Scavenging and anti-fatigue activity of Wu-Wei-Zi aqueous extracts

Chen Xiang* and Zhang Guohai

College of Physical Education, Wenzhou University, Wenzhou 325035, China.

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In this study, the radical scavenging properties and the anti-fatigue activity of Wu-Wei-Zi aqueous extracts (WAE) were evaluated, respectively. Forced swimming exercise of mice was carried out after 4 weeks of WAE administration and biochemical parameters related to fatigue, such as blood lactic acid (BLA), blood urea nitrogen (BUN), hepatic glycogen (HG), superoxide dismutase (SOD) and glutathione peroxidase (GPX) contents were determined. Results showed that WAE had strong scavenging activity to superoxide anion and 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical. And it had significant anti-fatigue activity, which could not only increase the hepatic glycogen, SOD and GPX contents but also extend the swimming time of the mice. It indicated that WAE is worthy of further study.

Key words: Scavenging, anti-fatigue, Wu-Wei-Zi, mice.

INTRODUCTION

Schisandra chinensis (Turcz.) Baill, a perennial lignifying liana, is mainly distributed in northeastern China. Fruits from S. chinensis (Turcz.) Baill, is called Wu-Wei-Zi in Chinese and is a traditional Chinese herb originally recorded in Shen Nong Ben Cao Jing (over 2000 year old Herbal Pharmacopoeia in China) (Xu et al., 2008; Kim et al., 2010). The main effective constituents of Wu-Wei-Zi are essentially oil and lignans (schisandrin A, deoxyisochisandrin, schisandrin B and schisandrin C) (Gao et al., 2003; Ma et al., 2007). Wu-Wei-Zi has been used for nourishing heart and stomach and strengthening immune function in traditional Chinese medicine (Huang et al., 2005; Ma et al., 2007). It is also used as a tonic for the treatment of chronic fatigue, night sweats, wasting disorders, irritability, palpitations and insomnia (Siwicki et al., 2004). Fatigue can be defined as the reversible decline in skeletal muscle contractile performance due to intense muscle activity (Mach et al., 2010). Fatigue can be divided into two categories: physical and mental fatigue. Physical fatigue is caused by such things as forced exercise or swimming, while mental fatigue is caused by sleep deprivation, etc (Chen et al., 2009).

There are several theories about the mechanisms of physical fatigue: “exhaustion theory”, “clogging theory”, “radical theory”, “homeostasis disturbance theory”, “protective inhibition theory” and “mutation theory” (Wang et al., 2008; You et al., 2011). The “radical theory” suggests that intense exercise can produce an imbalance between the body’s oxidation system and its anti-oxidation system. The accumulation of reactive free radicals will put the body in a state of oxidative stress and bring injury to the body by attacking large molecules and cell organs (Wang et al., 2008). Muscle cells contain several anti-oxidant defense mechanisms to protect themselves from free radical injury, including endogenous antioxidants and antioxidant enzymes. Moreover, many studies have indicated that exogenous dietary antioxidants can decrease the contribution of exercise-induced oxidative stress and improve the animal’s physiological condition. The reason may be that exogenous antioxidants can promote or interact with endogenous antioxidants to form a cooperative network of cellular antioxidants (Morillas-Ruiz et al., 2006; Mizuno et al., 2008; Muñoz et al., 2010). Reports from recent studies demonstrated that a large number of traditional Chinese herbs have been found to act as antioxidants by scavenging free radicals.
radicals/reactive oxygen species (ROS) and some of them have anti-fatigue activity (Yang et al., 2000; Morihara et al., 2006; Yu et al., 2010). In traditional Chinese medicine, Wu-Wei-Zi has been widely used for the treatment of chronic fatigue (Saito et al., 1974). However, this has not been validated by scientific approach. Therefore, the present study was designed to determine the radical scavenging properties of WAE and further the anti-fatigue activity of WAE was investigated through forced swimming exercise of mice.

