Fatigue is best defined as the difficulty in initiating or sustaining voluntary activities, and can be classified into mental and physical fatigue. Physical fatigue is thought to be accompanied by deterioration in performance (Tanaka et al., 2008). There are several theories about the mechanisms of exercise-induced fatigue: "exhaustion theory", "clogging theory", "radical theory", "homeostasis disturbance theory", "protective inhibition theory", and "mutation theory" (Wang et al., 2008). The "exhaustion theory" suggests that during exercise, many energy sources, such as glucose (GLU) and liver glycogen, will be exhausted, thus leading to physical fatigue. Several reports showed that post-exercise nutrition through the administration of proteins, peptides or amino acids can facilitate recovery from fatigue (Wang et al., 2008). Compared with proteins, peptides, as nutrition supplements, not only are absorbed quickly and easily without competition from amino acids, but also promote the use of amino acids, proteins and glucose (van Loon, Saris, Kruijshoop, & Wagenmakers, 2000). Therefore, peptides may be useful in assisting in counteracting and ameliorating physical fatigue. According to the "clogging theory", the over accumulation of serum lactic acid (LA) and blood urea nitrogen (BUN) will also result in metabolic disorders leading to fatigue.

Among the fatigue mechanisms, the "radical theory" has been attracting more interest. Free radicals are intermediate metabolites of many vital biochemical events in the body, and they are in a dynamic balance between their production and clearance. Harman's classical "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 complex endogenous cellular defense mechanisms to eliminate reactive oxygen species, such as superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) and catalase (CAT), and to protect among other things against exercise-induced oxidative injury. Moreover, some reports (Yu et al., 2006) showed that exogenous dietary antioxidants can also 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 (Mizuno et al., 2008). However, the mechanisms have not been fully elucidated. Nevertheless, consumers are seeking more natural antioxidant components in their diet to reduce oxidative damage and fight against fatigue, without the side effects of other anti-fatigue drugs. Such antioxidant components have been found in plants, such as red mold rice (Wang, Shieh, Kuo, Lee, & Pan, 2006) and tissue culture extracts of Saussurea (Jia & Wu, 2008), and in herbs, such as Trichopus zeylanicus (Tharakan, Dhanasekaran, & Manyam, 2005). However, there are few reports...
regarding antioxidant peptides from animal sources having anti-fatigue activity.

Loach (Misgurnus anguillicaudatus) is a common freshwater fish in East Asia. It has been familiar to the Chinese since ancient times, for its desirable taste and flavour. It has also traditionally been considered as a folk remedy for physical and mental fatigue in the southern part of China. However, the mechanisms of its anti-fatigue activity have not been explored.

In this study, we investigated the in vitro antioxidant activities and the in vivo anti-fatigue effect of loach peptide (LP) previously prepared by papain digestion. The in vitro antioxidant activities were studied by determining the 1,1-diphenyl-2-picrylhydrazyl (DPPH) and the hydroxyl radicals scavenging activities, the chelating ability with Cu²⁺ ions, and the lipid peroxidation inhibitory activity in a linoleic acid emulsion system, using glutathione (GSH) as a control. In the subsequent in vivo study, mice were fed with high or low doses of LP for 4 weeks. Then, an exhaustive swimming test was recorded and several biochemical parameters related to fatigue [GLU, liver glycogen, LA, BUN, lactate dehydrogenase (LDH), creatine kinase (CK), SOD, CAT, GSH-Px] were also determined to explore the anti-fatigue activity of loach peptides.

