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Effects of Administration of Licorice Flavonoid Supplement on Lipid Metabolism in Obese Dogs

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ABSTRACT

The efficiency of licorice flavonoid supplement as anti-obesity substance was investigated in 14 client-owned dogs. This flavonoid supplement reduced whole and visceral fat in overweight humans. All experimental dogs were given the supplement at a dose of 10 mg kg^{-1} day^{-1} in the evening with food for 8 weeks. After 8 weeks post-administration, the body weight was decreased in 11 animals (78.6%). The decreasing rate was 2.51±0.6% (Mean±SE). Serum glucose (GLU), Triglyceride (TG), Malondialdehyde (MDA) concentrations and aspartate aminotransferase (AST), Alanine aminotransferase (ALT), Glutathione peroxidase (GSHPx) activities were decreased but the changes were not statistically significant. On the contrary, serum adiponectin (ADN) concentrations, though statically significance was not seen, tended to increase after 8 weeks post-administration of the supplement compared to those in baseline. In conclusion, the licorice flavonoid supplement was suggested to show anti-obesity effect accompanied with improvement of lipid metabolism in tissues of dogs.

Key words: Dog, licorice flavonoid supplement, lipid metabolism

INTRODUCTION

Prevalence of overweight and obesity in dogs has increased in recent years (Burkholder and Toll, 2000). In obese dogs, hyperlipidemia is regarded as a common characteristic and a deteriorative factor for Metabolic Syndrome (MS) in dogs (Kawasumi *et al*., 2012). Improvement of obesity also leads to reduction of onset of MS and subsequent development of severe metabolic diseases such as diabetes mellitus and cardiovascular disorders in dogs. A licorice flavonoid supplement (KANEKA GLAVONOID™) derived from licorice, *Glycyrrhiza glabra* was developed for obese humans by KANEKA CORPORATION (Osaka, Japan) (Tominaga *et al*., 2006). The flavonoid supplement reduced whole and visceral fat in overweight humans (Tominaga *et al*., 2009). This flavonoid supplement also showed marked anti-obesity effect such as reduced hepatic cholesterol and plasma lipoprotein cholesterol concentrations in high-fat diet rats (Honda *et al*., 2013; Kamisoyama *et al*., 2008). It has been noticed that this flavonoid supplement suppressed abdominal fat accumulation in obese diabetic KK-A^y mice (Nakagawa *et al*., 2004). Furthermore, it was reported that this supplement enhanced acyl-CoA dehydrogenase activities for beta oxidation

of fatty acids, while it decreased acetyl-CoA synthetase 2, ATP citrate lyase, acetyl-CoA carboxylase and fatty acid synthase activities for fatty acid synthesis in mouse liver tissues (Aoki *et al*., 2007). The aim of this study was to investigate the effects of the flavonoid supplement on lipid metabolism in obese dogs, therefore, we measured changes in physiological variables such as body weight and body condition score and serum metabolite concentrations and enzyme activities in dogs after administration of the flavonoid supplement.

MATERIALS AND METHODS

Experimental animals: Twenty dogs from 7 different veterinary clinics around Tokyo were recruited to evaluate the effectiveness of flavonoid supplement in this study. Two Miniature Schnauzers were excluded from this study, since they have genetic problems on lipid metabolism. Four dogs did not complete experimental schedule. The remaining 14 dogs (6 females and 8 male, 1-12 years old) without clinical problems were used to evaluate the effectiveness of licorice flavonoid supplement (KANEKA GLAVONOID™) in the dogs. Each dog was given the same meal twice a day during the experimental period. The diet composition varied among examined dogs, since each dog's owner was permitted to give normal diet in this study. They were given the flavonoid supplement at a dose of 10 mg kg^{-1} day^{-1} in the evening every day for 8 weeks. Profiles of breed, age and sex of 14 dogs were shown in Table 1. The degree of obesity in the dogs was assessed by BCS on the basis of the following five-point scale (1-5): (1) Very thin, (2) Underweight, (3) Ideal, (4) Over-weight and (5) Obese commonly used in Japan (Mori *et al*., 2011). Dogs with BCS of 3.0-5.0 were used in this study. Ethical approval was obtained from the Nippon Veterinary and Life Science University Animal Research Committee (No. 13-92). Written informed consent was obtained from owners of the animals used in this study.

