ORIGINAL

Goishi tea leaf powder affects lower body weight and visceral fat accumulation during energy restriction in obese rats

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Abstract : Goishi tea is a unique Japanese post-fermented tea produced in Kochi prefecture. The aim of this study was to investigate whether the supplementation of energy-restricted diet with Goishi tea leaves affects body weight, visceral fat accumulation, and fecal lipids in diet-induced obese rats. 18 male Wistar rats were fed a high-fat diet for 12 weeks. Subsequently, the diet-induced obese rats were fed a low-energy diet containing 1% (G1 group) or 3% (G3 group) of Goishi tea leaf powder, or without any tea extracts (C group) for 4 weeks. After 4 weeks, body weight and body fat ratio were significantly lower in the G3 group than in the C group. Plasma insulin levels were significantly higher in the C group than in the G1 and G3 groups, whereas plasma leptin levels were significantly lower in the G3 group than in the C group. In addition, the lipid absorption rate was significantly lower in the G3 group than in the C and G1 groups. In conclusion, the administration of Goishi tea leaves under dietary restrictions might contribute to body weight reduction and inhibition of lipid absorption, as a diet therapy to help prevent obesity and metabolic syndrome. J. Med. Invest. 70 : 60-65, February, 2023

Keywords : *Goishi tea, obesity, fat accumulation, energy restriction*

INTRODUCTION

Obesity is regarded as a risk factor for lifestyle-related diseases such as dyslipidemia, type 2 diabetes, and hypertension, and its prevalence is increasing in several countries. Currently, approximately 33% of men and 22% of women aged ≥20 years in Japan are obese (body mass index ≥25 kg/m^2). Excessive adipose tissue accumulation in obesity occurs due to overeating or lack of exercise. Obesity is difficult to treat ; therefore, it is important to prevent it by increasing energy expenditure over intake. In recent years, various foods have been observed to have potential health benefits ; in particular, tea, as a beverage, has been recognized to prevent obesity and metabolic disorders (1, 2).

Tea is the most popular and widely consumed beverage worldwide and is classified into two groups according to the production method : non-fermented tea and fermented tea. Non-fermented tea, such as green tea, is produced by harvesting tea leaves along with the inactivating enzymes. Fermented tea is further subdivided into two types : pre-fermented (oolong and black teas) and post-fermented teas (Pu-erh tea). Pre-fermented tea is produced by the enzymes of tea leaves, whereas post-fermented tea deactivates the enzyme function of picked tea leaves and then fermented with microorganisms. Currently, various kinds of post-fermented teas, such as Batabata-cha, Ishizuchi-kurocha, Awa-bancha, and Goishi-cha (Goishi tea) are produced in Japan, although this traditional practice has been declining.

Previous studies have focused on beverages like green tea, which contains catechins, for weight loss and obesity management. However, it has been recently shown that post-fermented teas contribute to reduced visceral fat accumulation and modified body weight (3, 4). Goishi tea, a traditional post-fermented tea from Kochi Prefecture, Japan, is produced by a two-stage fermentation process, involving aerobic and anaerobic fermentation. The first stage of fermentation under aerobic conditions involves mold (*Aspergillus fumigatus, Penicillium sp*., and *Scopulariopsis brevicaulis*), and the secondary anaerobic fermentation involves lactic acid bacteria (*Lactobacillus plantarum*) (5). Since post-fermented teas involve microorganisms in the manufacturing process, the ingredients are different from those of non-fermented teas, and more beneficial effects can be expected compared to other teas. For example, Goishi tea contains many unique aroma compounds, such as 4-ethylphenol, aldehydes, and caproic acids, which are rarely found in green teas (6).

Several animal and human studies have shown that Goishi tea may inhibit health risks, including hyperlipidemia and arteriosclerosis (7, 8). Furthermore, a recent study has indicated that Goishi tea consumption is associated with lower adipose tissue weight and decreased concentrations of serum total cholesterol and blood glucose in obese mice (9). However, to the best of our knowledge, no research has been conducted on the effects of Goishi tea on weight during diet restriction. We hypothesized that adding Goishi tea to a low-energy diet could be expected to synergistically reduce body weight and visceral fat accumulation. Furthermore, it remains unclear whether oral ingestion of Goishi tea leaves, rather than its aqueous extracts, brings about the same obesity-suppressing effect. Recent studies have reported that oral feeding 1.5% and 4% green, oolong, black, and pu-erh tea leaves to SD rats for 30 weeks could lower the serum concentrations of total cholesterol and lipoprotein cholesterol levels (10). Our previous study also demonstrated that compared to a low-energy diet, oral ingestion of 3.0% Awa-bancha leaves combined with a low-energy diet significantly reduced body weight and prevented fat absorption in obese rats (11). It is very encouraging to find that Goishi tea leaves can further reduce body weight and fat mass during diet restriction. The purpose

