

Antioxidative Effect of Large Molecular Polymeric Pigments Extracted from Zijuan Pu-erh Tea *In Vitro* and *In Vivo*

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ABSTRACT

The antioxidative effect of large molecular polymeric pigments (LMPP) extracted from Zijuan Pu-erh tea was investigated *in vitro* and *in vivo*. The results showed that LMPP had significant scavenging activities on the hydroxyl radical and the 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical *in vitro* and showed strong reducing power. Moreover, the 50% inhibitory concentrations of LMPP for scavenging DPPH radical and hydroxyl radical were 0.217 mg.mL⁻¹ and 0.461 mg.mL⁻¹, respectively. *In vivo*, the LMPP-treated rat groups showed significantly increased serum superoxide dismutase (SOD) and glutathione peroxidase (GSH-PX) activities, reduced malondialdehyde (MDA) formation, increased nitric oxide (NO) production and significantly decreased rat endothelin-1(ET-1) concentrations compared with those in the hyperlipidemia model group ($P < 0.05$). The serum SOD and GSH-PX activities and NO concentration were 66.88, 29.09 and 55.11% higher, respectively, whereas, the serum ET-1 and MDA concentrations were 34.62 and 59.11% lower in the high-dose LMPP treatment group (1.215 g.kg⁻¹ body weight) than in the hyperlipidemia model group ($P < 0.05$). These results showed that LMPP has a good antioxidative function and can be considered as a natural antioxidant source.

Keywords: Zijuan Pu-erh tea, large molecular polymeric pigments, antioxidant, *in vitro*, *in vivo*

INTRODUCTION

Free-radical-initiated autoxidation of cellular membrane lipids can lead to cellular necrosis and a variety of pathological conditions such as aging, membrane damage, heart disease, stroke, emphysema and cancer in living organisms (Marx, 1987). Hence, a considerable number of investigations have focused on the prevention of

oxidative damage initiated by free radicals. Among these, investigations of natural antioxidants and bioactive compounds for certain human diseases have received much attention (Hsieh *et al.*, 2007).

The Zijuan tea plant is a cultivar developed from an individual plant of *Camellia sinensis* var. *assamica* (Masters) Kitamura obtained from Menghai county in China. It has

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purple stems, buds and leaves, a light purple calyx and pedicle, and a pale purple fruit skin. Its leaves are processed into Zijuan sun-dried green tea by multiple procedures, including fixation, rolling, and sun drying. The liquor of the resulting Zijuan green tea appears purple and tastes bitter, with indigo leaves residing at the bottom (Liang and Xia, 2003; Bao *et al.*, 2008; Yang *et al.*, 2009). Zijuan green tea can be further manufactured into the more valuable Zijuan Pu-erh tea by appropriate wetting and solid-state fermentation. Zijuan Pu-erh tea is brown, and its liquor appears thick and brightly red, with a rich taste without bitterness, and it has a unique fragrance. Because of these enjoyable characteristics, Zijuan Pu-erh tea is well accepted by consumers despite its high price (Wang *et al.*, 2012).

During the manufacture of Zijuan Pu-erh tea by solid-state fermentation, the composition of the tea leaves changes substantially. The concentration of tea polyphenols, total catechins, thearubigin and theaflavin all decreased sharply, whereas the concentration of large molecular polymeric pigments (LMPP) increased from 23.8 g.kg⁻¹ to 124.3 g.kg⁻¹. The substantial decrease in phenols and the increase in LMPP suggest that LMPP may be a unique component in Zijuan Pu-erh tea. A previous study showed that intragastric infusion of high-dose LMPP (1.215 g.kg⁻¹ body weight) extracted from Zijuan Pu-erh tea effectively suppressed the elevation in TC and LDL cholesterol ($P < 0.05$) and prevented a reduction in HDL cholesterol ($P < 0.05$) compared with the hyperlipidemia model group (Wang *et al.*, 2012). This proved that LMPP had a hypolipidemic function effect on rats consuming a high lipid diet; however the potential antioxidative effect of LMPP has not been intensively studied. Therefore, the aims of the present study were to investigate the antioxidative activity of LMPP *in vitro* and *in vivo*, to provide a basis for its application in functional foods, health care, cosmetics and natural pigments. For example, a better understanding of its biological functions

may guide a rational development of this natural substance into a new dietary supplement for antioxidant.

