

# The Anti-obesity and Anti-inflammatory Effects of “LICONINE™”, an Extract of *Glycyrrhiza Uralensis*, on Diet-induced Obese Mice and 3T3-L1 Mouse Adipocytes

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**Abstract** The anti-obesity effect of *Glycyrrhiza uralensis* (Ural licorice) extract at the intake level of low risk has not been clarified. In the present study, the anti-obesity effects of Ural licorice, its extracts, its fractions and Glycyrrhizic acid at intake levels of low risk were examined in high-fat high-sucrose (HFS) diet-induced obese mice. Ural licorice extract, which was given the brand name “LICONINE™,” was the most effective extract; it decreased body weight, visceral fat weight, number of adipocyte crown-like structures, and adipocyte size in visceral fat. Low doses of LICONINE also had anti-obesity effects. The terpenoid fraction, but not flavonoid fraction, of LICONINE had an anti-obesity effect that did not depend exclusively on Glycyrrhizic acid. Next, we examined the effects of Ural licorice extracts, LICONINE fractions, and constituents of LICONINE terpenoid fraction on production of monocyte chemoattractant protein-1 in 3T3-L1 mouse adipocytes. LICONINE and its terpenoid fraction had anti-obesity and anti-inflammatory effects in both HFS diet-induced obese mice and 3T3-L1 adipocytes. Our results demonstrate for the first time that Licorice saponin G2, Licorice saponin H2, and Glycyrrhizic acid (all constituents of LICONINE terpenoid fraction) had anti-inflammatory effects in 3T3-L1 adipocytes. In summary, at the intake level of low risk (human equivalent to less than 1 g licorice per day), LICONINE is an effective extract of Ural licorice and the terpenoid fraction is an effective fraction of LICONINE for treating obesity. Further research is needed to identify the mechanism underlying the anti-obesity and anti-inflammatory effects of LICONINE and its terpenoid fraction.

**Keywords:** *glycyrrhiza uralensis*, *ural licorice extract*, LICONINE, *high-fat high-sucrose diet-induced obese mice*, *obesity*, *3T3-L1 mouse adipocytes*, *monocyte chemoattractant protein-1*

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## 1. Introduction

The roots and stolons of licorice (*Glycyrrhiza uralensis* [Ural licorice], *Glycyrrhiza glabra*, etc.) have been used widely for 4000 years as food and medicine. Glycyrrhizic acid is the main constituent in licorice [1]. Glycyrrhizic acid, licorice, and licorice extract have been used as food and medicines for the treatment of inflammation, liver disease, digestive symptoms, and cough [2].

Recently, it was reported that *Glycyrrhiza glabra* extract has an anti-obesity effect [3,4,5,6]. Moreover, there are some studies about anti-obesity effects of Ural licorice or its extracts, but the anti-obesity effect of Ural licorice extract at the intake level of low risk has not been clarified.

Reports indicated that the anti-obesity effect can be achieved only by higher rather than the intake level of low risk [7,8,9,10], and that Ural licorice extract at the intake level of low risk was ineffective [11]. Several reports indicate that long-term excess intake of Glycyrrhizic acid (more than 500 mg per day) had side effects, such as hypokalemia, hypertension, edema, and pseudohyperaldosteronism [2]. Licorice (1-5 g per day), licorice extract (equivalent to 1-5 g licorice per day), or Glycyrrhizic acid (40-200 mg per day) has also risk of side effects [2], whereas licorice (less than 1 g per day), licorice extract (equivalent to less than 1 g licorice per day), or Glycyrrhizic acid (less than 40 mg per day), which is the intake level of low risk, may be consumed as a food with low risk of side effects [2].

In the present study, we examined 1) the anti-obesity effects of Ural licorice, its extracts, LICONINE fractions,

and Glycyrrhizic acid (at intake levels of low risk) in high-fat high-sucrose (HFS) diet-induced obese mice and 2) effects of Ural licorice extracts, LICONINE fractions, and constituents of LICONINE terpenoid fraction on the monocyte chemoattractant protein-1 (MCP-1) production in 3T3-L1 mouse adipocytes.