2. Materials and methods

2.1. Loach peptide preparation

Live loach (Misgurnus anguillicaudatus, 8.4 ± 1.1 g body weight and 9.8 ± 3 cm body length) were purchased from a local market in Guangzhou, China, and transported to the lab within 10 min. After killing, the meat (without head, tail, skin, bone and blood) was collected and ground twice through a meat grinder with a plate with 4 mm holes (MM12, Shaoquan Food Machine Co., Shaoquan, China). The ground meat was stored in a polyethylene bag at −18 °C until use, for a maximum of 4 months. Fifty grams of loach meat were mixed with 100 ml of distilled water and homogenised at a speed of 10,000 rpm for 1 min using a basic homogeniser (T25, Ika, Staufen, Germany). The homogenate was hydrolyzed with papain (6 × 10⁵ U/g) (Baiao Biochemistry Co., Jiangmen, China) at 55 °C for 4.5 h. The enzyme to substrate ratio was 3:1000 (w/w). The hydrolysis was conducted at pH 7.0 (SL1-PHS-3B pH-metre, Wuhan Midwest Instrument Co. Ltd., Wuhan, China) in a water bath shaker (New Brunswick Scientific C24, Jintan, China). After hydrolysis, the enzyme was inactivated by placing the samples in boiling water for 15 min. The hydrolysates were centrifuged in a GL-21M refrigerated centrifuge (Xiangyi Instrument Co. Ltd., Changsha, China) at 5000g for 20 min, and the supernatants were fractionated through ultrafiltration membranes using a bioreactor system (Vivafflo 200, Vivascience, Sartorius, Goettingen, Germany) with a molecular weight cutoff (MWCO) of 5 kDa. The fraction with molecular weight less than 5 kDa was lyophilised (R2L-100KPS, Kyowa Vacuum Engineering, Tokyo, Japan) and stored in a desiccator for further use. The estimated protein content of the LP powder was 85%, determined by the Kjeldahl method (AOAC, 1995) using a conversion factor of 6.25. The fractions in the LP with molecular weight <1 kDa, 1–3 kDa, 3–5 kDa and >5 kDa were 32%, 48%, 18% and 2%, respectively, determined by gel permeation chromatography, as described in our previous report (You, Zhao, Cui, Zhao, & Yang, 2008).

2.2. Animals

All the in vivo tests were carried by the School of Pharmaceutical Sciences of Southern Medical University (Guangzhou, China), which obtained the permission for performing the research protocols and all animal experiments conducted during the present study from the ethics committee of Southern Medical University. Fifty-four male NIH mice (18–22 g, specific pathogen-free grade, SPF, Approval No. 2007A058) were purchased from the Academy of Experimental Animal Center of Southern Medical University (Guangzhou, China). They were housed in a SPF level laboratory in the School of Pharmaceutical Sciences of Southern Medical University. All experimental procedures were conducted under the oversight and approval of the Academy of Experimental Animal Center of Southern Medical University and in strict accordance with the NIH Guide for the Care and Use of Laboratory Animals (NIH, 2002). Animals were allowed to adapt to their surroundings for 1 week before starting the experiments. Mice were housed 5–6 per cage at room temperature (22 ± 2 °C) and moderate humidity (50 ± 10%), with a 12/12-h light–dark cycle; noise was <60 dB. They were fed a balanced murine diet provided by the Academy of Experimental Animal Center of Southern Medical University, and had drinking water available ad libitum. After adaptation, the 54 mice were randomly divided into six groups: two control groups, two LP treatment groups at a high dose (LP-H), and two groups at a low dose (LP-L), with 9 mice each. One group from each set was used for the exhaustive swimming test. The other group was used for collecting the blood to determine biochemical parameters related to fatigue, after swimming for 30 min. For 4 week, the LP-H mice were given 5 mg/(g d) of LP, and the LP-L mice were given 1 mg/(g d) by intraperitoneal injection every day at 1:00–3:00 pm. The control groups received distilled water. After each treatment, all groups of the mice were allowed to rest 30 min and were forced to swim for 20 min to become accustomed to swimming (see below).

2.3. Amino acid analysis

The amino acid profile of LP was determined according to the method of You et al. (2008). The amino acid composition was determined by high-performance liquid chromatography (Waters, Milford, MA, USA) equipped with a PICO.TAG column. The total amino acids (except for tryptophan) were determined after hydrolysis at 110 °C for 24 h with 6 M HCl, prior to the derivatisation with phenyl isothiocyanate. Alkaline hydrolysis was also done for the determination of tryptophan level. The amino acid standards (Sigma–Aldrich, St. Louis, MO, USA) were analysed using the same conditions as for the samples.

