Hepatoprotective and Anti-fatigue Effects of Lactic Acid Bacteria (Lactobacillus acidophilus, Bifidobacterium bifidum and Streptococcus thermophilus)

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This study was carried out to investigate the effect of LAB (Lactic acid bacteria: Lactobacillus acidophilus, Bifidobacterium bifidum and Streptococcus thermophilus) on detoxication of damaged liver in carbon tetrachloride (CCl₄) and ethanol (25%)-treated rats. Rats had been daily (twice a day) pre-treated with saline (0.5 ml/kg: untreated group), CCl₄ (0.5 ml/kg: other groups) for 6 days. At seventh day, after treating rat with CCl₄ and then, mixture of LAB (10⁷/0.5 ml: LAB group), saline (0.5 ml/kg: untreated group, CCl₄ group), and bifenyldimethyl dicarboxylate (DDB) (50 mg/kg: DDB group) were treated orally with CCl₄ for 8 days. Ethanol is treated as the same manner instead of CCl₄. To investigate the hepatoprotective effect, rats treated with CCl₄ and ethanol were analyzed with serum GOT and GPT level. The GOT and GPT levels of LAB group was lower than the level of CCl₄ and DDB group. Especially, compared with data of CCl₄ group, GPT activity showed statistically significant result in the significance level of p < 0.05. The LAB group treated with ethanol also showed lower level of GOT and GPT than the other control groups treated with ethanol. The triglyceride level of serum decreased more in a group treated special materials (DDB and LAB group) than ethanol group. As weill, the effect of LAB on the antifatigue has been investigated. The animals (10/group) were divided into 4 groups (untreated group, Carrier group, Red-ginseng group, LAB group). Each group was given carrier (0.9 mg/0.2 ml), red ginseng extract (200 mg/kg), and mixture of LAB (10⁷/0.2 ml). Special materials were given for three weeks. After finishing treating through oral, horizontal wire test, rotarod test, and forced swimming test were performed. The time of resistance to fatigue of the group, fed with mixture of LAB, was longer than the time when mice treated with red-ginseng that the effect was already revealed. The result of this study revealed that LAB could decrease hepatocellular injury compared with rats treated orally with CCl₄ and ethanol, and could also decrease fatigue.

Key words: Antifatigue, Bifidobacterium bifidum, Carbon tetrachloride, Bifenyldimethyl dicarboxylate, Hepatoprotective, Lactobacillus acidophilus, Red-ginseng, Streptococcus thermophilus.

INTRODUCTION

In modern society, the chronic liver diseases, including HBV, alcoholic liver disease, liver cirrhosis, and hepatocellular carcinoma, is one of the reasons that causes many adults to die in Korea. Moreover, because of heavy responsibility and stresses on people in this society, the degradation of liver function and liver diseases are increasing, and once the liver function is degraded, people easily feel tired.

The reason of fatigue is innumerable; however, they are not easily detected in psyciochemical examination, such as hematological test and urinary test. But rather, the fatigue can be defined as a previous warning for physical disorder that cannot be detected by modern science. One of the most important things in the physical disorders is disorder of liver function.

The liver is an important organ that detoxicates alcohol, drugs, chemical products, and various pollutants by discharging them when they come into human body.
CCl₄ and alcohol are the most representative drugs. CCl₄, a drug that causes a disorder of liver, is used for a solvent of resin and rubber at the industrial places, and exposed CCl₄ from the places hinders protein synthesis in liver, decreases synthesizing glycogen, and increases GOT and GPT in blood (Jeon and Park, 2002; Jin et al., 2005; Kim et al., 2000; Suja et al., 2004).

Moreover, the more people take in alcohol due to stress, the more the alcoholic liver disease increases. Taking in alcohol causes liver function to get injured; therefore, alcohol has to be discharged, not restored. Since the liver has enzymes that intervenes in decomposition of alcohol, alcohol is broken down in the liver, and at the middle of the degrading process, alcohol is changed to acetaldehyde that can injure the cell of liver. Moreover, as a result of alcohol metabolism, much fatty acid is produced and accumulated at the liver, causing alcoholic fatty liver (Kim et al., 2003; Pramyothin et al., 2005; Yang et al., 2004).