## 2. Materials and Methods

### 2.1. Materials

Distilled water, 50% ethanol, or 99.5% ethanol was used for the extraction of Ural licorice. Ural licorice extracts were made by adding one volume of crushed Ural licorice to 10 volumes of distilled water and heating to 80°C or by treating the one volume with 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 distilled water, 50% ethanol, or 99.5% ethanol was approximately 20%, 30%, or 10% (w/w), respectively. The 50% ethanol extract of Ural licorice was given the brand name “LICONINE™” (MG Pharma, Osaka, Japan). LICONINE was separated into terpenoid and flavonoid fractions using high-performance liquid chromatography. The 8 main constituents of LICONINE included Glycyrrhizic acid, Licorice saponin G2, Licorice saponin H2, and 22 $\beta$ -acetoxyglycyrrhizin (the terpenoid fraction), and Isoliquiritigenin, Liquiritigenin, Isoliquiritin, and Liquiritin (the flavonoid fraction). Glycyrrhizic acid was the most abundant LICONINE constituent (6% [w/w]). In our experiments, 11% (w/w) of LICONINE and 9% (w/w) of LICONINE terpenoid fraction were 4 terpenoids, such as Glycyrrhizic acid, Licorice saponin G2, Licorice saponin H2 and 22 $\beta$ -acetoxyglycyrrhizin (Table 1). Furthermore, 6% of LICONINE and 13% of LICONINE flavonoid fraction were 4 flavonoids, such as Isoliquiritigenin, Liquiritigenin, Isoliquiritin and Liquiritin (Table 1). Glycyrrhizic acid (17217-44, Nacalai Tesque, Osaka, Japan), Licorice saponin G2, and Licorice saponin H2 (P25021 and P25031, TOKIWA Phytochemical Co., Ltd., Chiba, 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  $\pm$  2°C, 50  $\pm$  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 1: Comparison of Ural Licorice Extracts

C57BL/6J mice (age, 5 weeks; weight, 20.04  $\pm$  0.08 g [18.60–21.43]) were divided into the following six groups at random, with the number of mice per group shown in Table 2: normal, control, 1% Ural licorice, 0.2% Ural licorice water extract, 0.1% Ural licorice 99.5% ethanol extract, and 0.3% LICONINE. 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 Ural licorice, Ural licorice water extract, Ural licorice 99.5% ethanol extract, or LICONINE. The percentage of each test article in the diet was equivalent to 1% Ural licorice and was calculated based on the extraction rate of Ural licorice.

### 2.4. Animal Study 2: Effects of LICONINE Fractions and Glycyrrhizic Acid

C57BL/6J mice (age, 5 weeks; weight, 20.29  $\pm$  0.10 g [19.03–21.28]) were randomly divided into the following six groups of six mice each: normal, control, 0.38% LICONINE terpenoid fraction, 0.13% LICONINE flavonoid fraction, 0.018% Glycyrrhizic acid, and 0.3% LICONINE. Mice in the normal and control groups received the MF diet and HFS diet, respectively, for 8 weeks. Mice in each experimental group received the HFS diet containing either LICONINE terpenoid fraction, LICONINE flavonoid fraction, Glycyrrhizic acid, or LICONINE for 8 weeks. The percentage of each test article in the diet was equal to the amounts of 4 terpenoids, 4 flavonoids, or Glycyrrhizic acid in 0.3% LICONINE.

Table 1. Constituents' contents of LICONINE, LICONINE terpenoid fraction, and LICONINE flavonoid fraction

Constituents' contents	LICONINE	LICONINE	LICONINE
	% (w/w)	terpenoid fraction % (w/w)	flavonoid fraction % (w/w)
Terpenoids			
Glycyrrhizic acid	5.97	4.25	0.00
Licoricesaponin G2	0.76	0.65	0.00
Licoricesaponin H2	1.17	2.44	0.00
22 $\beta$ -acetoxyglycyrrhizin	1.73	1.52	0.00
Flavonoids			
Liquiritin	3.26	0.00	9.23
Liquiritigenin	0.27	0.00	2.48
Isoliquiritin	0.47	0.00	1.14
Isoliquiritigenin	0.07	0.00	0.46

## 2.5. Animal Study 3: Low Doses of LICONINE

C57BL/6J mice (age, 5 weeks; weight,  $21.29 \pm 0.18$  g [19.55–22.57]) were randomly divided into the following four groups of six mice each: normal, control, 0.1% LICONINE, and 0.2% LICONINE. Mice were fed the MF diet (the normal group), HFS diet (control group), or the HFS diet blended with either 0.1% LICONINE or 0.2% LICONINE (the 0.1% LICONINE or 0.2% LICONINE experimental groups) for 8 weeks.

