นิพนธ์ต้นฉบับ

Effects of garlic extract on cardiovascular complications in diabetic rats.

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By using streptozotocin-treated rats (STZ) (65 mg/kg of BW.,ip.), the effects of crude garlic extract on diabetic cardiovascular complications were studied. The animals were separated into three major groups of age-matched controls, STZ, and garlic-treated STZ rats (STZ-G). STZ-G received daily oral feeding of garlic extract with the dose of 100 mg/kg of BW, starting one day after STZ injections. The parameters of cardiovascular functions : common carotid arterial pressure (CAP), aortic flow rate (AFR), left ventricular isotonic contraction (LVIC), and coronary vascular resistance (CR) were determined for all groups. Including, the values of blood glucose, lipid profile, and proteinuria were also monitored.

The results indicated that at sixteen weeks after the STZ injection, all determined cardiovascular parameters obtained from STZ-G were significantly more improved than the STZ (STZ: CAP = 100. 56 $\pm$ 6.36 mmHg, AFR = 41.83 $\pm$ 7.68 ml/min, LVIC = 125.92 $\pm$ 70.9 gm/100 gm of heart weight, CR = 32.92 $\pm$ 0.93 mmHg/ml/min/gm; STZ-G : CAP = 78.33 $\pm$ 13.42 mmHg, AFR = 62.83 $\pm$ 10.96 ml/min, LVIC = 230.76 $\pm$ 71.2 gm/100gm of heart weight, CR = 20.38 $\pm$ 0.45 mmHg/ml/min/gm). Besides these cardiovascular effects, the abnormalities of plasma lilid (HDL and LDL) and the incidence of proteinuria were also prevented by the daily oral feeding of garlic extract. It is concluded that garlic extract might be used for prevention of cardiovascular complications in diabetic patients in the future.

Key words : *Diabetic rat, Cardiovascular complications, Garlic extract.*

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สุทธิลักษณ์ ปทุมราช, อัญชลี เจตะภัย, วสันต์ อุทัยเฉลิม. ผลของสารสกัดกระเทียมต่อภาวะแทรกซ้อนของหัวใจและหลอดเลือดในหนูที่เป็นเบาหวาน. จุฬาลงกรณ์เวชสาร 2538 สิงหาคม; 39(8): 571-581

การศึกษาครั้งนี้เป็นการศึกษาผลของสารสกัดกระเทียมต่อภาวะแทรกซ้อนของหัวใจและหลอดเลือดในหนูที่ทำให้เกิดเบาหวาน โดยใช้ streptozotocin ขนาด 65 มก./กก.ของน้ำหนักตัว โดยแบ่งสัตว์ทดลองเป็นสามกลุ่มคือ กลุ่มควบคุม (Control) กลุ่มหนูเบาหวาน (STZ) และกลุ่มหนูเบาหวานที่ได้รับสารสกัดกระเทียม (STZ-G) กลุ่ม STZ-G จะได้รับสารสกัดกระเทียมขนาด 100 มก./กก. ของน้ำหนักตัวทุกวัน โดยเริ่มตั้งแต่หนึ่งวันหลังการฉีด STZ ค่าพารามิเตอร์ของระบบหัวใจและหลอดเลือดที่ทำการศึกษาคือ ค่าความดันเลือดในหลอดเลือดแดงคอมมอนคาโรติดส์ (CAP) ค่าอัตราการไหลเวียนเลือดในเอออร์ตา (AFR) ค่าแรงหดตัวของหัวใจห้องล่างข้างซ้ายแบบไอโซโทนิค (LVIC) และค่าความต้านทานของหลอดเลือดโคโรนารีย์ ตลอดจนวัดค่าของระดับน้ำตาลและไขมันในเลือดรวมทั้งค่าโปรตีนในปัสสาวะสำหรับทุกกลุ่มของสัตว์ทดลอง

ผลการทดลองพบว่าที่ 16 สัปดาห์หลังการฉีด STZ ค่าพารามิเตอร์ของระบบหัวใจและหลอดเลือดซึ่งประเมินได้จากกลุ่ม STZ-G นั้น มีค่าดีขึ้นกว่าค่าต่าง ๆ ของ STZ (STZ : CAP = 100.56 $\pm$ 6.36 มม. ของปรอท, AFR = 41.83 $\pm$ 7.68 มล./นาที, LVIC = 125.92 $\pm$ 70.9 กรัม/100 กรัม นน.หัวใจ, CR = 32.92 $\pm$ 0.93 มม.ปรอท/มล./นาที/กรัมk; STZ-G : CAP = 78.33 $\pm$ 13.42 มม. ของปรอท, AFR = 62.83 $\pm$ 10.96 มล./นาที, LVIC = 230.76 $\pm$ 71.2 กรัม/100 กรัม นน.หัวใจ, CR = 20.38 $\pm$ 0.45 มม.ปรอท/มล./นาที/กรัม) นอกจากนั้นผลของสารสกัดกระเทียมยังช่วยป้องกันความผิดปกติของระดับไขมันและน้ำตาลในเลือดด้วย ดังนั้นจึงสรุปได้ว่าสารสกัดกระเทียมอาจนำมาใช้ในการป้องกันการเกิดภาวะแทรกซ้อนของหัวใจและหลอดเลือดในผู้ป่วยเบาหวานได้ในอนาคต