Supplement: Licorice flavonoid supplement, KANEKA GLAVONOID™ (KANEKA CORPORATION, Osaka, Japan) was made from licorice (*Glycyrrhiza glabra*) root extraction using ethanol. Then extraction was filtrated and concentrated. The ethanolic layer was mixed with Medium-Chain Triglycerides (MCT) consisting of C8:C10 fatty acids (99:1). The concentration of glabridin, a major constituent of the solution was adjusted to 3%. Glycyrrhizic acid,

Table 1: Profiles of the breed, age, sex in 14 dogs

Breeds	Age (years old)	Sex	Castration/Spay
American cocker spaniel	4	♀	○
Golden retriever	4	♀	○
Labrador retriever	6	♂	○
Miniature dachshund	12	♂	○
Miniature dachshund	7	♀	○
Miniature dachshund	10	♂	○
Miniature dachshund	7	♂	
Mix	1	♀	○
Mix	9	♂	○
Mix	3	♀	○
Pomeranian	3	♂	○
Schipperke	4	♂	○
Toy poodle	2	♀	○
Toy poodle	2	♂	○

a hydrophilic compound in licorice was almost eliminated (below 0.005%). Compounds in the licorice ethanolic extract were polyphenols including licorice prenylflavonoids. This solution was dispensed into capsules.

Blood collection, serum metabolite and enzyme activities assays: Serum metabolite and enzyme activities were measured before and at 4 and 8 weeks post administration of the supplement. Blood samples from the dog's jugular veins after overnight fasting (without any nutrients for >8 h after the last meal) were collected. Serum was recovered by centrifugation at 3,000 rpm for 15 min in each veterinary clinic and stored at -40°C until use. Levels of serum glucose (GLU), triglyceride (TG), Total Cholesterol (TC), Total Protein (TP), BUN, creatinine (CRE), alanine aminotransferase (ALT), aspartate aminotransferase (AST) activities were measured using an autoanalyzer (JCA-BM2250, JEOL Ltd., Tokyo, Japan) with manufacturer's reagents at Monolis Inc. (Tokyo, Japan). Non-Esterified Fatty Acid (NEFA) levels were measured using a commercial kit (NEFA-C test, Wako Pure Chemical Industries, Inc., Tokyo, Japan). Serum insulin (ISN) and adiponectin (ADN) concentrations were measured with commercial ELISA kits, Lbis dog insulin kit (SHIBAYAGI Co., Gunma, Japan), mouse/rat adiponection kit (Otsuka Pharmaceutical Co., Ltd, Tokyo, Japan), respectively. Serum superoxide dismutase (SOD) activities were measured using commercial kit, NWLSS™ Superoxide Dismutase Activity Assay (Northwest Life Science Specialties, LLC, Vancouver, Canada). Serum glutathione peroxidase (GSHPx) activities were measured using commercial kit, NWLSS™ Gluthathione Peroxidase Assay (Northwest Life Science Specialties, LLC, Vancouver, Canada).

Statistical analysis: Results are presented as Mean±SE (standard error). Statistical significance was determined by paired Student's t-test. The significance level was set at $p<0.05$.

RESULTS

Changes in body weight: Profiles of 14 dogs examined in this study were shown in Table 1. At baseline, 12 of 14(85.7%) dogs were overweight or obese with BCS of ≥4. As shown in Table 2 and

Table 2: Changes in body condition score (BCS) and body weight (BW) between pre and 8-week post administration of flavonoid supplement in 14 dogs

BCS		BW (kg)		
Pre	8-week post	Pre	8-week post	Decreasing rate (%)
4.0	4.0	11.66	11.50	-1.4
5.0	5.0	36.50	36.50	±0
4.0	3.5	32.80	32.60	-0.6
5.0	5.0	8.40	8.30	-1.2
5.0	5.0	8.17	7.90	-3.3
5.0	5.0	10.80	10.68	-1.1
5.0	5.0	8.00	7.90	-1.3
3.0	2.5	17.78	16.54	-7.0
3.0	3.0	10.80	10.50	-2.8
5.0	5.0	6.90	7.00	+1.4
5.0	5.0	10.80	11.20	+3.7
4.5	4.5	8.28	7.96	-3.9
4.0	3.5	4.00	3.85	-3.8
4.0	4.0	3.50	3.45	-1.4

+: Decreasing rate means increase of BW

Table 3: Changes in body weight (BW) after administration of flavonoid supplement for 8 weeks in 14 dogs

Parameters	BW↓
Occurrence rate (%)	11/14 (78.6)
Decreasing rate (%)	2.51±0.6

Decreasing rate was calculated as (BW (after 8 weeks)-BW (pre))/BW (pre)×100 and presented as Mean±SE

Table 4: Changes in serum biomarker levels between pre and post administration of flavonoid supplement in 11 dogs with decreased body weight

