Food and Nutrition Sciences, 2014, 5, 1202-1211
Published Online July 2014 in SciRes. http://www.scirp.org/journal/fns
http://dx.doi.org/10.4236/fns.2014.513131

Antihypertensive Properties on Spontaneously Hypertensive Rats of Peptide Hydrolysates from Silkworm Pupae Protein

Wei Wang[1*], Nan Wang[2], Yu Zhang[1]

[1]Institute of Quality and Standard for Agriculture Products, Zhejiang Academy of Agricultural Science, Hangzhou, China
[2]College of Biology and Environmental Engineering, Zhejiang Shuren University, Hangzhou, China
Email: [*]wangwei5228345@126.com

Received 21 April 2014; revised 25 May 2014; accepted 6 June 2014

Abstract

Peptide hydrolysates of silkworm pupae protein with molecular weight of less than 5000 Da were prepared by ultrafiltration. The extracted peptide hydrolysates of silkworm pupae protein had inhibitory action on angiotensin-I-converting enzyme activity *in vitro*. The hydrolysates were orally administered to spontaneously hypertensive rats (SHR) in one period and long-term (four weeks). The results showed that the systolic blood pressure (SBP) of the treatment groups decreased in a dose-related manner. After one oral administration of silkworm protein hydrolysates with doses of 60, 20 and 5 mg/kg, the SBP of SHR decreased by 21.5, 13.8, and 9.0 mmHg in 1.5 h. After four weeks of the treatment in 80 mg/kg, the SBP decreased by 25 mmHg, with the antihypertensive activity close to 4 mg/kg of captopril; the SBP of the 40 mg/kg dose group also decreased by 17.5 mmHg. The peptide hydrolysate did not affect the SBP in normal, non-hypertensive rats in one period and long-term treatments. The acute toxicity research showed that the peptide hydrolysates were safe and without side effects. This research would be helpful in exploring the silkworm protein peptides as functional components for the antihypertension treatment.

Keywords

Angiotensin-I-Converting Enzyme, Antihypertensive Effect, Hypertension, Systolic Blood Pressure, Silkworm Pupae Hydrolysate, Spontaneously Hypertensive Rat

[*]Corresponding author.

How to cite this paper: Wang, W., Wang, N. and Zhang, Y. (2014) Antihypertensive Properties on Spontaneously Hypertensive Rats of Peptide Hydrolysates from Silkworm Pupae Protein. *Food and Nutrition Sciences*, **5**, 1202-1211.
http://dx.doi.org/10.4236/fns.2014.513131

1. Introduction

Numerous studies have recently shown that several amino acid sequences in food proteins expressed broad bioactivities, such as promoting mineral absorption, antihypertension and improving immunity. In several sequences with some physiological effects after absorption, the bioactive peptides from exogenous protein sources show potential applications. However, the limitations for protein and peptide absorption in the digestive system of the body and the thing whether the peptides can reach their targets have become the focus point of researchers in peptide medicine and health care products [1].

Two independent absorption and transport mechanisms exist between small peptides (especially dipeptides and tripeptides) and amino acids. Free amino acids are transported into the human intestinal epithelial cells by brush border membrane in a special amino acid transport system, whereas small peptides are transported by a special peptide transport system [1]. The peptide transport system is located in the brush border membrane of the intestinal epithelial cells. The requirements of small peptide carrier are not strictly dependent on the amino acid composition of peptides. Thus, several peptides can be absorbed through this absorption method [2].

Among all kinds of bioactive peptides, angiotensin-I-converting enzyme inhibitory peptides (ACEIPs) have received an increasing attention because of their ability to lower blood pressure without any side effects. Angiotensin-I-converting enzyme (ACE, EC3.4.15.1) has an important function in regulating hypertension. ACE catalyzes the conversion of decapeptide (angiotensin I) to a potent vasoconstriction octapeptide (angiotensin II). Inhibited ACE activity results in decreased angiotensin II concentration, which reduces blood pressure [3]-[7].

Several studies have been performed to determine the antihypertensive effects of ACEIPs using spontaneously hypertensive rats (SHRs) [8]-[10]. However, no study on the antihypertension effects *in vivo* of silkworm pupae protein-derived peptides has been reported. In our previous studies, we reported on silkworm pupae protein hydrolysates with ACE inhibitory activity *in vitro* [11]-[13]. However, whether the peptides in the hydrolysates will have ACE inhibitory activity *in vivo* remains unknown. In the present paper, we fed SHRs with silkworm pupae protein hydrolysates and studied the effects of the short- and long-term feeding on the arterial pressure of SHR and the toxicological safety of the hydrolysates. The results of this study would be helpful in understanding the resistance ability of peptides to digestion and exploring novel safe functional foods in antihypertension.

2. Material and Methods

2.1. Preparation of Peptide Hydrolysates of Silkworm Pupae Protein

The silkworm pupae protein was distilled by alkali dissolution and acid precipitation. The protein content of the freeze-dried powder was 95.4%. The protein was hydrolyzed by acid protease (*Aspergillus usamii* NO. 537) under the following conditions: hydrolysis temperature of 35˚C, hydrolysis time of 5.0 h, protein concentration (protein/water, w/v) of 1:7, 3% acid protease (E = 3000 U/g), and pH 2.0. After the hydrolysis time reached the endpoint, the hydrolysates were heated to 90˚C and maintained for 20 min before the reaction was stopped. The hydrolysates were then cooled naturally to room temperature, and the pH was adjusted to 7.0. The hydrolysates were centrifuged at 3000 g for 20 min; the supernatant was ultrafiltered by 5000 Da; and the ultrafiltrate was concentrated and lyophilized. The molecular weight distribution of the silkworm pupae protein peptide hydrolysates was showed in **Figure 1**. There were mainly two parts in the hysrolysate, the molecular weight between 307 Da to 5000 Da was 88.2%, and less than 307 Da was 11.8%, it means the peptides mainly were short peptides.

