Evaluation of the Antidepressant Effect of the Functional Beverage Containing Active Peptides, Menthol and Eleutherosides, and Investigation of Its Mechanism of Action in Mice

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SUMMARY

Research background. Depression has become a global threat to human health. In order to solve it, researchers have conducted multi-faceted studies including diet. Many food-derived bioactive substances have shown antidepressant effects. However, there are few studies on the design of industrialized food with antidepressant effect. This study aimed to evaluate the antidepressant effect of a functional beverage made from several ingredients with potential antidepressant function and investigate its antidepressant mechanisms.

Experimental approach. The beverage consists of peppermint oil, active peptides derived from bovine milk casein and Acanthopanax senticosus extract (ASE) whose active ingredient is eleutheroside. Different amounts of ASE were evaluated to determine the optimal concentration of eleutheroside in this functional beverage to deliver best antidepressant effect through extensive behavioral testing including preliminary acute stress experiments and further chronic unpredictable...
mild stress test.

**Results and conclusions.** The results demonstrated that the beverage with 15.00 mg/kg of eleutheroside could significantly reduce the mice’s immobility time of tail suspension test and forced swimming test, recover mice’s sucrose preference and behavior changes in the open-field test, improve the contents of dopamine, norepinephrine, 5-hydroxytryptamine and the activity of superoxide dismutase and reduce the content of malondialdehyde in mice’s brains, which indicated that the improvement of monoamine neurotransmitter systems and antioxidation was one potential mechanism of antidepressant action.

**Novelty and scientific contribution.** This study provides a design of antidepressant functional beverage and an efficient way for the prevention and treatment of depression.

**Key words:** functional beverage, eleutheroside, behavioral testing, antidepressant mechanism, monoamine, antioxidation

**INTRODUCTION**

Major depressive disorder (MDD) is a serious mood disorder, which can be caused by a combination of biological, psychological, and social distress. Patients suffering from depression often have unstable emotions, with long-lasting symptoms that usually deprive the patient’s capabilities for work and logical communication (1). Depression could even lead to suicide. According to the World Health Organization (WHO), 850 000 people suicide every year due to depression (2). The WHO had predicted that depression would be one of the two top causes of global health and disability (3).

In addition to common nutrients, foods are also sources of bioactive substances that have a potential positive impact on human health (4). Diet may provide considerable benefits for moderate to severe depression and anxiety (5). According to reports, some Chinese herbal medicines and fruits also have certain antidepressant effects (6-8). Mint, the dried aerial part of the Lamiaceae plant *Mentha haplocalyx* Briq., is cool-natured, acrid flavour, and it can disperse stagnated liver in the theory of Traditional Chinese Medicine (9). L-menthol (LM) is the main active ingredient of the mint, and its...
content is up to 87% in the essential oil of mint (10). LM was able to induce an antidepressant-like effect in a mouse model of depressive behavior, and this effect might be partially mediated by dopaminergic (DAergic), 5-hydroxytryptaminergic and gamma-aminobutyric acidergic (9). Acanthopanax senticosus HARMS (ASH) root bark is also traditionally used to treat high blood pressure and mental disorders in China (11,12). ASH has an anxiolytic effect against not only mild anxiety, but also anxiety due to higher levels of stress, which is related to an increase in hippocampal brain-derived neurotrophic factor signaling (13). Bioactive peptides are small protein fragments derived from enzymatic hydrolysis and gastrointestinal digestion of food proteins, which are beneficial to living beings (14). Among the many protein foods, milk is a major research object (15). Kim et al. (16) have shown that the ingestion of alpha(S1)-casein hydrolysate can decrease the stress-related symptoms in females, particularly in intellectual and emotional problems. Guesdon et al. (17) demonstrated that in mice the tryptic bovine alpha(S1)-casein hydrolysate has protective effect on sleep during exposure to chronic mild stress conditions. In recent years, the demand for foods with additional functional benefits has been increasing (18). However, the design of industrialized food with antidepressant effect has been rarely explored.

In the present study, a beverage (referred as functional beverage below) was designed, which consisted of peppermint oil, active peptides derived from bovine milk casein and Acanthopanax senticosus extract (ASE) whose active ingredient was eleutheroside. In order to fully confirm antidepressant effect of the functional beverage in mice and determine the optimal concentration of eleutheroside in the functional beverage to deliver best antidepressant effect, we conducted extensive behavioral testing including preliminary acute stress experiments and further chronic unpredictable mild stress (CUMS) test. The potential antidepressant mechanisms were also investigated.

