Introduction

As is well known in Japan, lifestyle-related disease has been increasing markedly in the Japanese. It is well known that obesity can lead to an increase in lifestyle-related disease (Isomaa 2003). The prevention of lifestyle-related disease is one of the most important problems for public health. There is a close connection between daily dietary intake and lifestyle-related disease.

Diabetes is seriously increasing world-wide. It has become a public health problem in developing countries, because of its growing prevalence, economic and social costs and important cardiovascular complications (Wild et al. 2004). Diabetes is now one of the main threats to human health and is likely to remain a huge threat to public health in years to come (Zimmet et al. 2001). There is a close connection between daily dietary intake and lifestyle-related disease. Consumption of dietary fiber could play an important role in the prevention of diabetes (Weickert and Pfeiffer 2008; Jenkins et al. 2000).

Nostoc flagelliforme is an edible blue-green alga called “Facai” or “black moss” that has been used as a food delicacy as well as an herbal medicine in China and south-east Asia to which it is native for thousands of years. The safety of this alga was evaluated in the oral acute toxicity study and the 28-days oral subacute toxicity study (Takenaka et al. 1998). This alga has been reported to show various health benefits including anti-cancer, anti-viral and anti-infectious activities (Takenaka et al. 1997; Kanekiyo et al. 2005, 2007; Yamaguchi et al.

Beneficial effect of edible blue-green alga Nostoc flagelliforme (Cyanophyceae) on blood glucose level and serum lipid concentrations in spontaneously non-insulin-dependent diabetic rats

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Abstract: The edible blue-green alga Nostoc flagelliforme was evaluated for beneficial effect on blood glucose level and serum lipid concentrations in an experiment with SHR rats used as model animals for spontaneously non-insulin-dependent diabetes mellitus.

In both of N. flagelliforme powder administered rats and N. flagelliforme extract administered rats, the fasting glucose levels on 21st day, 35th day, 49th day and 63rd day were significantly lower than control rats. The concentrations of triglyceride in the serum of N. flagelliforme powder administered rats and total cholesterol and triglyceride in the serum of N. flagelliforme extract administered rats were significantly lower than those of control rats. The concentrations of triglyceride and total cholesterol in the liver of both of N. flagelliforme powder administered rats and N. flagelliforme extract administered rats were significantly lower than those of control rats.

These results indicate that N. flagelliforme has beneficial effects on blood glucose level and serum lipid concentrations in spontaneously non-insulin-dependent diabetic rats.

Keywords: blood glucose, cholesterol, diabetic rat, edible blue-green alga, Nostoc flagelliforme.
2009).

Although previous research has shown *N. flagelliforme* confers health benefits, no information is available on diabetes. In this study, we investigated the beneficial effect of *N. flagelliforme* on blood glucose level and serum lipid concentrations in spontaneously non-insulin-dependent diabetic rats.

**Materials and Methods**

**Alga**

*Nostoc flagelliforme* was harvested in Alxa, Inner Mongolia, China in the summer of 1996. After washing using water, the alga was dried in the sun and then powdered. The composition of the dried alga was 21.4% protein, 0.5% lipids, 56.8% carbohydrate (mostly polysaccharides), 1.9% dietary fiber and 4.4% ash.

**Extraction**

Ten grams of *N. flagelliforme* was extracted with 1000 mL of water at 90 °C for 60 min, and then filtered after cooling. This aqueous extract was concentrated and freeze-dried.

**Animals**

Male GK/Jcl-NIDDM rats (4 weeks old) were purchased from Kyushu Animal (Japan), and maintained in our laboratory for 1 week before use in the experiment. Rats were divided into three groups of eight rats each: control group, *N. flagelliforme* powder administered group and *N. flagelliforme* extract administered group, and were housed individually in a cages for rats in a temperature-controlled room (22 ± 2 °C) with a 12 hour interval of light (8:00 - 20:00) and dark. Rats were fed on each diet prepared as described below for 63 days. Rats were allowed free access to the diets and distilled-deionized water during this study. The amount of food intake and the body weight of rats were measured every day and every three days during this study, respectively. At the end of the experimental periods, the animals were fasted overnight and immediately sacrificed under ethyl ether anesthesia after blood was collected via the post-caval vein. In *N. flagelliforme* extract group, 3 mL head$^1$ of *N. flagelliforme* extract suspension was administered by oral gavage every morning for 48 days, consequently, the dosage level was calculated as the amount of *N. flagelliforme* powder intake of rats in *N. flagelliforme* powder group.