According to the experimental dose design, the lyophilized silkworm peptides were accurately weighed, fully dissolved in 0.9% saline solution, repacked, and then stored at −20˚C.

We detected the ACE inhibitory bioactivity of the peptide hydrolysates *in vitro* by the method that we have reported in reference 12 and 13 [12] [13]. And, the ACE inhibitory activity the peptide hydrolysates *in vitro* was 73.7% in 1.0 mg/mL.

2.2. Feeding Conditions of SHR

Male SHRs, 17 weeks old to 20 weeks old, weighing 300 g to 350 g (SHR/SLAC, Shanghai Laboratory Animal Center (SLAC), China) were used in this study. The animals were maintained at a temperature of 25˚C and humidity of 70%, with 12 h light/dark cycles in Animal Lab of Zhejiang Chinese Medical University. The animals

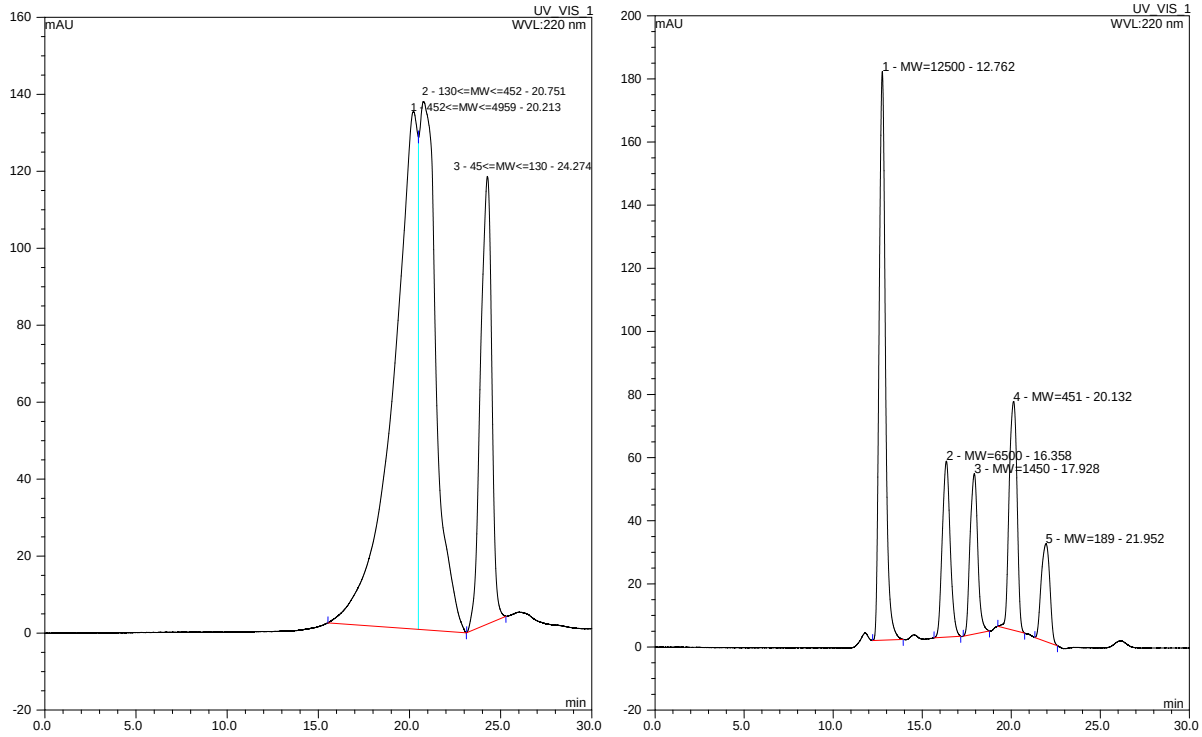

Figure 1. The molecular weight distribution of the silkworm pupae protein hydrolysate. **Note:** The molecular weight distribution detection conditions were as follows: Chromatogram column was TSKgel G2000 SWXL 300 mm × 7.8 mm; Mobile phase was Acetonitrile:water: Trifluoroacetic acid = 45:55:0.1 (Volume ratio); Wavelength was UV220 nm, Velocity was 0.5 mL/min; Column temperature was 30˚C; The injection volume was 10 μL. The standard molecular weight sample was Cyyochrome (MW = 12,400 Da), Aprotinin (MW = 6500 Da), Bacitracin (MW = 1450 Da), Octreotide acetate (MW = 1019 Da), Thymopentin (MW = 679 Da), Reduced glutathione (MW = 307 Da), they were all bought from Sigma-Aldrich (US).

consumed tap water and were given a standard diet (MO2, SLAC, China) during the experiments. All of the rats were conducted in accordance with international guidelines for animal care.

2.3. Method of Blood Pressure Measuring of SHR

The systolic blood pressure (SBP) of the rats was measured via the tail-cuff method [14]. SBP was measured three times, and the average was used as the rat-tail artery blood pressure. All of the SHRs were conducted in accordance with international guidelines for animal care.