MATERIALS AND METHODS

Preparation of functional beverage

We chose bovine milk casein hydrolysates, peppermint oil (Jinxing Spice, Dongtai, Jiangsu, China) and ASE (Hongda Plant Chemical, Xi’an, Shaanxi, China) as the ingredients of functional beverage
and their concentration settings referred to some reports and a Chinese national food standard (19-21). The functional beverage was made of peppermint oil (0.30 g/kg), bovine milk casein hydrolysates (690.00 g/kg) and ASE (0.25-2.00 g/kg), which contained 0.10 g/kg menthol, 25.05 g/kg active peptides, 3.75-30.00 mg/kg eleutheroside which consist of equal amount of eleutheroside B and eleutheroside E.

The preparation and determination of bovine milk casein hydrolysates referred to our previous study with same enzymes and reagents (22). The protein content of bovine milk casein hydrolysates was determined to be 4.06 % (by mass) by Kjeldahl method (23). The trichloroacetic acid (TCA) precipitation method (24) was used to measure the peptide concentration of the hydrolysates, which was 3.63 % (by mass). The hydroxyl radical-scavenging activity of bovine milk casein hydrolysates was determined to be 50.06 % by using the 2-deoxy-d-ribose oxidation method (22). The concentrations of menthol of peppermint oil and eleutheroside of ASE were respectively 33.33 % and 1.50 %, which were obtained from the guaranteed analysis provided by the manufacturer. Note that all concentration used in this study referred to the concentration of eleutheroside in the functional beverage.

**Animals**

Male Kunming mice (20-25 g) and fodder were provided by the experimental animal center of Fourth Military Medical University (Xi’an, China). The production license number of experimental animal was SCXK (Shaanxi) 2014-002. The fodder was standard mice pellet feed. The rearing environment was a SPF laboratory animal room with the license number of experimental animal SYXK (Shaanxi) 2014-001. All animals were housed under standard conditions of temperature (22±2) °C, humidity (55±4) % and light (12:12 h light/dark cycle), and free access to food and water. Clomipramine hydrochloride (CH) (Weimeng Biotech, Shanghai, China) was used in the present study. All animal use procedures were carried out in accordance with the Regulations of Experimental Animal Administration issued by the State Committee of Science and Technology of the People’s
Republic of China (25), with the approval of the Northwest A&F University Ethical Committee. All behavioral experiments were performed once.

Preliminary acute stress experiment

Treatments in acute stress experiment

Acute stress experiment in the present study included forced swimming test (FST) and tail suspension test (TST), which are used widely to measure the pharmacological effects of antidepressant drugs or changes in stress-evoked behavior in mice (26,27). They were carried out in order to determine the preliminary concentration with a better antidepressant effect. In each test, fifty experimental mice were randomly divided into 5 groups ($N=10$). They were fed with normal saline (control group), 40 mg/kg CH (CH group), and three functional beverage samples (7.50 mg/kg, 15.00 mg/kg and 30.00 mg/kg of eleutheroside). All animals in each group were fed twice a day with intragastric administration of 0.02 mL/g body mass every time for 5 days continuously. The mice were not anesthetized before gavage. Mice that were subjected to TST and FST were sacrificed by cervical dislocation.

Tail suspension test

The TST was conducted 1 h after the last intragastric administration on day 5. Mice were suspended for 6 min by placing an adhesive tape 1 cm away from the tip of the tail. Each mouse was suspended 50 cm away from the floor and was acoustically and visually isolated from other animals during test. The immobility time of each mouse was subsequently recorded. Immobility was defined as when the mouse’s four paws and head were all immobile or passive swinging (28).

Forced swimming test

The FST was conducted 1 h after the last intragastric administration on day 5. Each mouse was placed into an 80 L polypropylene basin (height 24 cm, diameter 65 cm) filled with 50 L of water (25±1 °C) and was kept for 6 min. The mouse was forced to swim for 6 min and judged to be immobile.
when it floated in an upright position and made only small movements to keep its head above water. The duration of immobility was recorded during the last 4 min of the 6 min testing period (29). Each mouse was conducted the FST individually.

