Journal of Food and Nutrition Research, 2018, Vol. 6, No. 8, 509-512
Available online at http://pubs.sciepub.com/jfnr/6/8/4

DOI:10.12691/jfnr-6-8-4

"LICONINE®", an Extract of Glycyrrhiza Uralensis, Normalizes the Fecal Microbiota Disturbance in Diet-induced Obese Mice

Yuka Sasakawa[1,2,#,*], Akari Kominami[1,2,#], Michiyo Abe[1,2], Kaori Yamamoto[2], Mayumi Nakao[2], Fumiko Nakaoka[2], Shigetoshi Okumura[2,3], Lekh Raj Juneja[2,3], Jun Kunisawa[1]

[1]Laboratory of Vaccine Materials, National Institutes of Biomedical Innovation, Health and Nutrition, Osaka, Japan
[2]MG Pharma Inc., Osaka, Japan
[3]ROHTO Pharmaceutical Co., Ltd., Osaka, Japan
[#]These authors contributed equally to this work.
*Corresponding author: y-sasakawa@mgpharma.co.jp

Received July 01, 2018; Revised August 20, 2018; Accepted August 28, 2018

Abstract It has been reported that *Glycyrrhiza uralensis* (Ural licorice) extract, with the brand name "LICONINE®", at an intake level of low risk (human equivalent to less than 1 g licorice per day) had anti-obesity and anti-inflammatory effects on high-fat high-sucrose (HFS) diet-induced obese mice, but the effect of LICONINE on the fecal microbiota has not been clarified. In the present study, effects of LICONINE versus Ural licorice at intake levels of low risk on the fecal microbiota *Bacteroidetes* and *Firmicutes* were examined in HFS diet-induced obese mice. The results demonstrated that consumption of LICONINE was associated with higher decrease in the body weight and visceral fat weight than those with Ural licorice. The results also demonstrated that the *Firmicutes* to *Bacteroidetes* ratio in the feces was decreased more with LICONINE than with Ural licorice, and that the body weight and visceral fat weight were correlated with the *Firmicutes* to *Bacteroidetes* ratio in HFS diet-induced obese mice. These results indicate that LICONINE, at an intake level of low risk, may reduce obesity through the normalization of the fecal microbiota disturbance. In summary, at the intake level of low risk, LICONINE is more effective than Ural licorice for treating obesity and fecal microbiota disturbance. Further research is needed to identify the mechanisms underlying the effects of LICONINE on fecal microbiota.

Keywords: *glycyrrhiza uralensis, ural licorice extract, LICONINE, high-fat high-sucrose diet-induced obese mice, obesity, microbiota*

Cite This Article: Yuka Sasakawa, Akari Kominami, Michiyo Abe, Kaori Yamamoto, Mayumi Nakao, Fumiko Nakaoka, Shigetoshi Okumura, Lekh Raj Juneja, and Jun Kunisawa, "'LICONINE®', an Extract of Glycyrrhiza Uralensis, Normalizes the Fecal Microbiota Disturbance in Diet-induced Obese Mice." *Journal of Food and Nutrition Research*, vol. 6, no. 8 (2018): 509-512. doi: 10.12691/jfnr-6-8-4.

1. Introduction

It has been reported that *Glycyrrhiza uralensis* (Ural licorice) extract, with the brand name "LICONINE®", at an intake level of low risk (human equivalent to less than 1 g licorice per day) had anti-obesity and anti-inflammatory effects on high-fat high-sucrose (HFS) diet-induced obese mice [1]. The roots and stolons of licorice (Ural licorice, *Glycyrrhiza glabra*, etc.) have been used widely for 4000 years as food and medicine. Licorice and licorice extract have been used as food and medicines for the treatment of inflammation, liver disease, digestive symptoms, and cough [2]. Licorice at less than 1 g per day and licorice extract at equivalent to less than 1 g licorice per day, which are intake levels of low risk, may be consumed as a food with low risk of side effects [2]. LICONINE is an effective extract of Ural licorice at an intake level of low risk for treating obesity [1].