2.4. Single Administration Treatment

According to blood pressure levels, 25 SHRs were randomly divided into five groups (n = 5): blank control, positive control (captopril), silkworm pupae protein peptide hydrolysates in high-dose, middle dose group, and low dose groups. The blank control group received the same volume of saline. The dose of each group was as follows: 0 mg/kg (blank control), 4 mg/kg (captopril), 60 mg/kg (high-dose group), 20 mg/kg (middle dose group), and 5 mg/kg (low dose group). The SBP of the rats in each group were measured by the tail-cuff method. SBP was measured three times, and the average was used as the rat-tail artery blood pressure.

We also feed 10 rats which were non-hypertensive rats in SBP at 115 ± 5.5 mmHg, and gave 5 rats the silkworm pupae protein peptide hydrolysates in dose of 60 mg/kg, the other five rats in normal feeding, and detected the 10 rats tail artery blood pressure after the single administration treatment.

2.5. Long-Term Administration Treatment

According to blood pressure levels, 45 SHRs were randomly divided into five groups (n = 9), namely, blank control, positive control (captopril), silkworm pupae protein peptide hydrolysates in high-dose, middle dose, and

low dose groups. The blank control group received the same volume of saline. The dose of each group was as follows: 0 mg/kg (blank control), 4 mg/kg (captopril), 80 mg/kg (high-dose group), 40 mg/kg (middle dose group), and 20 mg/kg (low dose group). The SBP and the body weight of the rats in each group administered with single oral dose were measured every week. Single daily administration was provided continuously for a month. SBP was measured three times, and the average was used as the rat-tail artery blood pressure.

We also feed 10 rats which were non-hypertensive rats in SBP at 118 ± 3.6 mmHg, and gave 5 rats the silkworm pupae protein peptide hydrolysates in dose of 80 mg/kg, the other five rats in normal feeding and detected the 10 rats tail artery blood pressure after each administration treatment.

2.6. Acute Toxicity of Peptides in Silkworm Pupae Protein Hydrolysates

2.6.1. Pre-Test

Five dose groups of 5.2, 3.9, 2.9, 2.2, and 1.6 g/kg were set according to the maximum solubility of peptide hydrolysates in silkworm pupae protein. Fifty Kunming mice, weighing from 18 g to 22 g, were randomly divided into five groups (n = 10), with equal numbers of male and female mice. The mice in each group with 0.4 mL/10 g weight were given single oral administration. Before administration, food, but not water, was prohibited for 12 h. The toxicity and the death of mice in 24 h after administration were repeatedly observed. The mice were subjected to continuous observation daily for 7 d, and the toxicity to mouse and the number of deaths in each dose group were recorded. The dose range of mortality in 0% and 100% were calculated. All of the mice were conducted in accordance with international guidelines for animal care.

2.6.2. Formal Test

The ratios among the groups were determined according to the dose range of mortality in 0% and 100% in the pre-test. Fifty Kunming mice, weighing from 18 g to 22 g, were randomly divided into five groups (n = 10), with equal numbers of male and female rats. Each group of mice was subjected to determinant doses of single intragastric administration. Before administration, food, but not water, was prohibited for 12 h. The animals were continuously observed for 7 d, and the animal weight changes, diet, appearance, behavior, secretions, excretions, death, and toxic reactions (symptoms of toxic reactions, severity, start time, duration, and reversibility) were recorded. Autopsies were performed on dying and dead animals. After the observation period, the other animals were anatomized. When changes on organ size, color, and texture were found, histopathological examination of the organs was performed, and the possible toxicity target organ was determined by preliminary test results.

When the experiment was finished, the death number of mice in each group was calculated. The median lethal dose (LD_{50}) was calculated by a modified Cole formula [15]. All of the animals were conducted in accordance with international guidelines for animal care.

2.7. Statistical Analysis

The experimental results were expressed as mean ± standard deviation. T-test and analysis of variance was performed to determine the difference between the two groups. SPSS 12.0 was used for statistical analysis.

3. Results

3.1. Effect of Single Administration Treatment on Blood Pressure of SHR

After the single administration treatment of each SHR group, we detected the blood pressure at 0 h, 1 h, 1.5 h, 2 h, 4 h, and 6 h (**Figure 2**). The results show that the blood pressure of SHR in the treatment groups in different degrees. The captopril group showed the most obvious changes in blood pressure, which decreased from 163.1 ± 4.1 mmHg to 136.2 ± 4.0 mmHg in 1 h, compared with the blank control. A significant difference of $p < 0.01$ exists between these two groups. At 1.5 h, the blood pressure decreased to its lowest value of 129.0 ± 1.6 mmHg, a decrease of 34.1 mmHg. At 2 h, the blood pressure was sustained at the lowest level of 130.4 ± 0.8 mmHg. After 6 h of single treatment, the blood pressure of the captopril group returned to its pre-gavage level.