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of this study was to examine whether the consumption of Goishi tea leaf powder combined with an energy-restricted diet can further reduce body weight, visceral fat accumulation, and fecal lipids in diet-induced obese rats.

MATERIALS AND METHODS

Polyphenol composition of the tea extracts

Crude Goishi tea leaf extract (Yamane-en Co., Kochi, Japan) was prepared by adding 3 g of tea leaves to 200 mL of boiling water for 5 min, according to a general preparation method. The crude extract was filtered using a 0.45 µm pore size syringe filter. Green tea samples (ITO EN Ltd, Tokyo, Japan) were also extracted and filtered using the same method. The catechin and caffeine compositions of these tea extracts were analyzed by high-performance liquid chromatography (HPLC) following a modified procedure, as mentioned in a previous study (12). The compounds were determined by HPLC (Prominence ; Shimadzu, Co., Kyoto, Japan), using a YMC-ODS column (YMC-Pack φ4.6 × 250 mm, S-5 µm ; YMC Co., Kyoto, Japan), UV detection at 270 nm, mobile solvents of acetonitrile and 10 mM sodium phosphate buffer pH 2.6 (0-6 min ; 7% acetonitrile, 6-20 min ; 7%→20% acetonitrile, 20-25 min ; 50% acetonitrile), at a flow rate of 1 mL/min and a column temperature of 40℃. The catechin and caffeine compositions are shown in Table 1.

Table 1. Catechin and caffeine compositions of Goishi tea and green tea.

(mg/100mL)	Goishi tea	Green tea
Catechins		
C	0.46	0.55
EC	0.23	17.74
ECg	0.56	14.81
EGC	0.19	17.00
EGCg	0.28	45.79
Total	1.72	95.89
Caffeine	4.13	6.43

C, catechin ; EC, epicatechin ; ECg, epicatechin gallate ; EGC, epigallocatechin ; EGCg, epigallocatechin gallate.

Animals and experimental protocols

Seven-week-old male Wistar rats (n = 18) purchased from Charles River Laboratories Japan (Atsugi, Kanagawa, Japan) were housed in individual stainless-steel cages under a controlled temperature of 23 ± 2℃ and relative humidity of 50%, with a 12-h-light-dark cycle. All rats were fed a preliminary diet for 1 week before the start of the experiments. After adaptation, the rats were allowed to freely consume a high-fat diet (48% energy from fat ; 100 kcal/day), in which lard was added to a standard stock diet (Oriental Yeast, Osaka, Japan) for 12 weeks. The diet-induced obese rats were then randomly divided into three groups of six rats each and fed a 20% casein control diet (C group) or a 20% casein diet supplemented with either 1% Goishi tea leaf powder (G1 group) or 3% Goishi tea leaf powder (G3 group). We administered 16 g of the experimental diets each day (11% energy from fat ; 60kcal/day), and they were consumed entirely by all the rats. These experimental diets were limited to 60% of the energy intake from the high-fat diet and were administered for 4 weeks. The nutritional compositions of the experimental diets are shown in Table 2. At the end of the experiment, all rats were fasted overnight and sacrificed under anesthesia. Blood was collected from the inferior vena cava, and the carcasses were stored frozen at -80℃ until analysis. Moreover, the liver, stomach, kidney, gastrocnemius muscle, and adipose tissue, including the perinephric, epididymal, and mesenteric, were removed and weighed. Feces were collected 3 days before dissection. All procedures were performed in accordance with the Guide for the Care and Use of Laboratory Animals at Shikoku University and were approved by the Committee of the Care and Use of Laboratory Animals at Shikoku University.