We previously found that consumption of LICONINE at an intake level of low risk was associated with decreased body weight, visceral fat weight, number of crown-like structures, and adipocyte size in visceral fat of HFS diet-induced obese mice [1], but the effect of LICONINE on the fecal microbiota has not been clarified. It has been reported that two divisions of beneficial bacteria are dominant in the gut microbiota of humans and mice, the *Bacteroidetes* and *Firmicutes* [3,4,5]. The *Firmicutes* to *Bacteroidetes* ratio in the gut microbiota was higher in obese humans and mice in comparison to lean humans and mice [3-5]. In the present study, effects of LICONINE versus Ural licorice at intake levels of low risk on the fecal microbiota, *Bacteroidetes* and *Firmicutes*, were examined in HFS diet-induced obese mice.

2. Materials and Methods

2.1. Materials

Ural licorice and Ural licorice extract were used. For the extraction of Ural licorice, 50% ethanol was used. Ural licorice extract was made by adding one volume of crushed Ural licorice to 10 volumes of ethanol at 20°C for 24 h. The extracted liquid was lyophilized and then dried under vacuum. The rate of extraction with 50% ethanol was approximately 30% (w/w). The 50% ethanol extract of Ural licorice was given the brand name "LICONINE®" (MG Pharma, Osaka, Japan).

2.2. Animals and Their Treatment

The present study conformed to the ethical guidelines for animal experimentation of MG Pharma Inc., which are in accordance with the Declaration of Helsinki. Healthy C57BL/6J strain male mice (age, 4 weeks) (Japan SLC, Shizuoka, Japan), with pelage in good condition, were used in our experiments. The mice were housed in an air-conditioned room (23 ± 2°C, 50 ± 10% RH) with a 12-h light and dark cycle (7:00–19:00 light hours). The mice were acclimatized in an experimental animal room for 7 days with free access to MF diet (Oriental Yeast, Tokyo, Japan; 5% fat, 55% carbohydrate [55% cornstarch], and 23% protein) and water before beginning the experiment.

2.3. Animal Study

C57BL/6J mice (age, 5 weeks; weight, 20.08 ± 0.20 g [18.60-21.21]) were divided into the following four groups at random, with the number of mice per group shown in Table 1: normal, control, 0.3% LICONINE, and 1% Ural licorice. Each group of mice was fed a special diet for 8 weeks: the normal group received the MF diet; the control group, the high-fat high-sucrose (HFS) diet (D12079BM, Research Diets, New Brunswick, NJ, USA; 21% fat, 50% carbohydrate [34% sucrose], and 20% protein); and the experimental groups, the HFS diet blended with either LICONINE or Ural licorice. The percentage of LICONINE in the diet was equivalent to 1% Ural licorice and was calculated based on the extraction rate from Ural licorice. The mice had free access to both diet and water. The body weight and food intake were measured over time. The feces of mice exerted in the cage for one day were collected at week 8. The fecal microbiota were analyzed using the terminal-restriction fragment length polymorphism analysis method of Techno Suruga Laboratory (Shizuoka, Japan). At the last day of the study after a 4-h fast, the mice were euthanized by exsanguination from the inferior vena cava. The visceral fat including epididymal fat, perirenal fat, and mesenteric fat, was weighed.

2.4. Statistical Analysis

The results are expressed as mean ± SEM (standard error of the mean). The coefficient of variation in each assay was less than 5%. The data fit a normal distribution, and the normality of the distribution was confirmed by Geary's test [6]. For statistical analysis, both analysis of variance (ANOVA) and multiple comparisons by Ryan's method were used [7,8]. Compared values were considered significantly different when p was less than 0.05. Relations between the body weight, visceral fat weight, *Bacteroidetes* ratio, *Firmicutes* ratio, and *Firmicutes* to *Bacteroidetes* ratio in the four groups were analyzed using Person's product moment correlation coefficient.

