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Effects of Corni Fructus Extract on the Progression of Diabetes and Renal Function in STZ-induced Diabetic Rats

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This study investigated the effect of Corni Fructus (*Cornus officinalis* Sieb. et Zucc.) extract on hyperglycemia and renal function in streptozotocin-induced diabetic rats. Male Sprague-Dawley rats were divided into three groups including normal control (NC), diabetic control (DC), and diabetic treatment with Corni Fructus (DCF). Over a 4-week experimental period, Corni Fructus aqueous extract was administered orally at 500 mg/kg BW/day. The final fasting serum glucose, serum urea nitrogen, triglyceride, urinary total protein level, and relative weight of the left kidney in the DCF group were significantly lower than the DC group. Serum insulin level in the DCF group was higher than the DC group by 23%. The renal xanthine oxidase and superoxide dismutase activities in the DCF group were significantly lower than the DC group. The renal catalase activity in the DCF group was significantly higher than the DC group. In conclusion, these results indicated that Corni Fructus can reduce glucose level and prevent or retard the development of diabetic complication via its antioxidative effect and protecting against diabetic renal damage in streptozotocin-induced diabetic rats.

Key Words: Corni Fructus, Streptozotocin, Renal function, Diabetic rat, Antioxidant effect

INTRODUCTION

Diabetes mellitus is a major endocrine disorder and growing health problem in most countries (Anderson et al., 2001). Diabetes is a chronic disease that cannot be completely cured may develop complications if not properly regulated. Diabetic nephropathy as a late complication in chronic diabetes occurs in 40% of human diabetes cases (Welta et al., 2007). Diabetic nephropathy is characterized by the proteinuria, loss of renal function, excessive deposition of protein in extracellular matrix. Recently, attention has been drawn to the theory that oxidative stress is involved in the development of complications associated with chronic diabetes (Maritim et al., 2003). Oxidative stress has been considered as a common pathogenetic factor in diabetic nephropathy and other complications (Baynes, 1991). An earlier study has shown that treatment with antioxidant reduces diabetic complications (Wohaieb & Godin, 1987).

In spite of the presentation of many hypoglycemic agents, diabetes and its related complications are still a major medical problem. Therefore, it is an increasing demand for natural products and traditional herbal medicines which have antidiabetic activities. Fructus of *Cornus officinalis* Sieb. et Zucc. (Corni Fructus) has been used as Korean traditional medicine. It represents one of the seven-component drugs in Yukmi-jihang-tang that has been used for the treatment of diabetes mellitus or diabetic complications in Korean traditional medicine (Jin et al., 2006). Major chemical constituents which have been identified by many investigators are saponins, phenolic acid (gallic acid, tannic acid) and loganin (Li et al., 1994). Saponins and phenolic acid are known to have antioxidant activities (Rong et al., 1995). Recently, it has been reported that Corni Fructus has beneficial effect on advanced glycation end product-mediated

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renal injury in STZ-treated diabetic rats (Yamabe et al., 2007). This study investigated the effects of Corni Fructus extract on the progression of diabetes, antioxidant enzyme activities and renal function in streptozotocin (STZ)-induced diabetic rats.

MATERIALS AND METHODS

1. Corni Fructus aqueous extraction

The fruits of *Cornus officinalis* were obtained from the Gunwi, Gyeongbuk, Korea and authenticated by a doctor of oriental medicine Y.G. Choi, Department of Oriental Medicine, College of Oriental Medicine, Sangji University (Gangwon-Do, Korea). Three-hundred gram of Corni Fructus (fruits of *Cornus officinalis*) with 5000 ml distilled water was boiled for 2 hours in a heating extractor (COSMOS-660, Kyungseo Machine Co., Korea) and concentrated to 1500 ml. Thereafter, the aqueous extract was distributed into pouches containing daily volume each and stored at cold temperature. The calculated yield of the aqueous extract was 25% (w/w) by a lyophilization method.