## 2.6. Animal Study Items

In all studies, the body weight and food intake were measured over time. The mice had free access to each diet and water. 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.

In study 1, histopathological analysis was performed by Applied Medical Research Laboratory (Osaka, Japan). The epididymal fat was fixed in 10% phosphate-buffered formalin, embedded in paraffin, sectioned (4  $\mu$ m thickness), and stained with hematoxylin-eosin for microscopic examination at 10-fold magnification. The number of crown-like structures (CLS) and size of adipocytes were measured per field of view (i.e., in an area 0.561596 mm<sup>2</sup>) and one field was assessed for each sample.

## 2.7. MCP-1 Production in 3T3-L1 Mouse Adipocytes

3T3-L1 mouse adipocytes (JCRB9014, JCRB Cell Bank, Osaka, Japan) were seeded at  $3.5 \times 10^4$  cells/well in a 12-well plate and incubated overnight at 37°C under 5% CO<sub>2</sub> in DMEM supplemented with 10% NCS. The following day, cells were cultured in DMEM supplemented with 10% FBS for 3 days and then cultured in adipocyte differentiation medium (0.5 mM Isobutyl-methylxanthine, 1  $\mu$ M Dexamethasone, 1  $\mu$ g/mL Insulin in DMEM supplemented with 10% FBS) for 2 days, cultured in DMEM supplemented with 10% FBS for 6 days, cultured in serum-free DMEM for a day, and then in serum-free DMEM with 5 ng/mL tumor necrosis factor (TNF)- $\alpha$  for 24 h. Test article (Ural licorice extracts, LICONINE fractions, or constituents of LICONINE terpenoid fraction) or DMSO was added at 18 h before TNF- $\alpha$  stimulation. The cell supernatant was collected at 24 h after TNF- $\alpha$  stimulation, and then stored frozen at -30°C until used. The level of MCP-1 in the cell supernatant was measured using a Mouse MCP-1 DuoSet ELISA Kit (DY479, R&D Systems, Minneapolis, MN, USA). Test article (dissolved in DMSO to 200 mg/mL), DMSO for vehicle control, and the TNF- $\alpha$  control were diluted more than 2000 times with serum-free DMEM. The cell viability rate was measured using Cell Count Reagent SF (07553-15, Nacalai Tesque, Osaka, Japan).

## 2.8. Statistical Analysis

The results are expressed as mean  $\pm$  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 [12]. For statistical analysis, both analysis of variance (ANOVA) and multiple comparison by Ryan's method were used [13,14]. Compared values were considered significantly different when  $p$  was less than 0.05. Dose response relationships were analyzed by Pearson's product moment correlation coefficient.

## 3. Results

### 3.1. Animal Study 1: LICONINE at the Intake Level of Low Risk was the Most Effective Extract for Treating Obesity

At first, we examined the effects of Ural licorice and its extracts (at intake levels of low risk) on HFS diet-induced obese mice. The percentage of each test article added to diet was equivalent to that added in 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 [15]. The human equivalent of 1 g/kg per day (the intake level of Ural licorice by animals) is 1 g per day, which is the intake level of low risk. The body weight and visceral fat weight were increased in the control group compared with the normal group (Figure 1 and Table 2); decreased in the Ural licorice, Ural licorice 99.5% ethanol extract, and LICONINE groups compared with the control group, and were similar between the Ural licorice water extract and control groups. The food intake by all experimental groups was similar to that by the control group (data not shown). The number of CLS and adipocyte size in the visceral fat were higher in the control group than the normal group (Table 2 and Figure 2). Compared with the control group, the Ural licorice, Ural licorice water extract, and LICONINE groups had fewer CLS; the LICONINE group had smaller adipocytes; however, the Ural licorice water extract and Ural licorice 99.5% ethanol extract groups had larger adipocytes. These data indicated that LICONINE at the intake level of low risk was the most effective extract because it decreased body weight, visceral fat weight, number of CLS, and size of adipocytes in the visceral fat.