MATERIALS AND METHODS

Chemicals and Materials

Zijuan sun-dried green tea was purchased from the Tea Research Institute of Yunnan Academy of Agricultural Sciences (Menghai, Yunnan, China). The 2, 2-diphenyl-1-picrylhydrazyl (DPPH) was purchased from Sigma–Aldrich (Buenos Aires, Argentina). Assay kits for superoxide dismutase (SOD), glutathione peroxidase (GSH-PX), malondialdehyde (MDA) and nitric oxide (NO) were obtained from the Nanjing Jiancheng Bioengineering Institute (Nanjing, Jiangsu, China). Rat endothelin-1 (ET-1) enzyme-linked immunosorbent assay (ELISA) kits were obtained from R&D Systems (Minneapolis, MN, USA). Lovastatin capsules were obtained from the Jiangsu D&Y Pharmaceutical Co., Ltd. (Suqian, Jiangsu, China). All other chemical reagents used were of analytical grade.

Preparation of large molecular polymeric pigments extracted from Zijuan Pu-erh tea

The Zijuan sun-dried green tea leaves were fermented using the following procedures. A sample of 10 kg of leaves (moisture content 85.0 g.kg⁻¹) were wetted with 9,000 mL of distilled water; they were covered with gas-permeable food-grade polyethylene film and kept in a fermentation oven (45 °C, relatively humidity 70%) for 40 d with constant rotation. The leaves were then dried at 60 °C and Zijuan Pu-erh tea leaves were obtained.

Extraction of LMPP from Zijuan Pu-erh tea was as follows. Zijuan Pu-erh tea (5,000 g) was extracted three times in advance with anhydrous ethanol (1:4 weight per volume, w/v) for 12 hr at 35 °C, after which the ethanol extraction was discarded. The tea residues were dissolved in distilled water (1:5 w/v) for 3 hr at 85 °C and

filtered three times. The resulting aqueous extract was extracted with chloroform (1:1 volume per volume, v/v), ethyl acetate (1:1 v/v), and n-butanol (1:1 v/v) at room temperature and the extract was discarded. Then, the residual tea aqueous extract was precipitated by anhydrous ethanol (1:4 v/v) for 12 hr at room temperature and the precipitation was collected by centrifugation. The water solution of the precipitation (10% w/v) was vacuum freeze-dried (FD-1PF vacuum lyophilizer; Detianyou Scientific; Beijing, China) into powder. The LMPP powder (ethanol precipitate) consisted of $740.0 \pm 8.5 \text{ g.kg}^{-1}$ LMPP, $131.1 \pm 14.8 \text{ g.kg}^{-1}$ protein residual and $101.4 \pm 17.8 \text{ g.kg}^{-1}$ carbohydrate residual.

Measurement of antioxidative activity of large molecular polymeric pigments extracted from Zijuan Pu-erh tea *in vitro*

The LMPP powder was dissolved using distilled water to make a series of different concentration samples (0.1, 0.2, 0.3, 0.4 and 0.5 mg.mL^{-1}); then the antioxidative activity *in vitro* was measured and determinations were performed in triplicate for each sample and the values were averaged.

Determination of reducing power

The reducing power of LMPP with different concentrations was determined by the method of Oyaizu (1986). Specifically, 1 mL of LMPP samples of different concentrations (0.1–0.5 mg.mL^{-1}) was mixed with phosphate buffer (2.5 mL, 2.0 mol.L^{-1} , pH 6.6) and potassium ferricyanide (2.5 mL, 1.0 g per 100 mL); the mixture was incubated at 50 °C for 20 min. A portion (2.5 mL) of trichloroacetic acid (10 g per 100 mL) was added to the mixture, which was then centrifuged at 3,000× for 10 min. The upper layer of the solution (2.5 mL) was mixed with distilled water (2.5 mL) and ferric chloride (2.5 mL, 0.1 g per 100 mL), and its absorbance was measured at 700 nm (UV-1700 spectrometer; Shimadzu Corp.; Tokyo, Japan). Increased absorbance of

the reaction mixture indicated increased reducing power.

