



Anti-fatigue effects of proteins isolated from *Panax quinquefolium*



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ARTICLE INFO

Article history:

Received 8 September 2013

Received in revised form

24 February 2014

Accepted 25 February 2014

Available online 4 March 2014

Keywords:

American ginseng proteins

Anti-fatigue

Forced swimming test

ABSTRACT

Ethnopharmacological relevance: American ginseng (*Panax quinquefolium*) is an obligate shade perennial plant that belongs to Araliaceae ginseng species, and is native to eastern USA and Canada. Ginseng proteins are reported to have several pharmaceutical properties. However, such properties of American ginseng proteins (AGP) have seldom been reported. Also, anti-fatigue properties of AGP have not been studied. Therefore, we examined the anti-fatigue effects of AGP in mice.

Materials and methods: The molecular weight and protein contents of AGP were determined by SDS-PAGE, while the amino acid composition was analyzed by HPLC. The mice were divided into four groups. The control group was administered distilled water by gavage every day for 28 days. The other groups, designated as AGP treatment groups, were administered 125, 250 and 500 mg/kg of body weight, respectively of AGP by gavage every day for 28 days. Anti-fatigue activity was estimated using forced swimming test, and biochemical indices were determined using available kits.

Results: The subunit molecular weight of AGP ranged from 8–66 kD and the protein content measured by Bradford assay was 1.86 mg/mL. The forced swimming time of low, intermediate and high groups were found to be longer as compared to the control group. AGP significantly decreased blood lactate (BLA) and serum urea nitrogen (SUN) levels, and increased hepatic glycogen (GLU) level. Additionally, AGP lowered malondialdehyde (MDA) content and increased the levels of glutathione peroxidase (GPx) and superoxide dismutase (SOD).

Conclusion: AGP shows anti-fatigue activity in mice, as measured by the physiological indices for fatigue.

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1. Introduction

American ginseng is the root of *Panax quinquefolium*, which is currently grown in Canada and eastern USA, similar to *Panax ginseng* C.A. Meyer. It is an obligate shade perennial plant. American ginseng is a traditional valuable herb, which belongs to Araliaceae ginseng species. It contains various active constituents such as ginsenosides, polysaccharides, polyacetylenes, phenolic compounds, peptides and essential oils (Lemmon et al., 2012; Yoo et al., 2012; Trammell et al., 2012). Much attention has been given to saponins present in ginseng and American ginseng. More than 20 saponins have been isolated from ginseng root (Liu, 2012). However, few researchers have investigated the biological activities of other constituents. It has been reported that ginseng proteins have various pharmaceutical activities, such as antioxidant, antifungal and antiviral (Wang and Ng, 2000; Ng and Wang,

2001; Lam and Ng, 2001; Yoon et al., 2002; Moon et al., 2010). In China, ginseng has been traditionally used for developing physical strength, especially in patients who suffered from severe fatigue (Saito et al., 1974). Fatigue is defined as a feeling of extreme physical or mental tiredness (Moriura et al., 1996; Kim et al., 2001). It is a complex phenomenon, which may be present in many illnesses. Fatigue is caused by the depletion of energy sources, including decrease in glycemic levels and liver glycogen consumption; accumulation of end products of fatigue, such as BLA; disorder of internal environment; and metabolic control disorders of nervous system, enzymes, and hormones, while performing sports (Tan et al., 2012). Since it is difficult to improve the available therapies for fatigue in modern medicine, potential alternatives from traditional medicine and their respective mechanisms of action are worth studying (Shimizu et al., 2010; Huang et al., 2011). According to literature reports, ginseng polysaccharides possess anti-fatigue activity. It was shown that the acidic polysaccharide had higher potency to induce anti-fatigue activity as compared to the neutral polysaccharide (Wang et al., 2010). But the anti-fatigue effects of American ginseng proteins have been less studied.

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In this study, we examined the anti-fatigue property of American ginseng proteins (AGP) using forced swimming test in order to understand the potential mechanism of *Panax quinquefolium*'s beneficial effects.

