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Full Length Research Paper

Evaluation of antioxidant properties and anti- fatigue effect of green tea polyphenols

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Free radical production during exercise contributes to fatigue and antioxidant treatment might be a valuable therapeutic approach. In this study, the antioxidant properties of green tea polyphenols (GTP) were evaluated *in vitro* through hydroxyl radical-scavenging activity, and ascorbic acid was used as reference compound. The study showed that GTP possessed more pronounced hydroxyl radical scavenging activity than ascorbic acid, and the scavenging activity increased with increasing of the concentration. The anti-fatigue effects of GTP were evaluated *in vivo* through a swimming exercise test. Forty male Kunming (KM) mice were randomly divided into 4 groups (n = 10 in each group) including one control group and three GTP administered groups (60, 120 and 240 mg/kg body weight). The GTP were administered to the mice every day for 4 weeks. The mice were submitted to weekly swimming exercise supporting constant loads (lead fish sinkers, attached to the tail) corresponding to 10% of their body weight. The study showed that GTP has an anti-fatigue effect and also prolongs the swimming time of mice with less fatigue. Although there could be several mechanisms of action of GTP for its effectiveness to combat fatigue, the antioxidant properties seem to be highly significant.

Key words: Antioxidan, anti- fatigue, green tea polyphenols, hydroxyl radical, swimming exercise test.

INTRODUCTION

Tea is second only to water in popularity as a beverage in the world, and its medicinal properties have been widely explored (Mukhtar and Ahmad, 2000; Wu and Wei, 2002; El-Beshbishy, 2005; Gomikawa et al., 2008). The tea plant, Camellia sinensis, is a member of the theaceae family. According to the manufacturing process, teas are classified into three major types: 'non-fermented' green tea, 'semi-fermented' oolong tea and 'fermented' black and red (*Pu-Erh*) teas (McKay and Blumberg, 2002; Cabrera et al., 2006). Green tea is produced from steaming fresh leaves at high temperatures, thereby inactivating the oxidizing enzymes and leaving the polyphenol content intact (Zaveri, 2006). Polyphenols account for up to 30% of the dry weight and serve as major effective components of green tea (Graham, 1992). Most of the polyphenols being flavanols are more commonly known as catechins. The primary catechins in green tea are epicatechin (EC), epicatechin-3-gallate (ECG), epigallocatechin (EGC), and epigallocatechin-3-gallate (EGCG) (Figure 1) (Fujiki et al., 2002).

A number of polyphenolic compounds from green tea have been found to have a variety of nutritional and pharmacological properties, including antioxidant (Cai et al., 2002), anti-carcinogenic (Yang and Wang, 1993), anti-diabetic(Matsumoto et al., 1993), anti-bacterial (Miura et al., 2001), anti-mutagenic (Wang et al., 1989; Gupta et al., 2002), anti-hypertensive (Potenza et al., 2007), antiviral (Song et al., 2005) and anti-atherogenic effects (Chyu et al., 2004). Consequently, there is growing interest in the use of green tea polyphenols for the treatment and prevention of diseases.

Exercise is known to promote good health and prevent various diseases. However, strenuous exercise can cause oxidative stress which leads to an imbalance between reactive oxygen species (ROS) production and antioxidant defense (You et al., 2009). Under normal circumstances, ROS are neutralized by an elaborate endogenous antioxidant system, comprising of enzymatic