MATERIALS AND METHODS

Plant materials

Wu-Wei-Zi was purchased from Dongfeng Medicinal Materials Factory, Wenzhou, China and judged by Chinese Traditional Medicine Research Institute in Zhejiang and fitted for Chinese Pharmacopoeia. The voucher specimen (Number: WU-KO 0231) was deposited in the Herbarium of Wenzhou University.

Chemicals and reagents

Butylated hydroxytoluene (BHT), DPPH and nitro blue tetrazolium (NBT) were purchased from Sigma Chemicals Company (St. Louis, MO). Methionine was purchased from Sangon Biotech Company Limited (Shanghai, China). Riboflavin was purchased from Huamei Biochemical Company (Shanghai, China). BUN reagent kit was purchased from Biosino Biotechnology and Science Incorporated. (Beijing, China). BLA, HG, SOD and GPX reagent kits were purchased from Jianchen Biological Engineering Institute (Nanjing, China). All other chemicals were of analytical grade and were purchased from Zhejiang Chemical Reagent Company Limited (Hang Zhou, China).

Experiment animal

Male Kunming mice (3 month old, weighing 18 to 22 g) were obtained from the Animal Center of the Wenzhou Medical College, Wenzhou, China. To avoid possible individual’s differences, only male mice were studied in this study. Because male animals have small individuals differences and there is no obvious physical characteristics when compared with female animals. The study was carried out according to the “Principles of Laboratory Animal Care” World Health Organization (WHO) (Chronicle, 1985). The mice were acclimatized for 1 week before being used for the experiment. Before and during the experiment the mice were housed under controlled environmental conditions of temperature (22 ± 2°C) and a 12 h light and dark cycle and maintained on (unless otherwise stated) standard food pellets and tap water ad libitum.

Preparation of Wu-Wei-Zi aqueous extracts

WAE was prepared by boiling the dried Wu-Wei-Zi with distilled water for 5 h. The extract was filtered, freeze-dried and kept at 4°C. The yield of extraction was approximately 11.62% (w/w). The direct extract was dissolved in distilled water before being used.

Superoxide anion radical scavenging assay

Superoxide anion radical scavenging activity of WAE was determined according to the method described by Prasad et al. (2010) with slight modifications. Briefly, all solutions were prepared in 0.2 M phosphate buffer (pH 7.4). The test samples at different concentrations (12.5, 25, 37.5 and 50 μg/ml) were mixed with 3 ml of reaction buffer solution (pH 7.4) containing 1.3 μM riboflavin, 0.02 M methionine and 5.1 μM NBT. The reaction solution was illuminated by exposing them to two 30 W fluorescent lamps for 20 min and the absorbance was measured at 560 nm. BHT was used as positive control. Superoxide anion radical scavenging activity (SRSA) was calculated by the following equation:

\[
SRSA(\%) = \frac{1 - A_{sample}}{A_{control}} \times 100
\]

Where \(A_{control}\) and \(A_{sample}\) represent the absorbance of blank control group and sample group under 560 nm.

DPPH radical scavenging assay

DPPH radical scavenging activity of WAE was determined according to the method described by Schlesier et al. (2002) with slight modifications. Briefly, 0.1 ml of the samples at different concentrations (25, 50, 75 and 100 μg/ml) was mixed with 1 ml of 0.2 mM DPPH (dissolved in methanol). The reaction mixture was incubated for 20 min at 28°C under dark. The control contained all reagents without the sample while methanol was used as blank. The DPPH radical scavenging activity was determined by measuring the absorbance at 517 nm. BHT was used as positive control. DPPH radical scavenging activity (DRSA) was calculated by the following equation:

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DRSA(\%) = \frac{1 - A_{sample}}{A_{control}} \times 100
\]

Where \(A_{control}\) and \(A_{sample}\) represent the absorbance of blank control group and sample group under 510 nm. In this study, scavenging activity of the sample was expressed as 50% effective concentration (EC50), which represented the sample concentration (μg/ml) inhibiting 50% of the DPPH radical activity.