**Further chronic unpredictable mild stress test**

Treatments in chronic unpredictable mild stress test

CUMS test was carried out in order to determine the optimal concentration of eleutheroside with the best antidepressant effect. Another 60 mice were divided into 6 groups (N=10) randomly. There was normal control group, model control group, CH (40 mg/kg) group, functional beverage (3.75 mg/kg, 7.50 mg/kg and 15.00 mg/kg of eleutheroside) groups. Note that the functional beverage concentrations were adjusted in CUMS test based on the results of the preliminary acute stress experiment. The functional beverage group (30.00 mg/kg) was deleted and new dose group (3.75 mg/kg) was added. Mice in normal control group and model control group were fed with normal saline. All mice were fed once a day by oral gavage of samples at 0.02 mL/g body mass for 3 weeks. The mice were not anesthetized before gavage.

Normal control group was not stimulated. Other mice were subjected to CUMS as described by Kaye et al. (30) with some modifications. Animals were subjected to stress paradigm randomly once a day over a period of 3 weeks. The order of stressors was showed in Table 1. After three weeks of stress, an open-field test and a sucrose preference test were conducted for each mouse.

Open-field test

A 40 cm high, 80 cm long and 80 cm wide case was prepared for this experiment. The case was separated into 25 equal areas (16 cm by 16 cm) by drawing black lines. Each mouse was placed at the center of the case at the beginning and the mouse was allowed to move freely in this open-field case. Within the 3 min test, the time that the mouse stays in the center area, the number of times that the mouse moves across lines, the number of stand-up times and the number of stools were recorded (31).
Sucrose preference test

Sucrose preference test was employed herein to determine the anhedonia, which is one of the core symptoms of major depression in humans (32). After chronic stress experiment, the mouse was offered two bottles of water (one is drinking water, the other one is drinking water with 29.24 mM sucrose). This experiment began at 4:00 pm after CUMS and ended at 8:00 am of the second day. Water and sucrose intakes were calculated by weighing each bottle. Then, the sucrose preference was calculated according to the Eq. 1:

\[
\text{w(sucrose preference)} = \frac{m(\text{sucrose})}{m(\text{sucrose}) + m(\text{water})}\]

where \(m(\text{sucrose})\) is the mass of sucrose intake in mice and \(m(\text{water})\) is the mass of sucrose intake in mice.

Determination of monoamine and matter of antioxidation

Mice after CUMS were sacrificed by cervical dislocation on the ice pack and their brains were isolated and weighed. Brain homogenates were prepared manually from normal saline and mice brain tissues in a ratio of 9:1 (by mass). The supernates were stored in a -80 °C environment after centrifugation at 15 000×g and 4 °C for 10 min (H1650; Xiangyi Centrifuge Instruments, Changsha, Hunan, China). Levels of dopamine (DA), 5-hydroxytryptamine (5-HT) and norepinephrine (NE) were measured by enzyme-linked immunosorbent assay (ELISA) kits (H710, H104, H096; Jiancheng Bioengineering Institute, Nanjing, Jiangsu, China) using a microplate reader (Model 680; Bio-Rad Laboratories, Redmond, WA, USA). Superoxide dismutase (SOD) and malondialdehyde (MDA) were determined by WST-1 method and thiobarbituric acid (TBA) colorimetry by kits (A001-3, A003-1; Jiancheng Bioengineering Institute, Nanjing, Jiangsu, China) using the above-mentioned microplate reader.

Statistical analysis

All the results are expressed as mean value±standard deviation (S.D.). The data were analyzed by one-way ANOVA test and Duncan’s test at \(p<0.05\) and \(p<0.01\) using the IBM SPSS Statistics version 20.0 software (33).
RESULTS AND DISCUSSION

Effects of functional beverage on mice behavior after preliminary acute stress experiment

The emotional despair is one of the core symptoms of depression and has causal relevance to committing suicide (34). TST is a desperate model induced by inability to overcome abnormal postures and FST induces despair through inability to escape the water environment (35,36). In addition, another symptom of depression is psychomotor retardation manifested as the reduction in locomotor activity in rodents, also known as immobility (26). Because the main measures of TST and FST are the reduction of locomotor activity and they contain a desperate environment, they are commonly used as rodent depression models (27).

The effect of functional beverage on the immobility time of TST and FST was shown in Fig. 1. The immobility time in TST and FST was very significantly reduced in the dose groups of 7.50 mg/kg and 15.00 mg/kg (p<0.01) as well as in the positive control CH group compared to the control group. The dose group of 30.00 mg/kg had no significant difference (p>0.05) from the control group, which suggested that the dose group of 30 mg/kg does not have antidepressant effect. The dose group of 7.50 mg/kg and 15.00 mg/kg in TST did not show significant difference (p>0.05) from the CH group, which suggested that the dose groups of 7.50 mg/kg and 15.00 mg/kg can reach the same antidepressant effect as CH group.

