Herbkines Increases Physical Stamina in Mice

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Herbkines has been used for the purpose of development of physical strength. In the present study, we investigated the effect of Herbkines on performance of the forced swimming test (FST) and on blood biochemical parameters related to fatigue: blood urea nitrogen (BUN), creatine kinase (CK), lactic dehydrogenase (LDH), glucose (Glc), and total protein (TP). Herbkines were orally administered to mice, 10 ml/kg, continuously once per day for 2 weeks using a feeding atraumatic needle. After 2 d, on FST, the immobility time was decreased in the Herbkines-fed group (178±8.2 s) in comparison with the control group (189±22 s); however, the statistical difference was very weak (p=0.596). After 2 weeks, the immobility time was significantly decreased in the Herbkines-fed group (196±4.5 s) in comparison with the control group (221±6.2 s). In addition, the content of BUN in the blood serum was significantly decreased. However, the levels of CK, LDH, Glc, and TP did not show a significant change. The results predict a potential benefit of Herbkines as an anti-fatigue treatment and for improving physical stamina.

Key words Herbkines; forced swimming test; blood biochemical parameters related to fatigue; physical stamina

Herbkines formula is a newly modified prescription for the purpose of development of physical strength, especially for those who have suffered from wasting diseases, like cancer, in South Korea. Herbkines is composed of eight Oriental medicinal herbs. The name of Herbkines originated from herbs and cytokines because Herbkines increases the production of cytokines such as interleukin-2 (IL-2), IL-4, IL-12 and tumor necrosis factor (TNF)-α from MOLT-4 cells or mouse peritoneal macrophages.

Forced swimming test (FST) is a behavioral test for rodents, which predicts the efficacy of antidepressant treatments.1–8) This test induces the development of immobility as a reflection of helplessness when subjected to an inescapable situation (tank of deep water). In this paradigm, mice are placed in the tank for an extended period. After an initial swimming period, the animal exhibits immobility behavior considered a depression-like response. FST is used to examine whether certain agents have an anti-fatigue effect9–12) and is also used as an endurance test.13,14)

Blood urea nitrogen (BUN), creatine kinase (CK), lactic dehydrogenase (LDH), glucose (Glc) and total protein (TP) are blood biochemical parameters related to fatigue. The BUN test is a routine test used primarily to evaluate renal function. Urea is formed in the liver as the end product of protein-metabolism. During digestion, protein is broken down into amino acids. Amino acids contain nitrogen, which is removed as NH4 + (an ammonium ion), while the rest of the molecule is used to produce energy or other substances needed by the cell. Serum CK and LDH are known to be accurate indicators of muscle damage.15,16) The normal function of CK in cells is to add a phosphate group to creatine, turning it into the high-energy molecule phosphocreatine. Phosphocreatine is burned as a quick source of energy by cells. However, the normal function of CK isn’t as relevant, in this case, as what happens to CK when muscle is damaged. During the process of muscle degeneration, muscle cells break open and their contents find their way into the bloodstream. Because most of the CK in the body normally exists in muscle, an increase in the amount of CK in the blood indicates that muscle damage has occurred, or is occurring. LDH catalyzes the interconversion of pyruvate and lactate. Exercising muscles convert Glc to lactate. Lactate is released into the blood and is eventually taken up by the liver. The liver converts lactate back to Glc and releases Glc into the blood. This Glc is then taken up by resting muscles, red blood cells, and other tissues. Energy for exercise is derived initially from the breakdown of glycogen, and later from circulating Glc released by the liver and from non-esterified fatty acids.17) As is commonly known, the Glc level is decreased immediately after exercise. TP is a rough measure of serum protein. Protein measurements can reflect nutritional state, kidney disease, liver disease, and many other conditions. Enzymes, some hormones, hemoglobin, LDL, fibrinogen, and immunoglobulins are some examples of proteins.

The aim of the present study is to examine the effect of Herbkines on FST. In addition, the contents of BUN, CK, LDH, Glc and TP in the blood serum were measured.

MATERIALS AND METHODS

Animals Male ICR mice weighing 10–12 g (Damul Science Center, Daejeon, South Korea) were used in these experiments. They were group housed under the following laboratory conditions: temperature 23±1 °C, humidity 40–60%, 12:12-L/D cycle, lights on at 07:00 h. Food and water were available ad libitum. All the experiments were carried out between 10:00 and 15:00 h in testing rooms adjacent to the animal rooms. Each experimental group consisted of five mice. Mice were treated in accordance with the current law and the NIH Guide for the Care and Use of Laboratory Animals.

Preparation of Herbkines The ingredients of 125 g Herbkines include 5 g of Glycyrrhiza uralensis Fisch (radix), 20 g of Atractylodes macrocephala Koidz (rhizoma), 20 g of Dioscorea batatas Decaisne (rhizoma), 20 g of Cinnamomum cassia Presl. (cortex), 10 g of Rubus coreanus Miq (fructus), 20 g of Liriope platyphylla Wang et Tang (radix), 20 g of Astragalus membranaceus Bunge (radix), and 10 g of Cornus

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officinalis S. et Z. (fructus). Herbkins, the dried prescription of herbs, was decocted with distilled water, then the extract was fermented (alcohol fermentation). The fermented extract was filtered and kept at 4 °C. The extract or saline was orally administered into mice, 10 ml/kg, once per day for 2 weeks using a feeding atraumatic needle.

