Physiological Effect of Hamcho Yogurt on Streptozotocin-Induced Diabetic Rats

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The present study was undertaken to investigate the hypoglycemic effect of Hamcho yogurt fermented by lactic acid bacteria containing Hamcho (*Salicornia herbacea*) extract powder in streptozotocin (STZ)-induced diabetic rats for 3 week period. The Hamcho yogurt given to the STZ-diabetic rats had significant effect in lowering the serum glucose concentrations and food intakes compared to the STZ-diabetic rats. The Hamcho yogurt administration increased the tolerance to oral glucose loading in the STZ-diabetic rats. The Hamcho yogurt and yogurt given to the STZ-diabetic rats were significantly decreased the serum total cholesterol concentration and the atherogenic index. Based on the beneficial effect against the blood glucose and cholesterol levels, we suggest the Hamcho yogurt can be used as functional food for the hypoglycemic and hypocholesterolemic effects.

Key words – Hamcho yogurt, hypoglycemic effect, streptozotocin, diabetes

Plants have been used as an important source of therapy. In accordance to the recommendations of the WHO Expert Committee on diabetes mellitus, it is important to investigate the hypoglycemic effect from plants which were originally used in traditional medicine[1]. The biological active components of the plants with hypoglycemic action include flavonoides, polysaccharides, peptidoglycans, dietary fibers[1, 11,23,35].

Seaweeds were also reported to have hypoglycemic activity components, polysaccharides and dietary soluble fiber in the streptozotocin and alloxan-induced diabetic rats[8,20, 21,34]. The seaweed foodstuff, the *Salicornia herbacea* is called "Hamcho" in Korea, has been regarded as a traditional Korean medicine used for thousands of years in the prevention and treatment of various human diseases, such as hypertension, diabetes mellitus, cancer, hepatitis, and obesity. Hamcho is reported to contain high contents of minerals such as Mg, Ca, K, and Fe and dietary fibers[12,13]. This seaweed has been reported to be biological and physiological effects, such as antioxidant and hypolipidemic effect[13,17]. The administration of Hamcho powder improved the hypoglycemia to animal models of insulin-dependent diabetes mellitus (IDDM)[3].

In many countries there is the wild spread belief that milk products fermented by lactic acid bacteria, such as yogurt, are beneficial toward hypertension, hypercholesterolemia, intestinal infections, and cancer[26,28,30,33]. However, their antidiabetic effects have hardly been studied relation to the lowering of glycated hemoglobin (HbA1c), the improved glucose tolerance in neonatally streptozotocin (nSTZ)-induced diabetic rats fed a diet containing *Lactobacillus* GG cells[32] and the lowered glycemic indexes in healthy subjects fed fermented milk product[27]. It is our hypothesis that the combination of milk products fermented by lactic acid bacteria, and Hamcho extracts, might induce hypoglycemic activity due to their hypoglycemic effect. The hypoglycemic effects of seaweeds are chiefly found in the water-extracts, which contain polysaccharides and dietary soluble fiber[8, 20,34]. The relationship between Hamcho yogurt and glycemic control has not been elucidated in diabetic animal models, even though Hamcho yogurt would be an ideal candidate for glycemic control. The present study was undertaken to investigate the effect of Hamcho yogurt administration on the blood glucose and lipid concentrations, and the oral glucose tolerance in STZ-diabetic rats.

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Materials and Methods

Materials

Hamcho obtained naturally grown in closed sea beach. The streptozotocin was purchased from Sigma Chemical Co., (Louis, MO, USA). The lactic acid bacteria used in the manufacture of the Hamcho yogurt were *Lactobacillus acidophilus, Streptococcus thermophillus* and *Bifidobacterium longum*. All other chemicals and reagents were of the best commercial grade available.