The high, middle, and low dose groups of silkworm pupa protein peptide hydrolysates showed that blood pressure decreased significantly in 1.5 h, at 139.0 ± 10.4 mmHg (−21.5 mmHg), 148.4 ± 6.3 mmHg (−13.8 mmHg), and 152.4 ± 4.0 mmHg (−9.0 mmHg), respectively. Compared with the blank control, the blood pressure

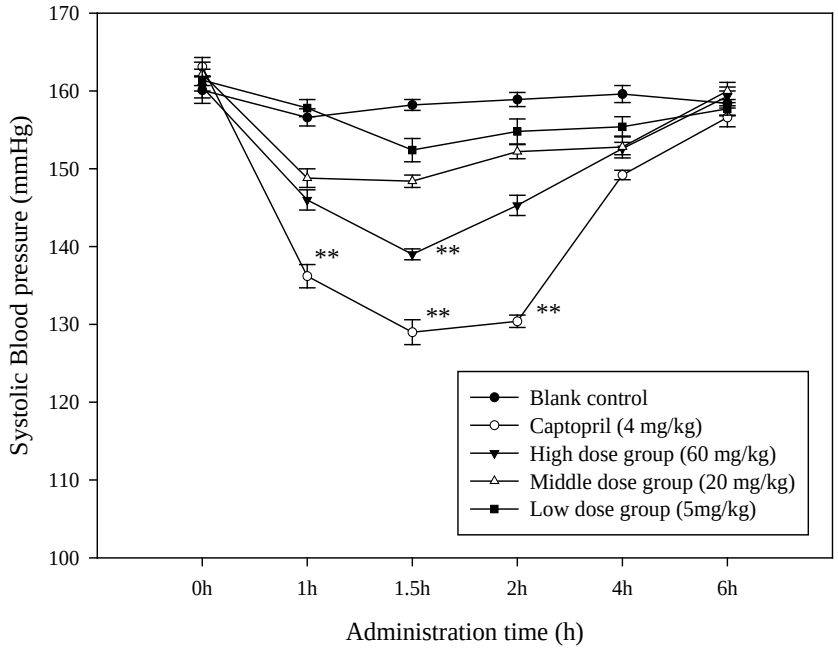

Figure 2. Effect of single administration treatment on blood pressure of SHR (Mean ± SD, n = 5). Note: Compared with blank control, $^{*}p < 0.05$; $^{**}p < 0.01$.

of SHR in the high dose group decreased and showed a significant difference of $p < 0.05$. At about 2 h, the blood pressure of the three peptide treatment groups began to rebound slowly. At 6 h, the blood pressure values recovered to their pre-gavage levels. These results showed that for peptides in silkworm pupae protein hydrolysates with good blood pressure lowering effect, the antihypertensive activity was closely related to the treatment dose.

During the single administration treatment on blood pressure of SHR, we also feed the non-hypertensive rats in high dose treatment, The SBP of non-hypertensive rats in high dose treatment with no differences to the rats in normal feeding. The results proved that the peptides did not affect the SBP of non-hypertensive rats in short time treatment.

Michio Muguruma [16] reported the antihypertensive effect of two kinds of peptides, **KRVIQY** (**M6**, a novel peptide) and **VKAGF** [17] (**A5**) on SHR. M6 could decreased SBP of SHR 12 mmHg and 23 mmHg in dose of 10 mg/kg, after the oral administration in 3 h and 6 h. After the oral administration of A5 in 10 mg/kg, the SBP of SHR decreased by 12 mmHg in 3 h and 17 mmHg in 6 h. In another study, after the oral administration of 60 mg/kg of **LKPNM**, the pro-drug type similar to M6, the SBR of SHR decreased by 23 mmHg in 6 h [18]. Jae-Young Je also reported that a kind of purified ACE inhibitor from fermented oyster sauce, *Crassostrea gigas*, exhibited antihypertensive effects of the SBP reduction of 12 mmHg in a dose of 10 mg/kg of body weight, 3 h after inhibitor administration, and this activity was maintained for 6 h [19].

Compared with our results, SBP of SHR decreased by 21.5 mmHg, 13.8 mmHg, and 9.0 mmHg at 1.5 h after the oral administration of silkworm protein hydrolysates at a dose of 60 mg/kg, 20 mg/kg, and 5 mg/kg, respectively. The results show that the high dose of silkworm protein hydrolysates have close effects as M6 (**KRVIQY**) and the middle dose have close effects as the puried peptide from fermented oyster sauce [19]. Hiroyuki Fujita have reported that the thermolysin digest of "Katsuobushi", a traditional Japanese food, enriched by ultra-filtration named "S-KO", with the reduction of SBP by 10 mm Hg required an oral dose of 290 mg/kg in SHR [20]. However, the peptides of silkworm protein have not been separated, they were mixed peptides, thus compared with M6. The results show that more bioactive peptides possibly exist in the mixture compared with M6.

3.2. Effect of Long-Term Administration Treatment on Blood Pressure of SHR

According to the conditions and the design of section 2.5, the five groups of different treatments were fed for four weeks, and we detected the blood pressure of each groups at the same time every week. The results are

shown in **Figure 3**. The blood pressure of SHR increased with period of disease course. The blood pressure of the treatment groups with peptides and captopril decreased in different degrees. The Captopril group decreased to 144.3 ± 2.5 mmHg (−20.2 mmHg) at two weeks, which showed a significant difference from the blank control group at $p < 0.01$; this blood pressure status was maintained until the end of our experiment. The blood pressure of the high dose group decreased to 149.6 ± 1.2 mmHg (**−13.0 mmHg**) at two weeks, with a significant difference of $p < 0.05$, and 147.8 ± 1.8 mmHg (**−14.8 mmHg**) at three weeks in a significant difference of $p < 0.05$. However, after four weeks of high dose treatment, the blood pressure decreased to 137.6 ± 1.2 mmHg (**−25.0 mmHg**), with a significant difference of $p < 0.01$. The results are noteworthy because the blood pressure of the high dose group was lower than that of the captopril group after four weeks of treatment. The blood pressure of the middle dose group decreased to 146.6 ± 1.8 mmHg (**−17.9 mmHg**) with a significant difference of $p < 0.05$ compared with the blank control group, and 147.0 ± 1.5 mmHg (**−17.5 mmHg**) at four weeks with a significant difference of $p < 0.05$. No significant difference was observed in the low dose group during the four weeks of treatment.