**Preparation of diets**

The diets were prepared according to the composition of the AIN-93G diet (Reeves et al. 1993), by replacing cornstarch with *N. flagelliforme* powder. The diets contained (in g kg diet$^{-1}$) vitamin free casein (Sigma), 200; soybean oil (no additives), 70; fiber, 50; mineral mixture (AIN-93G-MX), 3.5; vitamin mixture (AIN-93-VX), 10; L-cystine, 3; choline bitartrate, 2.5; tetra-butylhydroquinone, 0.014. These diets did not contain polyphenols. The amount of food intake of rats was recorded every day during this study.

**Serum biochemical tests**

The serum of rats on the final experimental day of this study was subjected to the following measurements (GOT, GPT, total protein, albumin, globulin, haemoglobin, blood urea nitrogen and creatinine) using by assay kits (Wako Pure Chemical Industries, Ltd., Japan).

**Measurement of triglyceride and total cholesterol concentrations**

The concentrations of triglyceride and total cholesterol of rats on the final experimental day of this study were determined by assay kits (Wako Pure Chemical Industries, Ltd., Japan).

**Measurement of blood glucose levels**

The blood glucose levels of rats were measured by Astanse III (Horiba, Ltd., Japan), using blood obtained from the tail vein on 0, 7, 21, 35, 49 and 63 days.

**Measurement of cholesterol concentrations and bile acid contents in feces**

The cholesterol and bile acid in the dried feces of rats on the final experimental day of this study were extracted by the method described previously (Ishibashi 2004) and were determined by assay kits (Wako Pure Chemical Industries, Ltd., Japan).
Oral glucose tolerance test

An oral glucose tolerance test was performed by administration of 1 g of basal diet and 50 mg of *N. flagelliforme* powder or *N. flagelliforme* extract by oral gavage (3 mL head⁻¹). Blood was taken from the tail vein at 0, 60 and 120 min for the measurement of blood glucose levels.

Sucrase and maltase inhibitory assay

A crude enzyme solution was prepared from 1 g of rat intestinal acetone powder (Sigma Aldrich, USA) added 9 mL of 50 mM sodium maleate buffer (pH 6). To 0.5 mL of 2% disaccharide (sucrose or maltose), 0.5 mL of sample (1% *N. flagelliforme* powder suspension, 1% *N. flagelliforme* extract or sodium maleate buffer as control) and 0.5 mL of crude enzyme solution were added. The reaction mixture was incubated at 37 °C for 15 min. The reaction mixture was heated for 2 min in a boiling water bath to stop the reaction, and then the amount of glucose produced was measured by assay kits (Wako Pure Chemical Industries, Ltd., Japan).

Statistical analysis

Statistical analyses were performed with SPSS for windows (version 10.0. 5J; SPSS Inc., USA). All results are expressed as means ± standard deviations. The results of each rats group were compared statistically using unpaired Student’s *t*-test. Group differences were considered to be significant when *p* < 0.05.

Results

There was no difference in the food intake among three groups (date not shown).

Table 1 shows the weight of body and organs of rats on the final experimental day. The weight of perirenal adipose tissue in *N. flagelliforme* powder administered rats and *N. flagelliforme* extract administered rats was significantly lower than that of control rats.

Figure 1 shows the change in the fasting glucose levels of rats. In both of *N. flagelliforme* powder administered rats and *N. flagelliforme* extract administered rats, the fasting glucose levels on 21st day, 35th day, 49th day and 63rd day were significantly lower than control rats. The glucose level of *N. flagelliforme* extract administered rats was smaller than that of *N. flagelliforme* powder administered rats.

Table 2 shows the concentrations of total cholesterol, free cholesterol, triglyceride and phospholipids in the serum of rats on the final experimental day. The concentrations of triglyceride in the serum of *N. flagelliforme* powder administered rats and total cholesterol and triglyceride in the sera of *N. flagelliforme* extract...
administered rats were significantly lower than those of control rats.