Preparation of Hamcho yogurt

The Hamcho yogurt was prepared by Biohub Co., Ltd, (Jinju, Korea). The Hamcho water-extract was extracted by hot water from dried Hamcho stream, freeze-dried and ground to powder of <1 mm in diameter using a cyclotec mill (Tecator, Hoganas, Sweden). The composition of fermented milk base was 8% skimmed milk powder and 2% sucrose. The Hamcho yogurt was produced using 0.1% Hamcho extract powder containing 1% (v/v) starter culture with lactic acid bacteria and 10% (w/v) skimmed milk powder. Both yogurts were fermented at 37℃ for 6 hr. We have chosen this study because Hamcho yogurt containing 0.1% Hamcho extract powder was relatively good in the assay of lactic acid bacterial numbers, pH, acidity, viscosity, and sensory test and its also exerted cholesterol lowering effect when fed to hypercholesterolemic rats[7,14].

Diets and animal experiments

The five week old male Sprague-Dawley rats were purchased from Hyochang Science (Daegu, Korea) and housed individually in suspended wire-mesh stainless cages in a temperature controlled animal room (21~24℃), with a 12 hr light/dark cycle (07:00~19:00). The STZ solution was prepared in 0.05 M citrate buffer (pH 4.5), immediately prior to an intraperitoneal dose injected at 50 mg/kg B.W. following overnight fasting[7]. Diabetes was defined as a blood glucose concentration above 300 mg/dl 48 hr after the STZ injection. The STZ-induced diabetic rats were randomly divided into 3 groups of 6 rats each with similar mean weights and blood glucose levels. All rats were *ad libitum* with the commercial powdered non-purified diet (Superfeed Co., Ltd, Wonju, Korea) in which was incorporated an equal volume of either Hamcho yogurt or yogurt at the levels of 10% (v/w) and had free access to drinking water for 3 weeks. The compositions of a commercial non-purified diet were crude protein 22.1%, crude fat 3.5%, dietary fiber 5.0%, ash 8.0%, calcium 0.6%, and phosphorus 0.4%. The normal and STZ-diabetic rats were fed a commercial powdered non-purified diet, supplemented with not fermented milk solution in equivalent amounts, instead of the Hamcho yogurt or yogurt. The body weights were recorded every week, and the water consumptions and food intakes were recorded every other day.

Analytical procedure

At the end of the treatment period, the animals were killed by withdrawing blood from the abdominal aorta, under light diethyl ether anesthesia, after an 12-hr fast. Their livers were quickly removed and weighed, the tissue weights being expressed as absolute or relative weights. The serum was separated onto the centrifugation for determination of glucose and lipid concentrations. The serum glucose and lipid concentrations were measured by a Fuji DRI-Chemiclinical Chemistry analyzer (FUJI DRI-CHEM 3500, Tokyo, Japan).

Oral glucose tolerance test (OGTT)

The OGTT was performed in the normal and the STZ-treatment rats fed the experimental diets after overnight fasting (water was allowed *ad libitum*). The blood glucose concentrations were measured on whole blood collected from the tail vein using a Lifescan glucose meter with One Touch test strips (Lifescan Inc., Milpitas, CA, USA), at 0, 30, 60, 90, and 120 min after the oral administration of a glucose solution (one g/kg B.W.).

Statistical analysis

The data from experiments are presented as the means± SEM, and were analyzed using a one way analysis of variance (ANOVA), with the differences analyzed using the Duncan's new multiple-range test[9]. A p value <0.05 was accepted as being a statistically significant difference.

Results

The lactic acid bacterial numbers, pH, and titratable acidity of both Hamcho yogurt and yogurt are shown in Table 1. We have chosen this study because Hamcho yogurt containing 0.1% Hamcho extract powder was relatively good on quality characteristics compared to other Hamcho extract supplemented concentrations, and its also exerted cholesterol lowering effect when fed to hypercholesterolemic rats[7].

The body weight changes are shown in Fig. 1. The body weights in the normal rats were significantly greater than in the STZ-treatment rats at the same time point. However, there were no significantly differences in the final body weights between the STZ-treatment rats.