Acute toxicity assay

Acute toxicity test of WAE was carried out on Kunming mice (weighing 18 to 22 g) of either sex. Animal were randomly divided into five equal groups (n = 10) and were orally administered with the WAE at 12.5, 25, 50, 100 and 200 g/kg body weight, respectively. The following profiles of animals were observed continuously for 2 h (Li et al., 2009). Behavioral profile: Alertness, restlessness, irritability and fearfulness; Neurological profile: Spontaneous activity, reactivity, touch response, pain response and gait; Autonomic profile: Defecation and urination. After a period of 24 and 72 h lethality or death was observed.

Anti-fatigue activity assay

Anti-fatigue activity of WAE was investigated through forced swimming exercise of mice. The model was a reliable measure of anti-fatigue treatment as established in both laboratory animals and humans (Tang et al., 2007; Zhang et al., 2009). WAE was given to mice at concentrations of 0, 5, 10 and 20 g/kg body weight, named as negative control dose group (CD group), low dose treatment group (LD group), middle-dose treatment group (MD group) and
high-dose treatment group (HD group), respectively. Distilled water was given to mice in CD group. Samples were orally administered into mice using a feeding atraumatic needle, once per day for 4 weeks. The doses of these treatments were chosen from literature references and pilot studies. After the final treatment with WAE, forced swimming exercise of mice was performed in acrylic plastic pool (50 × 50 × 40 cm) filled with water (25 ± 2°C) to a depth of 30 cm (Matsumoto et al., 1996). The mice were determined to be exhausted when they failed to rise to the surface to breathe after 10 s (Jung et al., 2007). This 10 s criterion was considered to correlate with exhaustion and was used as an indication of the maximum swimming capacity of the animal. Mice were removed at this point, before drowning. The exhaustive swimming time was observed. After the forced swimming exercise, the mice were allowed to rest for 30 min. Then they were anesthetized with ether and whole blood samples were collected in tubes by heart puncture to determine BLA, BUN, SOD and GPX contents using commercial kits. In addition, immediately after the blood had been collected, the liver was dissected out quickly from the mice, washed with physiological saline and dried with absorbent paper. Then the contents of HG were analyzed with commercial kits.

Statistical analysis

All experiments were carried out in triplicate and all the data were expressed as means ± SD (standard deviation). The significance of statistics was evaluated using Student’s t-test and \( P < 0.05 \) was taken as being significant.

RESULTS AND DISCUSSION

Superoxide anion radical scavenging activity of WAE

Among different ROS, \( \cdot O_2^\bullet \) is generated first. Although \( \cdot O_2^\bullet \) is a relatively weak oxidant, it may decompose to form stronger ROS, such as singlet oxygen and hydroxyl radical (\( \cdot OH^\bullet \)), which initiates peroxidation of lipids. \( \cdot O_2^\bullet \) is also known to initiate indirectly the lipid peroxidation as a result of the formation of \( H_2O_2 \), creating precursors of \( \cdot OH^\bullet \) (Qiao et al., 2009). Therefore, \( \cdot O_2^\bullet \) scavenging is extremely important to antioxidant work. Superoxide anion radical scavenging activity of WAE was presented in Figure 1. In this study, WAE exhibited an excellent superoxide anion scavenging activity, and the scavenging effects of WAE were significant stronger than that of BHT \( (P < 0.05) \). The maximum DPPH radical scavenging activity of WAE was 46.67±1.53%.

DPPH radical scavenging activity of WAE

DPPH free radical is a stable free radical, which has been widely accepted as a tool for estimating the free-radical scavenging activities of antioxidants (Hu et al., 2004). DPPH radical scavenging activity of WAE was presented in Figure 2, the EC50 of WAE and BHT were 21.29 and 23.23 µg/ml, respectively. In this study, WAE showed moderate DPPH radical scavenging activity. The maximum DPPH radical scavenging activity of WAE was 88.13±2.97%. Compared with the BHT, WAE performed higher activity on DPPH. In humans, muscular exercise promotes the production of ROS in the working muscle. Growing evidence indicates that ROS are responsible for exercise-induced protein oxidation and contribute highly to physical fatigue (Tharakan et al., 2005). Thus, treatments that reverse muscle fatigue may be acting through mechanisms that scavenge ROS. The present study established that WAE possessed superoxide anion and DPPH radical scavenging activity, which suggested that WAE may be beneficial to the alleviation of physical fatigue, so the WAE was used for the in vivo experiment in
mice to estimate the anti-fatigue activity.