Therefore, the research of functional food materials that can recover the liver function, suppress the injury of liver caused by alcohol, drugs, and chemicals, and decrease the fatigues required.

Lactic acid bacteria (LAB) currently used in the prevention and treatment of disease (Kim et al., 2003; Menino et al., 1993). It has important roles in human health specifically in the intestinal environment which includes the inhibition of harmful bacteria by lowering the pH of the intestines, improvement of diarrhea or constipation cases, synthesis of vitamins, lowering the level of blood cholesterol and used as medicine for intestinal disorders. LAB wards off disease by suppressing harmful bacteria in the intestines through the propagation of macrophage. Therefore, it is assumed that LAB have an effect on fatigue caused by taking in alcohol and drugs chronically (Yun et al., 2005).

In this research, we verified that LAB have anti-fatigue effect and the improvement in the liver function by observing the effect on serum GOT, GPT, total cholesterol and triglyceride levels when CCl₄ and ethanol are treated orally with LAB, and by using horizontal wire, rotarod, and forced swimming test, respectively.

MATERIALS AND METHODS

Animals and materials.

Hepatoprotective effects of lactic acid bacteria: Male Sprague-Dawley (SD) rats (150–200 g) were obtained from Hanlim experimental animal Co. (Hwasung, Korea). LAB was provided by Cellbiotech Co. (Gyeonggido, Korea). Biphenyl dimethyl dicarboxylate (DDB: Kwangdong Pham, Korea) solution was used as a positive control. All the animals had free access to food and water in an animal room that was maintained as a controlled environment (22 ± 2°C, 55 ± 5% humidity, 12 h/12 h alternate light/dark cycles with light on at 6:00 h) (Chattopadhyay, 2003).

Anti-fatigue effects of lactic acid bacteria: Male ICR mice (4–5 weeks of age) were obtained from Hanlim experimental animal Co. (Hwasung, Korea). LAB and carrier were provided by Cellbiotech Co. (Gyeonggido, Korea).

Red-Ginseng (RG) extract (KRG extract; Korean Ginseng Co., Seoul, Korea) (Kim and Roh, 2000; Kimura and Sumiyoshi, 2004) was used as a positive control. All the animals had free access to food and water in an animal room that was maintained as a controlled environment (22 ± 2°C, 55 ± 5% humidity, 12 h/12 h alternate light/dark cycles with light on at 6:00 h) (Kim and Roh, 2000; Kim et al., 2003).

Hepatocellular injury from CCl₄ and ethanol: The animals (6/group) were divided into 4 groups (untreated group, CCl₄ group, DDB group, and LAB group) after stabilization for a week in the animal room. Rats had been daily (twice a day) pre-treated with saline (0.9% NaCl, 0.5 ml/kg; untreated group), CCl₄ (olive oil: CCl₄ = 6 : 4, 0.5 ml/kg; other groups) for 6 days (Janbaz et al., 2002; Park et al., 2002; Sin et al., 2002; Suja et al., 2004; Wang et al., 2004). At seventh day, after treating rat with CCl₄ and then, mixture of LAB (10¹¹/0.5 ml: LAB group), saline (0.5 ml/kg: untreated group, CCl₄ group), and biphenyl dimethyl dicarboxylate (DDB) (suspension in 1% CMC, 50 mg/kg: DDB group) were treated orally with CCl₄ for 8 days (Janbaz et al., 2002; Jeon et al., 1999; Park et al., 2005; Sin et al., 2002). Ethanol (25%) is treated as the same manner instead of CCl₄ (Chiu et al., 2003).

Measurement of GOT, GPT activities in serum of rats: All animals were anaesthetized with ether, and blood was withdrawn from the heart. The blood was centrifuged at 3,000 rpm and 4°C for 10 min to obtain sera (Baek, 1995; Jin et al., 2005; Ravikumar et al., 2005). GOT and GPT activities were measured with a kit (Asan Pham, Korea; According to Reitman and Frankel (1976)'s colorimetric methods) (Bae et al., 1997; Choi and Kim, 2000; Hui et al., 2002; Kim et al., 2002).

Measurement of total cholesterol and triglyceride levels in serum of rats: We requested it to Samkwang Medical Laboratories.