2.4. Determination of in vitro antioxidant activities of LP

2.4.1. Hydroxyl radical scavenging activity assay

The hydroxyl radical scavenging activity was assayed according to the method of Li, Jiang, Zhang, Mu, and Liu (2008), with some modifications. A mix of 600 μl of 1.10-phenanthroline (5.0 mM), 600 μl of FeSO₄ (5.0 mM) and 600 μl of ethylenediaminetetraacetic acid (EDTA) (15 mM) were mixed with 400 μl of sodium phosphate buffer (0.2 M, pH 7.4). Then, 600 μl of LP (0–25 mg/ml) and 800 μl of H₂O₂ (0.01%) were added. The mixture was incubated at 37 °C for 60 min, and the absorbance was measured at 536 nm (UV754, Xianjian Scientific Instrument Co., Shanghai, China). GSH (Sigma–Aldrich, St. Louis, MO, USA) at 0–10 mg/ml was used as a control. The following equation was used:

\[
\text{Hydroxyl radical scavenging activity (\%)} = \left( \frac{A_0 - A_s}{A_0} \right) \times 100
\]

where \(A_s\) is the absorbance of the sample; \(A_0\) is the absorbance of the blank solution using distilled water instead of sample; and \(A_s\) is the absorbance of a control solution in the absence of \(H_2O_2\). The plot of scavenging activity against the concentration of the hydroly-
sate was prepared, and the IC_{50} (concentration of samples to decrease the scavenging activity by 50%) obtained.

2.4.2. DPPH radical scavenging activity assay

The DPPH radical scavenging activity was determined by the method of Wu, Chen, and Shiu (2003), with a slight modification. Two millilitres of LP (0-5 mg/ml) were mixed with 2 ml of 0.15 mM DPPH that was dissolved in 95% ethanol. The mixture was then shaken vigorously using a mixer (QT-1 Mixer, Tianchen Technological Co. Ltd., Shanghai, China), and was kept for 30 min in the dark. The absorbance of the resulting solution was recorded at 517 nm. GSH at 0–20 mg/ml was used as a control. The scavenging activity was calculated using the following equation:

\[
\text{Scavenging activity } (\%) = \left( \frac{A_{\text{DPPH blank}} - A_{\text{Sample control}}}{A_{\text{DPPH sample}}} \right) \times 100
\]

where \(A_{\text{DPPH sample}}\) is the value for the 2 ml of sample solution mixed with the DPPH solution; \(A_{\text{sample control}}\) is the value for the 2 ml of sample solution mixed with the 2 ml of 95% ethanol; and \(A_{\text{DPPH blank}}\) is the value for the 2 ml of 95% ethanol mixed with the DPPH solution. The plot of the scavenging activity against the concentration of sample was prepared, and the IC_{50} obtained.

2.5. In vivo anti-fatigue effect of LP

2.5.1. Exhaustive swimming test

After the final treatment with LP or distilled water, the mice were allowed to rest for 30 min. Then, they were placed in the swimming tank (50 cm × 50 cm × 40 cm) with 30 cm deep water at 25 ± 1 °C. The current in the pool was generated by circulating water with a pump, and the strength of the current was adjusted to 8 l/min with a water flow metre (type F45500, Blue White Co., Westminster, CA, USA). The water was agitated to make the mice limbs keep moving. The mice were determined to be exhausted when they failed to rise to the surface to breathe after 7 s (Jung, Han, Kwon, Lee, & Kim, 2007).

2.5.2. Measuring biochemical parameters related to fatigue

After the final treatment with LP or distilled water, the mice were allowed to rest for 30 min. Then they were placed in the swimming tank (50 cm × 50 cm × 40 cm) with 30 cm deep water (temperature: 25 ± 1 °C). After swimming for 30 min, they were taken out. Blood was collected from the orbital sinus to determine GLU, LA and BUN content and CK, SOD, GSH-Px, LDH and CAT activity (Jung et al., 2007). The livers of the mice were also taken to determine the content of liver glycogen (see below). All of the biochemical parameters were determined by using an automated Biochemistry Analyzer (7600-010, Hitachi, Japan).