Table 2. Composition of experimental diets

(g/100g diet)	Group		
	C	G1	G3
Casein	20.0	20.0	20.0
α-Corn starch	45.7	45.0	43.7
Sucrose	22.8	22.5	21.8
Corn oil	5.0	5.0	5.0
AIN-93 vitamin mixture	1.0	1.0	1.0
AIN-93G mineral mixture	3.5	3.5	3.5
Cellulose	2.0	2.0	2.0
Goishi tea powder[a]	0	1.0	3.0

[a]Commercially available dry leaves of Goishi tea (Yamane-en Co., Kochi, Japan) were powdered using a mixer.

Plasma biochemical parameters

Plasma glucose, triglyceride (TG), total cholesterol (TC), and high-density lipoprotein-cholesterol (HDL-C) levels were determined using Fuji Dri-Chem3030 (Fuji Film Med. Co., Ltd., Tokyo, Japan). Plasma insulin, leptin, and adiponectin levels were measured using rat insulin enzyme-linked immunosorbent assay (ELISA) kits, rat leptin ELISA kit, and rat adiponectin ELISA kit, respectively (Shibayagi, Gunma, and Wako, Osaka, Japan).

Whole body fat and fecal lipids

The fat ratios in the carcasses were determined with reference to a previous study (13) ; that is, the carcasses were dried at 105℃ for 24 h and then homogenized using a mixer. The fat content was measured by extraction with diethyl ether using a Soxhlet apparatus. Fecal lipid levels were measured using the same method.

Statistical analysis

Statistical analyses were performed using R 4.0.2 for Windows. All data are expressed as mean ± standard deviation (SD). All data were analyzed using a one-way analysis of variance (ANOVA). Significant differences between groups were established using Tukey's honestly significant difference (HSD) test. Differences were considered significant at $p<0.05$.

RESULTS

Body weight and body composition

After 12 weeks of administering the high-fat diet, the mean body weight was 532 ± 36 g in the C group, 536 ± 29 g in the G1 group, and 535 ± 41 g in the G3 group (Table 3). The weight loss and body fat ratios after consuming the experimental diets for 4 weeks are shown in Fig. 1. Body weight was significantly lower in the G3 group than in the C group ($p<0.05$). At the end of the experimental period, weight loss of 3.2%, 4.2%, and 7.3% was observed in the C, G1, and G3 groups, respectively. Moreover, the body fat ratio was significantly lower in the G3 group than in the C and G1 groups. However, there were no significant differences in the liver, stomach, kidney, and gastrocnemius muscles among the three groups (data not shown). The effects of the experimental diets for 4 weeks on visceral fat weight are shown in Table 4. The weight of the perinephric adipose tissue was significantly lower in the G3 group than in the C and G1 groups ($p<0.05$). The weight of the epididymal adipose tissue was significantly lower in the G3 group than in the G1 group ($p<0.05$). In addition, total adipose tissue weight was significantly lower in the G3 group than in the C and G1 groups ($p<0.05$).

Plasma glucose and lipid concentrations

Table 5 shows the plasma glucose and lipid concentrations after consuming the experimental diets for 4 weeks. Fasting blood glucose, TG, and TC concentrations in the plasma did not differ among the three groups, whereas plasma HDL-C concentrations in the G3 group were significantly lower than those in the C and G1 groups ($p<0.05$).

Plasma insulin, leptin, and adiponectin levels

The results of the comparison of plasma insulin, leptin, and adiponectin levels after consuming the experimental diets for 4 weeks are shown in Fig. 2. Plasma insulin levels were significantly higher in the C group than in the G1 and G3 groups ($p<0.05$). Plasma leptin levels were significantly lower in the G3 group than in the C group ($p<0.05$).

Lipid absorption

The effects of the experimental diet on the total amount of ingested and fecal lipids are shown in Table 6. Total fecal lipid weight was significantly higher in the G3 group than in the

Table 3. Growth parameters of three groups.

	Group		
	C	G1	G3
Initial body weight (g)	532 ± 36	536 ± 29	535 ± 41
Final body weight (g)	515 ± 31	513 ± 27	495 ± 37
Food intake (g/day)	16	16	16

Data are means ± SD (n = 6).