During the long-term treatment on blood pressure of SHR, we also feed the non-hypertensive rats in high dose treatment, The SBP of non-hypertensive rats in high dose treatment with no differences to the rats in normal feeding. The results proved that the peptides did not affect the SBP of non-hypertensive rats in long-term treatment.

Hiroshi Yoshii reported that the values for systolic, mean, and diastolic blood pressure were approximately 10% less in SHRs by oligopeptides of 1 kDa or less via hydrolysis of chicken egg yolks with a crude enzyme administered compared with controls [21]. Wang reported that a purified peptide with sequence VVYPWTQRF from oyster protein hydrolysate (fraction II) exhibited antihypertensive activity close to 2 mg/kg captopril when orally administered to SHR at a dose of 20 mg/kg, and SBP decreased by more than 20 **mmHg** [22]. Compared with our study, after four weeks of treatment in high dose, SBP decreased by 25 **mmHg**, the antihypertensive ability was close to 4 mg/kg of captopril, and the SBP of middle dose group also decreased by 17.5 **mmHg**. The results showed that the silkworm pupae protein hydrolysates with significant antihypertensive activity were similar to the purified peptides, reminding us that more highly antihypertensive activity peptides exist in the hydrolysates. These results showed that the silkworm protein peptides can become functional components for antihypertension.

3.3. Effect of Long-Term Administration Treatment on Body Weight of SHR

We observed the change of SHR body weight during the long-term administration treatment. After four weeks

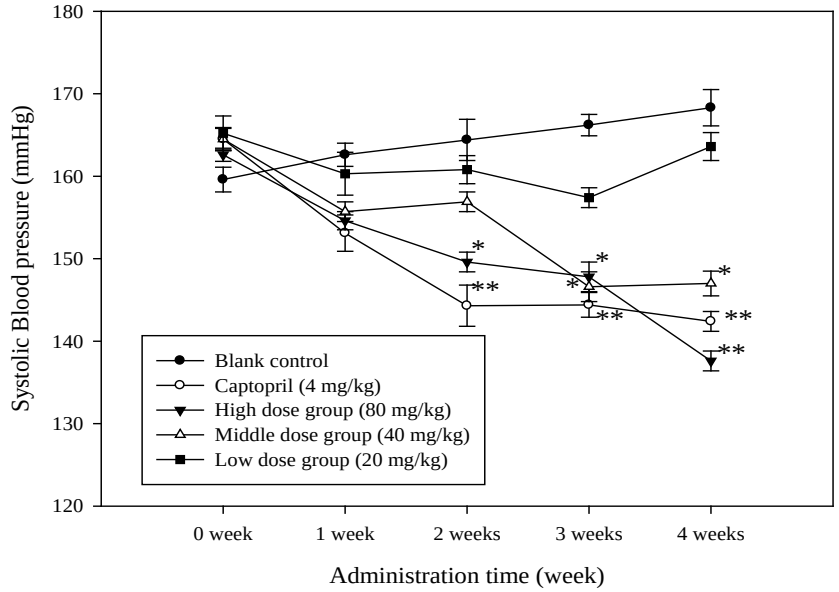

Figure 3. Effect of long-term administration treatment on blood pressure of SHR (Mean ± SD, n = 9). Note: Compared with blank control, $^{*}p < 0.05$; $^{**}p < 0.01$.

of experimental observation, the body weight of each group increased in different degrees, the body weight of blank control, Captopril, High dose group, Middle dose group and Low dose group were in 229.9 ± 3.1 g, 226.1 ± 5.3 g, 226.0 ± 5.2 g, 229.3 ± 7.8 g and 228.3 ± 11.5 g, respectively (**Table 1**). There was no significant difference between each treatment group and the blank control group ($p > 0.05$). This result shows that the peptide hydrolysates of silkworm pupae protein had no influence on body weight of SHR and did not affect the normal growth of SHR.

3.4. Safety Effect of Peptide Hydrolysates in Silkworm Pupae Protein

3.4.1. LD_{50}

After the pre-experiment, rats were administered with peptides in different doses. After 7 d of continuous observation, the psychosis and the physiology of treatment groups were all in good conditions, with normal increased body weights, and all of the experimental mice are still alive. After 8 d of treatment, the experimental mice were sacrificed; and their internal organs were examined. No significant difference was found among the treatment groups. These results showed that the peptide hydrolysates had no obvious toxicities, thus we cannot calculate LD_{50}.

3.4.2. Acute Toxicity of Peptide Hydrolysates in Silkworm Pupae Protein

We immediately observed the physiological state after the mice were administered with peptide hydrolysates. The mice showed no difference in movement and physical external performance. The excrement of some mice was in the same color and dilution, and did not exhibit abnormalities. After 14 d of continuous observation, diet, appearance, behavior, secretions, and excretions of the experimental mice were in good conditions. When the experiment was finished, the mice were killed by cervical dislocation. Autopsies were performed and the heart, liver, spleen, lungs, kidneys, and other major organs were carefully observed. The results showed that no organomegaly, atrophy, and necrosis anomalies of the organs occurred.