Assay for evaluating 2, 2-diphenyl-1-picrylhydrazyl radical scavenging activity

The determination of the scavenging effect on the DPPH radical by LMPP samples of different concentrations (0.1–0.5 mg.mL^{-1}) was carried out by the method of Von Staszewski *et al.* (2011). Briefly, 0.4 mL of the sample was mixed with 2.0 mL of DPPH in methanol prepared daily (0.1 mmol.L^{-1}). The mixture was shaken vigorously at room temperature for 30 min in darkness, and the absorbance at 517 nm was measured. The scavenging rate was determined from Equation 1:

$$\text{DPPH radical scavenging rate (\%)} = ((A_0 - A)/A_0) \times 100 \quad (1)$$

where, A_0 is the absorbance of the control solution (containing only DPPH) and A is the absorbance of the DPPH solution with the sample.

Assay for evaluating hydroxyl radical scavenging activity

The hydroxyl radical (OH•)-scavenging assay was carried out according to the method described by Halliwell *et al.* (1987), with a slight modification. First, the hydroxyl radical was generated by incubating a reaction mixture containing 20 $\mu\text{mol.L}^{-1}$ FeCl_3 , 1.4 mmol.L^{-1} H_2O_2 , 2.8 mmol.L^{-1} deoxyribose, 2 mmol.L^{-1} EDTA, and 100 $\mu\text{mol.L}^{-1}$ ascorbate in 1 mL of 10 mmol.L^{-1} KH_2PO_4 –KOH buffer (pH 7.4), and 1 mL LMPP samples of different concentrations (0.1–0.5 mg.mL^{-1}) for 60 min at 37 °C. Deoxyribose degradation by the hydroxyl radical was then determined with the aid of the thiobarbituric acid (TBA) method (Kikuzaki and Nakatani, 1993). The addition of 2.8 g per 100 mL trichloroacetic acid (TCA) caused the color to develop, after which the absorbance was measured spectrophotometrically at 535 nm. The scavenging rate was determined from Equation 2:

$$\text{OH}\cdot \text{scavenging rate (\%)} = ((A_0 - A_s)/A_0) \times 100 \quad (2)$$

where A_0 is the absorbance of the control solution and A_s is the absorbance of the LMPP sample.

Investigation of antioxidative activity of large molecular polymeric pigments extracted from Zijuan Pu-erh tea *in vivo*

Selection of experimental animals

Healthy adult male Sprague-Dawley (SD) rats (weighing 150 to 200 g and aged 4 mth) were obtained from the Animal Experimental Center of Kunming Medical College (license number SCXX [Dian 2005–0008]). All animal procedures were approved by the National Natural Science Fund of China, in accordance with guidelines from the China Council for Healthy Food (Yang, 2008).

Feeding method and management

A basal feed diet was obtained from the Animal Experimental Center of Kunming Medical College. The formulation was as follows: corn, 350 g.kg⁻¹; wheat bran, 250 g.kg⁻¹; bean pulp, 250 g.kg⁻¹; fish meal, 80 g.kg⁻¹; yeast, 20 g.kg⁻¹; bone meal, 20 g.kg⁻¹; whey powder, 10 g.kg⁻¹; salt sodium, 5 g.kg⁻¹; rape oil, 5 g.kg⁻¹; mineral mix, 1 g.kg⁻¹; vitamin mix, 0.3 g.kg⁻¹; methionine, 1.3 g.kg⁻¹; lysine, 0.7 g.kg⁻¹; and cod liver oil, 0.5 g.kg⁻¹. The formulation of the high lipid diet (basal feed, 788.0 g.kg⁻¹; lard oil, 100.0 g.kg⁻¹; yolk powder, 100.0 g.kg⁻¹; cholesterol, 1.0 g.kg⁻¹; and bile salt, 2.0 g.kg⁻¹) was based on the Technical Standards for Determination and Assessment of Health Foods issued by the China Ministry of Health in February 2003 (Wang *et al.*, 2012).