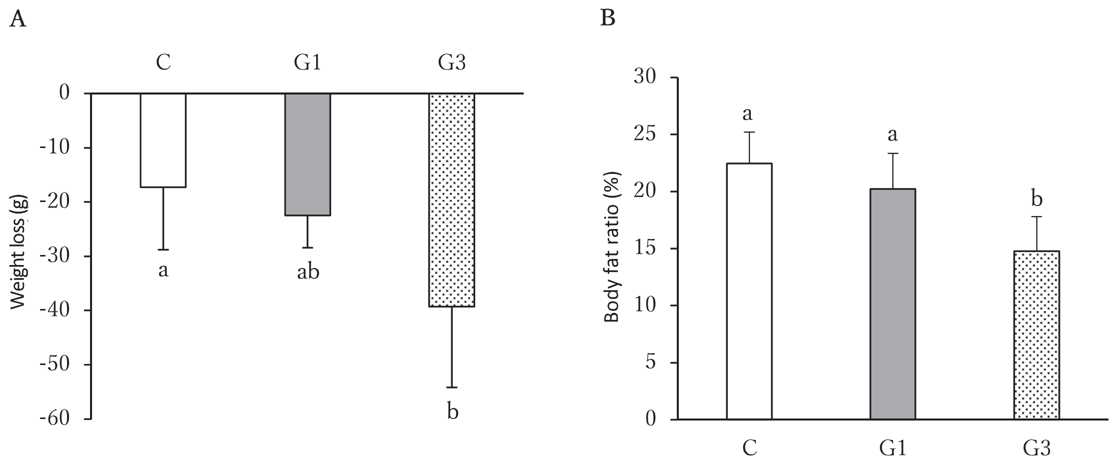

Fig 1. Comparisons of the effects of powder Goishi tea on (A) weight loss and (B) body fat ratio in obese rats fed a control, G1, and G3 diet for 4 weeks. Mean values within each group with different superscripts are significantly different ($p<0.05$).

Table 4. Comparisons of the effects of powder Goishi tea on visceral fat weight in obese rats fed a control, G1, and G3 diet for 4 weeks.

	Group		
	C	G1	G3
Perinephric (g)	12.52 ± 3.63^{a}	12.26 ± 2.08^{a}	7.36 ± 1.26^{b}
Epididymal (g)	10.91 ± 3.67^{ab}	12.23 ± 1.44^{a}	8.12 ± 2.63^{b}
Mesenteric (g)	4.67 ± 1.38	4.23 ± 0.82	3.54 ± 0.80
Total (g)	28.10 ± 8.48^{a}	28.72 ± 2.72^{a}	19.02 ± 4.37^{b}

Data are means ± SD (n = 6).
Mean values within each group with different superscripts are significantly different ($p<0.05$).

Table 5. Comparisons of the effects of powder Goishi tea on plasma glucose and lipids in obese rats fed a control, G1, and G3 diet for 4 weeks.

	Group		
	C	G1	G3
Fasting blood glucose (mg/dL)	136.1 ± 12.9	131.7 ± 18.1	139.0 ± 8.9
TG (mg/dL)	82.5 ± 25.9	75.8 ± 33.9	68.2 ± 28.4
TC (mg/dL)	84.4 ± 8.3	77.2 ± 12.4	75.6 ± 14.0
HDL-C (mg/dL)	51.6 ± 4.3^a	56.3 ± 4.0^a	45.3 ± 4.3^b

TG, triglyceride; TC, total cholesterol; HDL-C, high-density lipoprotein cholesterol
Data are means ± SD (n = 6).
Mean values within each group with different superscripts are significantly different (p<0.05).