4. Discussion

Generally, the most common mechanisms that the blood pressure-lowering effect of food peptides seems to be the inhibition of the activity of angiotensin-I-converting enzyme (ACE) and thus, the search for ACE inhibitory activity *in vitro* is a widespread strategy in the selection of antihypertensive hydrolysates and peptides [23]. The *in vivo* effects are usually tested in spontaneously hypertensive rats (SHR) which constitute an accepted model for human essential hypertension [24]. In general terms, the results of the *in vitro* ACE inhibitory activity dose not mean the same bioavailability *in vivo* action because of the physiological transformations. While, by the results we got from single and long-term administration, we are sure that there are enough peptides in the hydrolysates from silkworm pupae protein could resist the physiological transformations.

There were some reports such as Marta Miguel reported the egg white hydrolysate with pepsin (HEW) and its fraction with molecular mass lower than 3000 Da (HEW < 3000) have ACE-inhibitory activity *in vitro* and exert antihypertensive effects after single-oral administrations to SHR, the maximum reduction in SBP was observed 6 h after the administration of HEW < 3000 (decreased 28 mm Hg) in 100 mg/kg [25]. Marta Miguel also reported a bovine casein hydrolysate could decrease the SBP of SHR at 2 h decreased 20 mmHg in 400 mg/kg [26] while the high dose groups of silkworm pupa protein peptide hydrolysates showed that blood pressure decreased significantly at 1.5 h decreased 21.5 mmHg in 60 mg/kg. From the above comparation, the pupa protein peptide

Table 1. Effect of long-term administration treatment on body weight of SHR.

Treatments	Body weight (g)
blank control	229.9 ± 3.1
Captopril	226.1 ± 5.3
High dose group	226.0 ± 5.2
Middle dose group	229.3 ± 7.8
Low dose group	228.3 ± 11.5

hydrolysates obviously with more optimistic bioactivity on decreasing SBP.

Recently, literatures reports mostly focused on the bioactivities of the hydrolysates *in vivo* or *in vitro*, but few reports were on the safety of the hydrolysates. In this paper, the pepides hydrolysates from silkworm pupae protein do not affect the normal blood pressure, which was verified. And, the acute toxicity of the silkworm pupae protein hydrolysates was also considered, the results showed the pepides hydrolysates without any side effects, the experiments mice were all healthy with body weight and their physiological state in good conditions in the enough dosage.

In our previous studies, we found a novel peptide "APPPKK" from the silkworm pupae protein hydrolysates, The peptide inhibitory activity was 0.047 mg/mL in IC50. The peptide was bonded to Asp^{415}, Asp^{453}, Thr^{282}, His^{353}, Glu^{162} in hydrogen bond to ACE active pocket [13]. This time we also detected the ACE inhibitory *in vitro*, got the ACE inhibitory rate was 73.7% in 1.0 mg/mL. It was proved some mechanisms of the the silkworm pupae protein hydrolysates decreased the SBP on SHRs, it should because of a mount of peptides in the silkworm pupae protein hydrolysates with the bioactivity nearly to "APPPKK".

In order to produce antihypertensive effects *in vivo*, the peptides have to be absorbed intact through the intestine and reach the cardiovascular system in an active form. In this regard, specific structural properties play an important role. Most of the ACE inhibitory peptides are short peptides with only two to nine amino acids [27]. Some reports have showed that di- or tripeptides, especially those with C-terminal proline or hydroxyproline residues, are generally resistant to degradation by digestive enzymes [28] [29]. In addition, short peptides consisting of two or three amino acids are absorbed more rapidly than free amino acids [30] [31]. Larger peptides (10 - 51 amino acids) present in the diet can also be absorbed intact through the intestine and produce biological effects, although the potency of the peptides decreases as the chain length increases [32] [33]. In this research, the molecular weight distribution of the silkworm pupae protein peptide hydrolysates were mainly in two parts, the molecular weight between 307 Da to 5000 Da was 88.2%, and less than 307 Da was 11.8%, it means the peptides mainly were short peptides. The antihypertensive effects on SHR of the silkworm pupae protein peptide hydrolysates in one period and long-term treatment tipped us that some peptides could be absorbed intact through the intestine and reach the cardiovascular system in an active form and that the further more research on determining the structures of these peptides and the mechanisms needs our continuous efforts.

5. Conclusion

Considering the aforementioned effects of peptide hydrolysates in silkworm pupae protein on mice and SHR, we determined that the peptide hydrolysates in silkworm pupae protein exhibited the antihypertensive activity with safe and healthy characteristics. Our results would be helpful in exploring the functional foods or functional components for antihypertension.

Acknowledgements

This work was supported by the National Natural Science Foundation of China (No. 31101390) and the Zhejiang Provincial Natural Science Foundation of China (LY13C200015) and (LQ13C200005).

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this article.

References

[1] Lee, H.J. (1995) Biopharmaceutical Properties and Pharmacokineties of Peptide and Protein Drugs. In: Talor, M.D. and Amidon, G.L., Eds., *Peptide-Based Drugs Design*, ACS Professional Reference Book, American Chemical Society, Washington DC, 69.

[2] Fei, Y.J., Yoshikatsu, K., Stephan, N., Vadivel, G., Frederick, H.L., Michael, F.R., Satish, K.S., Walter, F.B. and Matthias, A.H. (1994) Expression Cloning of a Marnmaliam Proton-Coupled Oligopeptide Transporter. *Nature*, **368**, 563-566. http://dx.doi.org/10.1038/368563a0

[3] Dzau, V.J., Sasamura, H. and Hein, L. (1993) Heterogeneity of Angiotensin Synthetic Pathways and Receptor Subtypes: Physiological and Pharmacological Implications. *Journal of Hypertension. Supplement*, **11**, 13-18.