### 3.2. Animal Study 2: The Terpenoid Fraction of LICONINE had Anti-obesity Effect

Next, the effects of LICONINE fractions and Glycyrrhizic acid were examined in HFS diet-induced obese mice. The body and visceral fat weights were lower in LICONINE terpenoid fraction group than the control group (Table 3) but similar between LICONINE flavonoid fraction, Glycyrrhizic acid, and control groups. These data indicated that the terpenoid fraction, but not the flavonoid fraction, of LICONINE was effective, and that this anti-obesity effect in HFS diet-induced obese mice was not totally dependent on Glycyrrhizic acid.

### 3.3. Animal Study 3: Low Doses of LICONINE had Anti-obesity Effects

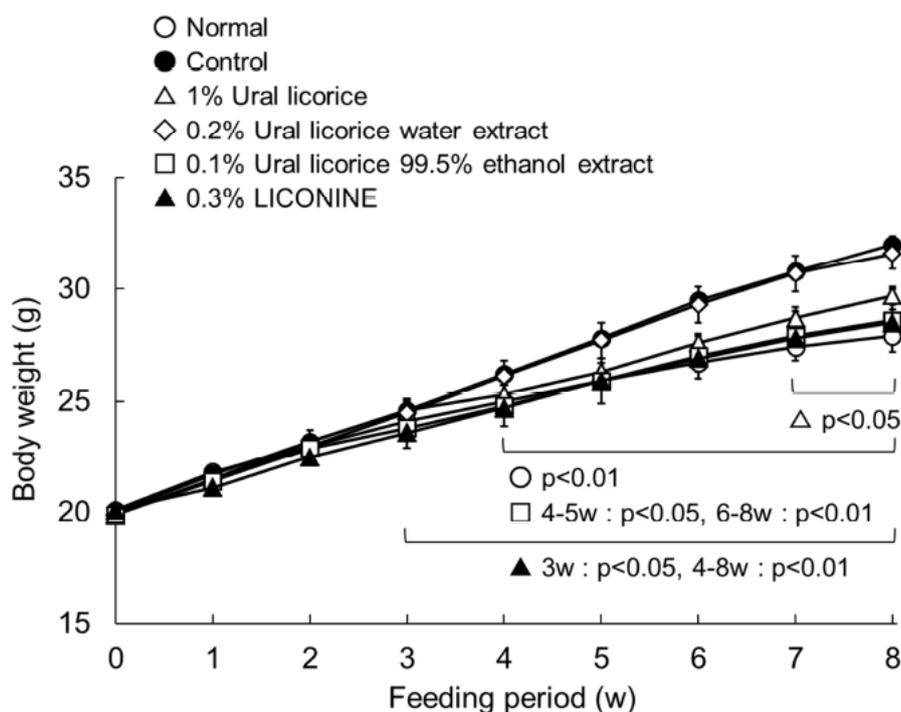
Effects of low doses of LICONINE on HFS diet-induced

obese mice were examined. The body and visceral fat weights of groups given low doses of LICONINE were decreased compared with the control group (Table 4). These data indicated that even low doses of LICONINE were effective in HFS diet-induced obese mice.

### 3.4. LICONINE and its Terpenoid Fraction had Anti-inflammatory Effects on 3T3-L1 Adipocytes

Effects of Ural licorice extracts, LICONINE fractions, and constituents of LICONINE terpenoid fraction on MCP-1 production in 3T3-L1 mouse adipocytes were examined. Cell viability fell to 16% in the presence of Ural licorice 99.5% ethanol extract (50  $\mu\text{g}/\text{mL}$ ), but not in the presence of Ural licorice 99.5% ethanol extract (20  $\mu\text{g}/\text{mL}$ ) or other test articles (50  $\mu\text{g}/\text{mL}$ ; data not

shown). The level of MCP-1 production was increased by stimulating cells with TNF- $\alpha$  (Figure 3) but decreased dose-dependently in TNF- $\alpha$ -stimulated cells, relative to the TNF- $\alpha$  control level, by adding LICONINE (Figure 3A and Figure 3B), Ural licorice 99.5% ethanol extract (Figure 3A), LICONINE terpenoid fraction (Figure 3B), and constituents of LICONINE terpenoid fraction, Licorice saponin G2, Licorice saponin H2, and Glycyrrhizic acid (Figure 3C), but not by adding Ural licorice water extract or LICONINE flavonoid fraction. These data indicated that LICONINE and its terpenoid fraction had anti-obesity and anti-inflammatory effects in HFS diet-induced obese mice and 3T3-L1 adipocytes. Licorice saponin G2, Licorice saponin H2, and Glycyrrhizic acid (all constituents of LICONINE terpenoid fraction) had anti-inflammatory effects in 3T3-L1 adipocytes.



