Effects of *Lycium chinense* Miller Fruit and its Constituent Betaine on Immunomodulation in Balb/c Mice

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**Abstract**

**BACKGROUND:** The aim of this study is to investigate the effects of *Lycium chinense* Miller fruit and its representative constituent betaine on reduction of immobility time and blood parameters in balb/c mice.

**METHODS AND RESULTS:** We investigated the immobility time and the changes in aspects of blood biochemical parameters by the administration of *L. chinense* Miller fruit and its representative constituent betaine, after the forced swimming test. The immobility time was significantly reduced about 41.3% and 53.6%, respectively, in the animal of *L. chinense* Miller fruit and its representative constituent betaine-administrated group for 7 days, in comparison with that of the control group. The level of blood urea nitrogen was significantly decreased in *L. chinense* Miller fruit and its representative constituent betaine-treated group compared with the control group (*P* < 0.05), respectively. In addition, the interlukin-2 levels of mice in *L. chinense* Miller fruit and betaine treated group was increased compared with the control group.

**CONCLUSION:** These results indicate that *L. chinense* Miller fruit and betaine might be helpful in the immune function improvement, enhance physical stamina, and fatigue recovery.

**Key words:** Betaine, Cytokine, Forced swimming test, *Lycium chinense* Miller fruit

**Introduction**

*Lycium chinense* Miller fruit (LF) is distributed in warm and subtropical regions of southeastern Asia and European countries, and it has been used as an agriculture source for anti-aging or hepatoprotective antioxidant (Ahn et al., 2014). A representative component of LF, betaine has reported that it is the natural amino acid and involved in the synthesis of methionine from homocysteine in the liver as an oxidative metabolite of choline (Zhao et al., 2013; Ahn et al., 2014). In addition, betaine exerts various physiologic functions such as anti-atherosclerosis, anti-osteoporosis and protective effect against chemical liver injury (Zhao et al., 2013).

Forced swimming test, which is a behavioral test for rodents to predict the efficacy of antidepressant treatments, is recently used to examine whether certain agents have an anti-fatigue effect (Porsolt et al., 1977; Moriura et al., 1996). Fatigue, defined as a
loss of force-generating capacity, may develop for a variety of reasons and involve both central and peripheral factors (Kim et al., 2008). The feeling of fatigue is also recognized in chronic medical illness including an inmate of a hospital in intensive care unit or enteral/parenteral feeding condition (Kim et al., 2008). Forced swimming test causes alterations in cellular and non-cellular immunity, lowers the ratio of lymphocytes, enhances the ratio of neutrophils in rat peripheral blood (Dubovik and Bogomazov, 1987; Delbende et al., 1994; Connor et al., 1997; Kim et al., 2010). In addition, Shin et al. (2004) have demonstrated on a relationship between immune function and immobility time after a forced swimming test by ICR mice. They have been made an effort in the development and research of natural products with those health benefits in many countries. The aim of this study was to investigate the effects of LF and its representative constituent betaine on reduction of immobility time, changes of blood parameters and the release of cytokines such as interferon (IFN)-γ and interlukin (IL)-2 in balb/c mice.

Materials and Methods

LF was purchased from Kyungdong traditional herb market (Seoul, Korea). For the preparation of LF, the 20 g of crude LF were washed and air-dried. An extract of LF was prepared by decocting with boiling distilled water and the duration of decoction was about 3 h. The decoction was filtered, lyophilized and kept at 4°C. The yield of dried extract from initial crude materials for the study was about 8.2%. After the samples were dissolved in saline, they were filtered through a 0.45 μm syringe filter and kept at 4°C. For LF or betaine treated group, it was dissolved in distilled water and administrated at dose of 10 mg/kg/day and 0.1 mg/kg/day for the study, respectively, once per day for 7 days using a feeding atraumatic needle. Fluoxetine (10 mg/kg/day) was used as a positive control of this study.