[4] Kim, S. and Iwao, H. (2000) Molecular and Cellular Mechanisms of Angiotensin II-Mediated Cardiovascular and Ren-

al Diseases. *Pharmacological Reviews*, **52**, 11-34.

[5] Roks, A., Buikema, H., Pinto, Y.M. and Van Gilst, W.H. (1997) The Renin-Angiotensin System and Vascular Function. The Role of Angiotensin II, Angiotensin-Converting Enzyme, and Alternative Conversion of Angiotensin I. *Heart and Vessels*, **12**, 119-124.

[6] Zimmerman, B.G. and Dunham, E.W. (1997) Tissue Renin-Angiotensin System: A Site of Drug Action. *Annual Review of Pharmacology and Toxicology*, **37**, 53-69. http://dx.doi.org/10.1146/annurev.pharmtox.37.1.53

[7] Ehlers, M.R., Fox, E.A., Strydom, D.J. and Riordan, J.F. (1989) Molecular Cloning of Human Testicular Angiotensin-Converting Enzyme: The Testis Isozyme Is Identical to the C-Terminal Half of Endothelial Angiotensin-Converting Enzyme. *Proceedings of the National Academy of Sciences of the United States of America*, **86**, 7741-7745. http://dx.doi.org/10.1073/pnas.86.20.7741

[8] Marta, M., Rosina, L.F., Mercedes, R. and Amaya, A. (2005) Short-Term Effect of Egg-White Hydrolysate Products on the Arterial Blood Pressure of Hypertensive Rats. *British Journal of Nutrition*, **5**, 731-737.

[9] Muguerza, B., Ramos, M., Sánchez, E., Manso, M.A., Miguel, M., Aleixander, A., Delgado, M.A. and Recio, I. (2006) Antihypertensive Activity of Milk Fermented by *Enterococcus faecalis* Strains Isolated from Raw Milk. *International Dairy Journal*, **16**, 61-69. http://dx.doi.org/10.1016/j.idairyj.2005.01.001

[10] Nakamura, Y., Yamamoto, N., Sakai, K., Okubo, A., Yamazaki, S. and Takano, T. (1995) Purification and Characterization of Angiotensin-I-Converting Enzyme Inhibitors from sour Milk. *Journal of Dairy Science*, **78**, 777-783. http://dx.doi.org/10.3168/jds.S0022-0302(95)76689-9

[11] Wang, W., Shen, S.R., Chen, Q.H., Ruan, H., He, G.Q. and Undurti, N.D. (2008) Hydrolysates of Silkworm Pupae (*Bombyx mori*) Protein Is a New Source of Angiotensin-I-Converting Enzyme Inhibitory Peptides (ACEIP). *Current Pharmaceutical Biotechnology*, **9**, 307-314. http://dx.doi.org/10.2174/138920108785161578

[12] Wang, W., Wang, N., Zhang, Y., Cai, Z., Chen, Q.H. and He, G.Q. (2013) A Convenient RP-HPLC Method for Assay Bioactivities of Angiotensin I-Converting Enzyme Inhibitory Peptides. *ISRN Biotechnology*, **2013**, Article ID: 453910. http://dx.doi.org/10.5402/2013/453910

[13] Wang, W., Wang, N., Zhou, Y., Zhang, Y., Xu, L.H. and Xu, J.F. (2010) Isolation of a Novel Peptide from Silkworm Pupae Protein Components and Interaction Characteristics to Angiotensin I-Converting Enzyme. *European Food Research and Technology*, **232**, 29-38. http://dx.doi.org/10.1007/s00217-010-1358-8

[14] Buñag, R.D. (1973) Validation in Awake Rats of a Tail-Cuff Method for Measuring Systolic Pressure. *Journal of Applied Physiology*, **34**, 279-282.

[15] Rone, G., Chappel, C.I. and Balazs, T. (1995) An Infarct-Like Myocardial Lesion and Other Toxic Manifestation Produced by Isoproterenal in the Rat. *Archives of Pathology Laboratory Medicine*, **67**, 443-455.

[16] Michio, M., Abdulatef, M.A., Kazunori, K., Satoshi, K., Masugi, M. and Toyoo, N. (2009) Identification of Pro-Drug Type ACE Inhibitory Peptide Sourced from Porcine Myosin B: Evaluation of Its Antihypertensive Effects *in Vivo*. *Food Chemistry*, **114**, 516-522. http://dx.doi.org/10.1016/j.foodchem.2008.09.081

[17] Ukeda, H., Matsuda, H., Osajima, K., Matsufuji, H., Matsui, T. and Osajima, Y. (1992) Peptides from Peptic Hydrolysate of Heated Sardine Meat That Inhibit Angiotensin I Converting Enzyme. *Nippon Nōgeikagaku Kaishi*, **66**, 25-29. http://dx.doi.org/10.1271/nogeikagaku1924.66.25

[18] Fujita, H., Yokoyama, K. and Yoshikawa, M. (2000) Classification and Antihypertensive Activity of Angiotensin I-Converting Enzyme Inhibitory Peptides Derived from Food Proteins. *Journal of Food Science*, **65**, 564-569. http://dx.doi.org/10.1111/j.1365-2621.2000.tb16049.x