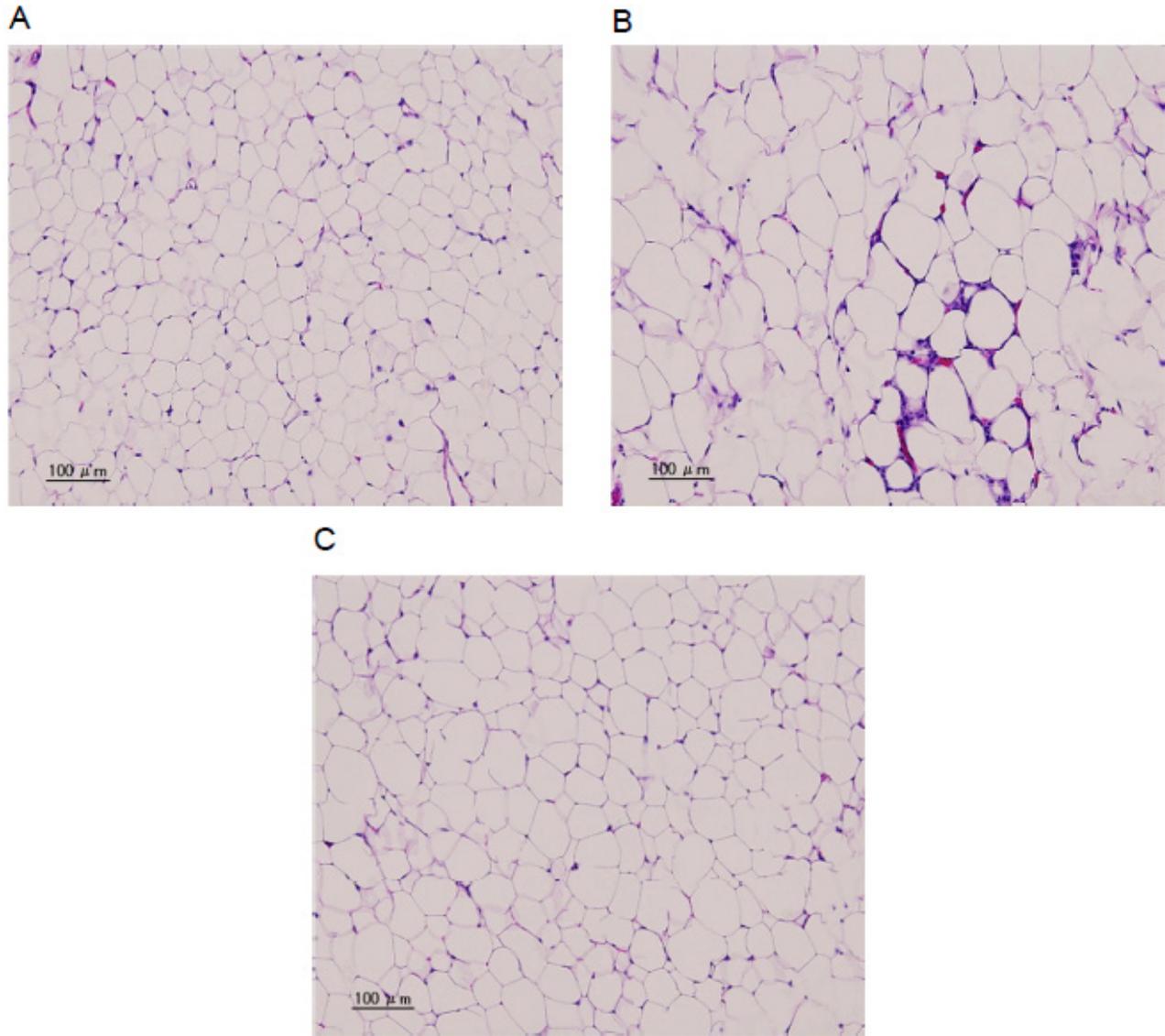
The body weight in mice fed MF or HFS diet with or without test articles for 8 weeks are shown. A significant difference was seen between the control group and the other group by ANOVA and Ryan's method.