Table 1. Effects of LICONINE and Ural licorice on the body weight and visceral fat weight in mice fed HFS diet

Group	N	Body weight (g)		Visceral fat weight (g)	
Normal	6	26.5 ± 0.3	**	0.80 ± 0.08	**
Control	5	31.1 ± 0.7	##	2.70 ± 0.20	##
0.3% LICONINE	6	28.3 ± 0.9	*	1.58 ± 0.18	** #
1% Ural licorice	5	29.3 ± 0.4	#	1.89 ± 0.11	* ##

The body weight and visceral fat weight in mice fed MF or HFS diet with or without test articles for 8 weeks are shown. A significant difference was seen between the control group (* $p<0.05$, ** $p<0.01$) or the normal group ($^{\#}$ $p<0.05$, $^{\#\#}$ $p<0.01$) and the test substance groups by ANOVA and Ryan's method.

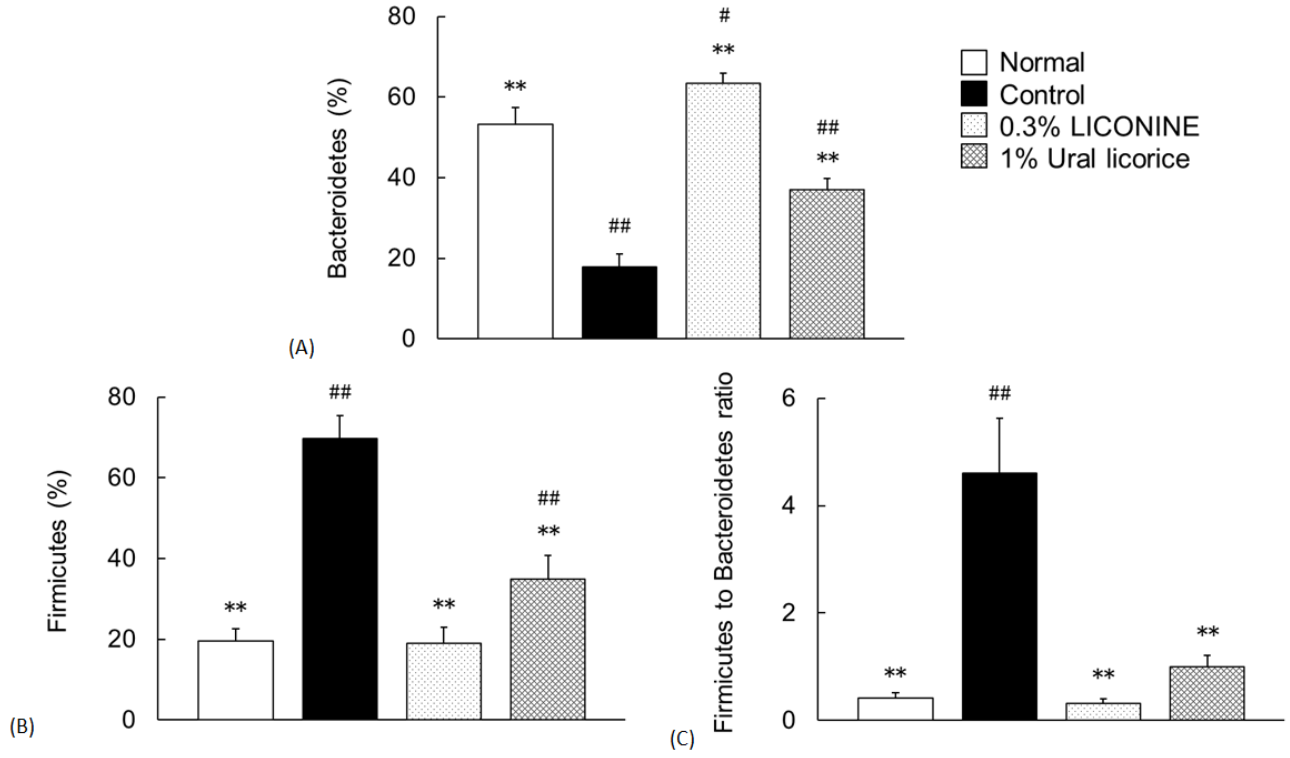
3. Results

Effects of LICONINE and Ural licorice at intake levels of low risk on the fecal microbiota *Bacteroidetes* and *Firmicutes* were examined in HFS diet-induced obese mice. The percentage of LICONINE added to diet was equivalent to 1% Ural licorice. The 1% Ural licorice was approximately 1 g/kg per day Ural licorice. The effective coefficient of conversion from the animal dose to the human dose of test articles is 1/60 [9]. The human equivalent of 1 g/kg per day (the intake level of Ural licorice by the animals) is 1 g per day, which is an intake level of low risk. The body weight was increased in the control group compared with the normal group (Table 1); decreased in the LICONINE group compared with the control group, and was similar between the Ural licorice and control groups. The visceral fat weight was increased in the control group compared with the normal group (Table 1); and decreased in the LICONINE and Ural licorice groups compared with the control group. LICONINE was associated with higher decrease in the body weight and visceral fat weight than those with Ural licorice. The food intake by all experimental groups was similar to that by the control group (data not shown). The *Bacteroidetes* ratio, *Firmicutes* ratio, and *Firmicutes* to *Bacteroidetes* ratio in the feces of the control group was decreased, increased, and increased compared with the normal group (Figure 1); and increased, decreased, and decreased in LICONINE and