The glucose, BUN, CK and LDH activities were determined using commercial diagnostic kits (Product Nos. 000060110, 000000280, 000000090 and 000000131, respectively, Biosino Bio-technology and Science Inc., Beijing, China). LA was determined using a commercial diagnostic kit (Product No. LC6351, Beijing Leadman Biochemistry Technology Co. Ltd., Beijing, China). The SOD activity was determined using SOD Assay Kit A001 (Institute of Biological Engineering of Nanjing Jianchen, Nanjing, China).

The GSH-Px activity was determined with a GSH-Px Assay Kit A005 (Institute of Biological Engineering of Nanjing Jianchen, Nanjing, China). The GSH-Px has the ability to decompose hydrogen peroxide (H_{2}O_{2}) and other organic hydroperoxides (ROOH). The reaction uses GSH to complete the reaction using H_{2}O_{2}, as the substrate. The consumption of nicotinamide adenine dinucleotide phosphate (NADPH) is used to determine the GSH-Px activity.

The catalase activity was determined colorimetrically with a CAT Assay Kit A007 (Institute of Biological Engineering of Nanjing Jianchen, Nanjing, China). The test is based on the decomposition of the H_{2}O_{2} optical density at 415 nm by CAT.

The livers of the mice were dissected immediately after taking them out, washed with 0.9% saline, and blotted dry with filter papers. Liver samples (~100 mg) were accurately weighed, and homogenised in 8 ml of homogenisation buffer from a Liver Glycogen Assay Kit A043 (Institute of Biological Engineering of Nanjing Jianchen, Nanjing, China). The liver glycogen was determined according to the recommended procedures.

2.6. Statistical analysis

All the tests were conducted in triplicate. The experimental data were expressed as mean ± standard error. The results were subjected to one-way analysis of variance (ANOVA). LSD and Dunnett’s T3 tests were performed to determine the significant difference between samples within the 95% confidence interval, using SPSS 13.0 software (SPSS Inc., Chicago, IL, USA).
3. Results and discussion

3.1. Amino acid composition of LP

The amino acid compositions of the protein hydrolysates are directly related to their bioactive activities. Histidine or histidine-containing peptides have chelating and lipid radical trapping ability due to the imidazole ring (Uchida & Kawakishi, 1992). Aromatic amino acids, such as tryptophan exhibit antioxidant activity since they can donate protons easily to electron-deficient radicals. Several amino acids, such as tyrosine, methionine, histidine, lysine, and tryptophan, have generally been considered as antioxidants (Chen, Muramoto, Yamauchi, & Nokihara, 1996). As shown in Table 1, the loach peptide contains 24.8% of the above five amino acids, suggesting that it might have potential antioxidant activity.

Amino acids also play an important role in the regulatory metabolism involved in muscular activity. Some amino acid, especially the branched chain amino acids, can improve the exercise capability and markedly retard the catabolism of protein in the muscle during exercise (Blomstrand & Newsholme, 1992). Bazzarre, Murdoch, Wu, Herr, and Snider (1992) reported that the amount of amino acids, especially alanine, glycine, valine, isoleucine, threonine, serine and tyrosine in the plasma will decrease rapidly during an endurance test. As shown in Table 1, the loach peptide contains 24.8% of the above five amino acids, suggesting that it might have potential antioxidant activity.

As shown in Table 1, the loach peptide contains 32.2% of the above amino acids, suggesting that it might enhance exercise capability. Glutamic acid was found to have a very positive effect on the nervous system and would also be helpful during exercise (Guzennec et al., 1998). Aspartic acid was considered to be helpful in the oxidative deamination and could lower the blood ammonia concentration, therefore delaying the occurrence of fatigue (Marquez, Roschel, Costa, Sawada, & Lancha, 2003). The loach peptide contains 16.7% glutamic acid and 8.74% aspartic acid, suggesting that it might have a potential anti-fatigue effect.