Figure 1. Effects of Ural licorice and its extracts on the body weight in mice fed HFS diet

Table 2. Effects of Ural licorice and its extracts on the body weight, visceral fat weight, number of CLS, and adipocyte size in the visceral fat in mice fed HFS diet

Group	Number of mice				Body weight (g)	Visceral fat weight (g)	Number of CLS	Adipocyte size ( $\mu\text{m}^2$ )
	Experiment number							
	1	2	3	Total				
Normal	5	6	8	19	27.9 $\pm$ 0.4 **	1.05 $\pm$ 0.10 **	2.0 $\pm$ 0.5 **	1883 $\pm$ 73 **
Control	5	5	6	16	32.0 $\pm$ 0.4 ##	2.57 $\pm$ 0.11 ##	13.3 $\pm$ 3.9 ##	3205 $\pm$ 109 ##
1% Ural licorice	5	-	-	5	29.7 $\pm$ 0.4 *	1.89 $\pm$ 0.11 ** ##	2.0 $\pm$ 0.9 *	2680 $\pm$ 68 ##
0.2% Ural licorice water extract	5	-	-	5	31.6 $\pm$ 0.7 ##	2.66 $\pm$ 0.15 ##	2.2 $\pm$ 0.7 *	5379 $\pm$ 55 ** ##
0.1% Ural licorice 99.5% ethanol extract	5	-	-	5	28.6 $\pm$ 1.4 **	1.87 $\pm$ 0.26 ** ##	5.6 $\pm$ 1.5	4344 $\pm$ 569 ** ##
0.3% LICONINE	-	6	8	14	28.5 $\pm$ 0.6 **	1.58 $\pm$ 0.13 ** ##	4.2 $\pm$ 1.3 *	2406 $\pm$ 127 ** #

The body weight, visceral fat weight, number of CLS, and adipocyte size in the visceral fat in mice fed MF or HFS diet with or without test articles at 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 other group by ANOVA and Ryan's method.



The histopathological image of visceral fat of the groups of the normal (A), control (B), and LICONINE (C) in mice fed MF or HFS diet with or without test articles at 8 weeks are shown (hematoxylin-eosin staining, 10×)

**Figure 2.** Effect of LICONINE on the visceral fat in mice fed HFS diet

**Table 3.** Effects of LICONINE fractions and Glycyrrhizic acid on the body weight and visceral fat weight in mice fed HFS diet