2. Animals and experimental design

Male Sprague-Dawley rats with body weight ranging from 200 to 220 g were obtained from a supply company, Daehan Biolink (Chungbook, Korea). Animals were housed in plastic cages at 22±1℃, with a relative humidity of 50 ±5%, an alternating 12-h light-dark cycle, and were allowed to have access to their respective diets *ad libitum*. The animals were allowed to acclimatize to the laboratory environment for 7 days and then were randomly assigned to one of three groups (seven animals each), for the 4-weeks experiment. The experimental groups were as follows: Group I, non-diabetic control rats (NC); Group II, diabetic control rats (DC); Group III, diabetic rats fed Corni Fructus extract (DCF). For repeated oral administration, rats were treated once daily for 4 weeks. Group I and II received distilled water, group III received the aqueous extract at 10 ml/kg BW/day (500 mg extracted powder of Corni Fructus /kg BW/day). Body weight and blood glucose level were monitored weekly between nine and ten o'clock in the morning. Daily food and water consumption were monitored weekly, and were determined by subtracting left-over amount from the total amount provided. The organs were weighed using an electronic balance to calculate weight. Each one of the removed kidneys was stored at -80℃ and another each one was fixed in 10% formalin solution.

3. Experimental induction of diabetes

Diabetes was induced by a single intraperitoneal injection of STZ (70 mg/kg BW; Sigma, USA) in freshly prepared citrate buffer (0.4 M, pH 4.5) after an overnight fast. The NC group was injected intraperitoneally with an equivalent amount of buffer (0.4 M citrate buffer, pH 4.5). Diabetic rats were confirmed by measuring the 4-h fasting blood glucose level from the tail vein at 72-h after injection with STZ. The animals with blood glucose level above 300 mg/dl were considered to be diabetic and included in the experiment. Blood glucose levels were determined using the glucose oxidase method with a Gluco card II^{TM} (ARKRAY, Japan).

4. Blood analytical measurement

At 28 days after beginning of the experiment, 4-h fasting blood samples were collected from the aorta of the abdomen under ether anesthesia. The serum glucose, urea nitrogen, creatinine, triglyceride, and total cholesterol levels were measured using a commercial available assay kit (EIKEN, Japan) with a Hitachi-7600 Analysis System (Hitachi, Japan) by the method of Brandstrup et al. (1957), Patton and Crouch (1977), Fossati et al. (1983), McGrown et al. (1983), Allain et al. (1974), respectively. Serum insulin was measured using a rat insulin ELISA kit (Mercodia, USA) with a microplate reader (Molecular devices, USA).

5. Urine analytical measurement

At 25 days after beginning of the experiment, the animals were transferred to metabolic cages individually and urine was collected for 24 h. The urinary creatinine was estimated using a commercial available assay kit (EIKEN, Japan) with a Hitachi-7600 Analysis System (Hitachi, Japan). The urinary total protein was estimated by the pryogallol red method using a commercial available assay kit (Auto kit Micro TP, Wako Pure Chemical, Japan) with ADVIA 1650

Table 1. Water and food intakes, body weight gain and food efficiency ratio of normal and diabetic rats

Items	Groups[a]		
	NC	DC	DCF
Water intake (ml/day)	49.40±3.85	294.29±20.64###	276.57±75.91###
Food intake (g/day)	27.40±2.30	51.00±3.65###	45.57±5.83###
Body weight gain (g/day)	3.15±0.29	-0.34±0.29###	0.07±1.01###
Food efficiency ratio[b] (%)	11.56±1.49	-0.67±0.61###	0.24±2.34###

[a]NC: Normal Control, DC: Diabetic Control, DCF: Diabetic Corni Fructus.
[b]Food efficiency ratio = (body weight gain/ food intake) × 100.
Values are means ± SD of 7 rats. The value with a sharp-note is significantly different from NC group by t-test (###; $P<0.001$).

