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Full Length Research Paper

Evaluation of antioxidant activity of polysaccharides isolated from *Camellia sinensis* (tea) in exhausting training mice

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We investigated the antioxidant effects of *Camellia sinensis* (tea) polysaccharides in exhausting training mice. Three groups of mice were fed with *C. sinensis* (tea) polysaccharides (100, 200 and 300 mg/kg, respectively). The results showed that plasma, lever and heart MDA level were reduced after 30 days of *C. sinensis* (tea) polysaccharides treatment. Compared with control mice, blood, liver and heart superoxide dismutase (SOD), catalase (CAT), GPx activities were significantly increased after 30 days of *C. sinensis* (tea) polysaccharides treatment. Overall, both extracts of *C. sinensis* (tea) polysaccharides possessed good antioxidant properties and can be developed as antifatigue medicine.

Key words: *Camellia sinensis*, polysaccharides, antioxidant, superoxide dismutase, exhausting training.

INTRODUCTION

Free radicals may play a pivotal role in the pathogenesis of a number of diseases. Reactive oxygen species are believed to be usually generated in aerobic cells and aerobic organisms are provided with antioxidant defense systems that could avert damage due to oxidative stress (Fridovich, 1978 and Sies, 1985).

The concentrations of ROSs are modulated by antioxidant enzymes and non-enzymatic scavengers (Saxena et al., 1993). Alterations in the antioxidant enzymes activities such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-Px), the three primary scavenging enzymes, have been demonstrated in different tissues of diabetic animals (Kakkar et al. 1995).

In 1982 Davies et al. (1982) provided the first direct proof, by electron spin resonance spectroscopy (ESR), that exercise at high intensity causes an increased free radical production. Davies and Hochstein (1982) then identified the ESR signal as mitochondrial ubisemiquinone. Interestingly, the intensity of the ESR signal could be modulated by vitamin E status, with vitamin E-deficient animals exhibiting significantly greater free radical 'concentrations' than vitamin E-sufficient animals (Davies et al., 1982). Further supporting this view (mitochondrial theory of reactive oxygen species production during exercise) is the adaptation to training of mitochondrial Mn-superoxide dismutase and glutathione peroxidase (Higuchi et al., 1985; Ji, 1999). Free radical damage to cell constituents during strenuous physical exercise was first suggested in 1978 by Dillard et al. (1978), who detected a substantial increase of exhaled pentane during exercise.

It is widely believed that consumption of antioxidant-rich foods will help to improve health status (Sun et al., 2010; Fan et al., 2009; Gao et al., 2010; Alim et al., 2009). Therefore, it is important to show that dietary antioxidants are bioavailable. The ability to absorb a significant amount of dietary antioxidants is a prerequisite for their bioavailability. Absorption and bioavailability studies on selected dietary antioxidants have been carried out in human and in *in vitro* preparations, such as cell culture and isolated organs, whereas studies involving *in vivo* or *in situ* animal models are relatively few. A simple and inexpensive *in vivo* or *in situ* animal model would be useful to allow for a relatively quick assessment of a large number of consumable extracts

with purported antioxidant properties (Liu et al., 2009; Oluduro and Aderiye, 2009).

Camellia sinensis (tea) is the most widely consumed beverage in the world, and its polyphenolic compounds have been found to possess widespread biologic functions and health benefits (Kao et al., 2006). There is a body of scientific evidence that indicates that green tea (*C. sinensis*) and its catechins, especially (–) epigallocatechin gallate, exhibit antiobesity and antidiabetic effects (Babu, Sabitha and Shyamaladevi, 2006; Sabu et al., 2002). Most of the polyphenols in green tea are flavonoids, commonly known as catechins. The major catechins in green tea are (–)-epicatechin (EC), (–)-epicatechin-3-gallate (ECG), (–)-epigallocatechin (EGC) and (–)-epigallocatechin-3-gallate (EGCG). The polyphenolic fraction of green tea has been reported to have multiple pharmacological actions (Liao et al., 2001). The polysaccharide of green tea has also been reported to have immunological, anti-radiation, anti-blood coagulation, anti-cancer, anti-HIV and hypoglycemic activities (Isiguki et al., 1992; Wang et al., 2000; Wang and Wang, 1992; Zhou et al., 1997). Great advances have been made in chemical and hypoglycemic studies of *C. sinensis* (tea) polysaccharide (Tadakazu et a., 1998; Wang et al., 2001). In this study, antioxidant activity of *C. sinensis* (tea) polysaccharides were investigated in exhausting training mice.