Betaine, avidine-peroxidase, and 2'-AZINO-bis (3-ethylbenzthiazoline-sullfonic acid) tablets substrate (ABTS) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Anti-human IFN-γ and IL-2 monoclonal antibodies, biotinylated anti-human IFN-γ and IL-2 and recombinant IFN-γ and IL-2 were purchased from R&D Systems (Minneapolis, MN, USA). Male balb/c mice (5-week-old, 19-21 g) were purchased from the Da-Mul Experimental Animal Center (Daejon, Korea). The animals were housed four to ten per cage in a laminar air-flow room maintained at a temperature of 22 ± 1°C, relative humidity of 55 ± 10%, 12:12 L/D cycle and light on at 07:00 h throughout the study. Food and water were available ad libitum. All manipulations were carried out between 09:00 and 16:00 h, and no animal was used more than once. The experiment was approved by the institutional review board and animal ethics committee (No. wku12-28) and conducted by in accordance with the internationally accepted principles for laboratory animal use and care as found in the US guidelines (NIH publication #85-23, revised in 1985).

During the 6 min of the forced swimming test, the duration of immobility was measured as previously described by Porsolt et al. (1977). 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. After the last forced swimming test, mice were anesthetized with an intraperitoneal injection of ketamine (80 mg/Kg) and xylazine (4 mg/Kg) and blood (1 mL) was withdrawn from the heart of mice into syringes. Then, blood was prepared by centrifugation at 1500 × g, 4°C for 10 min. Contents of blood urea nitrogen (BUN), creatine kinase (CK), lactate dehydrogenase (LDH), glucose, total protein, and albumin were determined by an autoanalyzer (Hitachi 747, Hitachi, Japan). Additionally, enzyme-linked immunosorbent assay (ELISA) was carried out for IFN-γ and IL-2 of serum in mice of experimental groups in duplicate using a 96-well format (Nunc, Denmark). After treating each antibodies and washing the wells, avidin-peroxidase was added and plates were incubated for 20 min at 37°C. Then, ABTS substrate was added. Color development was measured at 405 nm using an automated microplate ELISA reader. All results were
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statistically analyzed by SPSS 19.0 analysis program and presented as mean ± standard error of mean (SEM). Data were calculated by using student t-test, one-way analysis of variance and the post hoc test was performed via the Turkey’s test. \( p \)-values of less than 0.05 were used as the criterion for statistical significance.

**Results and Discussion**

The immobility time of mice in control group, fluoxetine group, LF group, and betaine group was 138.5 s, 73.8 s, 81.3 s, and 64.3 s, respectively (Fig. 1). Reduction of the immobility time during forced swimming test traditionally interpreted as a measure of ‘behavioral despair’ or ‘learned helplessness’ (Gao *et al.*, 2014). Many studies show the possibility that retrenchment the amount of time an animal spends immobile in the forced swimming test by natural compounds have the relation with cytokines and blood parameters (Shin *et al.*, 2004; Kim *et al.*, 2008; Kim *et al.*, 2010) However, it was not illuminated yet detail mechanism on its relation between immobility time and change of cytokines/ blood parameters. The immobility time of mice in fluoxetine administrated groups, LF administrated groups, and betaine administrated groups reduced significantly at the last day, compared with saline administrated group (\( P < 0.05 \)). These results indicated that administration of LF and betaine improve the body activity and decrease behavioral despair.

Previous studies demonstrated that the swimming exercise is to induce the biochemical changes in blood (De-Mello *et al.*, 1992). It was investigated in the changes of blood parameters such as BUN, CK, LDH, glucose, total protein, and albumin (Fig. 2). The contents of BUN of mice in fluoxetine, LF, and betaine treated groups were 20.9, 21.1, and 20.9 mg/dL, respectively, and decreased significantly in comparison with that in saline treated control group (\( P < 0.05 \)). The CK and LDH levels of saline, fluoxetine, LF, and betaine treated group did not represent significant difference compared with a control group, although they had tends to decrease. Additionally, the value of glucose, total protein, and...
Table 1. Body weight and the production of IFN-γ and IL-2