Table 3 shows the concentrations of total lipids, triglyceride, total cholesterol and phospholipids in the liver of rats on the final experimental day. Triglyceride and total cholesterol concentrations in the liver were significantly lower in \textit{N. flagelliforme} powder and \textit{N. flagelliforme} extract administered rats than in control rats.

The excretion of feces and the contents of cholesterol and bile acid in feces of three groups are shown in Table 4. The amount of feces excretion and the content of total cholesterol and bile acid of \textit{N. flagelliforme} powder administered rats and \textit{N. flagelliforme} extract administered rats were significantly higher than those of control rats.

Table 5 shows the change in the blood glucose levels (mg dL$^{-1}$) after administration of basal diet. Glucose levels at 60 and 120 mins were significantly lower in \textit{N. flagelliforme} powder and \textit{N. flagelliforme} extract administered rats than in control rats.

Table 6 shows the inhibitory effect of \textit{N. flagelliforme} powder or \textit{N. flagelliforme} extract on the rat small intestinal sucrase and maltase activity. Both of \textit{N. flagelliforme} powder and \textit{N. flagelliforme} extract inhibited the activity of intestinal sucrase and maltase.

**Discussion**

In this study, we demonstrated that \textit{N. flagelliforme} powder and/or extract significantly ameliorated hyperglycemia and hyperlipidemia by reducing the blood glucose levels in type 2 non-insulin-dependent diabetic rats. Diabetes mellitus is sub-divided into two main forms. Type 1 diabetes mellitus is mainly due to an autoimmune mediated destruction of pancreatic $\beta$-cell islets. On the other hand, type 2 diabetes mellitus is characterized by insufficient insulin secretion and insulin resistance (Goldstein 2002). Type 2 diabetes has been increasing worldwide (Guariguata 2014). In Japan, in particular, the

Most research on the association between type 2 diabetes risk and regional adiposity argues that the intra-abdominal fat depot has the most detrimental metabolic effects (Björntorp 1991, 1993; Despres 1993; Kissebah 1996; Boyko et al. 1995, 1996). The weight of body, liver and kidney in N. flagelliforme administered rats did not differ from those in the control rats in this study. However, the weight of perirenal adipose tissue in both of N. flagelliforme powder and extract administered rats was significantly decreased (Table 1). The perirenal adipose tissue is one of the visceral adipose tissue. The blood glucose levels in both of N. flagelliforme administered rats were significantly decreased as compared to control rats (Fig. 1).

N. flagelliforme depressed the serum and liver cholesterol elevation in rats fed a cholesterol diet (Takenaka and Ishibashi 2009). The present study also showed a significant decrease in triglyceride and total cholesterol concentrations in the serum and liver in N. flagelliforme powder and extract administered diabetic rats (Tables 2 and 3). The inverse association of dietary fiber intake with total cholesterol levels in adults with type 2 diabetes was reported (Narayan et al. 2014). We also found that the excretion of feces and the contents of cholesterol and bile acid in feces were increased by N. flagelliforme administered (Table 4). N. flagelliforme used this study contained 1.9% dietary fiber. A small dose of guar gum was shown to be as effective as larger doses in lowering the blood glucose response after meal (Jenkins et al. 1976; Holt et al. 1979; Leeds et al. 1981) and in long term studies (Aro et al. 1981; Smith and Holm 1982; Osilesi et al. 1985; Torsdottir et al. 1989) improved the control of diabetes. N. flagelliforme decreased the glucose levels in an oral glucose tolerance test (Table 5). This result indicates that the fiber in N. flagelliforme inhibits the absorption of glucose. N. flagelliforme powder and/or extract depress the blood glucose by both of promote the excretion in feces and inhibit the absorption of glucose. We found the inhibitory effect of N. flagelliforme on the rat small intestinal sucrose and maltase activity (Table 6). This effect may contribute to the inhibition of glucose absorption.

Although additional studies are needed to determine serum insulin, adiponectin and glycosylated hemoglobin (HbA1c) concentration, the results of the present study suggest that N. flagelliforme has beneficial effects on blood glucose level and serum lipid concentrations in spontaneously non-insulin-dependent diabetic rats. And, this study is in agreement with the novel nutraceutical idea “Phycopahgism”.

**References**


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