2. Materials and methods

2.1. Materials

2.1.1. Plant material and extraction

The roots of four-year old American ginseng (*Panax quinquefolium*) were obtained from the farms of Wisconsin, USA. These roots were homogenized with 0.05 M Tris–HCl buffer solution (pH 7.4) at 4 °C for 12 h before centrifugation. Ammonium sulfate was added to the supernatant to 80% saturation, while stirring constantly. Dialysis was performed to remove the ammonium sulfate, the precipitation was dissolved in water, applied to a hollow fiber membrane and the concentrated solution was lyophilized. The content of proteins in AGP determined by Bradford assay was 80% (w/w). The yield of AGP was 3.5%.

2.1.2. Chemicals and reagents

Assay kits for determination of blood lactate (BLA), serum urea nitrogen (SUN), hepatic glycogen (GLU), glutathione peroxidase (GPx), malondialdehyde (MDA) and superoxide dismutase (SOD) were purchased from Nanjing Jiancheng Biotechnology Institute (Nanjing, China). All other reagents used in this study were of analytical grade.

2.1.3. Experimental animals

Eighty Kunming mice (40 males and 40 females) weighing 20 ± 2 g were purchased from Pharmacology Experimental Center of Jilin University, China. The mice were housed at room temperature (23 ± 1 °C), with 12 h light and 12 h dark cycle (lights were kept on from 6:00 am to 6:00 pm). Food and water were available ad libitum. The mice were treated in compliance with the current law and the Guiding Principles for the Care and Use of Laboratory Animals approved by the Animal Ethics Committee of China.

2.2. Methods

2.2.1. Determination of molecular weight and protein content of AGP

AGP from American ginseng root was subjected to sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) using 12% separating gel (Bradford, 1976). 50 mg of each AGP sample was loaded in each lane. After electrophoresis, the bands were visualized by Coomassie brilliant blue R-250 staining. Protein content of AGP was determined by the Bradford method using BSA as a standard.

2.2.2. Analysis of amino acid composition of AGP

AGP (50 mg) from American ginseng root was hydrolyzed under vacuum in 6 mol/L HCl for 24 h at 110 ± 1 °C for general amino acid analysis. For cysteine analysis, AGP was hydrolyzed in 6 mol/L HCl for 24 h at 110 ± 1 °C after peroxidation treatment with formic acid:hydrogen peroxide (10:1). For tryptophan analysis, AGP was hydrolyzed in 4 mol/L each of methanesulfonic acid and NaOH. Amino acids converted to phenylisothiocyanate (PITC) derivatives were analyzed by high performance liquid chromatography (HPLC) (Agilent 1100 series; Agilent Technologies, USA) with Wondasil-C18 (4.6×150 mm, 5 μ m) (Pyo et al., 2011).

2.2.3. Experimental design

80 mice were randomly divided into four groups with 20 mice in each group comprising of 10 males and 10 females. The first group designated as control group (CG) was administered distilled water at 10 mL/kg of body weight by gavage. In other groups, American ginseng proteins (AGP) were administered to the mice at 125, 250 and 500 mg/kg of body weight, respectively and the three groups were accordingly designated as low-dose group (AGP-LG), intermediate-dose group (AGP-MG) and high-dose group (AGP-HG), respectively. Water/AGP was administered orally (at 8:00 am) for 28 days. The forced swimming test (FST) was conducted on the last day and corresponding biochemical parameters such as blood lactate (BLA), serum urea nitrogen (SUN), hepatic glycogen (GLU), glutathione peroxidase (GPx), malondialdehyde (MDA) and superoxide dismutase (SOD) were measured using appropriate kits.

2.2.4. Forced swimming test

The forced swimming test was carried out as described previously with some modifications (Uthayathas et al., 2007; Nozawa et al., 2009; Jin and Wei, 2011; Tan et al., 2012). After 28 days, 10 mice were taken from each group for the test. Briefly, 30 min after the final treatment with AGP or distilled water, the mice were placed individually in a swimming pool (50 cm \times 40 cm \times 40 cm), filled with water to a depth of 30 cm and maintained at 25 ± 1 °C. A lead sinker (10% of the body weight) was attached to the tail root of each mouse. Exhaustion was classified as the inability of the mice to rise to the surface within 10 s. The swimming time was recorded immediately. The mice were then removed from the pool, dried with a paper towel, and returned to their original cages. The pool water was replaced after each session.