Analysis System (BAYER, USA). The urinary total protein content was calibrated to the urinary volume (urinary protein/urinary volume). The creatinine clearance (CCr) was calculated according to the following formula: (urine creatinine/serum creatinine) × (total volume/(24 h×60 min)).

6. Antioxidant enzyme activities

The kidney tissue was homogenized in 0.25 M sucrose solution using a tissue homogenizer with a Teflon pestle at 4℃ to give 20% homogenate (w/v). The homogenate was centrifuged at 600 ×g for 10 minutes to remove any cell debris and then the supernatant was further centrifuged at 10000 ×g for 20 minutes to remove the mitochondria pellet. Finally, the supernatant was ultracentrifuged at 105000 ×g for 60 minutes to obtain the cytosol supernatant. The amounts of protein in the mitochondrial and cytosolic fractions were measured by the method of Lowry et al. (1951) with bovine serum albumin as the standard. The activities of superoxide dismutase (SOD), catalase (CAT), glutathione S-transferase (GST), and xanthine oxidase (XO) were measured by the methods of Martin et al. (1987), Aebi (1974), Habig et al. (1974) and Stripe and Della (1969), respectively.

7. Immunohistochemical analysis

Rabbit polyclonal TGF-β1 antibodies (Santa Cruz Biotechnology, USA) were used for immunostaining according to the following procedure. After being deparaffinized in xylene and rehydrated in ethanol, sections were heated in 0.01 M citrate buffer (pH 6.0) at 120℃ for 10 minutes to retrieve the antigen. After treatment in 0.3% H_2O_2, the sections were incubated with blocking solution (non-immune for 20 minutes to block nonspecific binding and then incubated with TGF-β_1 antibodies, diluted 1:50 in dilution buffer, at room temperature. The sections were incubated with rabbit IgG+HRP and visualized using diaminobenzidine tetrahydrochloride and counterstained in Mayer's hematoxylin. The negative control contained 1% BSA instead of primary antibody.

8. Statistical analysis

Values were given as means ± SD of 7 rats in each group. The data were analyzed by Student's t-test using SPSS-12.0. The limit of statistical significance was set at $P<0.05$.

RESULTS

1. Water and food intakes, body weight gain and efficiency ratio

Water and food intakes in diabetic groups were significantly higher ($P<0.001$) than the NC group by 478% and 76%, respectively and they did not differ between the two diabetic groups. Body weight gain and food efficiency ratio in diabetic groups were significantly lower ($P<0.001$) than the NC group and they were higher in the DCF group than the DC group by 121% and 136%, respectively (Table 1).

2. Blood glucose levels

Fig. 1 shows change in blood glucose levels of normal and diabetic rats. At 4 weeks after beginning of the experiment, blood glucose level in the DC group was significantly higher ($P<0.001$) than the NC group by 515%. However, it was lowered in the DCF group by 26% ($P<0.01$) compared to the DC group.

3. Fasting serum glucose, insulin, urea nitrogen, creatinine and lipid levels

A significant elevation ($P<0.001$) in final fasting serum glucose level was observed in diabetic groups compared to the NC group and it was significantly lower ($P<0.05$) in the DCF group than the DC group by 24%. Serum insulin level in the DC group was significantly lower ($P<0.001$) than the NC group and it was higher in the DCF group than the DC group by 23%. Serum urea nitrogen level in the DC group was significantly higher ($P<0.001$) than the NC group and it was significantly lower ($P<0.05$) in the DCF group than the DC group by 23%. Serum creatinine level in the DC group was significantly lower ($P<0.01$) than the NC group. However, they did not differ between the DC and DCF groups. Serum total cholesterol levels did not differ among the NC and diabetic groups. Serum triglyceride level in the DC group was higher than the NC group by 37%. However, it was significantly lowered ($P<0.05$) in the DCF group by 48% compared with the DC group (Table 2).