Ural licorice groups compared with the control group. LICONINE was associated with higher normalization of the fecal microbiota disturbance than that of Ural licorice. High positive correlation was found between the body weight, visceral fat weight, *Firmicutes* ratio, and *Firmicutes* to *Bacteroidetes* ratio, respectively. High negative correlation was found between the *Bacteroidetes* ratio and body weight, visceral fat weight, *Firmicutes* ratio, and *Firmicutes* to *Bacteroidetes* ratio (Table 2). These data indicated that the body weight and visceral fat weight were correlated with the *Firmicutes* to *Bacteroidetes* ratio in these HFS diet-induced obese mice.

Table 2. Relations between the body weight, visceral fat weight, *Bacteroidetes* ratio, *Firmicutes* ratio, and *Firmicutes* to *Bacteroidetes* ratio in the four groups

Correlation coefficient (r)	Body weight	Visceral fat weight	*Bacteroidetes* ratio	*Firmicutes* ratio	*Firmicutes* to *Bacteroidetes* ratio
Body weight	1.000				
Visceral fat weight	0.999	1.000			
Bacteroidetes ratio	-0.819	-0.809	1.000		
Firmicutes ratio	0.896	0.899	-0.954	1.000	
Firmicutes to *Bacteroidetes* ratio	0.847	0.858	-0.906	0.986	1.000

Relations between the body weight, visceral fat weight, *Bacteroidetes* ratio, *Firmicutes* ratio, and *Firmicutes* to *Bacteroidetes* ratio in the four groups were analyzed using Person's product moment correlation coefficient.



The *Bacteroidetes* (A), *Firmicutes* (B), and *Firmicutes* to *Bacteroidetes* ratio (C) in feces of mice fed MF or HFS diet with or without test articles for 8 weeks are shown. A significant difference was seen between the control group (** $p<0.01$) or the normal group (# $p<0.05$, ## $p<0.01$) and the test substance groups by ANOVA and Ryan's method.

Figure 1. Effects of LICONINE and Ural licorice on the fecal microbiota in mice fed HFS diet

4. Discussion

It has been reported that LICONINE at an intake level of low risk (human equivalent to less than 1 g licorice per day) had the anti-obesity and anti-inflammatory effects on HFS diet-induced obese mice [1], but the effect of LICONINE on the fecal microbiota has not been clarified.