The water consumptions and food intakes and liver weights are shown in Table 2. The water consumptions were significantly increased in the STZ-treatment rats compared to the normal rats. The amounts of food consumed were significantly increased in both the STZ-diabetic rats

Table 1. Lactic acid bacterial numbers, pH, acidity, and protein of Hamcho yogurt and yogurt fermented by lactic acid bacteria

Ingredient	Cell numbers (cfu/mL)	pH	Acidity (%)	Protein (mg/mL)
Yogurt	1.4×10^8	4.324	0.593	2.38
Hamcho yogurt	1.7×10^8	4.230	0.638	2.00

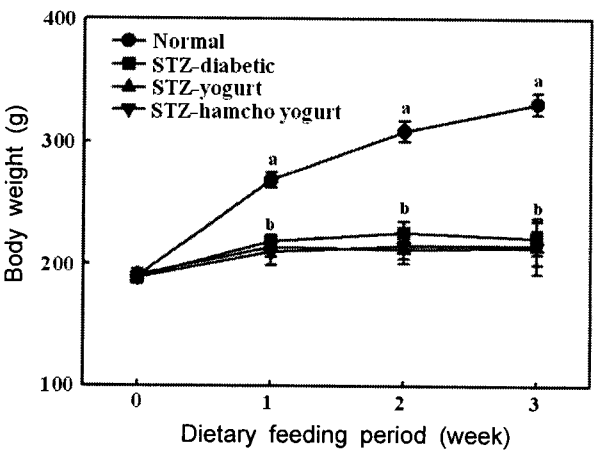

Fig. 1. Effect of Hamcho yogurt on the change of body weight in the STZ-treatment rats.
Values with different letters are significantly different at $p<0.05$. (mean±S.E., n=6).

and the STZ-yogurt fed rats and slightly increased in the STZ-Hamcho yogurt fed rats compared to the normal rats. The absolute liver weights were significantly reduced in the STZ-treatment rats compared to the normal rats, but there were no differences in the liver relative weights between the normal and STZ-treatment rats.

The concentrations of fasting serum glucose were significantly increased by approximately 4-fold, in the STZ-diabetic rats compared to the normal rats (Fig. 2). However, this rise was slightly decreased in the STZ-yogurt fed rats by 14% and was significantly decreased the STZ-Hamcho yogurt fed rats by 30% compared to the STZ-diabetic rats (Fig. 2). Fig. 3 show the results of the OGTT after treatment

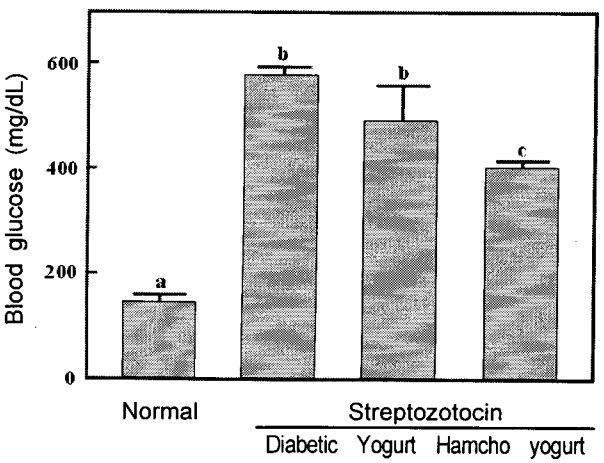

Fig. 2. Effect of Hamcho yogurt on the blood glucose levels in the STZ-treatment rats.
Values with different letters are significantly different at $p<0.05$. (mean±S.E., n=6).

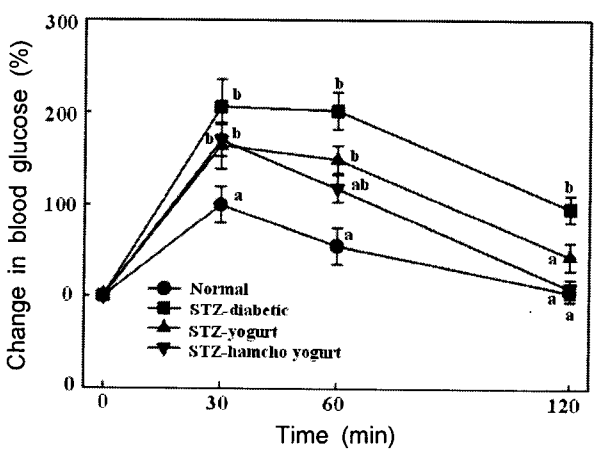

Fig. 3. Effect of Hamcho yogurt on the oral glucose tolerance test (OGTT) in the STZ-induced diabetic rats.
The results are given as the percentage increase in the fasting blood glucose concentrations. Values with different letters are significantly different at $p<0.05$. (mean±S.E., n=6).