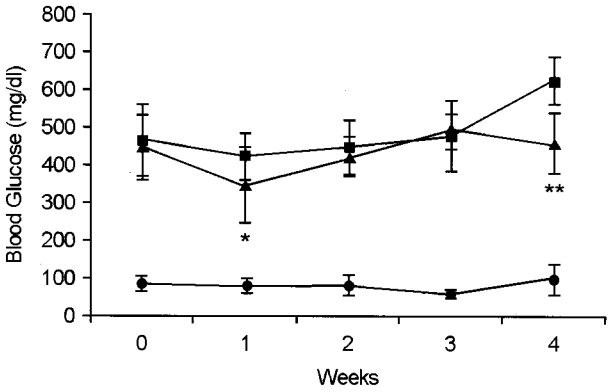

Fig. 1. Changes in blood glucose levels of normal and diabetic rats fed Corni Fructus extract for 4 weeks. Values are means ± SD of 7 rats. The value with an asterisk is significantly different from DC group by t-test (*; $P<0.05$; **; $P<0.01$). ●; NC, ■; DC, ▲; DCF.

4. Determination of urinary volume, total protein and creatinine clearance

Urinary volume and urinary total protein were significantly higher in the DC group than the NC group by 1171% ($P<0.001$) and 152% ($P<0.01$), respectively. Urinary volume did not significantly differ between the DC and DCF groups. Urinary total protein level in the DCF group was significantly lowered by 29% ($P<0.05$) compared with the DC group. Creatinine clearance in the DC group was lower than the NC group by 15% and it was higher in the DCF group

Table 2. Fasting serum glucose, insulin, urea nitrogen, creatinine and lipid levels of normal and diabetic rats

Items	Groups		
	NC	DC	DCF
Glucose (mg/dl)	167.00±7.04	619.67±124.95$^{\#\#\#}$	468.57±117.49$^{\#\#\#*}$
Insulin (pmol/l)	156.41±80.80	18.14±1.24$^{\#\#\#}$	22.38±4.48$^{\#\#\#}$
Urea nitrogen (mg/dl)	13.64±0.88	36.27±3.67$^{\#\#\#}$	27.65±7.43$^{\#\#*}$
Creatinine (mg/dl)	0.44±0.05	0.32±0.03$^{\#\#}$	0.31±0.03$^{\#\#}$
Total cholesterol (mg/dl)	90.60±6.62	89.83±14.22	88.14±18.93
Triglyceride (mg/dl)	69.80±18.66	95.50±37.35	49.17±15.77*

Values are means ± SD of 7 rats. The value with a sharp-note is significantly different from NC group by t-test ($^{\#\#}$; $P<0.01$, $^{\#\#\#}$; $P<0.001$). The value with an asterisk is significantly different from DC group by t-test (*; $P<0.05$)

Table 3. Urinary volume, total protein and creatinine clearance of normal and diabetic rats

Items	Groups		
	NC	DC	DCF
Urinary volume (ml/kg/day)	64.13±12.76	815.52±75.17$^{\#\#\#}$	772.91±304.76$^{\#\#}$
Urinary total protein (mg/day)	16.04±3.88	40.35±5.38$^{\#\#}$	28.75±7.60$^{\#*}$
Creatinine clearance[a] (ml/min)	3.00±0.80	2.54±0.75	3.27±0.85

[a] Creatinine clearance: (urine creatinine/serum creatinine)×(total volume/ (24 h × 60 min).
Values are means ± SD of 7 rats. The value with a sharp-note is significantly different from NC group by t-test ($^{\#}$; $P<0.05$, $^{\#\#}$; $P<0.01$, $^{\#\#\#}$; $P<0.001$). The value with an asterisk is significantly different from DC group by t-test (*; $P<0.05$)