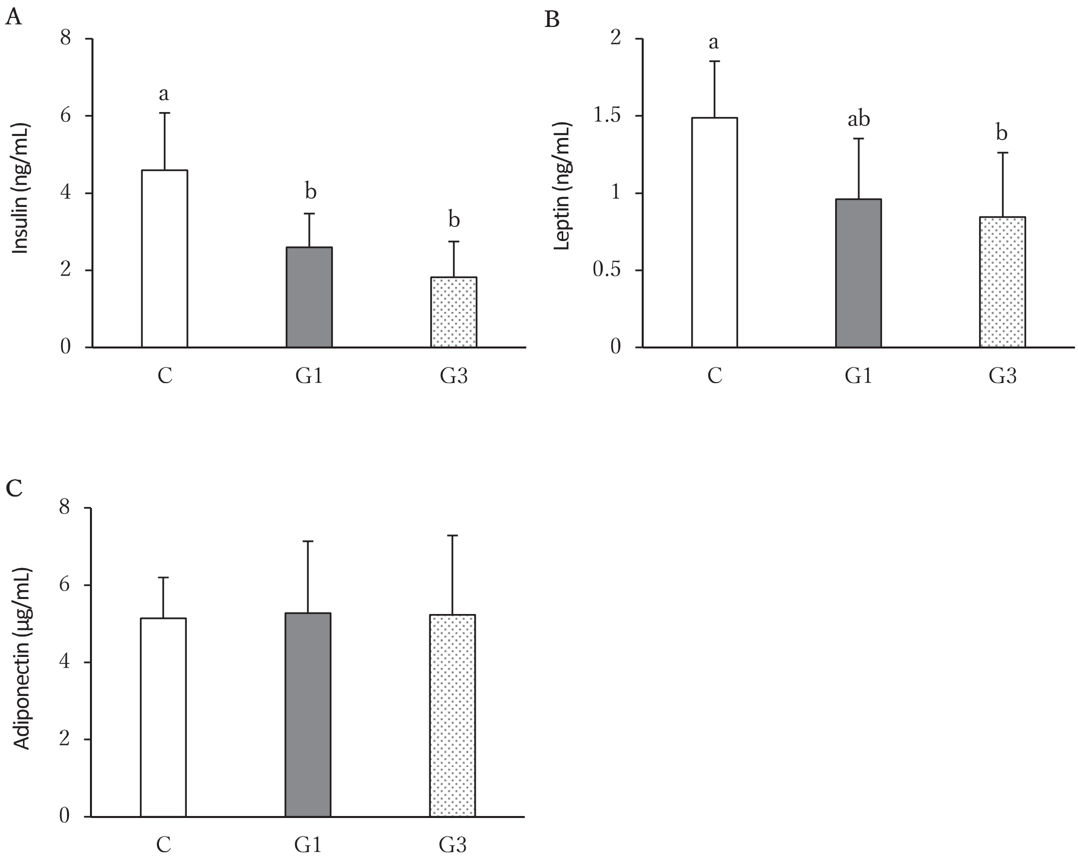

Fig 2. Comparisons of the effects of powder Goishi tea on (A) insulin, (B) leptin, and (C) adiponectin in obese rats fed a control, G1, and G3 diet for 4 weeks. Mean values within each group with different superscripts are significantly different (p<0.05).

Table 6. Comparisons of the effects of powder Goishi tea on lipid absorption in obese rats fed a control, G1, and G3 diet for 4 weeks.

	Group		
	C	G1	G3
Total amounts of ingested lipids (g/3d)	2.39	2.39	2.39
Total amounts of fecal lipids (g/3d)	0.11 ± 0.02^b	0.13 ± 0.03^b	0.18 ± 0.02^a
Lipid absorption rate (%)	95.35 ± 0.87^a	94.76 ± 1.22^a	92.36 ± 0.91^b

Data are expressed as mean ± SD (n = 6). Mean values within each group with different superscripts are significantly different (p<0.05).

C and G1 groups ($p<0.05$). The lipid absorption rate was significantly lower in the G3 group than in the C and G1 groups ($p<0.05$).

DISCUSSION

Evidence is mounting to suggest that tea regulates body weight and body fat accumulation, and may help prevent obesity and obesity-related complications. Hence, we conducted an animal study using diet-induced obese rats to investigate whether 4 weeks of Goishi tea leaf powder administration affects body weight and visceral fat accumulation. The present results showed that the G3 group had the highest body weight reduction and significantly reduced body fat ratio and visceral fat weight compared with the C group. Moreover, the total fecal lipid weight in the G3 group was significantly higher than that in the C group. Thus, we elucidated that diets supplemented with Goishi tea leaves enhanced body weight reduction and inhibited lipid absorption, supporting the hypothesis that ingesting Goishi tea leaves under dietary restrictions enhances the improvement of obesity.

Mitsuhiko Miyamura and his colleagues have reported that Goishi tea has potential health benefits in the prevention and treatment of many diseases, such as influenza infection, and hypercholesteremia (7, 14). Furthermore, this research group has indicated that Goishi tea consumption is associated with lower adipose tissue weight and decreased concentrations of serum TC and blood glucose in obese mice (9). Another study has also reported that the supplementation of Goishi tea in diet-induced obese mice resulted in the inhibition of the effect of serum insulin and tumor necrosis factor-alpha (TNF-α) on adipocytokines (15). Our study is in close agreement with the above studies ; total adipose tissue weight was lower in the G3 group than in the C group, and insulin concentration was also significantly reduced in the G3 and G1 groups. Importantly, our results showed that the body fat-reducing effect was enhanced by ingesting tea powder under dietary restrictions ; that is, Goishi tea leaves may help treat obesity while on a diet. In the future, it will be necessary to examine in more detail, the differences in body weight and visceral fat weight between individuals consuming diets supplemented with tea extract and leaves.