[19] Je, J.Y., Park, J.Y., Jung, W.K., Park, P.J. and Kim, S.K. (2005) Isolation of Angiotensin I Converting Enzyme (ACE) Inhibitor from Fermented Oyster Sauce, *Crassostrea gigas*. *Food Chemistry*, **90**, 809-814. http://dx.doi.org/10.1016/j.foodchem.2004.05.028

[20] Hiroyuki, F., Tomohide, Y. and Kazunori, O. (2001) Effects of an Ace-Inhibitory Agent, Katsuobushi Oligopeptide, in the Spontaneously Hypertensive Rat and in Borderline and Mildly Hypertensive Subjects. *Nutrition Research*, **21**, 1149-1158. http://dx.doi.org/10.1016/S0271-5317(01)00333-5

[21] Hiroshi, Y., Norihide, T., Riichiro, O., Osamu, S., Hidemaro, T. and Toru, I. (2001) Antihypertensive Effect of ACE Inhibitory Oligopeptides from Chicken Egg Yolks. *Comparative Biochemistry and Physiology Part C*, **128**, 27-33.

[22] Wang, J.P., Hu, J.E., Cui, J.Z., Bai, X.F., Dua, Y.G., Miyaguchi, Y.J. and Lin, B.C. (2008) Purification and Identification of a ACE Inhibitory Peptide from Oyster Proteins Hydrolysate and the Antihypertensive Effect of Hydrolysate in Spontaneously Hypertensive Rats. *Food Chemistry*, **111**, 302-308. http://dx.doi.org/10.1016/j.foodchem.2008.03.059

[23] Li, G.H., Le, G.W., Shi, Y.H. and Shrestha, S. (2004) Angotensin I-Converting Enzyme Inhibitory Peptides Derived from Food Proteins and Their Physiological and Pharmacological Effects. *Nutrition Research*, **24**, 469-486. http://dx.doi.org/10.1016/j.nutres.2003.10.014

[24] Fitzgerald, R.J., Murray, B.A. and Walsh, G.J. (2004) Hypotensive Peptides from Milk Proteins. *Journal of Nutrition*, **134**, 980-988.

[25] Miguel, M., Alonso, M.J., Salaices, M., Aleixandre, A. and López-Fandiño, R. (2007) Antihypertensive, ACE-Inhibitory and Vasodilator Properties of an Egg White Hydrolysate: Effect of a Simulated Intestinal Digestion. *Food Chemistry*, **104**, 163-168. http://dx.doi.org/10.1016/j.foodchem.2006.11.016

[26] Miguel, M., Contreras, M.M., Recio, I. and Aleixandre, A. (2009) ACE-Inhibitory and Antihypertensive Properties of a Bovine Casein Hydrolysate. *Food Chemistry*, **112**, 211-214. http://dx.doi.org/10.1016/j.foodchem.2008.05.041

[27] Erdmann, K., Cheung, B.W.Y. and Schröder, H. (2008) The Possible Roles of Food-Derived Bioactive Peptides in Reducing the Risk of Cardiovascular Disease. *The Journal of Nutritional Biochemistry*, **19**, 643-654. http://dx.doi.org/10.1016/j.jnutbio.2007.11.010

[28] Matsufuji, H., Matsui, T., Seki, E., Osajima, K., Nakashima, M. and Osajima, Y. (1994) Angiotensin I-Converting Enzyme Inhibitory Peptides in an Alkaline Protease Hydrolyzate Derived from Sardine Muscle. *Bioscience, Biotechnology, and Biochemistry*, **58**, 2244-2245. http://dx.doi.org/10.1271/bbb.58.2244

[29] Vermeirssen, V., Van Camp, J. and Verstraete, W. (2004) Bioavailability of Angiotensin I Converting Enzyme Inhibitory Peptides. *British Journal of Nutrition*, **92**, 357-366. http://dx.doi.org/10.1079/BJN20041189

[30] Gardner, M.L. (1988) Gastrointestinal Absorption of Intact Proteins. *Annual Review of Nutrition*, **8**, 329-350. http://dx.doi.org/10.1146/annurev.nu.08.070188.001553

[31] Webb, K.E. (1990) Intestinal Absorption of Protein Hydrolysis Products: A Review. *Journal of Animal Science*, **68**, 3011-3022.

[32] Masuda, O., Nakamura, Y. and Takano, T. (1996) Antihypertensive Peptides Are Present in Aorta after Oral Administration of Sour Milk Containing These Peptides to Spontaneously Hypertensive Rats. *Journal of Nutrition*, **126**, 3063-3068.

[33] Roberts, P.R., Burney, J.D., Black, K.W. and Zaloga, G.P. (1999) Effect of Chain Length on Absorption of Biologically Active Peptides from the Gastrointestinal Tract. *Digestion*, **60**, 332-337. http://dx.doi.org/10.1159/000007679

Abbreviations

ACE = Angiotensin-I-converting Enzyme
SBP = Systolic blood pressure
SHR = Spontaneously hypertensive rats
ACEIPs = Angiotensin-I-converting enzyme inhibitory peptides
LD_{50} = Lethal dose, 50%
MW = Molecular weight

Scientific Research Publishing (SCIRP) is one of the largest Open Access journal publishers. It is currently publishing more than 200 open access, online, peer-reviewed journals covering a wide range of academic disciplines. SCIRP serves the worldwide academic communities and contributes to the progress and application of science with its publication.

Other selected journals from SCIRP are listed as below. Submit your manuscript to us via either submit@scirp.org or Online Submission Portal.