RF-SG-PM on the activity of antioxidant enzymes in the serum and in the homogenates of heart, brain and liver of rats. Additionally, the antioxidant capacities were also determined. Effects of antioxidant activities of SG in rat serum are given in Table 1. The result of ANOVA showed that the activities of SOD, GSH-PX of rat serum in Group II, declined significantly than that of normal control (Group I) ($P < 0.05$). TBARS also significantly increased compared to that of the normal control (Group I) ($P < 0.05$). This indicated that the stimulated rat-ageing model is made successfully. On the other hand, in the rats fed with SG-PM, RF-SG-PM, activities of SOD and GSH-PX in the serum was enhanced ($P < 0.05$), and the concentration of TBARS decreased ($P < 0.05$) markedly. Tables 2–4 also show the similar effect of antioxidant activities in the heart, brain and liver tissues. Therefore, the results obtained using the present animal model successfully demonstrated the antioxidant activities of SG-PM, RF-SG-PM *in vivo*.

Table 1
Antioxidant activity of stilbene glycoside on the serum of rats

Group	Dose (mg/kg/d)	TBARS (nmol/mL)	SOD (U/mL)	GSH-PX (U/mL)
Normal control	–	5.72 ± 0.33^{a}	329.47 ± 20.23^{a}	663.72 ± 16.20^{a}
Model	–	7.02 ± 0.21^{b}	233.87 ± 12.47^{b}	421.64 ± 13.91^{b}
RF-PM-SG 1	20	6.45 ± 0.25^{c}	263.08 ± 15.33^{c}	597.37 ± 8.82^{c}
RF-PM-SG 2	40	5.84 ± 0.14^{a}	287.47 ± 11.10^{dc}	649.46 ± 18.0^{d}
PM-SG 1	20	5.48 ± 0.17^{ad}	311.03 ± 10.89^{ad}	623.73 ± 5.52^{e}
PM-SG 2	40	5.08 ± 0.37^{d}	318.90 ± 18.20^{a}	682.70 ± 10.8^{a}

Means in the same column with different superscript differed significantly ($P < 0.05$).

Table 2
The antioxidant activities of stilbene glycoside of PM in the rat heart ($X \pm$ SD, $n = 10$)

Group	Dose (mg/kg/d)	TBARS (nmol/mg prot)	SOD (U/mg prot)	GSH-PX (U/mg prot)
Normal control	–	6.76 ± 0.21^{a}	366.30 ± 10.79^{a}	221.09 ± 12.12^{a}
Model	–	9.45 ± 0.20^{b}	291.17 ± 11.45^{b}	185.30 ± 10.9^{b}
RF-PM-SG 1	20	7.62 ± 0.18^{c}	348.59 ± 14.08^{a}	215.87 ± 13.6^{a}
RF-PM-SG 2	40	6.75 ± 0.34^{a}	354.00 ± 13.42^{a}	229.32 ± 8.72^{a}
PM-SG 1	20	6.50 ± 0.16^{a}	370.29 ± 17.05^{a}	219.45 ± 6.71^{a}
PM-SG 2	40	5.80 ± 0.15^{d}	369.66 ± 4.31^{a}	233.06 ± 8.32^{a}

Means in the same column with different superscript differed significantly ($P < 0.05$).

Table 3
The antioxidant activities of stilbene glycoside of PM in the rat liver ($X \pm$ SD, $n = 10$)

Group	Dose (mg/kg/d)	TBARS (nmol/mg prot)	SOD (U/mg prot)	GSH-PX (U/mg prot)
Normal control	–	10.60 ± 0.39^{a}	328.67 ± 7.49^{a}	214.02 ± 14.38^{a}
Model	–	13.95 ± 0.22^{b}	263.72 ± 14.51^{b}	150.29 ± 8.45^{b}
RF-PM-SG 1	20	10.92 ± 0.25^{a}	336.17 ± 14.9^{ca}	212.78 ± 9.04^{a}
RF-PM-SG 2	40	9.73 ± 0.35^{c}	353.03 ± 16.35^{dc}	222.82 ± 9.90^{a}
PM-SG 1	20	10.81 ± 0.17^{a}	365.62 ± 10.72^{ed}	229.33 ± 14.59^{a}
PM-SG 2	40	8.97 ± 0.16^{d}	367.37 ± 11.64^{ed}	229.16 ± 10.60^{a}

Means in the same column with different superscript differed significantly ($P < 0.05$).

Table 4
The antioxidant activities of stilbene glycoside of PM in the rat brain ($X \pm$ SD, $n = 10$)

Group	Dose (mg/kg/d)	TBARS (nmol/mg prot)	SOD (U/mg prot)	GSH-PX (U/mg prot)
Normal control	–	8.77 ± 0.24^{a}	28.80 ± 1.40^{a}	16.78 ± 0.48^{a}
Model	–	10.84 ± 0.25^{b}	$22..38 \pm 0.84^{b}$	13.45 ± 0.72^{b}
RF-PM-SG 1	20	9.93 ± 0.23^{c}	24.42 ± 0.64^{bc}	14.79 ± 0.68^{ba}
RF-PM-SG 2	40	8.81 ± 0.35^{a}	27.10 ± 1.01^{ac}	$16.56 \pm 0.84_{a}$
PM-SG 1	20	9.19 ± 0.16^{a}	26.13 ± 1.72^{ac}	15.87 ± 0.79^{a}
PM-SG 2	40	8.34 ± 0.29^{ad}	29.03 ± 1.50^{a}	16.32 ± 0.97^{a}

Means in the same column with different superscript differed significantly ($P < 0.05$).

4. Discussion

Crucial components of the antioxidant defense system in the body are cellular antioxidant enzymes (superoxide dismutase, SOD and glutathione peroxidase, GSH-PX), which are involved in the reduction of reactive oxygen species (ROS) and peroxides produced in the living organism as well as in the detoxification of certain compounds of exogenous origin, thus playing a primary role in the maintenance of a balanced redox status. Hence, it can serve as a potential marker of susceptibility, early and reversible tissue damage, and of decrease in antioxidant defense.

SOD protects against oxygen free radicals by catalyzing the removal of superoxide radical, which damages the membrane and biological structures. The age-related decrease in the activity of SOD documented in our study is in agreement with earlier investigations (Arivazhagan, Thilakavathy, & Panneerselvam, 2000; Mo, Hom, & Andersen, 1995).

GSH-PX catalyzes the reduction of H_2O_2 to H_2O and O_2 at the expense of GSH. Our observation that SG-PM and RF-SG-PM have the ability to increase GSH peroxidase activity is in agreement with the reports of earlier investigators (Ito, Kajkenova, & Feuers, 1998).

Lipid peroxidation is a free radical induced process leading to oxidative deterioration of polyunsaturated lipids. Under normal physiological conditions, low concentrations of lipid peroxides are found in tissues. Free radicals react with lipids and cause peroxidative changes that result in enhanced lipid peroxidation (Girotti, 1985). These free radical species formed are capable of oxidizing sulphydryl moieties of proteins, thus leading to protein fragmentation and loss of cell viability. In our present study, a marked increase in TBARS was observed in model rats, and lower levels of the activity of antioxidant enzymes had been reported. Supplementation with SG brought the TBARS as well as antioxidant enzymes to the normal levels.

In conclusion, our observations suggest that SG-PM acts as a potent antioxidant in protection of rat tissues (heart, brain and liver) against oxidative stress induced by age. The results presented here also indicate that the antioxidant capacity of relatively impure RF-SG-PM is comparable to the pure compound, SG-PM.

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