Groups of animals

Sixty healthy male SD rats (150–200 g) were separately maintained in cages (20 ± 2 °C; relative humidity, 45–60%) with free access to water and food. After adaptive feeding with the basal feed diet and distilled water for 1 wk, a blood sample was collected from the orbit of each rat for hematological analysis. Subsequently, the rats were divided randomly into six groups (10 rats per group), consisting of: a normal control

group (Group I, basal feed diet and distilled water), a hyperlipidemia model group (Group II, high lipid diet and distilled water), a low-dose LMPP treatment group (Group III, high lipid diet and LMPP (0.135 g.kg⁻¹ BW)), a medium-dose LMPP treatment group (Group IV, high lipid diet and LMPP (0.405 g.kg⁻¹ BW)), a high-dose LMPP treatment group (Group V, high lipid diet and LMPP (1.215 g.kg⁻¹ BW)), and a positive control group (Group VI, high lipid diet and lovastatin (1.1 mg.kg⁻¹ BW)).

Selection of dosage

Gong *et al.* (2007) reported that the 50% lethal dose (LD₅₀) of LMPP was greater than 10 g.kg⁻¹ body weight and LMPP is a nontoxic substance. According to the requirements of the Technical Standards for Determination and Assessment of Health Foods (Ministry of Health, 2003) and the results of a preliminary study, the low-, medium-, and high-LMPP treatment groups were treated daily with LMPP at 0.135, 0.405, and 1.215 g.kg⁻¹ body weight, respectively, using the following procedures. Every day, an appropriate amount of LMPP powder was dissolved in 3 mL of distilled water and administered by intragastric infusion (once per day). The positive control group was treated daily with 1.1 mg.kg⁻¹ body weight of lovastatin by intragastric infusion, while the normal control and hyperlipidemia model groups were treated daily with the same volume of distilled water. After treatment for 45 d, the rats were deprived food for 12 hr and blood samples were collected from their orbits. Sera were separated from the blood samples and kept for subsequent analyses.

Measurement of serum oxidation indices

Total serum SOD activity, GSH-PX activity, MDA and NO concentrations were determined by the colorimetric methods using the instructions included with the assay kits. Briefly, total serum SOD activity was assayed at 550 nm using the hydroxylamine method, serum GSH-PX activity was assayed at 412 nm

with dithio-bis-nitrobenzoic acid, serum MDA concentrations were determined at 532 nm with thiobarbituric acid, and serum NO concentrations were determined at 550 nm using a nitrate reductase assay (Hou *et al.*, 2009). Serum ET-1 concentrations were determined by enzyme-linked immunosorbent assay (ELISA) and a microplate reader (Multiskan MK3; Thermo Fisher Scientific; Waltham, MA, USA).

Statistical analysis

Data were expressed as mean \pm SD. Data were analyzed by Grubbs' tests (Grubbs, 1950) to identify and remove potential outliers using the Microsoft Excel 2003 (Microsoft Corporation, USA) and Duncan's one-way ANOVA was carried out using the SPSS 16.0 software for Windows (SPSS Inc., Chicago, IL, USA). Statistical significance was determined as significant at $P < 0.05$ and highly significant at $P < 0.01$.