2.2.5. Analysis of biochemical parameters

After four weeks, GLU and blood biochemical parameters were analyzed in the other 10 mice from each group. 30 min after the last administration of AGP, the mice were forced to swim for 90 min without loads. After the mice rested for an hour, blood was collected through their eyeballs and serum was prepared by centrifugation at 4000 rpm at 4 °C for 15 min. Levels of BLA, SUN, SOD, MDA and GPx were determined according to the recommended procedures provided by the kits. The livers of the mice were immediately collected and homogenized to 10% solution with normal saline at 4 °C. GLU levels were determined using commercially available kits.

2.2.6. Statistical analysis

The results were expressed as mean \pm standard deviation (SD). Differences between groups were analyzed using analysis of variance and student's *t*-test. $P < 0.05$ was considered to be statistically significant.

3. Results

3.1. Determination of molecular weight and protein content of AGP

We determined the molecular weight of AGP in SDS-PAGE and found that the subunit molecular weight of AGP ranged from 8 kD to 66 kD (Fig. 1). The protein content determined by Bradford assay was 1.86 mg/mL.

3.2. Analysis of amino acid composition of AGP

Amino acid composition of AGP is summarized in Table 1. The top three amino acids present in AGP were arginine, alanine and

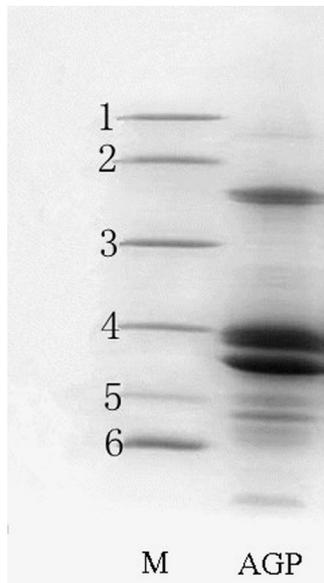


Fig. 1. Determination of molecular weight of AGP by SDS-PAGE. M represents molecular markers. Coomassie brilliant blue staining was used to stain protein moieties of AGP. The numbers represent standard proteins used: (1) rabbit phosphorylase b (97 kDa), (2) bovine serum albumin (BSA) (66 kDa), (3) rabbit actin (43 kDa), (4) bovine carbonic anhydrase (31 kDa), (5) trypsin inhibitor (20 kDa), (6) hen egg white lysozyme (14 kDa).

Table 1
Amino acid composition of AGP.

Amino acid	Amino acid composition of AGP (mg/g)
Asp	17.44
Glu	14.4
Ser	29.6
His	17.2
Gly	18.08
Cys	67.84
Thr	73.36
Arg	179.28
Ala	131.28
Pro	75.68
Tyr	16.8
Val	37.68
Met	8.8
Lle	57.52
Leu	5.36
Phe	35.12
Lys	76.08
Trp	2.51

lysine. There were eight essential amino acids for human, the ratio was 35.62%.

3.3. Effect of AGP on the forced swimming test performed on the mice

As shown in Fig. 2, the forced swimming time in low-dose AGP group (AGP-LG) was longer as compared to the control group (CG), and the difference was statistically significant ($P < 0.05$). The intermediate-dose group (AGP-MG) and the high-dose group (AGP-HG) also demonstrated significant increase in swimming time as compared to the CG ($P < 0.01$). Fig. 2 shows that the forced swimming time in the AGP-LG, AGP-MG, and AGP-HG increased by 9.21%, 27.11% and 59.54%, respectively, as compared to the CG.