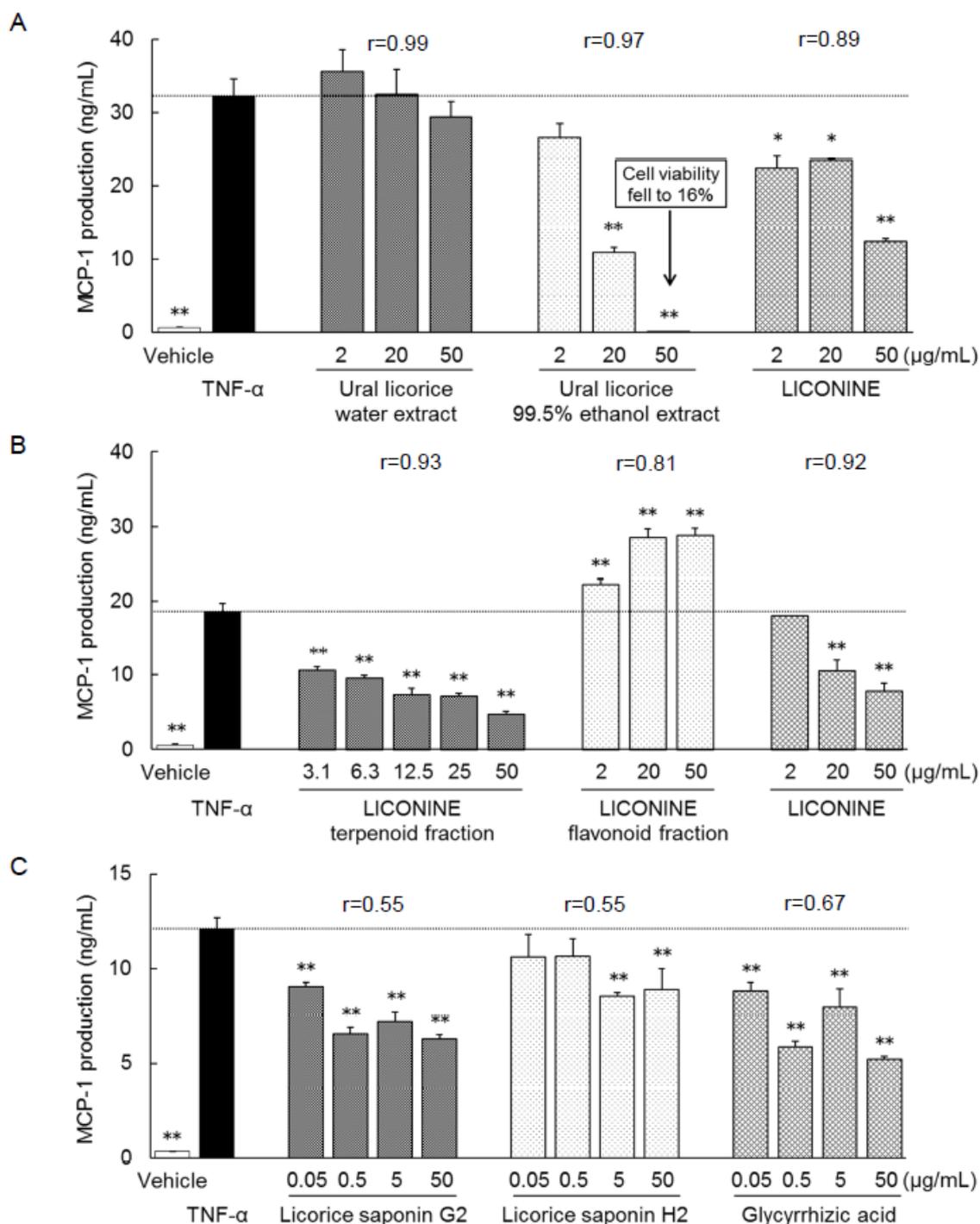
Group	Body weight				Visceral fat weight			
	(g)				(g)			
Normal	27.9	±	0.6	**	0.79	±	0.09	**
Control	31.7	±	0.8	##	2.27	±	0.17	##
0.38% LICONINE terpenoid fraction	28.1	±	0.1	*	1.31	±	0.10	**
0.13% LICONINE flavonoid fraction	31.1	±	0.8	#	1.85	±	0.34	##
0.018% Glycyrrhizic acid	31.7	±	0.9	##	2.50	±	0.23	##
0.3% LICONINE	27.4	±	0.7	**	1.13	±	0.15	**

The body weight and visceral fat weight in mice fed MF or HFS diet with or without test articles at 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 other group by ANOVA and Ryan's method.

**Table 4.** Effects of low doses of LICONINE on the body weight and visceral fat weight in mice fed HFS diet

Group	Body weight				Visceral fat weight			
	(g)				(g)			
Normal	27.7	±	0.8	**	1.01	±	0.09	**
Control	32.6	±	0.9	##	2.72	±	0.25	##
0.1% LICONINE	29.7	±	0.8	*	2.06	±	0.23	* ##
0.2% LICONINE	28.5	±	0.9	**	1.50	±	0.20	**

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



The effects of Ural licorice extracts (A), LICONINE fractions (B), and constituents of LICONINE terpenoid fraction (C) on MCP-1 production in 3T3-L1 mouse adipocytes are shown. A significant difference was seen between the TNF- $\alpha$  control value and the other value by ANOVA and Ryan's method ( $^* p < 0.05$ ,  $^{**} p < 0.01$ ). Dose response relationships were analyzed by Pearson's product moment correlation coefficient.

**Figure 3.** Effects of Ural licorice extracts, LICONINE fractions, and constituents of LICONINE terpenoid fraction on MCP-1 production in 3T3-L1 mouse adipocytes

## 4. Discussion

In the present study, we examined the anti-obesity effects of Glycyrrhizic acid and Ural licorice, its extracts, and LICONINE fractions (all at intake levels of low risk) in HFS diet-induced obese mice, and the effects of Ural licorice extracts and LICONINE fractions, and constituents of LICONINE terpenoid fraction on MCP-1 production in 3T3-L1 mouse adipocytes.