Table 4. Organ weight of normal and diabetic rats

Organs	Groups		
	NC	DC	DCF
Liver	10.80±1.12†	9.27±0.96#	9.24±1.31
	3.00±0.23‡	4.46±0.34###	4.30±2.78###
Kidney (right)	1.21±0.11	1.31±0.13	1.31±0.10
	0.34±0.03	0.63±0.05###	0.62±0.06###
Kidney (left)	1.21±0.11	1.39±0.12#	1.30±0.14
	0.34±0.03	0.67±0.04###	0.61±0.06###*
Heart	1.09±0.15	0.73±0.13##	0.76±0.12##
	0.30±0.04	0.35±0.03#	0.35±0.02#

†Absolute organ weight, Unit: g.
‡Relative organ weight, Unit: g/100 g body weight.
Values are means ± SD of 7 rats. The value with a sharp-note is significantly different from NC group by t-test (#; $P<0.05$, ##; $P<0.01$, ###; $P<0.001$). The value with an asterisk is significantly different from DC group by t-test (*; $P<0.05$)

Table 5. Effect of Corni Fructus on kidney XO, SOD, CAT and GST activities of normal and diabetic rats

Enzymes	Groups		
	NC	DC	DCF
XO[a]	1.64±0.35	2.08±0.52	1.52±0.19*
SOD[b]	2.18±1.34	6.59±0.49###	4.58±1.23#**
CAT[c]	4.21±0.59	3.08±0.90#	5.01±1.21**
GST[d]	21.48±3.52	27.55±3.72#	25.39±3.49

[a] Unit: nmole uric acid formed/mg protein/min.
[b] Unit: U (50% inhibition of autoxidation of hematoxylin) mg protein/min.
[c] Unit: H_2O_2 nmole reduced/mg protein/min.
[d] Unit: nmole 2, 4-dinitrobenzene-glutathione conjugate/mg protein/min.
Values are means ± SD of 7 rats. The value with a sharp-note is significantly different from NC group by t-test (#; $P<0.05$, ###; $P<0.001$). The value with an asterisk is significantly different from DC group by t-test (*; $P<0.05$, **; $P<0.01$)

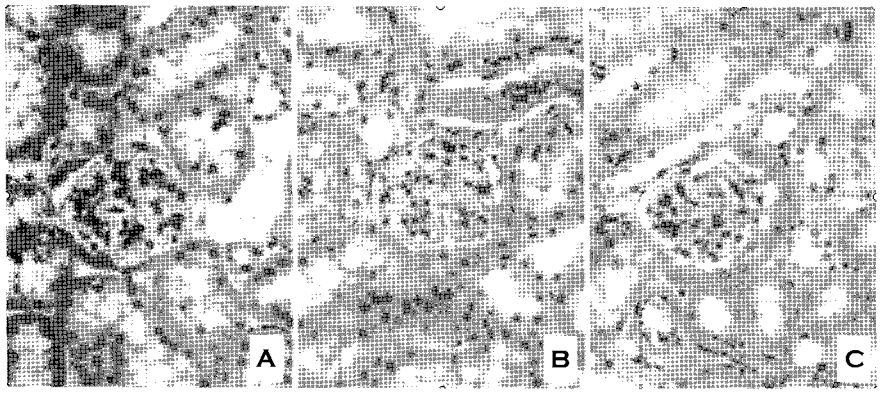

Fig. 2. Immunoperoxidase staining for TGF-β1 in glomerulus of normal and diabetic rats fed Corni Fructus extract for 4 weeks. **(A)** non-diabetic control, **(B)** untreated diabetic and **(C)** Corni Fructus treated diabetic rat. ×100.

than the DC group by 29% (Table 3).

5. Organ weight

Relative weight of liver ($P<0.001$), kidney ($P<0.001$) and heart ($P<0.05$) were significantly higher in the diabetic groups than the NC group. Relative weight of left kidney in the DCF group was significantly lower ($P<0.05$) than the DC group (Table 4).

6. Antioxidant enzyme activities

The activities of XO, SOD ($P<0.001$) and GST ($P<0.05$) in the DC group were higher than the NC group. However, they were lowered in the DCF group compared with the DC group by 27% ($P<0.05$), 31% ($P<0.01$) and 8%, respectively. Activity of CAT in the DC group was significantly lower ($P<0.05$) than the NC group. However, it was significantly higher ($P<0.01$) in the DCF group by 63% than the DC group (Table 5).