with the experimental diets, and are given as the percentage increase in the fasting blood glucose concentrations, as the

Table 2. Effect of Hamcho yogurt on the food and water intake, and liver weights in the STZ-treatment rats

Ingredient	Normal	Diabetes	Yogurt	Hamcho yogurt
		Streptozotocin-treatment rats		
Food intake (g/day)	26.8 ± 0.5^a	34.6 ± 0.6^b	35.0 ± 0.5^b	31.5 ± 0.5^c
Water intake (ml/day)	49.5 ± 3.0^a	161.3 ± 10.7^b	139.5 ± 17.5^b	155.6 ± 23.9^b
Liver weight				
Absolute (g)	13.36 ± 0.57^a	9.53 ± 0.76^b	8.51 ± 0.36^b	8.59 ± 0.39^b
Relative (%)	4.04 ± 0.16^{NS}	4.29 ± 0.15	4.01 ± 0.14	4.11 ± 0.28

Values are mean±S.E. of six rats per group. NS, not significant.
Values with different superscript letters are significantly different at $p<0.05$.

basal blood glucose concentration among the experimental groups were dissimilar before the glucose loading. The peak increase in the blood glucose was observed after 30 min in all the experimental groups. The blood glucose concentrations in the STZ-diabetic rats were significantly increased following the oral administration of glucose, and the treatment with the Hamcho yogurt partially restored glucose tolerance.

The serum total cholesterol concentration was significantly increased by 21% in the STZ-diabetic rats compared to the normal rats, however, this elevation was significantly reduced by 25% in the STZ-yogurt fed rats and was also significantly reduced by 23% in the STZ-Hamcho yogurt fed rats (Table 3). There were no significant differences in the triglyceride concentrations among the experimental rats. The changes in the atherogenic index (AI), which was calculated on the basis of the following expression: (total cholesterol - HDL cholesterol) / HDL cholesterol[7], were slightly increased in the STZ-diabetic rats compared to the normal rats. But this raise was significantly reduced by intake of the Hamcho yogurt and yogurt in the STZ-treatment rats (Table 3).

Discussion

We have chosen this study because Hamcho yogurt containing 0.1% Hamcho extract powder was relatively good on quality characteristics compared to other Hamcho extract supplemented concentrations[14], and exerted cholesterol lowering effect when fed to hypercholesterolemic rats[7].

As expected[10,22,31], the body weights in the normal rats were significantly greater than in the STZ-treatment rats at the same time point. However, there were no significantly differences in the final body weights between the STZ-treatment rats. It has been reported that the administration of STZ at 60 and 70 mg/kg body weight significantly reduced the body weight in STZ-diabetic rats compared to the normal rats, but body weight increased in 50 mg/kg diabetic rats [22]. Thus, the body weights in the STZ-treatment rats by 50 mg/kg body weight used in this study gradually slightly increased with age. The water consumptions (Table 2), as expected[10,31], were also significantly increased in the STZ-treatment rats compared to the normal rats. The amounts of food consumed were significantly increased in both the STZ-diabetic rats and the STZ-yogurt fed rats and slightly increased in the STZ-Hamcho yogurt fed rats compared to the normal rats. This result was consistent with previous reports, the food intakes in the STZ-diabetic rats were significantly increased due to the administration of Hamcho powder compared to the normal rats[3].