**Acute toxicity test**

Acute toxicity test revealed the non-toxic nature of the WAE. There was no lethality or any toxic reactions found at any of the doses selected until the end of the study period.

**Effect of WAE on the exhaustive swimming time of mice**

Swimming to exhaustion is an experimental exercise model to evaluate anti-fatigue activity; it works well for evaluating the endurance capacity of mice and gives a high reproducibility (Zhang et al., 2006; Yao and Li, 2010; You et al., 2011). Effects of WAE on the exhaustive swimming time of mice were presented in Figure 3. There
are significant differences in the exhaustive swimming time between the negative control group and each treatment group. The swimming time to exhaustion of the CD, LD, MD and HD groups were 7.4 ± 0.8, 11.7 ± 1.1, 14.9 ± 1.0 and 16.8 ± 1.4 min, respectively. Thus, the exhaustive swimming time of the LD, MD and HD groups were significantly longer than that of the CD group ($P < 0.05$). This result suggested that WAE had significant anti-fatigue activity. Fatigue is one of the most frequent physiological reactions. It often occurred in aging, cancer, depression, Human immunodeficiency virus (HIV) infection, multiple sclerosis and Parkinson’s disease (Tharakan et al., 2006). However, there were very few pharmacological drugs or therapies available for the treatment of fatigue (Uthayathas et al., 2007). Natural products not only could improve athletic ability, postpone fatigue and accelerate the elimination of fatigue in human beings, but also had few side effects (Kim et al., 2001).

Data from previous investigations indicated that some traditional Chinese herbs extracts have anti-fatigue activity, including *Radix Rehmanniae Preparata* (Tan et al., 2011), *Ganoderma lucidum* (Guo et al., 2011), *Cordyceps sinensis* (Kumar et al., 2011), *Acanthopanax senticosus* (Zhang et al., 2010; Huang et al., 2011), *Ginseng* (Wang et al., 1983; Zhao et al., 2009; Wang et al., 2010), *Eucommia* (Deyama et al., 2001), *Rhodiola rosea* (Panossian et al., 2007; Olsson et al., 2009), *Cynomorium songaricum* (Yu et al., 2010), *Morinda officinalis* (Zhang et al., 2009), etc. In the present study, it has also been shown that WAE enhanced the swimming capacity by lessening of fatigue in mice. To explore the mechanism of anti-fatigue activity, some biochemical parameters such as BLA, BUN, HG, SOD and GPX contents in the mice were determined after they have swam for 30 min.

**Effect of WAE on the BLA and BUN contents of mice**

BLA and BUN are important blood biochemical parameters related to fatigue (Xu and Luo, 2001). BLA is the glycolysis product of carbohydrate under an anaerobic condition and glycolysis is the main energy source for intense exercise in a short time (Ding et al., 2011). According to the study of Wilber (1959), violent swimming to exhaustion results in a significantly elevated BLA contents and the rate at which BLA accumulates in the blood showed an inverse relation to swimming time. Therefore, blood lactate acid represents the degree of fatigue after exercise and the condition of recovery (Wang et al., 2006). As shown in Figure 4, the BLA contents in the CD, LD, MD and HD groups were 12.48 ± 0.86, 10.13 ± 0.79, 9.47 ± 0.96 and 8.56 ± 0.84 mmol/l, respectively. Thus, the BLA contents in all treatment groups (LD, MD and HD groups) were lower than that in the CD group ($P < 0.05$). This result suggests that WAE can effectively retard and lower the BLA produced and postpone the appearance of fatigue. BUN, which is the metabolism outcome of protein and amino acid, is a sensitive index to evaluate the bearing capability when human bodies suffer from a physical load (Huang et al., 2011). Wu (1999) pointed out that the BUN in the blood rises significantly for a long-run athlete after exercise. In other words, the worse the body is adapted to exercise tolerance, the more significantly the BUN contents increase. Therefore, BUN is another index of fatigue status. As shown in Figure 4, the BUN contents in the CD, LD, MD and HD groups were 10.34 ± 0.71, 9.06 ± 0.86, 8.57 ± 0.85 and 8.25 ± 0.93 mmol/l, respectively. Thus, the BUN contents of the LD, MD and HD groups were significantly lower than that of the CD group ($P < 0.05$). This result suggests that WAE may reduce catabolic decomposition