7. Immunohistochemical analysis

Glomerular TGF-β_1 accumulation did not significantly differ between the DC and DCF groups (Fig. 2).

DISCUSSION

In this study we investigated the effect of the Corni Fructus extract (CFE) on blood glucose, antioxidant enzyme activities, renal TGF-β_1 expression, and renal function in STZ-induced diabetic rats (70 mg/kg body weight i.p.). Male Sprague-Dawley rats were divided into three groups of seven animals each. The oral administration of Corni Fructus aqueous extract (500 mg extracted powder/kg body

weight/day) was performed over a 4-week experimental period.

Increased food and water intakes and decreased body-weight gain were observed in diabetic rats compared with non-diabetic rats, which were attributed to a polyphagic condition and weight loss due to excessive break down of tissue protein (Chatterjea and Shinde, 2002). The treatment with CFE improved food efficiency ratio and increased body weight gain compared with diabetic control rats, indicating control over polyphagia and muscle wasting resulted in the hyperglycemic condition to some extent.

The treatment with CFE significantly reduced serum glucose and increased serum insulin to some extent. This may be beneficial in the amelioration of the diabetic state and could explain the lowered blood glucose levels in rats fed CFE. Serum triglyceride level of the diabetic group treated with CFE was significantly lower than the diabetic control group by 48%. Therefore, CFE seems to elicit a moderate, positive effect and may be useful in the prevention of some forms of diabetic hypertriglyceridemia and hyperglycemia.

Hyperglycemia also generates reactive oxygen species (ROS) that in turn cause lipid peroxidation and membrane damage (Hunt et al., 1988). Previous studies have reported that lipid peroxidation in the liver, kidney, and brain of diabetic rats was increased (Venkateswaran and Pari, 2002; Latha and Pari, 2003). Antioxidant enzymes are capable of eliminating ROS and lipid peroxidation products, thereby protecting cells and tissues from oxidative damage. Diabetic nephropathy is one of the most important microvascular complications of diabetes mellitus. Recent studies have indicated that ROS plays a key, intermediate role in the development of diabetic nephropathy. High glucose level directly increases hydrogen peroxide production of mesangial cells and lipid peroxidation of glomerular mesangial cells (Anjaneyulu and Chopra, 2003).

XO has been proposed to be a major source of ROS in diabetes mellitus (Butler et al., 2000). SOD accelerates dismutation of superoxide radicals to hydrogen peroxide that in turn is removed by CAT and GPx (Deisseroth and Dounce, 1970). Endogenous antioxidant enzymes (SOD, CAT and GST) are responsible for the detoxification of deleterious oxygen radicals (Del Maestro, 1980). In our study, the activity of XO was increased in the kidneys of diabetic rats, which indicates increase in the generation of ROS. The activities of SOD and GST were increased in the kidneys of diabetic rats, which could be due to the compensatory reaction against increase in ROS. Data presented in our investigation indicate that the progression of diabetes results in augmentation of oxidative stress accompanied by impaired enzymatic antioxidative defense system in the kidneys of diabetic rats. The treatment with CFE reduced oxidative stress as evidenced by the restoration of the enzymatic antioxidative defense system. The activity of CAT was significantly decreased in diabetic control rats compared with the normal control rats. This could be due to the increased utilization for scavenging of free radicals. The activity of CAT in diabetic group treated with CFE was significantly increased compared with the diabetic control group. These results indicate that treatment with CFE restored the SOD, CAT, GST, and XO activities and reduced oxidative stress in diabetic rats.