MATERIALS AND METHODS

Preparation of *C. sinensis* (tea) polysaccharides

Samples of *C. sinensis* (tea) were harvested and air-dried at room temperature for 5 days. The dried tea was ground to powder to pass through a 1 mm screen. 3 L of water was added to 1 kg powder, boiled and simmered for 3 h. The extraction was repeated, and two extracts were combined and filtered. The filtrate was concentrated to 300 ml in a vacuum desiccator at 70°C and 95% ethanol was added so as to yield a 60% ethanol solution in order to obtain the polysaccharide precipitation. The proteins in the product were removed using the Sevag reagent (Navarini et al., 1999) several times. After removal of the Sevag reagent, the final product was vacuum-dried, and the resulting powder was tea polysaccharides. The total polysaccharides of *C. sinensis* (tea) polysaccharides were measured. The content (%) of total polysaccharide was 87.9%.

Experimental groups and protocols

Balb/c mice (30 to 40 g) were obtained and maintained in the Central Animal Facility. Animals were housed in polypropylene cages at a room temperature of 21 ± 2°C with12 h light/12 h dark cycles and had free access to standard pellets and water *ad libitum.* All mice received exhausting training for 30 days. All animals were then randomly divided into four groups. group 1 (control) mice received exhausting training every day and fed with a standard diet; group 2, 3 and 4 mice received exhausting training every day and were fed with *C. sinensis* (tea) polysaccharides (100, 200 and 300 mg/kg b.w., respectively). The experiment last for 30 days.

At the end of the experimental period, the mice were sacrificed by cervical dislocation, and blood samples were taken from the orbital venous congestion to determine the plasma biomarkers. Plasma was prepared by centrifugation of the blood at 10,000 g for 5 min at 4°C and stored at –70°C until analysis. All animal experiments were approved by the Institutional Animal Care and Use Committee (IACUC).

Biochemical analysis

The tissues MDA concentration was determined using the method described by Jain et al. (1989) based on TBA reactivity. Briefly, 0.2 ml supernatant obtained from tissues, 0.8 ml phosphate buffer (pH 7.4), 0.025 ml BHT and 0.5 ml 30% TCA were added to the tubes and mixed. After 2 h incubation at –20°C, the mixture was centrifuged (4000 × g) for 15 min. After this, 1 ml supernatant was taken and added to each tube, and then 0.075 ml of 0.1 M EDTA and 0.25 ml of 1% TBA were added. These tubes with Teflon-lined screw caps were incubated at 90°C in a water bath for 15 min and cooled to room temperature. The optical density was measured at 532 for tissues MDA concentration.

The superoxide dismutase level was determined using a method of Asada et al. (1974). SOD activity was assayed by measuring its ability to inhibit the photoreduction of NBT (nim). One millilitre of homogenate supernatant is combined with 50 mm phosphate buffer (pH 7.8), 39 mM methionine, 2.6 mM NBT, and 2.7 mM EDTA. To obtain a concentration of 0.26 mM, riboflavin was added as the last, and switching on the light started the reaction. The changes in absorbance at 560 nm were recorded after 20 min. One unit of SOD is defined as the amount that inhibits the NBT reaction by 50%. Specific activity was defined as U/mg protein.

CAT activities were assayed using the method described by Claiborne (1986). The reaction mixture (1 ml) consisted of 50 mM potassium phosphate (pH 7.0), 19 mM H_2O_2, and a 20 to 50 µl sample. The reaction was initiated by the addition of H_2O_2, and absorbance changes were measured at 240 nm (25°C) for 30 s. The molar extinction coefficient for H_2O_2 is 43.6 $M^{-1}cm^{-1}$. The CAT activity was expressed as the unit that is defined as µmol of H_2O_2 consumed per min per gram of wet tissue. GSH-Px was analyzed by the method of Flohe and Gunzler (1984). 50 µl of 0.1 M phosphate buffer (pH 7.0), 100 µl enzyme sample, 100 µl glutathione reductase (0.24 units) and 100 µl of 10 mM GSH were mixed. The mixture was pre-incubated for 10 min at 37°C followed by the addition of 100 µl of 1.5 mM NADPH in 0.1% $NaHCO_3$. The overall reaction was started by adding 100 µl of pre-warmed hydrogen peroxide and the decrease in absorption at 340 nm monitored for 3 min.

Statistical analysis

Data for the Morris water maze and passive avoidance tests were expressed as mean ± S.E.M. The activity of acetylcholinesterase and the levels of antioxidant values were expressed as mean ± S.D. Antioxidant values were analyzed by one-way ANOVA. The data were considered to be statistically significant if the probability had a value of 0.05 or less.