Anti-fatigue test.
The animals (10/group) were divided into 4 groups
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(untreated group, Carrier group, Red ginseng group, LAB group) after stabilization for a week in the animal room.

Mice had been daily (once a day) treated orally with carrier solution (0.9 ng/0.2 ml: carrier group), red-ginseng extract (5 mg/0.2 ml: red-ginseng group) and LAB solution (10^7/0.2 ml: LAB group) for 3 weeks.

**Horizontal wire test.**

After giving supplementary materials for 3 weeks, anti-fatigue effect of supplementary materials was evaluated by using a horizontal wire. A horizontal wire test was carried out, according to the method described by Hui et al. with minor modifications (Hui et al., 2002). Mice were lifted by the tail and allowed to grasp a horizontally strung wire (5 mm diameter, 150 cm long, and placed 80 cm above the table) with their forepaws, and then released. The time spent on the horizontal wire was evaluated as endurance ability (Hui et al., 2002; Lee et al., 2004).

**Rotarod test (60 rpm).**

After horizontal wire test was conducted, performing rotarod (Ugo Basile, Italy) test right away did anti-fatigue effect of supplementary materials. The mouse was allowed to walk on a rotating rod of fixed diameter (3.5 cm) for a period of time until the mouse can no longer maintain its position. The rotarod apparatus employed by this laboratory consists of a central drive rod connected to a stepper motor (Jones and Roberts, Ugo Basile), which is divided into five testing stations with a timer. The speed rotatea can be ramped up from 0 rpm 60 rpm. The time spent (sec.) on the rotarod with 60 rpm was measured to evaluate motor coordination and enduring ability (Lee et al., 2004).

**Forced swimming test (FSTs).**

After rotarod test, anti-fatigue effect of supplementary materials was evaluated by performing Swimming test in mice. The swimming pool (Purchased from Sci. Tech. Korea Co.), is a large circular pool (diameter: 150 cm, height: 50 cm) made of stainless steel. The pool was filled with water with a depth of 15 cm (maintained at 8 ± 4°C) (Kim et al., 2002; Kimura and Sumiyoshi 2004; Lee et al., 2004). The mice were allowed to swim in the pool until they’re exhausted and looked like drowning.

**Statistical analysis.**

Data were expressed as mean ± S.E. (Standard Errors). The significant differences between the groups were assessed by standard or paired t-test (Germano et al., 2005; Porchezhan and Ansari, 2005; Shah et al., 2005; Yang et al., 2005). Differences were considered significant if p < 0.01.

**RESULTS AND DISCUSSION**

**Hepatoprotective effects of lactic acid bacteria.**

The effect of CCl4 on the weight and weight of the liver: Table 1 showed the increase of subject’s weight and percentage of the liver’s weight to the subject’s weight. The increase of the weight in untreated group was greater than other groups treated with CCl4 orally. Moreover, the groups where the livers were damaged due to CCl4 showed more increase in the percentage of liver’s weight than the untreated group.

The effect on serum glutamic oxaloacetic transaminase (GOT) and glutamic pyruvic transaminase (GPT): Fig. 1 showed the results of the serum GOT, GPT result graph of experimental groups. This graph showed a decrease of GOT in the group treated with LAB than the group treated with CCl4. The serum GPT also decreased in the LAB treated group than in the group treated with CCl4 (p < 0.05).

The effect of ethanol on the weight and the weight of the liver: Table 2 showed the increase of subjects’ weight and the percentage of the weight to the

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**Table 1. Effects of the LAB (mixture of lactic acid bacteria: *L. acidophilus*, *B. bifidum* and *S. thermophilus*) on body weight and liver weight/ body weight (%) in CCl4-treated rats**

<table>
<thead>
<tr>
<th>Group</th>
<th>Liver (g)</th>
<th>L/BW (%)</th>
<th>Body weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Weight</td>
<td>Initial</td>
<td>Final</td>
</tr>
<tr>
<td>Untreated</td>
<td>10.94 ± 1.44</td>
<td>3.02 ± 0.42</td>
<td>223.57 ± 6.10</td>
</tr>
<tr>
<td>CCl4</td>
<td>11.18 ± 1.61</td>
<td>4.22 ± 0.71</td>
<td>196.50 ± 5.68</td>
</tr>
<tr>
<td>DDB</td>
<td>12.86 ± 0.51</td>
<td>4.65 ± 0.40*</td>
<td>204.48 ± 2.62</td>
</tr>
<tr>
<td>LAB</td>
<td>12.81 ± 1.38</td>
<td>4.53 ± 0.71*</td>
<td>212.95 ± 1.36</td>
</tr>
</tbody>
</table>

*Untreated group: saline, CCl4 group: CCl4, DDB group: CCl4 + DDB, LAB group: CCl4 + *L. acidophilus*, *B. bifidum*, *S. thermophilus* group.