3.2. In vitro antioxidant activities of LP

As one of reactive oxygen species generated in the human body, hydroxyl radicals can react easily with biomolecules, such as amino acids, proteins and DNA. This can lead to physiological disorders (Cacciottolo, Triinh, Lumpkin, & Rao, 1993). DPPH has been used extensively as the free radical to evaluate reducing substances. As shown in Table 2, LP had a hydroxyl IC$_{50}$ value of 17.0 ± 0.54 mg/ml and a DPPH IC$_{50}$ value of 2.64 ± 0.29 mg/ml, which was 2.6-fold and 2.4-fold higher than that of GSH, respectively. The IC$_{50}$ value for the chelation activity of Cu$^{2+}$ ion was 2.89 ± 0.33 mg/ml, which was 1.8-fold higher than that of GSH. The lipid peroxidation is thought to proceed via a radical-mediated abstraction of hydrogen atoms from methylene carbons in polyunsaturated fatty acids (Rajapakse, Mendis, Jung, Je, & Kim, 2005). The lipid peroxidation inhibitory activity of LP of the linoleic acid system gave an IC$_{50}$ value of 12.3 ± 0.98 mg/ml, which was not statistically different from that of GSH ($P > 0.05$). Nevertheless, the average molecular weight of LP was four times more than that of GSH (data not shown). That is to say that LP had stronger antioxidant activities than GSH at the same molecular concentration. Therefore, LP has in vitro antioxidant activities, which indicates that it may have the potential to reduce the oxidative stress in vivo and to fight fatigue.

3.3. LP prolonged the exhaustive swimming time

A direct measure of an anti-fatigue effect is the increase in exercise tolerance. Swimming to exhaustion is an experimental exercise model to evaluate anti-fatigue; it works well for evaluating the endurance capacity of mice, and gives a high reproducibility (Zhang, Yao, Bao, & Zhang, 2006). Reduced susceptibility to fatigue is correlated with longer swimming times. Both the high dose (5 mg/(g d)) and low dose (1 mg/(g d)) of LP treatments prolonged the swimming time of the mice. As shown in Fig. 1, the median exhaustion time of the LP-H mice was 241 min (28% greater than that of the water-injected control group); the median exhaustion time of the LP-L mice was 226 min (20% greater than that of the control) ($P < 0.05$), indicating that LP possesses an anti-fatigue effect. To explore the mechanism(s), some biochemical parameters, such as GLU, liver glycogen, LA, BUN contents and LDH, CK, SOD, CAT, GSH-Px activities were determined in the mice after they swam for 30 min.

3.4. LP increased blood GLU and liver glycogen

The homeostasis of blood glucose plays an important role in prolonging endurance exercise (Wagenmakers et al., 1991). Hypoglycemia can suppress the active functioning of the brain during exercise, and this often leads to the inability to continue exercise (Wang et al., 2006). Thus, the amount of blood glycogen can illustrate the speed and degree of fatigue development (Wang et al., 2008). As shown in Table 3, the blood GLU levels of LP-H and LP-L mice were both significantly higher than that of the control group ($P < 0.05$); 28% and 42% higher, respectively. However, there is no obvious dose-dependence between the high and low doses groups.

### Table 1

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Concentrationa (mg amino acid residues/g loach peptide powder)</th>
<th>Compositionb (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspartic acid</td>
<td>69.7</td>
<td>8.74</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>133</td>
<td>16.7</td>
</tr>
<tr>
<td>Serine</td>
<td>28.1</td>
<td>3.52</td>
</tr>
<tr>
<td>Glycine</td>
<td>46.9</td>
<td>5.88</td>
</tr>
<tr>
<td>Histidine</td>
<td>31.1</td>
<td>3.90</td>
</tr>
<tr>
<td>Arginine</td>
<td>35.4</td>
<td>4.43</td>
</tr>
<tr>
<td>Threonine</td>
<td>37.7</td>
<td>4.72</td>
</tr>
<tr>
<td>Alanine</td>
<td>55.9</td>
<td>7.01</td>
</tr>
<tr>
<td>Proline</td>
<td>43.7</td>
<td>5.48</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>27.9</td>
<td>3.5</td>
</tr>
<tr>
<td>Valine</td>
<td>29.4</td>
<td>3.69</td>
</tr>
<tr>
<td>Methionine</td>
<td>19.6</td>
<td>2.45</td>
</tr>
<tr>
<td>Cystine</td>
<td>0.2</td>
<td>0.02</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>31.0</td>
<td>3.88</td>
</tr>
<tr>
<td>Leucine</td>
<td>55.9</td>
<td>7.01</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>65.7</td>
<td>8.23</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>33.0</td>
<td>4.14</td>
</tr>
<tr>
<td>Lysine</td>
<td>53.7</td>
<td>6.73</td>
</tr>
<tr>
<td>Total</td>
<td>798</td>
<td>100</td>
</tr>
</tbody>
</table>