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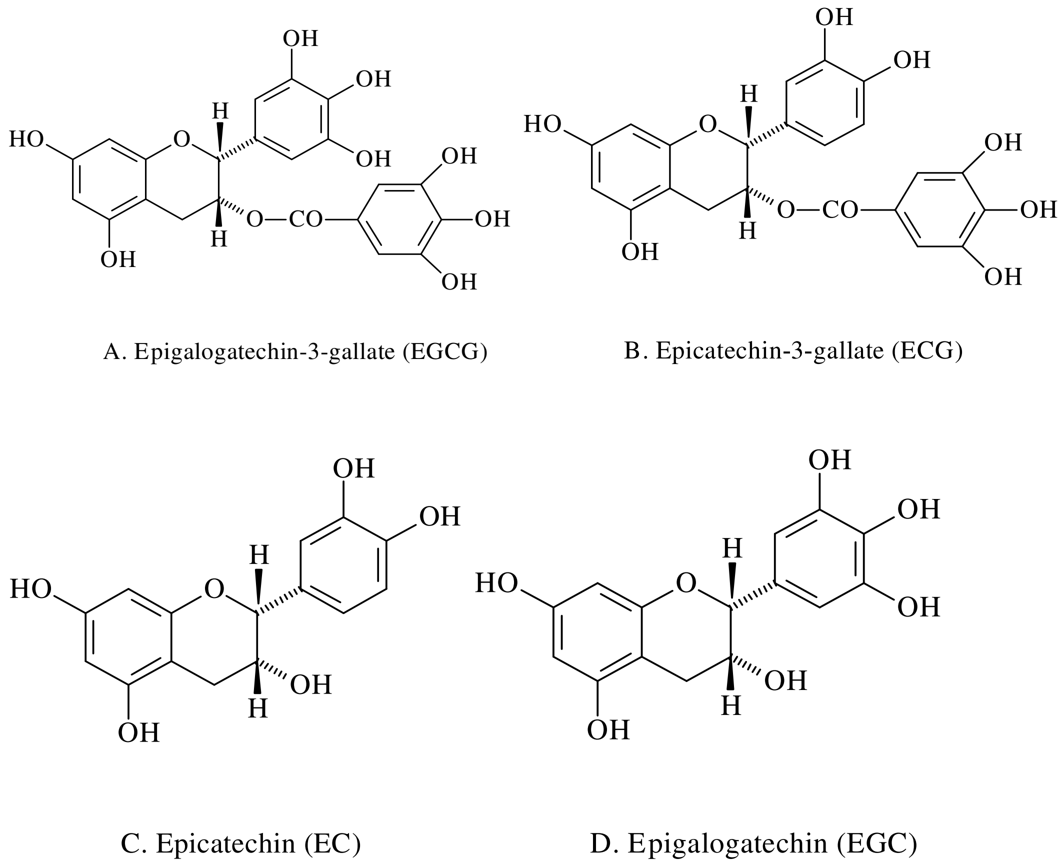

Figure 1. Catechins in green tea extracts.

and non-enzymatic antioxidants (Gohil et al., 1986; Radák et al., 2001; Urso and Clarkson, 2003; Keong et al., 2006). However, during strenuous exercise, the rate of ROS production may overwhelm the body's capacity to detoxify them, which can lead to increased oxidative stress. And free radical production reaches the highest level when exercise is exhaustive (Sjodin et al., 1990; Ji et al., 1998; Keong et al., 2006; Rosa et al., 2007; Prigol et al., 2009). There is evidence that free radical production during exercise contributes to fatigue and oxidative stress and it has been suggested to reduce endurance performance during exhaustive exercise (Novelli et al., 1990; Coombes et al., 2002; Keong et al., 2006). In this study, the anti-fatigue effects of green tea polyphenols were investigated through swimming exercise of Kunming (KM) mice. Also, their antioxidant properties were determined through scavenging activity to hydroxyl radicals.

MATERIALS AND METHODS

Reagents

All chemicals and media were purchased from Xuzhou Chemical Reagents Co., Ltd (Xuzhou, China) unless otherwise indicated. Fresh green tea was purchased from Jiangsu Zhongfu Tea Co., Ltd (Yixing, China).

Green tea polyphenols preparation

Green tea polyphenols (GTP) was prepared using microwave assisted extraction according to Quan et al. (2006). 100 g of fresh green tea were dried overnight at 40℃ and ground through a 1-mm sieve, then immerse in solvents (1:5 to 1:15 g/ml) for a certain time (0 to 90 min). Then it was transferred to flask, adjusted pH, and brewed in microwave oven (450 W) (Time: 300 to 420 s), radiation is done at regular intervals (30 s interval) to keep temperature from rising above 70℃. After that, the infusion was let cool down to room temperature, filtered to separate solid and concentrated by rotary vacuum evaporation. Final GTP was stored in refrigerator at 4℃.