Parameters	No. of dogs	Pre	4 week	8 week
GLU (mg dL^{-1})	(11)	86.0±7.0	87.5±7.6	76.2±7.3
AST (IU L^{-1})	(11)	34.9±4.8	28.4±3.6	26.6±3.3
ALT (IU L^{-1})	(11)	74.9±19.7	61.6±13.0	58.1±11.4
CHO (mg dL^{-1})	(11)	230.0±24.3	216.2±17.0	223.1±23.8
TG (mg dL^{-1})	(11)	210.9±78.3	116.3±24.3	147.1±55.5
MDA (μmol L^{-1})	(11)	5.8±2	4.4±0.6	3.5±0.5
NEFA (mEq L^{-1})	(11)	0.809±0.145	0.660±0.094	0.870±0.103
INS (ng mL^{-1})	(11)	1.5±0.3	1.8±0.5	1.9±0.5
ADN (μg mL^{-1})	(10)	11.4±4.5	14.2±5.7	15.3±5.9
BUN (mg dL^{-1})	(11)	16.6±2.1	16.9±1.6	15.8±1.6
CRE (mg dL^{-1})	(11)	0.8±0.1	0.8±0.0	0.8±0.0
SOD (U mL^{-1})	(11)	24.3±4.2	29.6±7.4	24.9±4.4
GSHPx (mU mL^{-1})	(9)	54.6±8.4	50.7±3.4	47.3±5.4

Data is presented as Mean±SE. Numbers in parentheses indicate the number of animals examined, significant ($p<0.05$) when compared the pre-administration values (paired t-test)

Table 3, in 11 of 14 dogs (78.6%), BW decreased after administration of the supplement for 8 weeks. Their decreasing rate was 2.51±0.6% (Mean±SE), however, BW of 2 dogs (No. 10 and 11) increased by average of 2.6% and BW of 1 dog (No. 2) did not change.

Changes in serum biomarker levels: As depicted in Table 4 in 11 dogs with decreased BW, serum GLU, TG, MDA concentrations and AST, ALT, GSHPx activities tended to decrease after administration of the supplement for 8 weeks but the changes were not statically significant. On the contrary, serum adiponectin (ADN) concentrations tended to increase after administration of the supplement.

DISCUSSION

The current experiment was set to evaluate the anti-obesity efficiency of flavonoid supplement in obese dogs. Flavonoid supplements showed Body Weight (BW) reducing effect in 11 of 14(78.6%) of obese (overweight) dogs at a dose of 10 mg kg^{-1} day^{-1} for 8 weeks administration. Although, BW reducing rate in experimental dogs with flavonoid supplement was only 2.5%, the body weight reducing effect of flavonoid was proven for these animals. As toxicity of flavonoid is considered to be low in animals (Aoki *et al*., 2007), the dose and duration for administration for dogs can be increased. Higher dose and longer duration of the supplement administration may show distinct effect for obese dogs. In overweight human, significant decreases in visceral fat area, BW, BMI and LDL-cholesterol were seen with administration at a dose of 900 mg day^{-1} (corresponding to 300 mg day^{-1} of 3% glabridin solution which was used in this study) for

8 weeks (Tominaga *et al*., 2009) and clinically significant adverse effects were not observed with administration at a dose of 1800 mg day^{-1} (corresponding to 600 mg day^{-1} of 3% glabridin solution which was used in this study) for 4 weeks (Tominaga *et al*., 2006).

Results of the current investigation revealed that activities of ALT as fatty liver marker in humans decreased in serum of the dogs administered with flavonoid supplement. It is reported that flavonoid shows significant effect in improving lipid metabolism in liver of mice and human (Aoki *et al*., 2007; Tominaga *et al*., 2009). Decreased activities of ALT and AST may suggest that flavonoid supplement improved hepatic lipid metabolism also in dogs.

Serum TC concentrations did not change in 11 dogs with decreased body weight. However, other researchers reported a contrary result which showed that α 2-cholesterol lipoprotein fractions in obese dogs were higher than those in healthy animals (Mori *et al*., 2011). Improvement of these cholesterol lipoprotein profiles after administration of the flavonoid supplement is expected. We need to investigate changes in cholesterol lipoprotein profiles after administration of flavonoid supplement in dogs fed on the same food as previously described (Kawasumi *et al*., 2014).

Accumulated lipid in obese animals induces hyperlipidemia and activation of beta-oxidation of fatty acids in mitochondria of liver and adipose tissues. Elevation of beta-oxidation of fatty acids produces excess amount of Reactive Oxygen Species (ROS) followed by increases in lipid peroxidation in tissues (Lipotoxicity) (Schrauwen *et al*., 2010). At early stages of obesity, serum SOD activities increase as compensatory effect to reduce ROS in animals. As MDA is an end-product of lipid peroxidation, serum MDA concentrations decreased when lipid metabolism was improved (Li *et al*., 2014). After flavonoid administration, serum MDA concentrations decreased in obese dogs. This finding may indicate flavonoid supplement improved lipid metabolism in liver of dogs. In addition, this flavonoid supplementation may also have improvement effect of glucose metabolism in dogs.

This study has some limitations. The number of animals was small and client-owned dogs were studied. The food for each dog varied. In 2 dogs, BW increased after flavonoid administration due to hepatic function improvement and appetite increase. In this study, the dose of flavonoid supplement was set at only one dose. Further studies are necessary to clarify accurate mechanism of lipid metabolism improvement by flavonoid in obese animals.

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