In vivo Anti-fatigue activity of total flavonoids from sweetpotato [Ipomoea batatas (L.) Lam.] leaf in mice

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Received 05 January 2013; revised 24 May 2013

The in vivo anti-fatigue activity of the total flavonoids from sweet potato [Ipomoea batatas (L.) Lam.] leaf was investigated in male Kunming mice. The total flavonoids from sweet potato leaf (TFSL) were orally administered at doses of 50, 100 and 200 mg/kg for 4 weeks and the anti-fatigue effect was studied using a weight-loaded swimming test, along with the determination of serum urea nitrogen (SUN), blood lactic acid (BLA) and hepatic and muscle glycogen contents. The results showed that TFSL had significant anti-fatigue effects. TFSL extended the exhaustive swimming time, effectively inhibited the increase of BLA, decreased the level of SUN and increased the hepatic and muscle glycogen content of mice. Thus, TFSL may have potential as an anti-fatigue agent.

Keywords: Flavonoids; Sweet potato; Anti-fatigue activity, Male Kunming mice, Ipomoea batatas (L.) Lam.

The plant sweet potato [Ipomoea batatas (L.) Lam.] ranks as the seventh most important staple crop in the world and the fifth in developing countries1. Its tubers are extensively utilized both as food and for the production of beverages, pasta, alcohol drink and natural colorants2,3. The sweet potato leaves are consumed as a leafy vegetable in many parts of the world and can be harvested many times during a season and are considered to be a rich source of flavonoids4,5. Flavonoids are the polyphenolic phytochemicals with inconsistent phenolic structures; they consist of flavones, flavanone, flavanols, flavonols and flavanonols that comprise a large group of secondary metabolites in plants6. The flavonoids have aroused considerable interest in recent years because of their beneficial effects on human health. They have been reported to have antiviral, anti-allergic, anti-platelet, anti-inflammatory, antitumor and antioxidant activities7,8.

Fatigue is a complex phenomenon that can be described as a time-dependent exercise-induced reduction in the maximal force generating capacity of a muscle9. Alteration in performance tends to vary across sports that are influenced more or less by factors like decreased muscular power, endurance, motor skill performance and mental lapses10. Since the available therapies for fatigue in modern medicine are very limited, potential alternatives from traditional medicine and their respective mechanisms of action are worth investigating11,12.

The present study has been undertaken to evaluate the anti-fatigue activity of total flavonoids from sweet potato leaf (TFSF) using a forced swimming test in male Kunming mice, along with the determination of serum urea nitrogen (SUN), blood lactic acid (BLA) and hepatic and muscle glycogen contents.

Materials and Methods

Chemicals

Rutin was purchased from Sigma-Aldrich Co. Ltd (St Louis, MO, USA). All other chemicals used were of analytical grade and obtained from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). Reagent kits for determination of serum urea nitrogen (SUN), blood lactic acid (BLA) and hepatic and muscle glycogen content were purchased from Jiancheng Biotechnology Co. (Nanjing, China).

Extraction of total flavonoids from sweet potato leaf (TFSF)

The leaves of sweet potato were collected in October 2010 from Shandong province district. The leaves were air-dried and ground to fine powder prior to experiments. Approximately 10 g of leaf powder was macerated in 200 ml of 70% (v/v) ethanol for 6 h at room temperature and homogenized at 30 MPa by a valve homogenizer. A deep brown extract was obtained which was filtered by using the filter. The extract was evaporated by using a rotary evaporator under reduced pressure at 40°C to obtain the flavonoids. The TFSF were stored at 4°C until used.