Selection of animals and care

Male Kunming (KM) mice (Grade 2, Certification No. 86047, weighing 18 to 22 g) used in this study were purchased from the Laboratory Animal Center of Xuzhou Medical College (Xuzhou, China.). The animals were housed in the animal care centre of China University of Mining and Technology (Xuzhou, China). They were kept in wire-floored cages under standard laboratory conditions of 12 h/12 h light/dark, 25 ± 2°C with free access to food and water. All animals received humane care in compliance with the Jiangsu Province guidance on experimental animal care. The protocol was approved by local animal study committee.

Table 1. Absorbance of green tea polyphenols and ascorbic acid at 532 nm.

Concentration (µg/ml)	Absorbance		
	Green tea polyphenols	Ascorbic acid	Control
10	0.692±0.008	0.956±0.009	1.054
20	0.526±0.011	0.883±0.007	
50	0.413±0.005	0.847±0.006	

Results are presented as mean ± SD (n = 3).

Hydroxyl radical-scavenging assay

The radical scavenging activity of GTP against hydroxyl radicals was measured using the method described previously with some modifications (Ohkawa et al., 1979; Kunchandy and Rao, 1990; Guan et al., 2007). Inhibitory effects of GTP on deoxyribose degradation were determined by measuring the competition between deoxyribose and GTP for the hydroxyl radicals generated from the Fe^{3+}/ascorbate/ EDTA/H_2O_2 system. The attack of the hydroxyl radical on deoxyribose leads to TBARS formation (Guan et al., 2007; Yi et al., 2008). Solutions of the reagents were made up in deaerated water before being used. The reaction mixture, containing test sample (10 to 50 µg/ml), was incubated with deoxyribose (3.75 mM), EDTA (100 µM), ascorbic acid (100 µM), H_2O_2 (1 mM), and FeCl3 (100 µM) in phosphate buffer (20 mM, pH 7.4) for 60 min at 37°C (Halliwell et al., 1987; Wu et al., 2007). The reaction was terminated by adding TBA (1%, w/v and 1 ml) and TCA (2%, w/v, 1 ml), then the tube was heated in a boiling water bath for 15 min. After the mixtures were cooled to room temperature, their absorbances at 532 nm were measured against a blank containing deoxyribose and buffer. Mixture without sample was used as control (Wu et al., 2007; Yi et al., 2008). Ascorbic acid was used as reference compound. Hydroxyl radical-scavenging activity (HRSA) was calculated using the following equation:

$$HRSA (\%) = [(A_c-A_s) / (A_c)] \times 100.$$

Where A_c is the absorbance with control, and A_s is absorbance with sample.

Swimming exercise test

The mice were allowed to adapt to the laboratory housing for at least 1 week. Forty male Kunming (KM) mice were randomly divided into 4 groups (n = 10 in each group): The first group designated as control dose group (CD) was administered with distilled water by gavage every day for 4 weeks. The second group designated as low dose group (LD) was administered with GTP of 60 mg/kg body weight day for 4 weeks. The third group designated as middle-dose group (MD) was administered with GTP of 120 mg/kg body weight day for 4 weeks. The fourth group designated as high-dose group (HD) was administered with GTP of 240 mg/kg body weight day for 4 weeks. The doses used in this study were confirmed to be suitable and effective in tested mice according to preliminary experiments. Samples were administrated in a volume of 150 ml. The tails of the mice were colored with a magic marker for individual recognition and the mice were submitted to weekly swimming exercise supporting constant loads (lead fish sinkers, attached to the tail) corresponding to 10% of their body weight (Ikeuchi et al., 2006; Zhang et al., 2007). The mice were assessed to be fatigued when they failed to rise to the surface of the water to breathe within 5 s and the time was immediately recorded (Ikeuchi et al., 2005; Lu et al., 2009). The swimming exercise was carried out in a tank (26×30×30 cm), filled with water to 24 cm depth and maintained at a temperature of 30±1°C.