These two doses (7.50 mg/kg and 15.00 mg/kg) were chosen to be the reference doses for follow-up experiments. Since the highest concentration (30.00 mg/kg) didn’t have antidepressant effect, it was necessary to explore the effect of lower concentration in order to obtain the most appropriate concentration of eleutheroside. Therefore, the dose group of 3.75 mg/kg was added in follow-up experiments.

Effects of functional beverage in mice after chronic unpredictable mild stress test

Effects on mice behavior

The chronic unpredictable stress model was proposed by Katz et al. (37). The mice suffered a series of different stress stimuli including tail-clip, cold-water swimming, and day-night reversal within 21 days, and these stimuli were randomly arranged. After the stimulation, the mice showed a series
of emotional behavioral changes, such as reducing horizontal activity and the ability of exploration (38). Lu et al. (39) demonstrated that CUMS-induced depression-like behaviors are coupled with DAergic hyperfunction in the nucleus accumbens and serotonergic hypofunction in the hippocampus and prefrontal cortex.

The results of the open-field test and the sucrose preference test were shown in Table 2. All measurements of model group were very significantly different from the control group (p<0.01), which proved the validity of this chronic mild stress model. The model control group showed significant differences from the CH group, the dose groups of 7.50 mg/kg and 15.00 mg/kg in sucrose preference, the number of the lattice moved, the stand-up times, immobility time, and the number of stool grains (p<0.05), except the dose group of 3.75 mg/kg in the stand-up times.

Contents of dopamine, norepinephrine and 5-hydroxytryptamine

After accidental finding that monoamine oxidase can inhibit iproniazid, monoamine hypothesis of depression was formulated, which stated that deficiency of monoamine neurotransmitters underlies clinical depression and depressive symptoms can be alleviated by increased monoamine (40-42). Currently, levels of monoamine such as NE, 5-HT and DA are often used as indicators in antidepressant research.

The effect of functional beverage on the contents of DA, NE, and 5-HT in mice’s brains was summarized in Table 3. Compared to the normal control group, the contents of DA, NE and 5-HT in mice’s brains of the model control group decreased highly significantly (p<0.01), which denoted again that the mice model of depression was established successfully. The contents of DA, NE, 5-HT of the dose groups of 7.50 mg/kg and 15.00 mg/kg significantly increased in comparison with the model group (p<0.05). The dose group of 3.75 mg/kg did not show significant difference (p>0.05) from the model control group. These results were consistent with effects on sucrose preference.

Changes of superoxide dismutase activity and malondialdehyde content

Oxidative alterations are recognized as a critical route of brain damage in the pathophysiology of stress-induced psychiatric disorders (43). In stress disorders, oxidative stress triggers or exacerbates
several routes of damage such as mitochondrial dysfunction, dysregulation of calcium homeostasis, disruption of energy pathways, damage to neuronal precursors, impairment of neurogenesis and induction of signaling events in apoptotic cell death (44). Oxidative stress is caused by an imbalance between levels of free radicals production and efficiency of the antioxidant enzyme system to neutralize and eliminate reactive oxygen species (ROS). Free-radical damage by ROS, such as the superoxide anion and hydrogen peroxide, is the primary source of oxidative stress (45). Two main antioxidant systems exist. The nonenzymatic system relies on molecules that can directly quench ROS and the enzymatic system is composed of specific enzymes that detoxify ROS. Among the latter, the SOD family is important in oxidative stress modulation (46). In addition, ROS levels are associated with lipid antioxidant defenses. The specific reduction in lipid-targeted antioxidant defenses may contribute to increased ROS levels and oxidative damage to lipid membranes (lipid peroxidation) including to polyunsaturated fatty acids. Lipid hydroperoxide chain reactions eventually cause the formation of reactive aldehydes, the end-product of lipid peroxidation, as indicated by increased levels of MDA (47).

Therefore, SOD activity and MDA content in the brains of mice were measured to reflect oxidative alterations. Whether SOD or MDA, all the functional beverage groups had significantly difference compared with the model control group (p<0.05) (Table 4). The dose group of 15.00 mg/kg have the best effect in increasing SOD activity.

CONCLUSION

In conclusion, the treatment using functional beverage with 15.00 mg/kg eleutheroside had the best antidepressant effect, which significantly reduced the immobility time of mice in the TST and FST, recovered sucrose preference degree of mice. It also significantly improved the content of DA, NE, 5-HT and the activity of SOD and decreased the content of MDA in mice’s brains, which indicated that the improvement of monoamine neurotransmitter systems and antioxidantion was potential mechanisms of antidepressant action. The results implied that the functional beverage made of
eleutheroside, active peptides and menthol may be consumed by human to achieve antidepressant effect.