At the intake level of low risk, licorice (less than 1 g per day) or licorice extract (equivalent to less than 1 g

licorice per day) is regarded as a food and can be used as medicine without requiring notification of side effects [2]. Although the anti-obesity effect of Ural licorice or its extract has been studied, the anti-obesity effect at the intake level of low risk remains unclear. It has been reported that intake of Ural licorice at 3.5 g per day for 2 months reduced body fat mass in humans [7]. Body weight in HF diet-induced obese mice has been decreased by the administration of Ural licorice extract at 80 mg per day [8] and by high-dose dietary intake of 0.5%

Isoliquiritigenin for 20 weeks [9,10]. Ural licorice 17% (human equivalent to 17 g per day approximately) contains 0.5% Isoliquiritigenin, which is present in low quantity in Ural licorice 0.03% [16]. These studies indicated that Ural licorice or its extract has anti-obesity effects but at levels disqualifying its use as a food. Also, the administration of 1 mg/kg (human equivalent to 1 mg per day approximately) Ural licorice extract for 4 weeks had no effect on body weight in diabetic mice [11]. In the present study, Ural licorice extract ingested at the intake level of low risk had no anti-obesity effect. We found that LICONINE at the intake level of low risk decreased body weight, visceral fat weight, number of CLS, and adipocyte size in the visceral fat of HFS diet-induced obese mice, making it the most effective extract tested. We also found that low doses (and therefore safer doses) of LICONINE also had anti-obesity effects.

In our study, the effect in HFS diet-induced obese mice was identified with the terpenoid fraction but not the flavonoid fraction of LICONINE and did not depend exclusively on Glycyrrhizic acid. The other constituents, such as Licorice saponin G2, Licorice saponin H2, and 22 $\beta$ -acetoxyglycyrrhizin in LICONINE terpenoid fraction, and Isoliquiritigenin, Liquiritigenin, Isoliquiritigenin, and Liquiritigenin in LICONINE flavonoid fraction, at intake levels of low risk also did not have anti-obesity effects in HFS diet-induced obese mice (data not shown). These results indicate that multiple constituents of LICONINE terpenoid fraction may be cooperating to exert anti-obesity effects in HFS diet-induced obese mice.

Our results demonstrate for the first time that the terpenoids Licorice saponin G2, Licorice saponin H2, and Glycyrrhizic acid have anti-inflammatory effects in 3T3-L1 adipocytes. We also confirmed that the terpenoid 22 $\beta$ -acetoxyglycyrrhizin failed to suppress MCP-1 production in 3T3-L1 adipocytes (data not shown). Thus, the combination of Licorice saponin G2, Licorice saponin H2, and Glycyrrhizic acid may be key to anti-obesity effectiveness in HFS diet-induced obese mice.

In our study, LICONINE and its terpenoid fraction had anti-obesity and anti-inflammatory effects in HFS diet-induced obese mice and 3T3-L1 adipocytes. LICONINE suppressed both of the number of CLS in HFS diet-induced obese mice and MCP-1 production in 3T3-L1 adipocytes. CLS are the histological hallmark of low-grade chronic inflammation (which is characterized by accumulation of lymphocytes, macrophages, and other immune cells around adipocytes in the adipose tissue of obese humans and mice) and leads to metabolic disease [17,18,19]. Enlarged adipocytes release proinflammatory cytokines such as interleukin (IL)-6, IL-8, and MCP-1. Additionally, inflammatory macrophages release TNF- $\alpha$  [20]. TNF- $\alpha$  activates nuclear factor- $\kappa$ B (NF- $\kappa$ B) signaling pathways, and NF- $\kappa$ B upregulates the expression of TNF- $\alpha$  and MCP-1 genes [21]. It has been reported that HF diet-induced macrophage accumulation in adipose tissue is reduced in MCP-1 knockout mice [22]. LICONINE may suppress the formation of CLS through the suppression of MCP-1 production in adipocytes. Mice lacking the p50 subunit of NF- $\kappa$ B were resistant to HF diet-induced fat accumulation and adipose tissue inflammation [23]. LICONINE and its terpenoid fraction may reduce obesity through anti-inflammatory effects in adipocytes.

In summary, LICONINE is an effective extract of Ural licorice at the intake level of low risk (human equivalent to less than 1 g licorice per day), and the terpenoid fraction is an effective fraction of LICONINE for treating obesity. Further research is needed to identify the mechanism underlying the anti-obesity and anti-inflammatory effects of LICONINE and its terpenoid fraction.

## Conflicts of Interest

The authors declare that there are no conflicts of interest.

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