Alterations in the blood glucose, serum lipids and renal oxidative stress in diabetic rats by supplementation of onion (*Allium cepa. Linn*)*

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Abstract

This study examined the anti-diabetic effect of onion (*Allium cepa. Linn*) in the streptozotocin (STZ)-induced diabetic rats. Male Sprague-Dawley rats were divided into normal rats fed control diet or supplemented with onion powder (7% w/w) and diabetic rats fed control diet or supplemented with onion powder. Diabetes was induced by a single injection of STZ (60 mg/kg, ip) in citrate buffer. The animals were fed each of the experimental diet for 5 weeks. Blood glucose levels of rats supplemented with onion were lower than those of rats fed control diet in the diabetic rats. Onion also decreased the total serum lipid, triglyceride, and atherogenic index and increased HDL-cholesterol/total cholesterol ratio in the diabetic rats. Glutathione peroxidase, glutathione reductase and glutathione S-transferase activities were high in the diabetic rats compared to normal rats and reverted to near-control values by onion. These results indicate that onion decreased blood glucose, serum lipid levels and reduced renal oxidative stress in STZ-induced diabetic rats and this effect might exert the anti-diabetic effect of onion.

Key Words: Diabetes, onion (*Allium cepa. Linn*), blood glucose, serum lipid profiles, renal antioxidant enzymes

Introduction

Oxidative stress induced by high glucose concentration plays a central role in complications of diabetes (Medvedeva *et al*., 2002), though it is not clear yet whether increased oxidative stress has a primary role in the pathogenesis of diabetic complications, or it is simply a consequence of them (Miranda *et al*., 2007). Oxidative stress induces the production of highly reactive oxygen radicals that are toxic to cells and has been attributed to protein glycation and/or glucose auto-oxidation owing to a hyperglycemic environment (Kesavulu *et al*., 2001). Oxygen radicals also interact with the lipid bilayer and produce lipid peroxides particularly in cell membranes (Haygaard, 1968; Miranda *et al*., 2007). Lipid peroxidation of cellular structures is thought to play an important role in atherosclerosis and late complications of diabetes (Kesavulu *et al*., 2001). Hence, it is likely that a substance known to reduce hyperglycemia and oxidative stress in vivo would reduce progression of cell damage in clinical diabetes. Although controlling blood glucose level is the most important approach in the management of complications of diabetes, abnormalities in lipid metabolism are known to contribute to the pathogenesis of progressive renal disease (Feldt-Rasmussen *et al*., 1986). Change in the plasma lipid concentration is a frequent complication in patients with diabetes mellitus (Hartnett *et al*., 2000; Howard *et al*., 1978; Nikkila & Kekki, 1973; Qureshi *et al*., 2002). The important role of lipid abnormalities in the pathogenesis of glomerular injury is understood by the almost invariable presence of hyperlipidemia in patients with renal disease (Babu & Srinivasan, 1999; Moorhead, 1991).

Onion (*Allium cepa. Linn*), commonly used in our daily diet, has been extensively studied for its therapeutic uses. Onion oil has been reported to effectively decrease the lipid levels in experimental animals (Bordia *et al*., 1975; Bordia *et al*., 1977). It also has flavonoids, such as quercetin and quercetrin, which have antioxidant and anticancer effects (Carson, 1987; Reddy *et al*., 1993). Aqueous extraction of onion showed an antithrombotic effect in stretozotocin (STZ)-induced diabetic rats (Jung *et al*., 2002). However, there is a little information about the effect of onion on the complication of diabetes.

The present study was designed to extend the current information on the hypoglycemic and hypolipidemic effects of onion as well as to determine its effects on renal antioxidant enzyme systems in diabetic rats to establish the mechanism of the anti-diabetic effects of onion.

Materials and Methods

Animals

Weanling male Sprague-Dawley rats (180~230 g) were purchased from Samyouk Animal Center (Seoul, Korea). The animals were housed in the cages under standard conditions (12:12 h light/dark cycle, 50% relative humidity at 21℃) and given free access to a semi-purified diet (Table 1) and water. After 7-d acclimation, experimental diabetes was induced by a single intraperitoneal injection of streptozotocin (Sigma) in citrate buffer at a dose of 60 mg/kg body weight. Blood samples were obtained from the tail vein of the animals at 72 hours after STZ injection and fasting blood glucose levels were determined. Rats with fasting blood glucose levels above 250 mg/dl were used as the diabetic animals. Just after STZ injection, two groups of diabetic rats and two groups of normal rats in parallel were fed experimental diet and maintained on the diets for 5 weeks. The onion-supplemented diet was made of 7% of freeze-dried onion powder added into control diet (Table 1). Food and water consumptions were measured every day and weight gain was measured every week. At the end of 5 weeks, the animals were decapitated after overnight fasting and the kidneys were quickly excised and processed for enzyme assays.