Interestingly, the G3 group had the highest total 3-day fecal lipid content despite the three groups having the same dietary intake and consistent total lipid intake. This observation suggests that Goishi tea inhibits the absorption of lipids in the intestinal tract. Muramatsu *et al*. reported that the fecal excretion of total lipids and cholesterol increased in rats that were fed a lard-cholesterol diet containing 2% tea catechins, compared with the control diet group (16). Tea catechins inhibit gastric and pancreatic lipases in vivo and in vitro (17, 18). In particular, the most abundant catechin found in green tea is epigallocatechin gallate (EGCg), which decreases plasma cholesterol and inhibits intestinal absorption of lipids (2, 19). However, the experimental results showed that Goishi tea contains low quantities of catechins. It is generally recognized that fermented teas such as black, oolong, and Pu-erh teas also have fewer catechins than green tea, but several animal studies have shown that fermented teas may also affect lipid absorption. Previous study reported that rats with a high-fat diet that were orally administered black tea decoction significantly increased fecal triglyceride level compared to those given green tea decoction (20). Another study reported that aqueous extract of Chinese post-fermented tea dose-dependently raises the levels of total cholesterols and triglycerides in the feces of hyperlipidemic rats (21). It was demonstrated that Chinese post-fermented teas increased the expression of carnitine palmitoyltransferase 1 (CPT1), which indicated the increase of β-oxidation of fatty acid (21). Furthermore, an in vitro cell study indicated that black tea theaflavins seem to suppress the activity of fatty acid synthase (FAS), which is an important enzyme in energy metabolism (22). Further research is needed to elucidate the detailed mechanism of Goishi tea fat oxidation.

The fermentation process for producing Goishi tea mainly involves *Lactobacillus plantarum*, *Aspergillus fumigatus*, and *Penicillium sp.* (5). Similarly, yogurt, kimchi, and pickles are fermented foods produced by lactic acid bacteria, which are closely associated with the prevention of obesity and metabolic diseases. For example, the supplementation of yogurt fermented by *Lactobacillus plantarum* Q180 in the diet of rats fed a high-fat diet for 8 weeks decreased adipocyte size and leptin (23). In a recent clinical crossover study, obese patients consuming fermented kimchi (300 g/day) for 4 weeks had significantly lower percent body fat, waist-hip ratio, serum TC levels, and fasting blood glucose levels than patients who consumed fresh kimchi (24). In addition, Wang *et al*. showed that in high-fat diet-induced obese rats fed *Lactobacillus paracasei subsp. Paracasei* NTU 101-fermented green tea, body weight gain and body fat content reduced after 8 weeks of feeding, compared to the unfermented tea groups (25). Our previous animal studies have also found that the administration of Awa tea leaves fermented by lactic acid bacteria enhanced the effect of diet restriction in obese rats (11). Among various bacterial species, lactic acid bacteria have been widely studied, and they offer multiple health benefits as probiotics (26). According to Yonejima *et al*., lactic acid bacteria reduced the relative expression of hepatic fatty acid synthase mRNA and downregulated the mRNA expression of inflammatory cytokines, such as TNF-α, in the adipose tissues of a high-fat diet mouse model (27). Lactic acid bacteria may play a role in the anti-obesity effect of Goishi tea, but the mechanism remains unclear.

One of the limitations of this study was that it did not consider the amount of dietary fiber in the experimental group's diet. It is well known that tea leaves are a potential good source of dietary fiber, which has health benefits such as improving serum lipid profile and promoting the excretion of lipids. For example, green tea leaves (Gyokuro) contain 43.9g of dietary fiber per 100g (28). In other words, the experimental diets in this study are expected to contain 0.4g/100g for the G1 group and 1.3g/100g for the G3 group. In future studies, this concern should be addressed.

In conclusion, an energy-restricted diet supplemented with Goishi tea leaf powder was effective in reducing body weight and visceral fat accumulation in diet-induced obese rats. We propose that the administration of Goishi tea leaves under dietary restrictions may be used as a diet therapy to prevent obesity and metabolic syndrome.

CONFLICTS OF INTEREST

We have no conflicts of interest to declare.

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