Diabetic nephropathy is characterized by increased urinary protein, loss of renal function, excessive deposition of extracellular matrix proteins in mesangium. TGF-β_1 has broad effects on the extracellular matrix leading to glomerulosclerosis (Flanders and Burmester, 2003). Several studies reported on the potential role of TGF-β_1 as a mediator of diabetic nephropathy (Gilbert et al., 1998; Hill et al., 2000). Glomerulosclerosis or increase in mesangial matrix was not detected in our study because of the limited, 4 weeks duration of the experiment. In immunohistochemical analysis, glomerular TGF-β_1 accumulation was not significant in the diabetic control group because of the short time period of the induced diabetes.

It has been reported that an increase in kidney weight in STZ-induced diabetic rats. It also has been hypothesized that this may be due to an increase in urinary output and renal blood flow (Zatz et al., 1986). A similar finding was observed in this study, treatment with CFE leading to significant decreased relative kidney weight of the STZ-induced diabetic rats. Microalbuminuria or evident proteinuria is the result of this change within the kidneys. In this study, serum urea nitrogen and urinary total protein in

the diabetic group treated with CFE were significantly lower than the diabetic control group. These data suggest that treatment with CFE can prevent the development of diabetic complication, such as diabetic nephropathy, by a reduction in renal damage though the restoration of enzymatic anti-oxidative defense system.

In conclusion, these results indicated that Corni Fructus can prevent or retard the development of diabetic complication via its beneficial effects for correcting the hyperglycemia, antioxidant enzyme system and renal dysfunction in the kidneys of diabetic rats. Thus present study suggests that Corni Fructus is a potential natural product for the prevention of diabetic complication.

REFERENCES

Aebi H. Catalase. In Methods of Enzymatic Analysis. Edited by Bergmeyer HU. 1974. Vol. 2. Academic Press. New York, USA.

Allain CC, Poon LS, Chan CS, Richmond W, Fu PC. Enzymatic determination of total serum cholesterol. Clin Chem. 1974. 20: 470-475.

Anderson RJ, Freedland KE, Clouse RE, Lustman PJ. The prevalence of comorbid depression in adults with diabetes: a meta-analysis. Diabetes Care. 2001. 24: 1069-1078.

Anjaneyulu M, Chopra K. Nordihydroguairetic acid, a lignin, prevents oxidative stress and the development of diabetic nephropathy in rats. Pharmacology. 2004. 72: 42-50.

Baynes JW. Role of oxidative stress in development of complications in diabetes. Diabetes. 1991. 40: 405-412.

Brandstrup N, Kirk JE, Bruni C. Determination of hexokinase in tissues. J Gerontol. 1957. 12: 166-171.

Butler R, Morris AD, Belch JJF, Hill A, Struthers AD. Allopurinol normalizes endothelial dysfunction in type 2 diabetics with mild hypertension. Hypertension. 2000. 35: 746-751.

Chatterjea MN, Shinde R. Text book of medical biochemistry, 5th ed. 2002. pp. 317. Jaypee Brothers, Medical Publishers (P) Ltd. New Delhi, India.

Deisseroth A, Dounce AL. Catalase physical and chemical properties, mechanism of catalysis and physiological role. Physiol Rev. 1970. 50: 3-24.

Del Maestro RF. An approach to free radicals in medicine and biology. Acta Physiol Scand. 1980. 492: 153-168.

Flanders KC, Burmester JK. Medical applications of transforming growth factor-beta. Clin Med Res. 2003. 1: 13-20.

Fossati P, Prencipe L, Beti G. Enzymatic creatinine assay: a new colorimetric method based on hydrogen peroxide measurement. Clin Chem. 1983. 29: 1494-1496.

Gilbert RE, Cox A, Wu LL, Allen TJ, Hulthen UL, Jerums G. Expression of Transforming growth factor-beta 1 and type IV collagen in the renal tubulointerstitium in experimental diabetes. Diabetes. 1998. 47: 414-422.

Habig WH, Pabst MJ, Jakoby WB. Glutathione S-transferase. The first enzymatic step in mercapturic acid formation. J Biol Chem. 1974. 249: 7130-7139.