*Liver weight/body weight %. Mean ± S.D. (n = 5–6). Statistically significant compared with data of untreated group (*, p < 0.005). Compared with data of CCl4 group (**, p < 0.5).
Fig. 1. Effects of the LAB on serum glutamic oxaloacetic transaminase (GOT) and glutamic pyruvic transaminase (GPT) activities in CCl4/olive oil (60:40, 0.5 ml/kg)-treated rats. Values are Mean ± S.D. of six rats/group; (*) statistically significant compared with data of CCl4-treated group (p < 0.05); (**) statistically significant compared with data of CCl4-treated group (p < 0.5); LAB: Lactic acid bacteria (L. acidophilus, B. bifidum and S. thermophilus).

Liver's weight. We can see that the livers of groups treated with ethanol were damaged by the ethanol since the increase of weight in the untreated group was larger than the groups treated orally with ethanol.

![Diagram showing GOT and GPT levels](image)

Fig. 2. Effects of the LAB on serum GOT and GPT activities in ethanol (25%)-treated rats. Values are Mean ± S.D. of six rats/group; (*) statistically significant compared with data of Ethanol-treated group (p < 0.1); (**) statistically significant compared with data of Ethanol-treated group (p < 0.5); LAB: Lactic acid bacteria (L. acidophilus, B. bifidum and S. thermophilus).

**Influence on serum GOT and GPT:** In the Fig. 2, we can find a decrease of serum GOT in the LAB treated group than the groups treated with ethanol and DDB; serum GPT also decreased in the group treated with LAB rather than the groups treated orally with ethanol.

Table 2. Effects of the LAB (mixture of lactic acid bacteria: L. acidophilus, B. bifidum and S. thermophilus) on body weight and liver weight/body weight (%) in ethanol (25%)-treated rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Liver (g)</th>
<th>Liver weight/body weight (%)</th>
<th>Body weight (g)</th>
<th>Final</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Weight</td>
<td>Initial</td>
<td></td>
<td>Final</td>
</tr>
<tr>
<td>Untreated</td>
<td>9.77 ± 0.90</td>
<td>207.18 ± 4.06</td>
<td>341.17 ± 10.28</td>
<td></td>
</tr>
<tr>
<td>Ethanol</td>
<td>8.28 ± 1.06</td>
<td>190.13 ± 5.87</td>
<td>309.00 ± 17.97</td>
<td></td>
</tr>
<tr>
<td>DDB</td>
<td>8.18 ± 0.24</td>
<td>198.63 ± 0.21</td>
<td>316.83 ± 9.11*</td>
<td></td>
</tr>
<tr>
<td>LAB</td>
<td>9.20 ± 1.54</td>
<td>200.85 ± 1.12</td>
<td>319.00 ± 22.56*</td>
<td></td>
</tr>
</tbody>
</table>

*(*) Untreated group: saline, Ethanol group: ethanol, DDB group: ethanol + DDB, LAB group: ethanol + L. acidophilus, B. bifidum, S. thermophilus.

**Liver weight/body weight %. Mean ± S.D. (n = 5–6).**

Statistically significant compared with data of Untreated group (*, p < 0.005; ***, p < 0.1).