Blood glucose and serum lipids

Blood glucose was measured from the blood of all the animals, taken from the tail vein using a kit (Medisense 2, Korea) every

week. The concentrations of serum total lipid, total cholesterol, HDL-cholesterol and triglyceride were determined using a commercial kit (Asan, Korea) at the end of the experiment. Atherogenic index and HDL-cholesterol / total cholesterol ratio were calculated from the data.

Antioxidant enzyme activities in kidney

An appropriate portion of kidney was homogenized in ice-cold Tris buffer, pH 7.4. The homogenates were centrifuged at 12,000xg for 20 min at 4℃, then again at 105,000xg for 1hr at 4℃. The supernatant (cytosol) was used for measuring the activities of glutathione peroxidase (Tapple, 1978), glutathione reductase (Carlberg & Mannervick, 1985), and glutathione S-transferase (Habig *et al.*, 1974). Glucose 6-phosphatase activity (Banginski *et al.*, 1983) and TBARS contents were measured in the microsome. Protein content was measured according to the Lowry method (Lowry *et al.*, 1951).
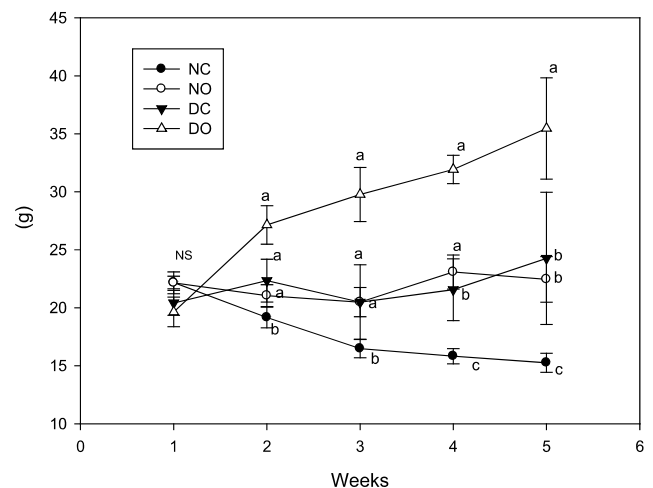
Statistical analysis

All data are expressed as the mean ± SE. The statistical analyses were performed on an SPSS program (version 12). The group comparisons were done using a variance analysis followed by Duncan's multiple range test. Statistical significance was considered at *P* < 0.05.

Results

Body weight, food intake and water intake

Diabetic rats fed the control diet showed no significant difference in food intake compared to normal rats. However, the

Table 1. Composition of experimental diets (Unit: g/100 g diet)

Component	Control diet	Onion powder diet
Corn starch	54.7	47.7
Casein	20.0	20.0
α-Cellulose	5.0	5.0
Mineral mixture[1]	4.0	4.0
Vitamin mixture[2]	1.0	1.0
DL-methionine	0.3	0.3
Corn oil	15.0	15.0
Onion powder[3]	-	7

[1] AIN 76 Mineral mixture. Nutritional Biochemicals, ICN Life Science Group, Cleveland, Ohio Composition of mineral mixture, g/kg mixture ; Calcium phosphate dibasic 500.00 g, Sodium chloride 74.00 g, Potassium citrate monohydrate 220.00 g, Potassium sulfate 52.00 g, Magnesium oxide 24.00 g, Manganous carbonate (43-48% Mn) 3.50 g, Ferric citrate (16-17% Fe) 6.00 g, Zinc carbonate (70% ZnO) 1.06 g, Cupric carbonate (53-55% Cu) 0.30 g, Potassium iodide 0.01 g, Sodium selenite 0.01 g, Chromium sulfate 0.55 g, Sucrose, finely powdered 118.0 g
[2] Nutritional Biochemicals, ICN Life Science Group, Cleveland, Ohio Vitamin mixture is composed of; Vit.A acetate (500,000 IU/g) 1.8 g, Vit.D conc. (850,000 IU/g) 0.125 g, α-Tocopherol (250 IU/g) 22.0 g, Ascorbic acid 45.0 g, Inositol 5.9 g, Choline chloride 75.0 g, Menadione 2.25 g, P-Aminobenzoic acid 5.0 g, Niacin 4.25 g, Riboflavin 1.0g, Pyridoxine hydrochloride 1.0 g, Calcium pantothenic acid 3.0 g, Biotin 0.02 g, Folic acid 0.09 g, Vit.B12 0.00135 g, and Dextrose to 1 kg
[3] Onion, growing Muan-gun, was sliced and frozen for 24hr at -20℃. The next day, frozen sliced onion was loaded into a freeze-dryer (ilshin Co. Korea). Drying was carried out with a shelf temperature of -35℃ and a chamber pressure of 〈100 mTorr for 48 h and then the chamber pressure was increased to 200 mTorr. Shelf temperature was held at 0℃ for 4 h. When prepared Diet, grind frozen onion to powder.