The concentrations of fasting serum glucose were significantly increased by approximately 4-fold, in the STZ-diabetic rats compared to the normal rats (Fig. 2). However, this rise was slightly decreased in the STZ-yogurt fed rats by 14% and was significantly decreased the STZ-Hamcho yogurt fed rats by 30% compared to the STZ-diabetic rats (Fig. 2). There were reported that the crude polysaccharides isolated from the seaweeds, *Laminaria ochroleuca, Saccorhiza polyshides, Fucus vesiculosus, Himanthalia elongate,* and *Codium tomentosum* caused a significant reduction in blood glucose in normal rabbits and alloxan-diabetic animals[21].

The seaweeds were also reported to have hypoglycemic activity components, polysaccharides and dietary soluble fiber such as carrageenan, in the streptozotocin- and alloxan-induced diabetic animals and diabetes mellitus patients [8,20,21,23,34]. The seaweed foodstuff, Hamcho, has been regarded as a traditional Korean medicine used for thousands of years in the prevention and treatment of diabetes mellitus. It was recently reported that the administration of Hamcho powder improved the hypoglycemia to the STZ-

Table 3. Effect of Hamcho yogurt on the serum lipid concentration and atherogenic index (AI) in the STZ-treatment rats

Ingredient	Normal	Diabetes	Yogurt	Hamcho yogurt
		Streptozotocin-treatment rats		
		(mg/dL)		
Total cholesterol	86.08±4.37^{ab}	103.80±6.73^{a}	78.14±2.98^{b}	80.00±4.91^{b}
HDL-cholesterol	46.78±2.52^{ns}	50.46±1.87	45.37±1.23	46.22±3.37
AI[1)]	0.84±0.04^{ab}	1.06±0.09^{a}	0.72±0.03^{b}	0.73±0.03^{b}
Triglyceride (mg/dL)	78.95±7.10^{NS}	74.04±6.41	65.26±6.24	68.42±5.41

Values are mean±S.E. of six rats per group.
[1)]AI (atherogenic index) = (total cholesterol - HDL cholesterol) / HDL cholesterol NS, not significant.
Values with different superscript letters are significantly different at $p<0.05$.

induced diabetic rats[3]. In addition, sea tangle has been reported to show hypoglycemic effect on the STZ-induced diabetic rats[23,24]. Previous studies have also shown that the fasting blood glucose concentrations in genetically diabetic mice and STZ-induced diabetic mice, fed on diet containing hot water-soluble polysaccharide from mushroom fruit bodies, were significantly decreased[19]. We previously reported that the mushroom yogurt containing water soluble polysaccharides having antidiabetic activity from *Ganoderma lucidum* was shown to have a potential hypoglycemic effect, by increasing blood insulin levels in the STZ-diabetic rats [6], and antiobesity and hypotriglyceridemic effects in Otsuka Long-Evans Tokushima Fatty (OLETF) rats and in the STZ-diabetic rats[15,16]. The antidiabetic effects of seaweeds and mushrooms are chiefly found in the water-extract, which contain polysaccharides, fiber, and minerals and are recognized as having various biologically active components[8, 20,21,34,36].

Hamcho has been reported to mainly contain about 70% dietary fiber[12,13], and also high content of minerals, 2,083 mg K, 110 mg Mg, 20 mg Ca per 100 g dry weights[17]. A previous study has also demonstrated that soluble fiber decreases the availability of glucose by delaying its absorption in the proximal small intestine, thus reducing the postprandial glucose levels[23,29]. These results suggest that the administration of Hamcho yogurt containing Hamcho fiber have an important potential in improving the hyperglycemia of STZ-induced diabetes. Thus, the hypoglycemic effect of Hamcho yogurt is supported by the fact that Hamcho yogurt product was produced using water-extract powder containing polysaccharides and/or dietary fiber having hypoglycemic activity from Hamcho powder. Furthermore, Hamcho yogurt partially restored glucose tolerance in the STZ-induced diabetes (Fig. 3). It has been reported that the nSTZ-diabetic rats fed diets containing *Lactobacillus* GG cells showed an improved glucose tolerance compared with nSTZ-diabetic control rats[32]. A previous study has also demonstrated that fermented milk products improve the blood glucose responses in healthy subjects fed yogurt, such as Filmjolk or Ropy milk[27]. The improvement of the glucose tolerance by lactic acid bacteria has been attributed to an enhanced activity of the β-cells of the pancreas, resulting in the secretion of a large amount of insulin 30 min after glucose solution administration[32]. Thus, these results indicated that lactic acid bacteria, Hamcho extract powder and/or Hamcho yogurt containing both of these could improve glucose tolerance in the diabetic rats.