RESULTS

Antioxidative activity of large molecular polymeric pigments extracted from Zijuan Pu-erh tea *in vitro*

In this study, the reducing power and the scavenging rates of the DPPH radical and of the hydroxyl radical of LMPP were measured in a concentration range from 0.1 mg.mL⁻¹ to 0.5

mg.mL⁻¹ (Table 1). As expected, the reducing power and the scavenging rates of the DPPH and hydroxyl radicals increased with an increasing concentration of LMPP. Of the three antioxidative indices, LMPP at 0.5 mg.mL⁻¹ showed the highest effect. Furthermore, the differences in the scavenging rates of the DPPH radical and hydroxyl radical at different concentrations all appeared to be significant ($P < 0.01$). In addition, the IC₅₀ concentrations of the DPPH radical and hydroxyl radical were 0.217 mg.mL⁻¹ and 0.461 mg.mL⁻¹, respectively. These results suggested that LMPP extracted from Zijuan Pu-erh tea had a good scavenging effect on free radicals, and possible protective effects against oxidative damage diseases through the intake of LMPP.

Antioxidative activity of large molecular polymeric pigments extracted from Zijuan Pu-erh tea *in vivo*

Serum superoxide dismutase and glutathione peroxidase activities

At the end of adaptive feeding, all groups had similar serum superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) activities (Table 2, $P > 0.05$). After subsequent treatment for 45 d, the hyperlipidemia model group had a significantly ($P < 0.05$) lower SOD activity than that in all the other groups. The SOD activities

Table 1 Antioxidative activity of large molecular polymeric pigments (LMPP) with different concentrations *in vitro*.

LMPP (mg.mL ⁻¹)	Reducing power (Absorbance)	DPPH radical scavenging rate (%)	Hydroxyl radical scavenging rate (%)
0.1	0.195 \pm 0.02 ^a	31.44 \pm 4.53 ^a	16.41 \pm 1.37 ^a
0.2	0.339 \pm 0.03 ^b	56.05 \pm 5.04 ^b	28.95 \pm 0.56 ^b
0.3	0.567 \pm 0.05 ^c	72.28 \pm 4.18 ^c	40.85 \pm 2.80 ^c
0.4	0.619 \pm 0.06 ^c	79.76 \pm 1.59 ^d	47.58 \pm 1.24 ^d
0.5	0.835 \pm 0.09 ^d	88.15 \pm 0.65 ^e	61.86 \pm 1.59 ^e
IC ₅₀	na	0.217 mg.mL ⁻¹	0.461 mg.mL ⁻¹

na = Not applicable. All data are presented as mean \pm SD, n = 9.

Values with the same lowercase superscript in the same column are significant at $P < 0.01$.

IC₅₀ = 50% inhibitory concentration. IC₅₀ values of DPPH and hydroxyl radical were calculated by Probit, regression, SPSS 16.0 software (Cai *et al.*, 2013; Tao *et al.*, 2011; Yang *et al.*, 2010).

of the low-, medium-, and high-dose LMPP treatment groups were increased by 58.21, 56.89 and 66.88%, respectively, compared with those of the hyperlipidemia model group. Furthermore, the positive control group and all three LMPP treatment groups had significantly ($P < 0.05$) higher SOD activity than that in the normal control group. These results suggested that similar to lovastatin, LMPP enhanced the activity of SOD.

After the 45 d treatment, the hyperlipidemia model group and the low-dose LMPP treatment group had similar GSH-PX activity to that in the normal control group ($P > 0.05$). The positive control group and the medium- and high-dose

LMPP treatment groups had significantly ($P < 0.05$) higher GSH-PX activity than that in the normal control and hyperlipidemia model groups. These findings showed that intragastric infusion of LMPP dose-dependently enhanced the SOD and GSH-PX activities in rats consuming a high-lipid diet.

Serum malondialdehyde, nitric oxide and endothelin concentrations

At the end of adaptive feeding, all groups had similar malondialdehyde (MDA), nitric oxide (NO), and endothelin (ET-1) concentrations (Table 3, $P > 0.05$). After subsequent treatment for 45 d, the hyperlipidemia model group showed

Table 2 Effect of large molecular polymeric pigments on serum superoxide dismutase (SOD) and glutathione peroxidase (GSH-PX) activities in rats.