Hill C, Flyvbjerg A, Gronbeak H, Petrik J, Hill DJ, Thomas CR, Sheppard MC, Logan A. The renal expression of TGF- isoforms and their receptors in acute and chronic experimental diabetes in rats. Endocrinology. 2000. 141: 1196-1208.

Hunt JV, Dean RT, Wolff SP. Hydroxyl radical production and autooxidative glycosylation. Glucose autooxidation as the cause of protein damage in the experimental glycation model of diabetes and aging. Biochem J. 1988. 256: 205-212.

Jin UH, Kim DI, Lee TK, Lee DN, Kim JK, Lee IS, Kim CH. Herbal formulation, Yukmi-jihang-tang-Jahage, regulates bone resorption by inhibition of phosphorylation mediated by tyrosine kinase Src and cyclooxygenase expression. J Ethnopharmacol. 2006. 106: 333-343.

Latha M, Pari L. Preventive effects of Cassia auriculata L. flowers on brain lipid peroxidation in rats treated with streptozotocin. Mol Cell Biochem. 2003. 243: 23-28.

Li KM, Yang XJ, Yu MQ, Xie C, Xu LZ. Determination of loganin in Cornus officinalis Sieb. et Zucc. by TLC scanner. Zhongguo Zhong Yao Za Zhi. 1994. 19: 738, 763.

Lowry OH, Rosenbrough NJ, Far AL, Randall RJ. Protein measurement with the folin phenol reagent. J Biol Chem. 1951. 193: 265-275.

Maritim AC, Sanders RA, Watkins III JB. Effect of alpha lipoic acid on biomarkers of oxidative stress in streptozotocin-induced diabetic rats. J Nutr Biochem. 2003. 14: 288-294.

Martin JP, Dailey M, Sugarman E. Negative and positive assays of superoxide dismutase based on hematoxylin autoxidation. Arch Biochem Biophys. 1987. 255: 329-336.

McGrowan MW, Artiss JD, Strandbergh DR, Zak B. A peroxidase-coupled method for the colorimetric determination of serum triglycerides. Clin Chem. 1983. 29: 538-542.

Patton CJ, Crouch SR. Spectrophotometeric and kinetics investigation of the Berthelot reaction for determination of ammonia. Anal Chem. 1977. 49: 464-469.

Rong Y, Li L, Shah V, Lau BHS. Pycnogenol protects vascular endothelial cells from t-butyl hydroperoxide-induced oxidant injury. Biotechnol Ther. 1995. 5: 117-126.

Stirpe F, Della CE. The regulation of rat liver xanthine oxidase. Conversion in vitro of the enzyme activity from dehydrogenase (type D) to oxidase (type O). J Biol Chem. 1969. 244: 3855-3863.

Venkateswaran S, Pari L. Antioxidant effect of Phaseolus vulgaris in streptozotocin-induced diabetic rats. Asia Pac J of Clin Nutr. 2002. 11: 206-209.

Welta K, Weissa J, Martinb R, Hermsdorfc T, Drewsa S, Fitzla G. Ginkgo biloba extract protects rat kidney from diabetic and hypoxic damage. Phytomedicine. 2007. 14: 196-203.

Wohaieb SA, Godin DV. Alterations in free radical tissue-defence mechanisms in streptozotocin-induced diabetes in rat. Effect of insulin treatment. Diabetes. 1987. 36: 1014-1018.

Yamabe N, Kang KS, Goto E, Tanaka T, Yokozawa T. Beneficial effect of Corni Fructus, a constituent of Hachimi-jio-gan, on advanced glycation end-product-mediated renal injury in streptozotocin-treated diabetic rats. Biol Pharm Bull. 2007. 30: 250-526.

Zatz R, Dunn BR, Meyer TW, Anderson S, Rennke HG, Brenner BM. Prevention of diabetic glomerulopathy by pharmacological amelioration of glomerular capillary hypertension. J Clin Invest. 1986. 77: 1925-1930.