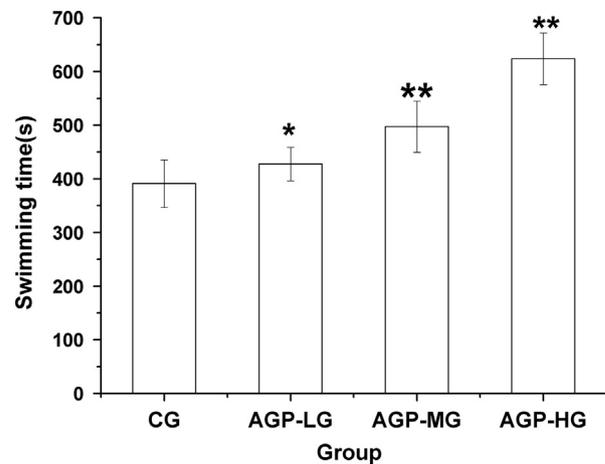


Fig. 2. Effect of AGP on the forced swimming time in mice. * $P < 0.05$ and ** $P < 0.01$ as compared to the CG.

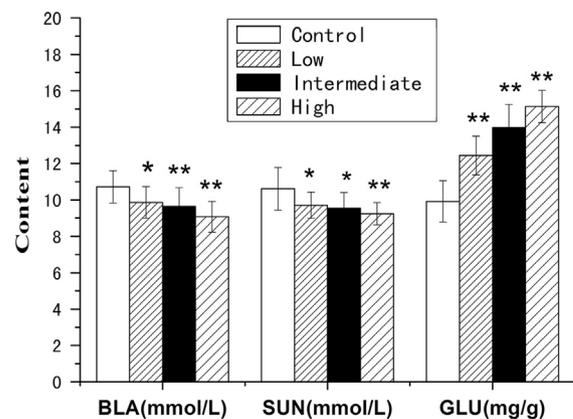


Fig. 3. Effect of AGP on blood lactate (BLA), serum urea nitrogen (SUN) and hepatic glycogen (GLU) in mice. * $P < 0.05$ and ** $P < 0.01$ as compared to the CG.

Table 2
Effect of AGP on GPx, SOD activities and MDA content in serum of mice.

Group	Dose (mg/kg)	GPx (U/L)	SOD (U/mL)	MDA (nmol/L)
CG		632.97 ± 18.63	75.73 ± 9.48	12.41 ± 1.12
AGP-LG	125	730.97 ± 13.15 ^a	88.04 ± 6.99 ^a	11.05 ± 1.16 ^b
AGP-MG	250	792.75 ± 15.84 ^a	98.96 ± 7.63 ^a	10.28 ± 1.04 ^a
AGP-HG	500	816.46 ± 14.68 ^a	104.76 ± 11.04 ^a	9.43 ± 0.64 ^a

^a $P < 0.01$ compared with CG.

^b $P < 0.05$ compared with CG.

3.4. Effects of AGP on blood lactate, serum urea nitrogen and hepatic glycogen in mice

Fig. 3 shows that the levels of BLA in the AGP treatment groups were lower ($P < 0.05$ for AGP-LG, and $P < 0.01$ for AGP-MG and AGP-HG) as compared to the CG after the forced swimming test. The decrease ratios were 8.02% (low), 9.98% (intermediate) and 15.39% (high), respectively.

As demonstrated in Fig. 3, SUN levels in AGP-HG were much higher as compared to the CG ($P < 0.01$). The SUN levels were also higher in AGP-LG and AGP-MG as compared to the CG ($P < 0.05$). The levels of GLU in the low, intermediate and high groups were $12.44 ± 1.07$, $13.99 ± 1.25$, $15.14 ± 0.88$ mg/g, respectively, as compared to $9.92 ± 1.14$ mg/g in the CG ($P < 0.01$). So, the increase in GLU may be one of the pathways contributing to the anti-fatigue properties of AGP.

3.5. Effect of AGP on glutathione peroxidase, malondialdehyde and superoxide dismutase in mice

As shown in Table 2, MDA in serum of mice treated with AGP was significant lower as compared to the CG ($P < 0.05$ for AGP-LG, and $P < 0.01$ for AGP-MG and AGP-HG). SOD and GPx in serum of control mice were much lower as compared to the treated groups ($P < 0.01$).