a One gram of loach protein from 0 to 5 kDa fraction contained 850 mg of total estimated protein. Thus, the approximate yield of the identified amino acid residues (amino acids minus H$_2$O) accounted for 798/850, or approximately 94% of the nominal protein.

b Normalised so that the observed amino acid residues add up to 100% of the total amino acid residues.

d The lipid peroxidation inhibition activity in a linoleic acid emulsion system.

c The chelating activity of Cu$^{2+}$ ion.

### Table 2

<table>
<thead>
<tr>
<th>Sample</th>
<th>Antioxidant activity (IC$_{50}$) (mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Hydroxylac</td>
</tr>
<tr>
<td>LP</td>
<td>17.0 ± 0.54</td>
</tr>
<tr>
<td>GSH</td>
<td>4.7 ± 0.63</td>
</tr>
</tbody>
</table>

a The scavenging activity for the hydroxyl radical.
b The scavenging activity for the DPPH radical.
c The chelating activity of Cu$^{2+}$ ion.
d The lipid peroxidation inhibition activity in a linoleic acid emulsion system.
Urea is formed in the liver as the end product of protein metabolism. During digestion, protein is broken down into small peptides and amino acids. The amino acid nitrogen is removed as NH₃, while the rest of the molecule is used to produce energy or other substances needed by the cell (Koo et al., 2004). Serum urea nitrogen, which is a product of energy metabolism, is another sensitive index of fatigue status. There was a positive correlation between urea nitrogen in vivo and exercise tolerance. The less an animal is adapted to exercise, the more the urea nitrogen level increases (Zhang et al., 2006). As shown in Table 3, the BUN levels of the mice were significantly lower by 17.5% in the LP-H group compared to the control (P < 0.05), while the decrease of 8.6% in the LP-L group was not significantly different to the control (P > 0.05). The reduced protein metabolism of the high dose of LP is indicative of enhanced endurance (Jia and Wu, 2008; Wang et al., 2008).

3.6. LP increased LDH activity but decreased CK activity

Serum CK and LDH are known to be accurate indicators of muscle damage. The normal function of CK in cells is to add a phosphate group to creatine, turning it into the high-energy molecule phosphocreatine, which is burned as a quick source of energy by the cells (Coombes & McNaughton, 2000). However, the normal function of CK is not as relevant here as what happens to CK when the muscle is damaged. During the process of muscle degeneration, the muscle cells lyse and their contents find their way into the bloodstream. Because most of the CK in the body normally exists in the muscle, an increase in CK in the blood indicates that muscle damage has occurred or is occurring. LDH catalyses the interconversion of pyruvate and lactate (Koo et al., 2004).

As shown in Fig. 2, the LDH level of mice was significantly increased for LP-H mice (P < 0.05), while the serum CK level decreased (P < 0.05) compared to the control. There were no significant changes for the LDH and CK levels of LP-L. However, the statistical differences were significant between the high and low LP (P < 0.05) for these two biochemical parameters.

3.7. LP enhanced the antioxidative enzymes in mice

Growing evidence indicates that reactive oxygen species are responsible for exercise-induced protein oxidation, and contribute strongly to muscle fatigue (Powers, DeRuisseau, Quindry, & Hamilton, 2004). Muscle cells contain two major classes (non-enzymic and enzymic) of endogenous cellular defense mechanisms to elim-

---

**Table 3**

The contents of glucose, liver glycogen, lactic acid and blood urea nitrogen in the mice (mmol/l).