Determination of total flavonoids

Total flavonoids were measured by the previously reported method13,14. Briefly, the dry TFSL powder was...
dissolved in distilled water and 0.5 ml of TFSL solution was transferred to 10 ml colorimetric tube. Then 0.5 ml of 5% (w/v) NaNO₂, 0.5 ml of 10% (w/v) Al(NO₃)₃ and 4 ml of 4% (w/v) NaOH were added in the order stated. The final volume was adjusted to 10 ml with 95% (v/v) ethanol. The mixture was allowed to stand for 15 min at room temperature and the absorbance was measured at 510 nm against a blank solution. The amount of total flavonoids was expressed as rutin equivalents (µg rutin/g sample) through the calibration curve of rutin. A calibration curve was constructed with different concentrations of rutin (10-50 µg/ml) as the standard. All determinations were performed in triplicate.

Animals

Male Kunming mice (weighting between 18-20 g) were procured from Libo Laboratory Animal Breeding Center (Jinan, China). They were given water ad libitum and fed with standard mouse pellets food for one week before drug administration in the following environment: under 21-25°C, with (50 ± 5)% relative humidity and ventilation at 15 air renewal cycles every hour and a 12 h-light and 12-dark cycle. 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.

Experimental design

Mice were randomly divided into four groups with 20 mice in each group. TFSL was given to the mice at doses of 0, 50, 100 and 200 mg/kg and the four groups were named accordingly as Groups I, II, III and IV. Samples were administered by gastric intubation using a feeding atraumatic needle once per day for 4 consecutive weeks. The TFSL solutions used in treatment groups were prepared by dissolving TFSL in distilled water.

Changes in the body weight of mice during the test, the average swimming time, as well as the SUN, BLA, hepatic and muscle glycogen contents were measured in the experiment.

Weight-loaded swimming test

The weight-loaded swimming test was performed as described previously with some modifications. Briefly, 30 min after the last oral administration of ‘TFSL solution, the mice were dropped individually into an acrylic plastic pool (50 × 50 × 40 cm, containing 40 cm of water maintained at 25 ± 1°C). A tin wire (5% of body) was loaded on the tail root of each mouse. The swimming period was regarded as the time spent by the mouse floating in the water with struggling and making necessary movements until exhausting its strength. The mice were considered to be exhausted, when they failed to rise to the surface of water to breathe within a 10 s period. The longest swimming time was recorded. At the end of session, the mice were removed from the water, dried with a paper towel and placed back in their home cages.

Determination of hepatic glycogen, SUN and BLA

Mice were forced to swim for 90 min without a load. After resting for 30 min, the mice were anesthetized with pentobarbital sodium. The blood samples were collected from eye sockets and then the mice were sacrificed and the liver and gastrocnemius muscle were immediately dissected, frozen in liquid nitrogen and kept at −80°C until analysis of glycogen concentration was performed. Levels of SUN, BLA and hepatic and muscle glycogen were determined according to the recommended procedures provided by the commercial diagnostic kit.

Statistical analysis

The results were expressed as mean ± SD. Differences among means were determined by analysis of variance (ANOVA) with Tukey’s post-hoc test, which were analyzed with SPSS.

Results and Discussion

Effect of TFSL on body weight in mice

Changes of body weight during the experimental period are shown in Table 1. The body weights of mice increased during the experimental period. There was no significant difference in the body weights of mice in the three TFSL treatment groups, compared with the control group during the experiment (P > 0.05). So, the TFSL had no significant effect on body weight.

Effect of TFSL on the weight-loaded swimming time of mice

The forced swimming test is one of the most commonly used animal models of behavioral despair that has been used extensively for the evaluation of
anti-fatigue properties of novel compounds\textsuperscript{16,17}. To standardize the workload and reduce the swimming time, weights at specific body weight percentages are added to the chest or tail of the animal\textsuperscript{18}. The length of the swimming time to exhaustion indicates the degree of fatigue. The improvement of exercise endurance is the most powerful representation of anti-fatigue effect. As shown in Fig. 1, the TFSL treatment groups (Groups II, III and IV) had a significant increase in swimming time to exhaustion, compared with the control group. This indicated that TFSL had anti-fatigue activity and could increase the exercise tolerance.