Statistical analysis

The results are presented as mean ± SD. Statistical analysis was performed using ANOVA following Mann-Whitney U-test. $P<0.05$ were considered statistically significant.

RESULTS AND DISCUSSION

Scavenging of hydroxyl radicals of green tea polyphenols

It is well known that hydroxyl radicals are highly reactive-oxygen species. They are considered to cause the ageing of human body and some diseases (Siddhuraju and Becker, 2007), interact with the purine and pyrimidine bases of DNA as well as abstract hydrogen atoms from biological molecules (example, thiol compounds), leading to the formation of sulphur radicals which are able to combine with oxygen to generate oxysulphur radicals, a number of which damage biological molecules (Halliwell et al., 1987; Huang et al., 2009). When hydroxyl radical generated by the Fenton reaction attacks deoxyribose, deoxyribose degrades into fragments that react with TBA on heating at low pH to form a pink color, which can be quantified spectrophotometrically at 532 nm (Yi et al., 2008). So, one can calculate the inhibition effect from the changes of absorption. Absorbance of green tea polyphenols and ascorbic acid at 532 nm were shown in Table 1 and hydroxyl radical-scavenging activity of GTP and ascorbic acid were shown in Figure 2. The results suggested that GTP possessed more pronounced hydroxyl radical scavenging activity than ascorbic acid, and the scavenging activity increased with increasing concentration. It suggested that the GTP might be beneficial to the alleviation of physical fatigue, so GTP was used for the *in vivo* experiment in mice to estimate the anti-fatigue effect.

Anti-fatigue effect of green tea polyphenols

Recently, forced swimming of animals has been widely used for anti-fatigue and endurance tests (Wang et al., 1983; Kim et al., 2002; Sakata et al., 2003; An et al., 2006; Shin et al., 2006; Koo et al., 2008; Feng et al., 2009; Jing et al., 2009). Other methods of forced exercise such as the motor driven treadmill or wheel can cause animal injury and may not be routinely acceptable (Orlans, 1987; Lapvetelainen et al., 1997; Wu et al., 1998; Misra et al., 2005). In this study, the mice loaded

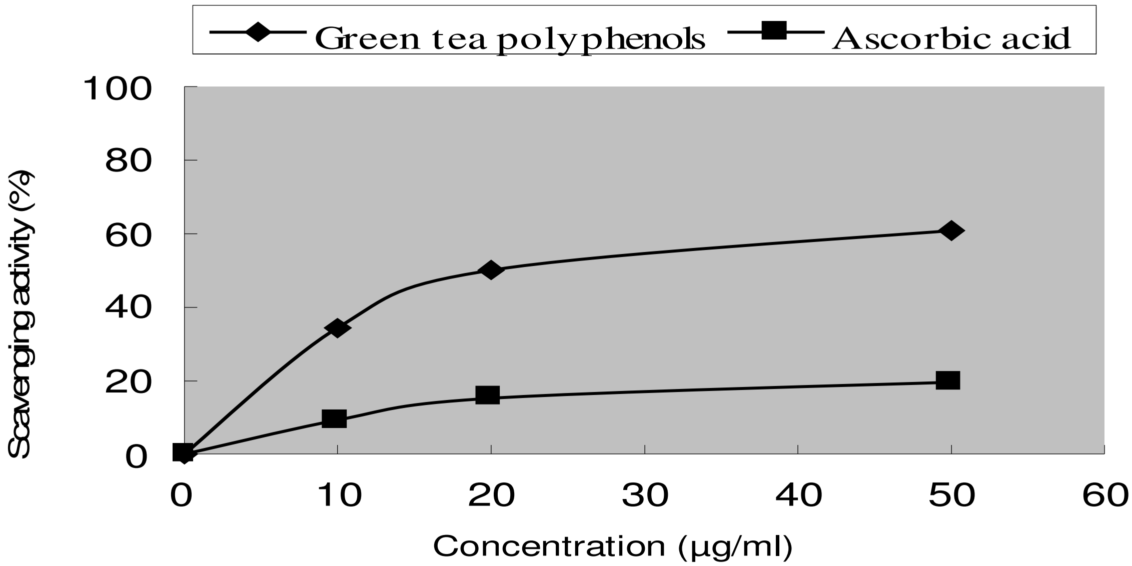

Figure 2. Hydroxyl radical-scavenging activity of GTP and ascorbic acid.