Fig. 1. Effect of supplementation of onion on the food intakes in the rats (g/day/rat). Values in the same period with same letter are not significantly different by Duncan's multiple range test (*P* < 0.05). NC : Normal rats fed control diet, NO: Normal rats fed onion powder diet (7%, w/w), DC: Diabetic rats fed control diet, DO: Diabetic rats fed onion powder diet (7%, w/w)

Table 2. Effect of onion powder on body weight, food intakes and water intakes in rats

Groups		No. of animals	Body weight gain (g/5 weeks)	Food Intake (g/day)	Water Intake (ml/day)	FER[1]
Normal	NC	8	78.97 ± 13.45[a]	17.79 ± 0.59[b]	20.94 ± 1.04[b]	4.33 ± 0.55[a]
	NO	10	68.58 ± 8.55[a]	22.04 ± 1.17[bc]	32.13 ± 6.91[b]	3.110 ± 0.40[a]
Diabetics	DC	8	-31.06 ± 8.66[b]	21.82 ± 2.71[bc]	114.52 ± 6.87[a]	-1.77 ± 0.47[c]
	DO	10	6.52 ± 2.93[b]	28.84 ± 1.88[a]	124.54 ± 7.45[a]	0.11 ± 0.74[b]

Experimental conditions are same as Fig. 1.
Values are Mean ± SE.
Values within the same row with same letter are not significantly different by Duncan's multiple range test ($P < 0.05$).
[1] Feed efficiency ratio

Table 3. Levels of serum triglyceride, total lipid, total cholesterol, HDL-cholesterol, LDL-cholesterol, HTR, and atherogenic index in normal and diabetic rats

| Groups		Total lipid (mg/dl)	Triglyceride (mg/dl)	Total cholesterol (mg/dl)	HDL-cholesterol (mg/dl)	HTR [1]	AI[2]
Normal	NC	174.6 ± 16.9[bc]	82.0 ± 8.7[b]	96.6 ± 7.3[cd]	54.6 ± 5.2[b]	0.56 ± 0.03[ab]	0.80 ± 0.08[b]
	NO	207.3 ± 14.2[bc]	116.76 ± 15.8[b]	86.1 ± 5.8[b]	48.1 ± 2.7[b]	0.57 ± 0.03[ab]	0.80 ± 0.09[b]
Diabetic	DC	409.6 ± 78.8[a]	225.2 ± 28.3[a]	131.2 ± 12.4[a]	60.0 ± 2.8[ab]	0.47 ± 0.03[b]	1.17 ± 0.13[a]
	DO	272.1 ± 24.2[b]	92.0 ± 13.2[b]	110.4 ± 5.5[ab]	73.0 ± 6.8[a]	0.67 ± 0.07[a]	0.58 ± 0.11[b]