RESULT

The effect of *C. sinensis* (tea) polysaccharides on blood, heart and liver MDA level in mice are shown in Figure 1. Treatment of animals with different doses of *C. sinensis* (tea) polysaccharides (groups II–V; 100 to 300 mg/kg b.w.) for 30 days significantly reduced the levels of blood, heart and liver MDA, as compared with the group of

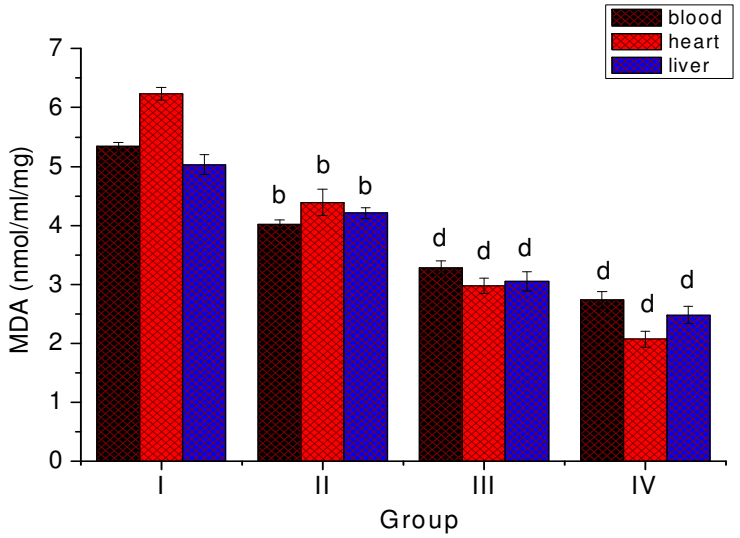

Figure 1. Effect of *C. sinensis* (tea) polysaccharides on blood MDA level $^{b}p < 0.01$, compared with group I; $^{d}p < 0.01$, compared with group II.

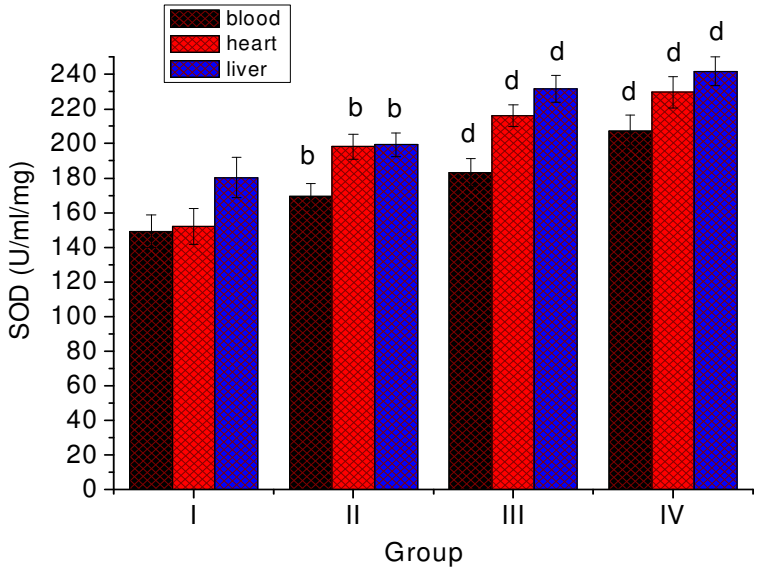

Figure 2. Effect of *C. sinensis* (tea) polysaccharides on blood SOD activity, $^{b}p < 0.01$, compared with group I, $^{d}p<0.01$, compared with group II.

control mice (group I) ($p < 0.05$). The effect was found to be dose-dependent.

Variance of blood, heart and liver SOD, CAT and GPx activities were showed in Figures 2 and 3 and 4. The three groups of mice were treated with the *C. sinensis* (tea) polysaccharides (100, 200 and 300 mg/kg b.w.) significantly elevated blood, heart and liver SOD, CAT and GPx activities in a dose-dependent manner. There was marked statistical difference between polysaccharides-treated groups and control group.

DISCUSSION

Antioxidants are closely related to their biofunctionalities, such as the reduction of cellular abnormalities like DNA damage, mutagenesis, carcinogenesis and which is often associated with free radical propagation in biological systems (Zhu et al., 2002). Recent reports show that dietary phytochemicals act as excellent free radical scavengers in different experimental systems (Soobrattee et al., 2005). Lipid peroxidation is a well-established