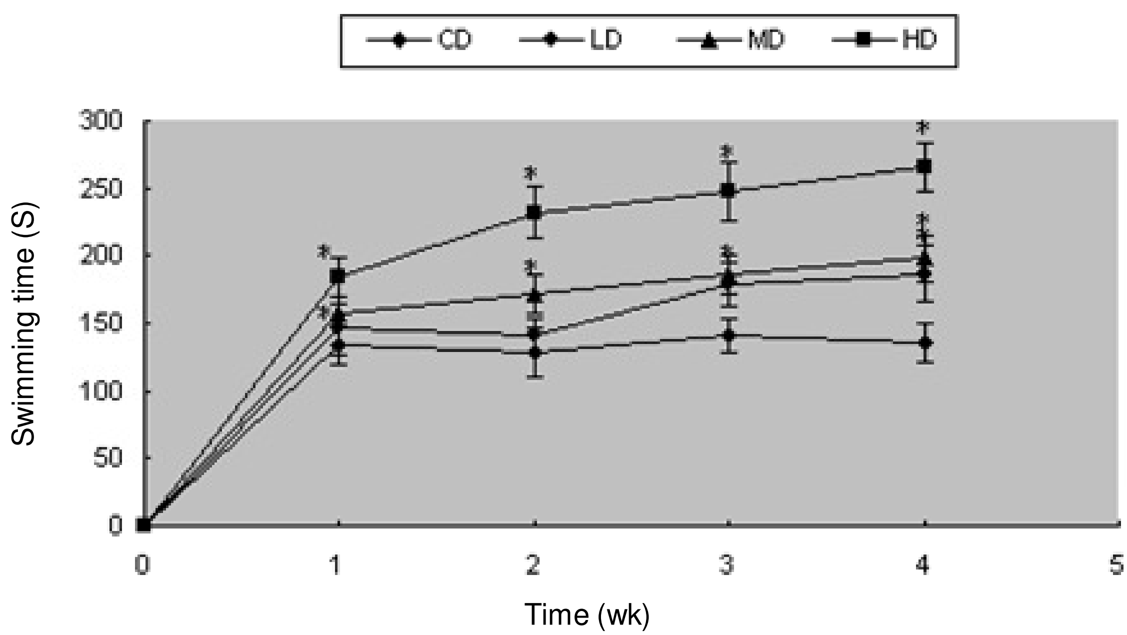

Figure 3. Effect of green tea polyphenols on swimming exercise in mice. Results are presented as mean ± SD (n = 10). * $p < 0.05$ vs. control.

with 10% of their body weight were placed in the water at room temperature (30±1 □) to swim and the mice were assessed to be fatigued when they failed to rise to the surface of the water to breathe within 5 s. As shown in Figure 3, the MD (120 mg/kg) and HD (240 mg/kg) groups showed a significant increase in swimming time to exhaustion as compared to the CD group from the first week. In the LD (60 mg/kg) group, a significant increase in swimming time to exhaustion as compared to the CD group was evident after 2 weeks. From these results, a conclusion can be drawn that GTP has an anti-fatigue

effect and also prolongs the swimming time of mice with less fatigue.

Conclusions

The present study established that green tea polyphenols possessed significant antioxidant properties through scavenging activity to hydroxyl radicals. In addition, green tea polyphenols showed an anti-fatigue effect on forced swimming of animals. Although there could be several mechanisms of action of green tea polyphenols for its effectiveness to combat fatigue, the antioxidant properties seem to be highly significant. Further studies on the mechanisms of action are under investigation.

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