Hyperlipidemia is a major risk factor leading to lifestyle-related diseases, obesity, arteriosclerosis and hypertension, and much attention has focused on improving serum lipids by the intake of functional foods[4,5]. The seaweeds are an ideal diet for the dietetic prevention of atherosclerosis due to their ability to lower the serum cholesterol and triglyceride concentrations because of their high contents of physiological components, dietary fiber, polysaccharides, carrageenan, alginate, and fucoidan[8,12,13,20,21,34]. Recently, the hypolipidemic effects, due to the consumption of Hamcho and their extracts, were observed in STZ-induced diabetic rats and diet-induced hyperlipidemic rats[3,17]. The serum total cholesterol concentration was significantly increased by 21% in the STZ-diabetic rats compared to the normal rats, however, this elevation was significantly reduced by 25% in the STZ-yogurt fed rats and was also significantly reduced by 23% in the STZ-Hamcho yogurt fed rats (Table 3). There were no significant differences in the triglyceride concentrations among the experimental rats. This result was also supported by another recent study, where the serum total cholesterol levels were significantly decreased in the STZ-diabetic rats given sea tangle powder, but changes in the triglyceride concentration did not take place[6].

Mann and Spoerry were the first to report that fermented milk consumption reduced the serum cholesterol levels in the Maasai tribes[25]. Numerous studies reports have shown the potential hypocholesterolemic activity of fermented milk in humans and animal experiments[2,18]. It is possible that the hypocholesterolemic effect of lactic acid bacteria and fermented milk in animal experiments with rats was mainly due to the action of the fermented milk in combination with lactic acid bacteria[18]. However, the Hamcho yogurt by the combination of lactic acid bacteria and Hamcho extract on the cholesterol-lowering effect has not yet been study. Present study was also confirmed the cholesterol-lowering effect by Hamcho yogurt or yogurt administration.

The changes in the atherogenic index, which was caculated on the basis of the following expression: (total cholesterol - HDL cholesterol) / HDL cholesterol. The atherogenic index was slightly increased in the STZ-diabetic rats compared to the normal rats. But this raise was significantly reduced by intake of the Hamcho yogurt and yogurt in the STZ-treatment rats (Table 3). This result is consistent with a previous study that the atherogenic index lowered significantly by the intake of fermented milk with *Lactobacillus casei* and

Streptococcus thermophilus[18].

Thus, the present study has demonstrated for the first time that the administration of fermented milk products (Hamcho yogurt) supplemented with Hamcho extract decreased blood glucose and total cholesterol concentrations in the STZ-treatment rats.

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초록 : Streptozotocin-유발 당뇨쥐에 대한 함초 요구르트의 생리학적 효능

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함초 추출물 분말을 첨가하여 유산 발효시킨 함초 요구르트를 streptozotocin- 유발 당뇨쥐에 투여하여 항당뇨 효과 및 콜레스테롤 저하 효과를 규명하기 위하여 3주간 실험하였다. 당뇨 대조군에 비해 함초 요구르트 투여군에서 혈당치 농도 및 식이 섭취량이 현저히 감소하였다. 경구당부하 실험에서도 당뇨 대조군에 비해 함초 요구르트 투여군에서 현저한 혈당상승 억제작용을 나타내었다. 당뇨쥐에 함초 요구르트와 일반 요구르트 섭취에 의해 혈중 총 콜레스테롤 농도 감소와 더불어 동맥경화지수도 감소하였다. 따라서, 함초 요구르트에 의한 혈당치 및 콜레스테롤 농도 감소효과는 고혈당증 및 고콜레스테롤혈증에도 유익한 작용을 할 수 있는 기능성식품 개발 가능성이 있음을 시사하였다.