<table>
<thead>
<tr>
<th>Groups</th>
<th>Control</th>
<th>LP-L</th>
<th>LP-H</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>5.06 ± 1.00a</td>
<td>6.47 ± 0.50a</td>
<td>7.20 ± 0.98a</td>
</tr>
<tr>
<td>Liver glycogen</td>
<td>3.44 ± 0.28b</td>
<td>11.4 ± 1.32b</td>
<td>13.8 ± 1.74b</td>
</tr>
<tr>
<td>Lactic acid</td>
<td>11.4 ± 1.11a</td>
<td>10.1 ± 0.82b</td>
<td>8.23 ± 0.59c</td>
</tr>
<tr>
<td>Blood urea nitrogen</td>
<td>9.98 ± 0.88a</td>
<td>9.12 ± 0.54b</td>
<td>8.23 ± 0.36c</td>
</tr>
</tbody>
</table>

* Different letters indicate significant differences between groups for the same.
The primary antioxidant enzymes include SOD, GSH-Px and CAT (Tharakan et al., 2005). SOD dismutates superoxide radicals to form H\textsubscript{2}O\textsubscript{2} and O\textsubscript{2}. GPH-Px is an enzyme responsible for reducing H\textsubscript{2}O\textsubscript{2} or organic hydroperoxides to water and alcohol, respectively. CAT catalyses the breakdown of H\textsubscript{2}O\textsubscript{2} to form water and O\textsubscript{2}. These antioxidant defense mechanisms become weaker during chronic fatigue and other disease conditions (Powers & Lennon, 1999). So, the improvement in the activities of these defense mechanisms can help to fight against fatigue. As shown in Fig. 3, the CAT activities of the LP treatment groups (both high and low doses) were significantly higher than that of the control (49% and 36% greater, respectively) (P < 0.05), while there was no statistical difference between LP-H and LP-L. The GSH-Px and SOD activities of LP-H increased by 27% and 8.5%, respectively, compared to the control (P < 0.05), while those for the low dose of LP treatment increased by 13% and 1.7%, respectively; however, both were without statistical significance (P > 0.05), compared to the control. The results show that LP can promote increases in the activities of these antioxidant enzymes, again supporting that LP has an anti-fatigue effect.

Fig. 3. Effect of loach peptide on the activities of antioxidant enzymes in mice. SOD refers to superoxide dismutase; GSH-Px refers to glutathione peroxidase; and CAT refers to catalase. The error bars represent one standard deviation. Different letters indicate significant differences between groups within the same procedure (P < 0.05).

4. Conclusions

The results show that a loach peptide prepared by papain digestion has not only in vitro antioxidant activities, but also an in vivo anti-fatigue effect in mice. It contained the amino acids, which were expected to contribute to its antioxidant and anti-fatigue activities. It acted as the scavenger for DPPH and hydroxyl radicals. It had the ability to chelate Cu\textsuperscript{2+} ion and to inhibit lipid peroxidation in a linoleic acid emulsion system. The in vivo study showed that LP prolonged the exhaustion swimming time of the mice. It improved the metabolic control of exercise and activated the energy metabolism by increasing the levels of blood GLU and liver glycogen. It helped to eliminate the accumulated products of metabolism by decreasing the levels of LA and BUN. It also improved the endogenous cellular antioxidant enzymes in mice by increasing the activities of SOD, CAT and GSH-Px. Therefore, LP can elevate the endurance capacity and facilitate recovery from fatigue. The results provide an important basis for developing the loach peptide as a novel antioxidant and anti-fatigue compound. However, further research needs to be carried out to evaluate its anti-fatigue activity on humans and its anti-fatigue mechanism(s) at the cellular and molecular levels.

Acknowledgements

We are grateful for the financial support from the National High Technology Research and Development Program of China (863 Program) (No. 205706083) and the 11th 5-year National Key Technologies R&D Program of China (Nos. 2006BAD27B03 and 2006BAD27B04).

References