Experimental conditions are same as Fig. 1.
Values are Mean ± SE.
Values within the same row with same letter are not significantly different by Duncan's multiple range test ($P < 0.05$).
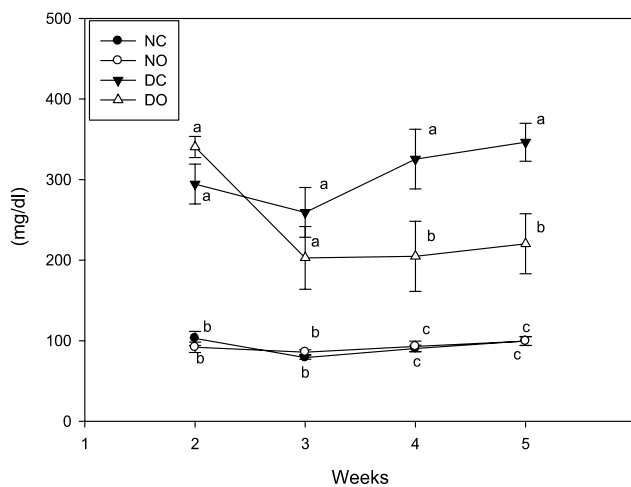[1] HTR: HDL-cholesterol/Total cholesterol ratio
[2] AI: atherogenic index: (Total cholesterol-HDL-cholesterol)/HDL-cholesterol
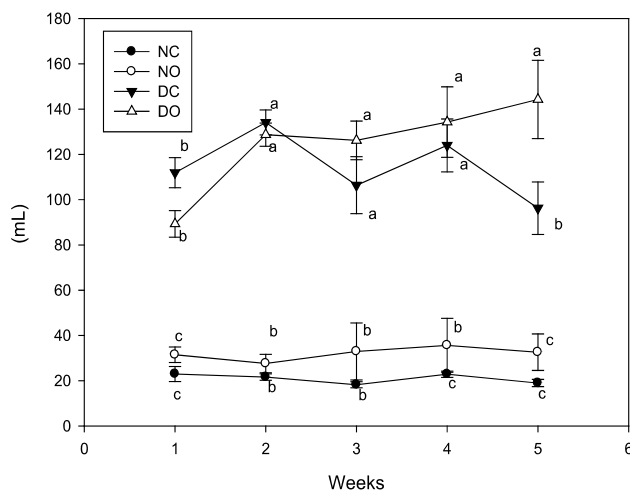
Fig. 2. Effect of supplementation of onion on the water intakes in the rats (ml/day/rat). Experimental conditions are same as Fig. 1. Values in the same period with same letter are not significantly different by Duncan's multiple range test ($P < 0.05$).

Fig. 3. Effect of supplementation of onion on the fasting blood sugar in the rats. Experimental conditions are same as Fig. 1. Values in the same period with same letter are not significantly different by Duncan's multiple range test ($P < 0.05$).

food intakes of diabetic rats supplemented with onion were significantly higher than those of other groups (Fig. 1). Diabetic rats showed low body weight gain regardless of diet. Onion increased the feed efficiency ratio in the diabetic rats, even though it was still lower than those of normal groups (Table 2). Water intakes of the diabetic rats were higher than those of the normal rats. Onion did not show any appreciable influence on the water intakes in the diabetic animals (Fig. 2, Table 2).

Fasting blood glucose and serum lipids

Levels of fasting blood sugar in the diabetic rats were significantly higher than those of the normal rats throughout the experimental period. Blood glucose in the diabetic rats supplemented with onion became lower when the rats had been fed onion for 3 weeks and was significantly lower than that of diabetic rats fed the control diet at the end of experiment (Fig. 3).

Onion did not affect serum total lipid, triglyceride and HDL-cholesterol in the normal rats. HDL-cholesterol/total cholesterol ratio and atherogenic index of the normal rats were also not affected by onion. Serum total lipid, triglyceride, total cholesterol and atherogenic index of the diabetic rats fed control diet were increased by 235%, 275%, 136% and 146%, respectively compared to normal rats. However, serum total lipid, triglyceride and the atherogenic index of the diabetic rats were decreased by onion and not significantly different from those

Table 4. Renal biochemical parameters in the normal and diabetic rats

Groups		GPx (nmoles NADPH oxidized/min/mg protein)	GR (nmoles NADPH oxidized/min/mg protein)	GST (nmoles CDNB conjugated/min/mg protein)	G6Pase (nmole Pi liberated/min/mg protein)	TBARS (nmole/mg protein)
Normal	NC[3]	363.29 ± 21.43[c]	95.62 ± 4.30[b]	134.85 ± 0.51[b]	441.22 ± 26.43	0.41 ± 0.02
	NO	346.23 ± 12.16[c]	73.74 ± 3.77[c]	159.23 ± 8.98[b]	425.35 ± 18.71	0.42 ± 0.02
Diabetics	DC	735.15 ± 58.72[a]	111.12 ± 3.16[a]	324.55 ± 2.30[a]	424.49 ± 19.30	0.40 ± 0.03
	DO	600.13 ± 57.73[b]	94.73 ± 4.11[b]	166.58 ± 9.85[b]	403.63 ± 19.90	0.40 ± 0.02

Experimental conditions are same as Fig. 1.
Values are Mean ± SE.
Values within the same row with same letter are not significantly different by Duncan's multiple range test ($P < 0.05$).

of normal rats fed the control diet (Table 3). Serum total cholesterol of the diabetic rats was decreased by onion but not significantly different compared to diabetic rats fed the control diet (Table 3).