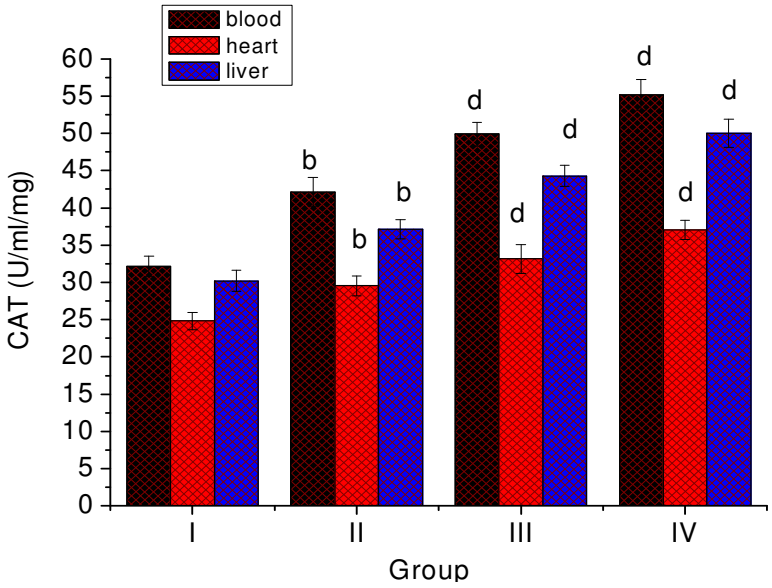

Figure 3. Effect of *C. sinensis* (tea) polysaccharides on blood CAT activity, $^{b}p < 0.01$, compared with group I, $^{d}p < 0.01$, compared with group II.

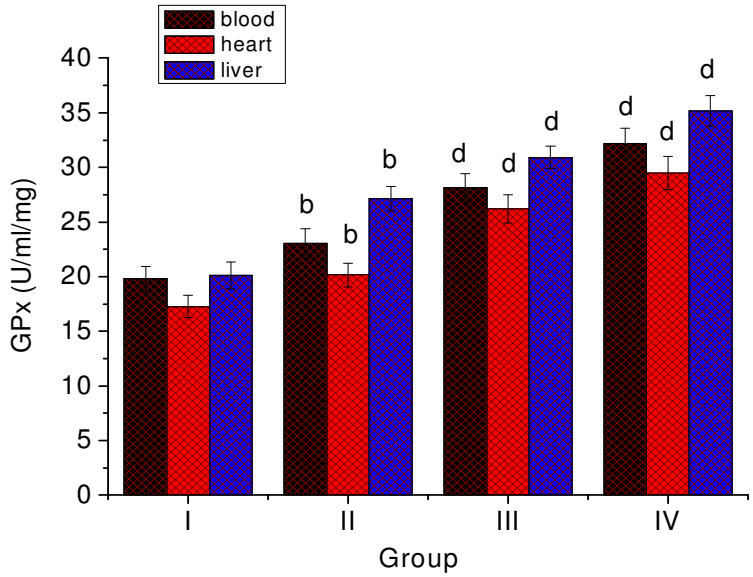

Figure 4. Effect of *C. sinensis* (tea) polysaccharides on blood GPx activity, $^{b}p < 0.01$, compared with group I, $^{d}p < 0.01$, compared with group II.

mechanism of oxidative damage caused by reactive oxygen species, and the increase in lipid peroxide levels is one of the most important contributing factors in the development of diabetes-related complications (Zigler and Hess, 1985). Antioxidants play a major role in protecting biological systems against reactive oxygen species and reflect the antioxidant capacity of the system (Irshad and Chaudhuri, 2002). MDA is an end-product of lipid peroxidation, and thus, MDA production reflects the peroxidation of polyunsaturated phospholipids (Hsieh et al., 2006; Luo et al., 2006). Our results show that *C. sinensis* (tea) polysaccharides markedly reduced tissues MDA level in a concentration dependent manner.

The effect of *C. sinensis* (tea) polysaccharides on the activity of SOD in red cells of mice was studied. The result showed that the activity of SOD in red cells of mice was increased obviously by feeding *C. sinensis* (tea) polysaccharides (Deng and Xu, 1998). The impacts of

C. sinensis (tea) polysaccharides on the antioxidant function and the morphology changes of liver and kidney in diabetic rats were studied. It was found that after four weeks' administration of the *C. sinensis* (tea) polysaccharides in diabetic rats, the liver and kidney SOD and GSH-PX activity were significantly improved while the MDA content decreased greatly, which in turn enhanced the antioxidant capacity (Ni et al., 2003). In the present study, the activity of SOD increased significantly in animals treated with *C. sinensis* (tea) polysaccharides for 30 days compared to the control control. In addition, the activity of CAT exhibited an similar effect in tea polysaccharides treated animals. An increase in SOD activity of SOD may be stimulated by excess ROS to eliminate superoxide radicals. The decrease in SOD amounts of SOD over time with *C. sinensis* (tea) polysaccharides treatment reflects the elimination of superoxide radicals and the excessive generation of hydrogen peroxide to stimulate an increase in CAT activity. At last, 30 days of *C. sinensis* (tea) polysaccharides treatment significantly enhanced tissues GPx activity in a dose-dependent manner.

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