Group	SOD (units.mL ⁻¹)		GSH-PX (units.mL ⁻¹)	
	After 7 d adaptive feeding	After 45 d treatment	After 7 d adaptive feeding	After 45 d treatment
Group I	297.29±24.46 ^a	270.28±36.94 ^b	4486.5±521.04 ^a	4121.0±446.91 ^a
Group II	292.28±22.18 ^a	215.92±64.76 ^a	4583.1±484.09 ^a	4211.6±266.87 ^a
Group III	294.62±16.38 ^a	341.60±27.52 ^c	4572.4±475.28 ^a	4592.0±377.16 ^{ab}
Group IV	291.15±24.92 ^a	338.74±23.84 ^c	4589.3±488.07 ^a	4742.7±502.65 ^b
Group V	287.22±23.77 ^a	360.33±34.31 ^c	4580.2±482.11 ^a	5437.0±392.74 ^c
Group VI	293.27±14.73 ^a	344.92±37.34 ^c	4591.4±490.06 ^a	4882.0±383.50 ^b

All data are presented as mean ± SD, n = 10.

Values with the same lowercase superscript in the same column are significant at $P < 0.05$.

Table 3 Effect of large molecular polymeric pigments on serum malondialdehyde (MDA), nitric oxide (NO) and endothelin-1 (ET-1) concentrations in rats.

Group	MDA(nmol.mL ⁻¹)		NO(μmol.L ⁻¹)		ET-1(ng.L ⁻¹)	
	After 7 d adaptive feeding	After 45 d treatment	After 7 d adaptive feeding	After 45 d treatment	After 7 d adaptive feeding	After 45 d treatment
Group I	3.78±0.27 ^a	3.65±0.43 ^b	168.43±38.47 ^a	156.91±26.33 ^{ab}	128.17±8.55 ^a	122.04±11.95 ^a
Group II	3.79±0.27 ^a	5.18±0.37 ^c	171.23±25.93 ^a	114.16±22.75 ^a	128.46±4.40 ^a	169.40±22.68 ^b
Group III	3.78±0.25 ^a	3.48±0.22 ^b	158.31±40.54 ^a	143.48±29.88 ^{ab}	130.53±6.39 ^a	126.26±19.74 ^a
Group IV	3.87±0.23 ^a	3.26±0.11 ^b	153.93±22.18 ^a	153.48±60.19 ^{ab}	128.46±4.40 ^a	124.08±24.03 ^a
Group V	3.84±0.23 ^a	2.11±0.66 ^a	163.37±37.08 ^a	177.08±22.60 ^b	131.39±5.40 ^a	110.76±18.29 ^a
Group VI	3.85±0.25 ^a	3.27±0.31 ^b	149.21±17.56 ^a	172.02±36.53 ^b	129.33±5.52 ^a	153.50±20.14 ^b

All data are presented as mean ± SD, n = 10.

Values with the same lowercase superscript in the same column are significant at $P < 0.05$.

significantly ($P < 0.05$) higher serum MDA concentrations than those in all the other groups. The MDA concentrations of the low-, medium-, and high-dose LMPP treatment groups and the positive control group were decreased by 32.82, 37.06, 59.27 and 36.87%, respectively, compared with that in the hyperlipidemia model group. The high-dose LMPP treatment group had significantly ($P < 0.05$) lower serum MDA concentrations than those in all the other groups, which suggested that intragastric infusion of a high dose of LMPP effectively enhanced the capability of rats consuming a high-lipid diet to clear serum MDA, a product of lipid peroxidation.

In the hyperlipidemia model group, the NO concentrations were 27.24% lower than those in the normal control group. In the low-, medium-, and high- LMPP treatment groups and the positive control group, the serum NO concentrations were 25.68, 34.44, 55.11 and 50.68% higher ($P < 0.05$), respectively, compared with the hyperlipidemia model group. These results showed that intragastric infusion of LMPP increased the serum NO concentrations in rats, suggesting that LMPP has protective cardiovascular effects. The hyperlipidemia model and positive control groups had significantly ($P < 0.05$) higher serum ET-1 concentrations than those in the normal control group. The low-, medium-, and high-dose LMPP treatment groups had significantly ($P < 0.05$) lower serum ET-1 concentrations than those in the hyperlipidemia model group and similar concentrations to those in the normal control group ($P > 0.05$). The ET-1 concentrations of the low-, medium-, and high-dose LMPP treatment groups, were decreased by 25.47, 26.75 and 34.62%, respectively, compared with that in the hyperlipidemia model group. These results demonstrated that intragastric infusion of LMPP effectively prevented serum ET-1 elevation in rats consuming a high-lipid diet.