4. Discussion and conclusion

Fatigue is one of the most frequent physiological reactions. It often occurs in depression, aging, tumor, HIV infection, multiple sclerosis and Parkinson's disease. It is possible to get tired when the body is in an oxygen-deficient state after strenuous exercise. The complex mechanism of fatigue has been described (Tan et al., 2012). Natural products, which have no side effects, could possibly delay fatigue.

The forced swimming test is one of the most valid models for evaluating anti-fatigue properties in animals (Tang et al., 2008; You et al., 2012). Our results revealed that AGP had notable effect on the anti-fatigue properties of mice. Previous studies have demonstrated that BLA is the product of glycolysis under anaerobic conditions, and glycolysis is the primary energy source during high intensity physical exercise. With the accumulation of BLA, the pH in muscle tissue and blood reduces, which is harmful to some organs and also causes fatigue (Cairns, 2006; Ma et al., 2008; Kim et al., 2012). Consequently, BLA is determined as an important parameter for gauging the extent of fatigue. Our study showed that AGP could prevent the accumulation of BLA, and this possibly delays the onset of fatigue. SUN is another blood biochemical index related to fatigue, which is formed in the liver as the end product of protein metabolism. When the body is unable to obtain sufficient energy from sugar and fat catabolism, it utilizes proteins and amino acids that have a stronger catabolism (Grassi and Passatore, 1991; Wang et al., 2008; Li et al., 2009). After prolonged physical activity, the level of SUN will significantly increase. Our study indicated that AGP could delay the production of SUN after exercise. The usage of stored hepatic glycogen to perform prolonged physical activity has been previously confirmed (Jung et al., 2007). Hepatic glycogen consumption could be an important element in the development of fatigue because with the consumption of hepatic glycogen during the course of exercise, the level of blood glucose needs to be maintained in the physiologic range; otherwise the consequential hypoglycemia could harm nervous function (Jung et al., 2004). With the exhaustion of energy sources such as glucose and hepatic glycogen, fatigue will set in.

Intense exercise may cause an imbalance between the body's oxidation and anti-oxidation systems, thereby producing more reactive oxide species (ROS). ROS could attack polyunsaturated fatty acids (PUFA), which will lead to lipid peroxidation. MDA is one of the degradation products in the lipid peroxidation process (Ding et al., 2011). Enzymatic antioxidant systems, such as GPx and SOD, are important in scavenging free radicals and their metabolites (Elias et al., 2008). Our results indicated that the anti-fatigue effect of AGP probably occurs through protection of corpuscular membrane by preventing lipid oxidation via modifying activities of several enzymes. These results are in accordance with the findings by Wang et al. (2010), which demonstrated similar effects of ginseng polysaccharides on MDA and GPx levels.

Aromatic amino acid residues, such as phenylalanine and tyrosine residues, are the main targets for oxidation. In biological systems, methionine is an important free radical scavenger in proteins. Methionine residues are very unstable during oxidation and can obliterate radicals before they attack other amino acid residues (Stadtman and Levine, 2003; Elias et al., 2008). Our

results showed that AGP could promote the activities of the antioxidant enzymes, and has anti-fatigue effects.

In conclusion, this is the first study to examine the anti-fatigue effects of AGP. AGP improved the swimming ability of mice by retarding the accumulation of BLA and SUN, and enhancing GLU levels. Additionally, AGP could improve the ability of antioxidant enzymes to eliminate ROS, which could be a likely pathway for the anti-fatigue activities of AGP. However, further study is required to ascertain the nature of AGP, and to evaluate its anti-fatigue effects at cellular and molecular levels.

Acknowledgments

This work was supported by the National Natural Science Foundation of China (No. 81274038), the National Science and Technology Major Project of the Ministry of Science and Technology of China (No. 2010ZX09401-305) and the National Key Technology Research and Development of the Ministry of Science and Technology of China (No. 81274038).