Compared with data of Ethanol group (*, p < 0.5).
Table 3. Level of serum cholesterol and triglycerides in control and experimental groups of rat

<table>
<thead>
<tr>
<th>Group</th>
<th>Karasen unit</th>
<th>Total cholesterol</th>
<th>Triglyceride</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated</td>
<td>55.00 ± 5.48</td>
<td>24.50 ± 3.70</td>
<td></td>
</tr>
<tr>
<td>Ethanol</td>
<td>56.00 ± 1.41</td>
<td>35.00 ± 7.28</td>
<td></td>
</tr>
<tr>
<td>DDB</td>
<td>56.00 ± 10.61</td>
<td>30.50 ± 7.94</td>
<td></td>
</tr>
<tr>
<td>LAB</td>
<td>60.25 ± 10.23</td>
<td>26.25 ± 5.91</td>
<td></td>
</tr>
</tbody>
</table>

1^Mean ± S.D. (n = 4–6).
2^Untreated group: saline, Ethanol group: ethanol, DDB group: ethanol + DDB, LAB group: ethanol + L. acidophilus, B. bifidum, S. thermophilus.
3^Statistically significant compared with data of Untreated group (*, p < 0.05; **, p < 0.5).

Table 4. Effect of the lactic acid bacteria on wet weight of liver in ICR mice

<table>
<thead>
<tr>
<th>Experimental group</th>
<th>Liver weight(g)</th>
<th>Weight</th>
<th>L/BW (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated</td>
<td>1.98 ± 0.19</td>
<td>5.29 ± 0.26</td>
<td></td>
</tr>
<tr>
<td>Carrier</td>
<td>1.95 ± 0.32</td>
<td>5.24 ± 0.42</td>
<td></td>
</tr>
<tr>
<td>Red-ginseng</td>
<td>1.77 ± 0.33</td>
<td>5.08 ± 0.40</td>
<td></td>
</tr>
<tr>
<td>LAB</td>
<td>1.92 ± 0.24</td>
<td>5.22 ± 0.33</td>
<td></td>
</tr>
</tbody>
</table>

1^Mean ± S.D. (n = 9–11).
2^Compared with data of normal group (*, p < 0.1).

Antifatigue effects of lactic acid bacteria.

Body weight: As shown in Fig. 3, we measured the change of weight of each group from the fifth week to the last week. Comparing to the untreated group, oral treating does not give any effect on stress and fatigue since the weight of all groups did not show any change.

Weight of liver: Table 4 shows the proportion of increase in the liver weight to body weight and liver weight. Since there was no difference in L/BW between untreated group and special material group, we can see that special materials (carrier, red-ginseng or LAB) do not give any effect on the liver of mice.

Anti fatigue effect of horizontal wire test, rotarod test, forced swimming test (FSTs): Anti-fatigue effect of the red-ginseng and LAB in mice was shown in Fig. 4, 5 and 6. Horizontal wire test, rotarod test and forced swimming test were measured to evaluate motor coordination and enduring ability. As shown in Fig. 4, the time of resistance to fatigue of the mice treated orally with LAB was longer than the time when mice were treated orally with red-ginseng. Fig. 5 shows the level of

Fig. 3. Effects of the lactic acid bacteria on body weight in ICR mice. Body weight change between 5 and 8 weeks in each experimental groups.

Fig. 4. Effects of the LAB on horizontal wire test in ICR mice. Each bar represents mean ± S.D. (n = 9–11) of endurance time. Mice were given water containing carrier, red ginseng extract and Lactic acid bacteria. Carrier group (carrier 0.9 mg/0.2 ml), Red-ginseng group (red ginseng extract: 5 mg/0.2 ml), LAB group (mixture of L. acidophilus, B. bifidum and S. thermophilus 10^9/0.2 ml).
motor coordination and exhaustion. Endurance and motor coordination abilities of LAB group were also longer than the enduring time of red-ginseng group. As shown in Fig. 6, the swimming time of LAB group in mice until exhaustion was significantly longer than the swimming time (p < 0.001) of untreated group. The time of resistance to fatigue of the LAB group was longer than the time red-ginseng group.

CONCLUSION

In this study, we observed the effect on serum GOT, GPT, total cholesterol and triglyceride level when treated orally with LAB and CCl₄. And, we tried to find out anti-fatigue effect and improvement on liver function by using horizontal wire, rotarod, and forced swimming test.

This study shows that LAB (mixture of Lactobacillus acidophilus, Bifidobacterium bifidum and Streptococcus thermophillus) have an excellent effect on restraining liver damage, and it can be used as a functional food that can constrain the liver damage caused by exposure of hazardous articles, habitual alcohol intake, and taking an overdose of medicines, and fatigue caused by the liver damage.

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