Renal antioxidant enzymes and TBARS content

Diabetes induced high activities of glutathione peroxidase (GPx), glutathione reductase (GR) and glutathione S-transferase (GST) in the rats fed the control diet (Table 4). Increased activities of these enzymes in the diabetic rats were significantly decreased by onion. TBARS content and glucose 6-phosphatase (G6Pase) activity showed no difference between the control and the diabetic rats.

Discussion

Streptozotocin (STZ) induced diabetes in animals caused weight loss due to abnormal carbohydrate and lipid metabolism and increased catabolism of protein. For this reason, weight reduction is being used as a marker of diabetes mellitus induced by STZ (Ghosh *et al.*, 1994). In the present study, onion decreased weight loss in the diabetic rats by the increase of feed efficacy ratio. Onion did not show any appreciable influence on the water intakes in the diabetic animals.

Hyperglycemia can induce lipid peroxidation and thereby atherosclerosis (Medvedeva *et al.*, 2002). It also induces gene expression of angiotensinogen in the kidney and insulin receptor resistance (Zhang *et al.*, 2000). In the present study, onion significantly decreased blood glucose in the diabetic rats. Onion might play an important role to prevent complications of diabetes by maintaining blood glucose.

We observed onion lowered serum lipids in the diabetic rats. The lowering effect on serum lipids in the diabetic animals might be associated with a good glucose control by onion. A good glucose control was reported to improve the parameters of serum lipid profile in patients with diabetes mellitus (Medvedeva *et al.*, 2002). Onion itself was also reported to have a serum lipid lowering effect in hyperlipidemic experimental animals (Bordia *et al.*, 1975; Bordia *et al.*, 1977; Carson 1987). Serum lipid lowering effect of onion can be one of the mechanisms of

anti-diabetic effects and contribute to the prevention of diabetic nephropathy. Hyperlipidemia is a risk factor for declining kidney function in patients with diabetic nephropathy (Bordia *et al.*, 1975). It was also reported that patients with low serum cholesterol concentration exhibited a lower degree of kidney lesions than those with high serum cholesterol concentration (Mulec *et al.*, 1990).

The increase of free radical mediated-toxicity is well documented in clinical diabetes (Nourooz-Zadeh *et al.*, 1997) and STZ-induced diabetic rats (Wohaie & Godin, 1987). Diabetes-induced changes in antioxidative defense enzyme activities are known to be tissue specific (Irizar & Ioannides, 1995; Obrosova *et al.*, 1998). In the kidney, SOD, GPx, GR, and GST, but catalase, appeared up-regulated in short term diabetes, which is consistent with a number of reports (Irizar & Ioannides, 1995; Kakkar *et al.*, 1997; Mak, 1996). Glutathione related antioxidant enzymes such as GPx, GR, and GST are the most important enzymes of the cellular antioxidant system (Dincer *et al.*, 2002). In the present study, enzyme activities of GPx, GR and GST in the kidneys were increased in diabetic animals. GPx activity might be increased in response to increased oxidative stress. TBARS content showed no difference between the control and the diabetic rats in the present study. It might be correlated to increased GPx activity. Kakkar *et al.* (1997) reported that increased GPx activity was observed in the 2nd, 3rd, and 4th weeks with progression of diabetes and correlated to the lack of rise in TBARS levels at this period. Most of the diabetes-induced changes were reverted to near normal values by onion in this study. The possibility that modulation of the hyperglycemic status by onion contributed to the amelioration of renal lesions cannot be ruled out. Another possibility is that onion itself acted as an antioxidant and could attribute to the amelioration of free radical induced toxicity in the diabetic kidneys. A variety of sulfur-containing compounds and their precursors are the main compounds, which attribute to the antioxidant activity of *Allium* plants (Nuutila *et al.*, 2003). In addition to sulfur-containing compounds, onion has a large amount of quercetin, which has antioxidant effects (Jung *et al.*, 2002; Reddy *et al.*, 1993). Therefore, we can conclude that oxidative stress was induced in the kidneys of diabetic animals and effectively prevented by onion.

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