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**Figure 4.** Effect of WAE on the blood lactic acid and blood urea nitrogen contents of mice.
Effect of WAE on the hepatic glycogen contents of mice

The liver converts lactate back to glycogen and releases glycogen into the blood. Energy for exercise is derived initially from the breakdown of glycogen and later from circulation glycogen released by the liver and from non-esterified fatty acids (Dorchy, 2002). So increasing the HG storage conduces to enhancing the endurance capacity and locomotory capacity (Tang et al., 2008). HG is a sensitive parameters related to fatigue. Effects of WAE on the HG contents of mice were presented in Figure 5. There are significant differences in the HG contents between the negative control group and each treatment group. The HG contents of the CD, LD, MD and HD groups were 7.35 ± 1.21, 10.48 ±2.17, 15.69±3.19 and 16.74±2.82 mg/g, respectively. Thus, the HG contents of the LD, MD and HD groups were significantly higher than that of the CD group (P < 0.05). This result suggests that the anti-fatigue activity of WAE may be related to the improvement in the metabolic control of exercise and the activation of energy metabolism (Wang et al., 2006).

Effect of WAE on the SOD and GPX contents of mice

It has been demonstrated that ROS are responsible for exercise-induced protein oxidation and contribute strongly to muscle fatigue (You et al., 2009). To protect against exercise-induced oxidative injury, muscle cells contain complex endogenous cellular defense mechanisms (enzymatic and non-enzymatic antioxidants) to eliminate ROS (Powers et al., 2004). Antioxidant agents such as reduced glutathione (GSH), vitamin C, E and enzymes such as SOD, catalase (CAT) and GPX, are important factors (Hassan and Schellhorn, 1988). SOD reduces superoxide to hydrogen peroxide; and GPX reduces hydrogen peroxide from the SOD reaction to water. In addition, GPX can reduce lipid peroxides directly (Finaud et al., 2006). Growing evidence indicates that the improvement in the activities of antioxidant enzymes can help to fight against fatigue (You et al., 2011). As shown in Figure 6, the SOD contents of the CD, LD, MD and HD groups were 96.54 ± 7.84, 146.81 ± 8.93, 163.48 ±11.26 and 171.29 ± 13.21 U/mg.pro, respectively. And the GPX contents of the CD, LD, MD, and HD groups were 4.68 ±0.94, 8.37 ±1.23, 10.45 ±1.17 and 12.67 ±1.36 U/mg.pro, respectively. Thus, SOD and GPX contents of the LD, MD and HD groups were significantly higher than that of the CD group (P < 0.05). This result suggests that WAE can promote increase in the activities of these antioxidant enzymes and again supporting that WAE has anti-fatigue activity.

Conclusions

WAE had strong scavenging activity to superoxide anion and DPPH radical. And it had significant anti-fatigue
activity, which could not only extend the swimming time of the mice, increase the hepatic glycogen and antioxidant enzymes (SOD and GPX) contents, but also decrease the BLA and BUN contents. However, further research needs to be carried out to evaluate its antioxidant and anti-fatigue activity at cellular and molecular levels.

REFERENCES