References

- Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* 72, 248–254.
- Cairns, S.P., 2006. Lactic acid and exercise performance: Culprit or friend? *Sports Med.* 36, 279–291.
- Ding, J.-F., Li, Y.-Y., Xu, J.-J., Su, X.-R., Gao, X., Yue, F.-P., 2011. Study on effect of jellyfish collagen hydrolysate on anti-fatigue and anti-oxidation. *Food Hydrocoll.* 25, 1350–1353.
- Elias, J.R., Kellerby, S., Decker, A.E., 2008. Antioxidant activity of proteins and peptides. *Crit. Rev. Food Sci. Nutr.* 48, 430–441.
- Grassi, C., Passatore, M., 1991. Anti-fatigue action exerted by the sympathetic nervous system in the rabbit digastric muscle. *Funct. Neurol.* 6, 255–258.
- Huang, L.Z., Huang, B.K., Ye, Q., Qin, L.P., 2011. Bioactivity-guided fractionation for anti-fatigue property of *Acanthopanax senticosus*. *J. Ethnopharmacol.* 133, 213–219.
- Jin, H.M., Wei, P., 2011. Anti-fatigue properties of tartary buckwheat extracts in mice. *Int. J. Mol. Sci.* 12, 4770–4780.
- Jung, K.A., Han, D., Kwon, E.K., Lee, C.H., Kim, Y.E., 2007. Anti-fatigue effect of *Rubus coreanus* Miquel extract in mice. *J. Med. Food* 10 (4), 689–693.
- Jung, K., Kim, I.H., Han, D., 2004. Effect of medicinal plant extracts on forced swimming capacity in mice. *J. Ethnopharmacol.* 93, 75–81.
- Kim, K.M., Yu, K.W., Kang, D.H., Koh, J.H., Hong, B.S., Suh, H.J., 2001. Anti-stress and anti-fatigue effects of fermented rice bran. *Biosci., Biotechnol., Biochem.* 65, 2294–2296.
- Kim, N.H., Moon, P.D., Pak, S.C., Kim, H.M., Jeong, H.J., 2012. Anti-fatigue effect of *Zizania caudiflora* (Turczaninow) Nakai. *Am. J. Chin. Med.* 40, 111–120.
- Lam, S.K., Ng, T.B., 2001. Isolation of a small chitinase-like antifungal protein from *Panax notoginseng* (sanchi ginseng) roots. *Int. J. Biochemistry & Cell Biol.* 33, 287–292.
- Lemmon, H.R., Sham, J., Chau, L.A., Madrenas, J., 2012. High molecular weight polysaccharides are key immunomodulators in North American ginseng extracts: characterization of the ginseng genetic signature in primary human immune cells. *J. Ethnopharmacol.* 142, 1–13.
- Li, X., Zhang, H., Xu, H., 2009. Analysis of chemical components of shiitake polysaccharides and its anti-fatigue effect under vibration. *Int. J. Biol. Macromol.* 45, 377–380.
- Liu, Z.Q., 2012. Chemical insights into ginseng as a resource for natural antioxidants. *Chem. Rev.* 112, 3329–3355.
- Ma, L., Cai, D., Li, H., Tong, B., Song, L., Wang, Y., 2008. Anti-fatigue effects of salidroside in mice. *J. Med. Coll. PLA* 23, 88–93.
- Moon, J.K., Han, B.K., Kim, T.D., Jo, D.H., 2010. Distribution of chitinases and characterization of two chitinolytic enzymes from one-year-old Korean Ginseng (*Panax ginseng* C.A. Meyer). *BMB Sports* 43, 726–731.
- Moriura, T., Matsuda, H., Kubo, M., 1996. Pharmacological study on *Agkistrodon blomhoffii blomhoffii* BOIE.V. anti-fatigue effect of the 50% ethanol extract in acute weight-loaded forced swimming-treated rats. *Biol. Pharm. Bull.* 19, 62–66.
- Ng, T.B., Wang, H., 2001. Panaxagin, a new protein from Chinese ginseng possesses anti-fungal, anti-viral, translation-inhibiting and ribonuclease activities. *Life Sci.* 68, 739–749.
- Nozawa, Y., Yamada, K., Okabe, Y., Ishizaki, T., Kuroda, M., 2009. The anti-fatigue effects of the low-molecular-weight fraction of bonito extract in mice. *Biol. Pharm. Bull.* 32, 468–474.
- Pyo, M.K., Choi, S.H., Hwang, S.H., Shin, T.J., Lee, B.H., Lee, S.M., Lim, Y.H., Kim, D.H., Nah, S.Y., 2011. Novel glycolipoproteins from ginseng. *J. Ginseng Res.* 35, 92–103.