In the present study, LICONINE was also associated with higher decrease in the body weight and visceral fat weight than those with Ural licorice in the HFS diet-induced obese mice. It has been reported that two divisions of beneficial bacteria are dominant in the gut microbiota of humans and mice, the *Bacteroidetes* and *Firmicutes* [3,4,5]. The *Firmicutes* to *Bacteroidetes* ratio in the gut microbiota was increased in obese humans and mice by comparison with lean humans and mice [3,4,5]. The relative proportion of *Bacteroidetes* was increased and the relative proportion of *Firmicutes* was decreased in obese people with weight loss on low-calorie diet [3]. Transplantation of the microbiota from mice with diet-induced obesity to lean germ-free recipients produced a significantly greater increase in adiposity [5]. These studies indicated that treating the disturbance of the gut microbiota may offer new possibilities for treating obesity [3,10]. Our results demonstrated that the *Firmicutes* to *Bacteroidetes* ratio in the feces of HFS diet-induced obese mice was decreased more with LICONINE than with Ural licorice, and that the body weight and visceral fat weight were correlated with the *Firmicutes* to *Bacteroidetes* ratio. These results indicate that LICONINE at an intake level of low risk may reduce obesity through the normalization of the fecal microbiota disturbance. In summary, at the intake level of low risk, LICONINE is more effective than Ural licorice for treating obesity and fecal microbiota disturbance. Further research is needed to identify the mechanisms underlying the effects of LICONINE on fecal microbiota.

Conflicts of Interest

The authors declare that there are no conflicts of interest.

References

[1] Sasakawa, Y., Kominami, A., Abe, M., Yamamoto, K., Nakao, M., Nakaoka, F., Okumura, S., Juneja, L.R. and Kunisawa, J., "The Anti-obesity and Anti-inflammatory Effects of "LICONINE™", an Extract of Glycyrrhiza Uralensis, on Diet-induced Obese Mice and 3T3-L1 Mouse Adipocytes," *Journal of Food and Nutrition Research*, 5(10). 781-788. 2017.

[2] Ministry of Health, Labour and Welfare, Japan, "About handling of medical supplies contain glycyrrhizic acid," The MHLW Ministerial Notification No. 158, February. 1978 (in Japanese).

[3] Ley, R.E., Turnbaugh, P.J., Klein, S. and Gordon, J.I., "Microbial ecology: human gut microbes associated with obesity," *Nature*, 444 (7122). 1022-1023. 2006.

[4] Ley, R.E., Bäckhed, F., Turnbaugh, P., Lozupone, C.A., Knight, R.D. and Gordon, J.I., "Obesity alters gut microbial ecology," *Proceedings of the National Academy of Sciences of the United States of America*, 102 (31). 11070-11075. 2005.

[5] Turnbaugh, P.J., Bäckhed, F., Fulton, L. and Gordon, J.I., "Diet-induced obesity is linked to marked but reversible alterations in the mouse distal gut microbiome," *Cell Host & Microbe*, 3 (4). 213-223. 2008.

[6] Geary, R.C., "Testing for normality," *Biometrika*, 34 (3-4). 209-242. 1947.

[7] Ryan, T.A., "Multiple comparisons in psychological research," *Psychological Bulletin*, 56 (1). 26-47. 1959.

[8] Ryan, T.A., "Significance tests for multiple comparison of proportions, variances, and other statistics," *Psychological Bulletin*, 57. 318-328. 1960.

[9] Ozaki, M., Fujita, T., Kumagai, Y. and Otani, Y., "A retrospective study for determining the starting dose in first-in-human studies on Japanese healthy volunteers," *Japanese Journal of Clinical Pharmacology and Therapeutics*, 37 (3). 119-126. 2006.

[10] López-Cepero, A.A. and Palacios, C., "Association of the Intestinal Microbiota and Obesity," *Puerto Rico Health Sciences Journal*, 34 (2). 60-64. 2015.