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<th>CON</th>
<th>Flu</th>
<th>LF</th>
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<tr>
<td><strong>Body weights</strong></td>
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<tr>
<td>Initial (g)</td>
<td>19.8 ± 0.2</td>
<td>19.6 ± 0.7</td>
<td>19.5 ± 0.5</td>
<td>19.7 ± 0.5</td>
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<tr>
<td>Final (g)</td>
<td>21.6 ± 0.5</td>
<td>21.4 ± 0.6</td>
<td>21.1 ± 0.2</td>
<td>21.4 ± 0.7</td>
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<td><strong>Cytokine levels</strong></td>
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<td>IFN-γ (pg/mL)</td>
<td>415.0 ± 19.8</td>
<td>415.0 ± 11.2</td>
<td>431.3 ± 8.0</td>
<td>430.8 ± 5.7</td>
</tr>
<tr>
<td>IL-2 (pg/mL)</td>
<td>112.3 ± 6.7</td>
<td>113.5 ± 13.1</td>
<td>134.3 ± 6.1*</td>
<td>143.0 ± 5.3*</td>
</tr>
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CON, saline-administrated control group; Flu, fluoxetine (10 mg/kg/day, p.o.)-administrated group; LF, *Lycium chinense* Miller fruit (10 mg/kg/day, p.o.)-administrated group; Bot, Botaine (0.1 mg/kg/day, p.o.)-administrated group. Values represent the mean ± SEM. *P < 0.05 vs. significantly different from CON.

albumin had no effect in LF and betaine treated groups, compared with a control group. The level of BUN is standard metrics used to diagnose and monitor kidney injury (Ferguson et al., 2000). CK and LDH are generally known accurate indicators of muscle damage (Coombes and McNaughton, 2000). Glucose plays an important role as main substrates for energy during exercise or starvation (Rose and Sampson, 1982). The value of total protein is known as an indicator of nutritional state, kidney disease and chronic liver disease (Costill and Fink 1974). Albumin level indicates also immune or nutrition states in the body (Whicber and Spence 1987). Thus, the reduction of BUN values by LF and betaine indicated that they could have a play in alleviating kidney injury by forced swimming test.

Immunoregulatory cytokines play an important role in immune response (Bernabei et al., 2003; Kim et al., 2010). Of these immunoregulatory cytokines, they are known that IFN-γ produced by T and natural killer cells is considered the principal effector cytokine of cell-mediated immunity, and IL-2 is a T cell growth factor, can augment natural killer cell cytolytic activity, or promotes immunoglobulin production by B cells (Kim et al., 2006; Lenardo, 1991). It was evaluated that whether LF and its constituent betaine have enhanced the IFN-γ and IL-2 levels in blood of mice after forced swimming test compared with control groups (Table 1). The levels of IL-2 in betaine treated cells were significantly increased compared to that of control (P < 0.05), and IFN-γ levels also tends to increase by LF and betaine treatment, respectively. The contents of released IL-2 from mice of each group in the saline, fluoxetine, LF, betaine treated groups was 112.3, 113.5, 134.3, and 143.0 pg/mL, respectively. The oral administration of fluoxetine and LF during 7 days showed no significant results compared with control group, but tended to increase IFN-γ and IL-2 levels by LF. These results show that it regulates IL-2 levels which reduced after forced swimming test, by its constituent betaine than LF itself.

Finally, it is shown that LF and betaine decrease the immobility time of mice in forced swimming test and tend to decrease BUN or enhance the level of IFN-γ and IL-2 levels. From this, it is indicated that LF and betaine could have the possibility as a useful agriculture and herbal source in the immune function improvement, enhance physical stamina, and fatigue recovery. However, it needs the further research on approach more detailed mechanisms of the relationship between the immobility time and cytokine production in mice model.

**Conclusion**

Present results provide evidences that the administration of LF and its constituent betaine decrease the immobility times after a forced swimming test in mice. Moreover, this treatment tends to improve the levels of several blood biochemical parameters, after a forced swimming test. In addition, the levels of IFN-γ and IL-2 tend to enhance by LF and betaine treatments. These results indicate that LF and betaine might be helpful in the immune function improvement, enhance physical stamina, and fatigue recovery.

**References**