In summary, the results showed that treatment with LMPP increased the NO concentrations and reduced those of MDA and ET-1, and the effects were positively related to

the dose of treatment.

DISCUSSION

Reactive oxygen species (ROS) and free radicals such as hydrogen peroxide and the hydroxyl radical are constantly formed in the human body by normal metabolic action and have been implicated in the pathogenesis of certain human diseases, including cancer, aging, diabetes and atherosclerosis (Moskovitz *et al.*, 2002). Their actions are opposed by a balanced system of antioxidant defenses including antioxidant compounds (such as ascorbic acid) and some enzymes (such as SOD and GSH-Px) and upsetting this balance causes oxidative stress, which can lead to cell injury and death (Ames *et al.*, 1993). SOD plays a critical role in the anti-oxidation system as its concentrations in tissues or blood reflect the conditions of oxidative stress and anti-oxidative capacity (Beckman *et al.*, 1993). GSH-PX is an important peroxidase that is widely found in the human body and it specifically catalyzes the decomposition of hydrogen peroxide by glutathione and thus contributes to the structure and function of cell membranes (Hafeman *et al.*, 1974). MDA is a final product of lipid peroxidation initiated by the attack of radicals on polyunsaturated fatty acids in biomembranes and the MDA concentration reflects the rate or intensity or lipid peroxidation in the host, and an abnormal elevation of MDA concentration may result in damage to cells (Draper and Hadley, 1989). With increasing blood lipid concentrations, the serum concentration of lipid peroxides increases correspondingly and thus causes damage to endothelial cells and blood vessels which then release ET-1 (a potent vasoconstrictor) in response to the damage (Pinzani *et al.*, 1996; Rockey *et al.*, 1998). ET-1 and NO (a vasodilator) are important factors that work in coordination to regulate the contraction of the vascular endothelium and to regulate the blood circulation in local tissues (Potenza *et al.*, 2005).

The present study was the first to demonstrate the antioxidative effects from daily oral administration of aqueous Zijuan Pu-erh tea LMPP in a hyperlipidemia rat model. The main outcome was the finding that LMPP substantially reduces the oxidation of lipids in the blood, thereby reducing pro-atherogenic processes.

During the last decade, the effects of tea and tea polyphenols have been extensively investigated and studies have shown that tea is capable of exerting an antioxidant and anti-aging effect (Cooper *et al.*, 2005; Duh *et al.*, 2004). However, the fermentation process lowers the polyphenol levels in Zijuan Pu-erh tea; conversely, levels of LMPP greatly increase after the fermentation process (Wang *et al.*, 2012). LMPP is a polymer resulting from the oxidative polymerization of polyphenols, such as theaflavins and thearubigins. During the manufacture of Pu-erh tea, the LMPP concentration increases and becomes a critical determinant of the quality of the final product (Wang *et al.*, 2011). Therefore, LMPP is a kind of main bioactive component in Zijuan Pu-erh tea. The data presented in this investigation indicated that Zijuan Pu-erh tea LMPP, like aqueous Pu-erh tea extract, could scavenge DPPH radicals and hydroxyl radicals and showed a strong reducing power; furthermore, it reduced lipid peroxidation to protect the cardiovascular system.

CONCLUSION

LMPP may consist of polymeric substances comprising pigments, polysaccharides, lipids and proteins. LMPP showed significant scavenging activities on the hydroxyl radical and the DPPH radical and also had strong reducing power. Intra-gastric infusion of LMPP effectively enhanced SOD and GSH-PX activities, reduced MDA formation and ET-1 release, and increased NO concentrations in rats consuming a high-lipid diet. The potential application of LMPP as a natural antioxidant source could be predicted.

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