FUNDING

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CONFLICT OF INTEREST

There are no conflicts of interest.

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Table 1. The arrangement of random stress for animals

<table>
<thead>
<tr>
<th>Weeks</th>
<th>Day-1</th>
<th>Day-2</th>
<th>Day-3</th>
<th>Day-4</th>
<th>Day-5</th>
<th>Day-6</th>
<th>Day-7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Week-1</td>
<td>F</td>
<td>E</td>
<td>T</td>
<td>O</td>
<td>W</td>
<td>S</td>
<td>C</td>
</tr>
<tr>
<td>Week-2</td>
<td>W</td>
<td>O</td>
<td>C</td>
<td>S</td>
<td>E</td>
<td>T</td>
<td>F</td>
</tr>
<tr>
<td>Week-3</td>
<td>C</td>
<td>E</td>
<td>W</td>
<td>F</td>
<td>T</td>
<td>S</td>
<td>O</td>
</tr>
</tbody>
</table>

F=Food deprivation for 24 h, E=Exposure to empty water bottles for 24 h, T=Tail pinch (60 s), O=Overnight illumination, W=Exposure to wet caging (200 mL of water into the sawdust bedding) for 24 h, S=Cold water swimming for 5 min at 4 °C, C=Tilted cage at 45 degree for 24 h.
Table 2. Effect of the functional beverage on the open-field test and the sucrose preference test in mice

<table>
<thead>
<tr>
<th>Group</th>
<th>w/(mg/kg)</th>
<th>w(sucrose preference)/%</th>
<th>Number of lattice moved</th>
<th>Stand-up times</th>
<th>t(immobility)/s</th>
<th>Number of stool grains</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>(68.22±6.24)\textsuperscript{abA}</td>
<td>(91.00±5.23)\textsuperscript{bC}</td>
<td>(24.71±3.20)\textsuperscript{abA}</td>
<td>(13.23±2.75)\textsuperscript{cD}</td>
<td>(1.43±0.98)\textsuperscript{bC}</td>
<td></td>
</tr>
<tr>
<td>Model control</td>
<td>(42.99±4.36)\textsuperscript{cB}</td>
<td>(56.86±3.89)\textsuperscript{C}</td>
<td>(10.43±3.15)\textsuperscript{cB}</td>
<td>(34.89±8.86)\textsuperscript{aA}</td>
<td>(3.86±1.07)\textsuperscript{aB}</td>
<td></td>
</tr>
<tr>
<td>CH</td>
<td>(75.96±12.81)\textsuperscript{dA}</td>
<td>(98.80±9.74)\textsuperscript{aDA}</td>
<td>(26.71±2.75)\textsuperscript{aDA}</td>
<td>(10.80±3.57)\textsuperscript{cD}</td>
<td>(1.14±0.69)\textsuperscript{cB}</td>
<td></td>
</tr>
<tr>
<td>Functional beverage</td>
<td>3.75</td>
<td>(48.53±8.18)\textsuperscript{bB}</td>
<td>(69.71±8.56)\textsuperscript{dB}</td>
<td>(12.57±2.76)\textsuperscript{cB}</td>
<td>(22.94±4.80)\textsuperscript{bB}</td>
<td>(2.71±0.95)\textsuperscript{bB}</td>
</tr>
<tr>
<td></td>
<td>7.50</td>
<td>(66.87±8.90)\textsuperscript{abA}</td>
<td>(88.00±7.16)\textsuperscript{cA}</td>
<td>(21.86±4.45)\textsuperscript{bA}</td>
<td>(13.29±4.50)\textsuperscript{cD}</td>
<td>(1.29±1.60)\textsuperscript{cB}</td>
</tr>
<tr>
<td></td>
<td>15.00</td>
<td>(71.91±14.24)\textsuperscript{abA}</td>
<td>(100.43±7.89)\textsuperscript{aA}</td>
<td>(26.14±6.52)\textsuperscript{abA}</td>
<td>(10.99±3.35)\textsuperscript{cD}</td>
<td>(1.14±0.90)\textsuperscript{cB}</td>
</tr>
</tbody>
</table>

Data were expressed as mean value±S.D. (N=10).

CH=clomipramine hydrochloride.

Different superscripted lowercase letters in the same column denote significant differences according to Duncan’s test (p<0.05).