- Saito, H., Yoshida, Y., Takagi, K., 1974. Effect of *Panax ginseng* root on exhaustive exercise in mice. *Jpn J. Pharmacol.* 24, 119–127.
- Shimizu, T., Hoshino, H., Nishi, S., Nozaki, S., Watanabe, Y., 2010. Anti-fatigue effect of dicethiamine hydrochloride is likely associated with excellent absorbability and high transformability in tissues as a vitamin B(1). *Eur. J. Pharmacol.* 635, 117–123.
- Stadtman, E., Levine, R., 2003. Free radical-mediated oxidation of free amino acids and amino acid residues in proteins. *Amino Acids* 25, 207–218.
- Tan, W., Yu, K.Q., Liu, Y.Y., Ouyang, M.Z., Yan, M.H., Luo, R., Zhao, X.S., 2012. Anti-fatigue activity of polysaccharides extract from *Radix rehmanniae preparata*. *Int. J. Biol. Macromol.* 50, 59–62.
- Tang, W., Zhang, Y., Gao, J., Ding, X., Gao, S., 2008. The anti-fatigue effect of 20(R)-ginsenoside Rg3 in mice by intranasally administration. *Biol. Pharm. Bull.* 31, 2024–2027.
- Trammell, R.A., Cox, L., Pikora, J., Murphy, L.L., Toth, L.A., 2012. Evaluation of an extract of North American ginseng (*Panax quinquefolius* L.) in *Candida albicans*-infected complement-deficient mice. *J. Ethnopharmacol.* 139, 414–421.
- Uthayathas, S., Karuppagounder, S.S., Tamer, S.I., Parameshwaran, K., Degim, T., Suppiramaniam, V., Dhanasekaran, M., 2007. Evaluation of neuroprotective and anti-fatigue effects of sildenafil. *Life Sci.* 81, 988–992.
- Wang, H.X., Ng, T.B., 2000. Quinqueginsin, a novel protein with anti-human immunodeficiency virus, antifungal, ribonuclease and cell-free translation-inhibitory activities from American ginseng roots. *Biochem. Biophys. Res. Commun.* 269, 203–208.
- Wang, L., Zhang, H.L., Lu, R., Zhou, Y.J., Ma, R., Lv, J.Q., Li, X.L., Chen, L.J., Yao, Z., 2008. The decapeptide CMS001 enhances swimming endurance in mice. *Peptides* 29, 1176–1182.
- Wang, J., Li, S., Fan, Y., Chen, Y., Liu, D., Cheng, H., Gao, X., Zhou, Y., 2010. Anti-fatigue activity of the water-soluble polysaccharides isolated from *Panax ginseng* C. A. Meyer. *J. Ethnopharmacol.* 130, 421–423.
- Yoo, K.M., Lee, C., Lo, Y.M., Moon, B., 2012. The hypoglycemic effects of American red ginseng (*Panax quinquefolius* L.) on a diabetic mouse model. *J. Food Sci.* 77, H147–H152.
- Yoon, J.Y., Ha, B.H., Woo, J.S., Lim, Y.H., Kim, K.H., 2002. Purification and characterization of a 28-kDa major protein from ginseng root. *Comp. Biochem. Physiol. Part B* 132, 551–557.
- You, L., Ren, J., Yang, B., Regenstein, J., Zhao, M., 2012. The anti-fatigue activities of loach protein hydrolysates with different antioxidant activities. *J. Agric. Food Chem.* 08, 1–40.