**Forced Swimming Test** During the 6 min of the forced swimming test, the duration of immobility was measured as previously described by Porsolt, Bertin, and Jalfre (1977).1) The apparatus consisted of two Plexiglas cylinders (height: 25 cm, diameter: 10 cm) placed side by side in a Makrolon cage filled with water (10 cm height) at 23—25 °C. Two mice were tested simultaneously for a 6 min period inside vertical Plexiglas cylinders; a nontransparent screen placed between the two cylinders prevented the mice from seeing each other. The total duration of immobility, after a delay of 2 min, was measured during a period of 4 min. Each mouse was considered to be immobile when it ceased struggling and remained floating motionless in the water, making only those movements necessary to keep its head above water.

**Preparation and Ingredient Analysis of Blood Serum**
Mice were anesthetized with an intraperitoneal injection of ketamine (80 mg/kg) and xylazine (4 mg/kg). After anesthesia, blood was withdrawn from the heart of forced swimming-treated mice into syringes. Then, serum was prepared by centrifugation at 3000 rpm at 4 °C for 10 min. Contents of BUN, CK, LDH, Glc and TP were determined by an autoanalyzer (Hitachi 747, Hitachi, Japan).

**Statistical Analysis** Results were expressed as the means±S.E. The significance of the mean difference was determined by Student’s t-test for independent data. All statistical analyses were performed using SPSS v10.0 statistical analysis software. A value of p<0.05 was considered to indicate statistical significance.

**RESULTS AND DISCUSSION**

**Effect of Herbkins on Immobility** Young ICR mice weighing 10—12 g were used in this study. According to some other experiments requiring a long experimental period, like 1 month, young mice weighing 10—12 g were usually used.18) We selected young mice since this study took about 3 weeks. First, we investigated the effect of Herbkins on FST. Mice, when placed into the cylinders for the first time, swim around vigorously, apparently searching for an exit. After 2 to 3 min their activity subsided, replaced by periods of immobility of increasing duration where the mice passively remain floating in the water in a semi-horizontal position with their heads just above the water. After the first measurement of immobility time, the mice were divided into a control group and a Herbkins group to match the swimming time in each group. The second measurement of immobility was performed 2 d after saline or Herbkins treatment. As a result of test, the immobility time was decreased in the Herbkins (10 ml/kg/d, p.o.)-treated group (178±8.2 s) in comparison with the saline (10 ml/kg/d, p.o.)-treated group (189±22 s); however, the statistical difference was very weak (p=0.596). (Fig. 1A). Figure 1B showed the effect of the Herbkins-treated group for 2 weeks. The immobility time was significantly decreased in the Herbkins-treated group (196±4.5 s) in comparison with the saline-treated group (221±6.2 s).

**FST** is generally used for assessing antidepressive effects. Recently, the FST has also been used as an anti-fatigue test and endurance test. As shown in Fig. 1, the duration of immobility on the 2nd and 14th days was shortened by feeding of Herbkins. These phenomena suggest that the decreased duration of immobility in mice may be caused by a change of certain metabolites in the system.

**Effect of Herbkins on Blood Biochemical Parameters**
In order to clarify its mechanisms, we assessed the levels of several blood biochemical parameters in mice after FST. Blood was withdrawn from the hearts of forced swimming-treated mice into syringes and then blood serum was prepared by centrifugation. Contents of BUN, CK, LDH, Glc and TP in the serum. Each level was determined by the autoanalyzer. Each data value indicates the mean±S.E. *p<0.05 versus saline-treated group.

| Table 1. Effect of Herbkins on Blood Biochemical Parameters in Mice19) |
|-----------------|-----------------|---------------|
| BUN (mg/dl)     | Herbkines (10 ml/kg/d) | Statistics |
| Saline (10 ml/kg/d) | F=0.50, p=0.03, df=9 | |
| 21.9±1.01       | 18.9±0.70       |               |
| 0.3±0.02        | 0.3±0.02        |               |
| 985.6±159       | 899.7±189       |               |
| 213±12.7        | 233±14.0        |               |
| 4.5±0.09        | 4.8±0.10        |               |

*a) Herbkins (10 ml/kg/d, p.o. for 2 weeks) was orally administered to mice, then we measured the levels of BUN, CK, LDH, Glc and TP in the serum. Each level was determined by the autoanalyzer. Each data value indicates the mean±S.E. *p<0.05 versus saline-treated group. F, Levene's test for equality of variances; p, p value; and df, degree of freedom.
blood biochemical changes. The BUN, CK, LDH, Glc and TP are blood biochemical parameters related to fatigue. Therefore, our results indicate that the fatigue metabolism of mice was influenced by Herbkines treatment. However, further studies are needed to clarify the detailed mechanisms involved in the anti-fatigue-like properties of Herbkines in order to support present findings.

In conclusion, the Herbkines-fed group decreased their immobility time during FST, and the metabolites related to fatigue were changed. Therefore, the present results suggest that Herbkines may be useful for the development of physical strength. In addition, it might be suggested that Herbkines has an antidepressive-like effect. However, this suggestion, based on our preliminary results, should be confirmed by further experimental studies.

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REFERENCES