Effects of TFSL on BLA of mice

BLA is the glycolysis product of carbohydrate under anaerobic conditions and glycolysis is the main energy source for intense exercise in a short time. The accumulation of BLA is a reason for fatigue during physical exercise and rapid removal of BLA is beneficial to relieving fatigue\textsuperscript{19,20}. Therefore, BLA is one of the important indicators for judging the degree of fatigue. As shown in Fig. 2, after swimming, the BLA level of the Groups II, III and IV decreased by 5.9%, 22.6% and 40.0%, respectively. Thus, these results suggested that different doses of TFSL administration could inhibit the increase of BLA of mice after swimming, indicating that TFSL could postpone the appearance of fatigue.

Effects of TFSL on SUN

SUN, a product of energy metabolism, is another sensitive index of fatigue status\textsuperscript{21}. 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 NH\textsubscript{4}\textsuperscript{+} ion, while the rest of the molecule is used to produce energy or other substances needed by the cell. There is a positive correlation between the urea nitrogen \textit{in vivo} and the exercise tolerance\textsuperscript{22}. As can be seen from Fig. 3, the SUN of Groups II, III and IV was lower than that of control group after swimming. The data indicated that the TFSL possessed the ability to lower or retard the formation of SUN after exercise.

Effects of TFSL on hepatic and muscle glycogen of mice

Enhancement of exercise capacity could be accounted for by a reduced rate of hepatic and muscle glycogen breakdown and by a greater potential for fatty acid metabolism\textsuperscript{23}. Liver glycogen depletion might be an important factor in the development of fatigue, since there is an inability to maintain blood glucose level when the live glycogen is depleted during exercise and the ensuing hypoglycemia could result in impaired nervous function\textsuperscript{24}. Also, the importance of muscle glycogen

Fig. 1—Effect of TFSL on the weight-loaded swimming time in male Kunming mice. [Results are mean ± S.D. of three parallel measurements. *P < 0.05 compared with control group. **P < 0.01 compared with control group]

Fig. 2—Effect of TFSL on blood lactate of mice [Results are mean ± S.D. of three parallel measurements. *P < 0.05 compared with control group. **P < 0.01 compared with control group]

Fig. 3—Effect of TFSL on serum urea nitrogen of mice [Results are mean ± S.D. of three parallel measurements. *P < 0.05 compared with control group. **P < 0.01 compared with control group]
levels in endurance exercise has been demonstrated and it is suggested that there is a depletion of muscle glycogen in fatigue and exhaustion\(^25\). As shown in Table 2, after swimming, the hepatic and muscle glycogen content of the TFSL treatment groups was higher than that of the control group. These data indicated that treatment with TFSL could significantly increase the level of liver and muscle glycogen of mice after swimming.

In conclusion, the total flavonoids extracted from sweet potato leaf could extend the swimming time to exhaustion of the mice, increase the hepatic and muscle glycogen content and decrease the blood lactate acid and serum urea nitrogen contents. These results indicated that TFSL exhibited significant anti-fatigue activity and thus may have a potential as anti-fatigue agent.

**Acknowledgements**

We are grateful to the Federation of Social Science Project of Dezhou city (No. 10YD054) for financial support.

**References**


**Table 2—Effect of TFSL on hepatic and muscle glycogen of mice**

[Values expressed as mean ± S.D (n = 10)]

<table>
<thead>
<tr>
<th>Groups</th>
<th>Glycogen (mg/g)</th>
<th>Hepatic</th>
<th>Muscle</th>
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<tbody>
<tr>
<td>I</td>
<td>8.27 ± 2.01</td>
<td>1.42 ± 1.30</td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>11.83 ± 2.39*</td>
<td>2.02 ± 1.28*</td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>17.35 ± 2.28**</td>
<td>2.59 ± 1.42*</td>
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</tr>
<tr>
<td>IV</td>
<td>20.34 ± 2.17**</td>
<td>3.11 ± 1.38**</td>
<td></td>
</tr>
</tbody>
</table>

*P < 0.05, compared with control group. **P < 0.01, compared with control group.