Different superscripted capital letters in the same column denote highly significant differences according to Duncan’s test (p<0.01).

In this table, w/(mg/kg) meant mg(eleutheroside)/kg(functional beverage).
Table 3. Effect of the functional beverage on the content of dopamine (DA), norepinephrine (NE) and 5-hydroxytryptamine (5-HT) in brain of mice

<table>
<thead>
<tr>
<th>Groups</th>
<th>w/(mg/kg)</th>
<th>γ(DA)/(pg/mL)</th>
<th>γ(NE)/(pg/mL)</th>
<th>γ(5-HT)/(pg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>(22.33±2.20)^abAB</td>
<td>(70.52±8.51)^bcdBC</td>
<td>(95.64±15.66)^aA</td>
<td></td>
</tr>
<tr>
<td>Model control</td>
<td>(16.67±2.12)^dC</td>
<td>(56.02±5.09)^eD</td>
<td>(71.37±2.85)^cC</td>
<td></td>
</tr>
<tr>
<td>CH</td>
<td>(23.06±2.77)^AB</td>
<td>(86.13±9.63)^JA</td>
<td>(97.95±6.31)^aA</td>
<td></td>
</tr>
<tr>
<td>Functional</td>
<td>3.75</td>
<td>(18.59±1.79)^dBC</td>
<td>(62.19±9.19)^dC</td>
<td>(80.16±9.00)^bC</td>
</tr>
<tr>
<td>beverage</td>
<td>7.50</td>
<td>(21.47±2.68)^abcABC</td>
<td>(73.31±8.77)^bcABC</td>
<td>(88.38±11.39)^abAB</td>
</tr>
<tr>
<td>Functional</td>
<td>15.00</td>
<td>(23.75±3.88)^JA</td>
<td>(79.21±11.05)^abAB</td>
<td>(98.96±10.33)^aA</td>
</tr>
</tbody>
</table>

Data were expressed as mean value±S.D. (N=10).

CH=clomipramine hydrochloride.

Different superscripted lowercase letters in the same column denote significant differences according to Duncan’s test (p<0.05).

Different superscripted capital letters in the same column denote highly significant differences according to Duncan’s test (p<0.01).

In this table, w/(mg/kg) meant mg(eleutheroside)/kg(functional beverage).
Table 4. Effect of active peptides beverage to the content of superoxide dismutase (SOD) and malondialdehyde (MDA) in brain of mice

<table>
<thead>
<tr>
<th>Groups</th>
<th>w/(mg/kg)</th>
<th>γ(SOD)/(U/mg prot)</th>
<th>γ(MDA)/(mmol/g prot)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td></td>
<td>(402.60±73.18)$^A$</td>
<td>(66.54±6.15)$^BC$</td>
</tr>
<tr>
<td>Model control</td>
<td></td>
<td>(203.07±12.73)$^BC$</td>
<td>(157.73±18.01)$^A$</td>
</tr>
<tr>
<td>CH</td>
<td></td>
<td>(402.53±47.24)$^A$</td>
<td>(94.44±11.84)$^B$</td>
</tr>
<tr>
<td>3.75</td>
<td></td>
<td>(277.20±51.10)$^{BC}$</td>
<td>(89.66±17.08)$^{BC}$</td>
</tr>
<tr>
<td>Functional beverage</td>
<td>7.50</td>
<td>(313.14±66.33)$^B$</td>
<td>(77.75±10.60)$^{DBC}$</td>
</tr>
<tr>
<td></td>
<td>15.00</td>
<td>(417.08±58.74)$^A$</td>
<td>(86.52±10.49)$^{BC}$</td>
</tr>
</tbody>
</table>

Data were expressed as mean value±S.D. (N=10).

CH=clomipramine hydrochloride.

Different superscripted lowercase letters in the same column denote significant differences according to Duncan’s test (p<0.05).

Different superscripted capital letters in the same column denote highly significant differences according to Duncan’s test (p<0.01).

In this table, w/(mg/kg) meant mg(eleutheroside)/kg(functional beverage).
Fig. 1. Effect of functional beverage on the immobility time of tail suspension test (TST) and forced swimming test (FST) in mice

Data were expressed as mean value±S.D. (N=10).

ch=clomipramine hydrochloride.

Different superscripted lowercase letters in the same half region (TST or FST) denote significant differences according to Duncan’s test (p<0.05).

Different superscripted capital letters in the same half region (TST or FST) denote highly significant differences according to Duncan’s test (p<0.01).