Cardiovascular complications are a major cause of morbidity and mortality in insulin dependent diabetes mellitus (IDDM), especially, in those who have poor glycemic control. A number of studies, both human and animal models, indicated the deficiency of aortic output, cardiac output, and the thickenning of left ventricular wall.[1-5] In addition, there appear to be a number of relatively specific changes in the microvasculature of the diabetic heart, especially in the intramural coronary arteries.[6-7] At present, it appears that the risks of diabetic cardiovascular complications involve many factors such as hyperglycemia, dyslipidemia, hypertension, decrease of fibrinolytic activity, and increase of platelet aggregation. Interestingly, there are many reports have indicated that garlic *(Allium sativum)*, one of the traditional medicinels of various cultures, seems to have the potential to normalize each one of these risk factors. However, there are no reports that directly address the effects of garlic on diabetic cardiovascular complications. Therefore, the major purpose of this investigation is to evaluate the effects of garlic on diabetic cardiovascular functional changes by using streptozotocin-induced diabetic rats as a studied model and report the findings here.

Materials and Methods

Garlic Extraction

The dry outer skins of fresh garlic cloves were removed. Then the cloves were washed and dried. Each 100 gram of cloves was mashed in a blender with 120 ml of chloroform until a good mixture was obtained. After filtering out the residue, the chloroform was extracted from the mixture by using a Rota Vaporizer at 55 °C. Finally, the filtrate, which was a yellowish oily liquid, was obtained. In this study, this oily garlic extract were prepared every week, therefore, the use of preservative, povidone, was not required.

Experimental Procedures

Male Wistar-Furth rats (n=54) weighing 100-150 gm each with ages of 4-5 weeks were used. All rats were fasted overnight and then 36 rats received intraperitoneal injections of streptozotocin (STZ) at 65 mg/kg of BW. Control group (n=18) received placebo injections of normal saline in stead. Blood glucose concentration was determined by hemoglucostrip and glucometer (Reflolux S). Then after the test of this criteria (performed at 36-48 hours after the STZ injection), eighteen of STZ-injected rats were randomly selected for daily oral feeding of crude garlic extract with the dose of 100 mg/kg of BW, and these rats were referred as STZ-G group.

On the day of determining cardiovascular parameters, the values of blood glucose were monitored again and also urine samples were collected for determining of protein content. Proteinuria were determined by sulfosalicylic acid turbidity test.[8]

The cardiovascular parameters including : common carotid arterial pressure (CAP), aortic flow rate (AFR), coronary vascular resistance (CR), and left ventricular isotonic contraction (LVIC) were determined at 8, 12, and 16 weeks after the STZ injections. All together, there were 6 rats in each of these three different aged groups. The values of CR (mmHg/ml/min/gm) were mathematically estimated by diving constant pressure by coronary flow rate (CFR) per gram of heart weight. The total volume of perfusate that come out from the isolated heart in one minute was an estimated value of CFR.

In each experiment, each rat was weighed and then anesthetized by sodium pentobarbital (i.p., 30 mg/kg of BW.). After tracheotomy, the animals were ventilated with small animal respirators (Haward Rodent Model 683). The chest was opened exposing the heart and three major vessels : the right subclavian artery, the innominate artery, and the ascending aorta. AFR was measured by a flow probe (Nikhon model FE-020T) placed on the ascending aorta. Blood pressures were monitored via a catheter (PE-180) cannulated common carotid artery connected to a pressure transducer (Nikhon model TP-300T) coupled to a polygraph (Nikhon RM 6000). Then hearts were carefully isolated by the modified Langendroff's method.[9-10] Fifteen minutes after the heart were isolated, the values of left ventricular contraction were measured via the wire hooked at the apex of left ventricle connected to an isotonic transducer and to a polygraph recorder. The preload was equal to 5 grams. In each experiment, after the right atrium were removed, blood sample were immediately collected for determining of lipids. Lipid profiles : cholesterol, triglyceride, high density lipoprotein (HDL), and low density lipoprotein (LDL) were determined by enzymatic colorimetric technique.[11-14]

Statistics

The unpaired Student's t-test was used to analyze the difference of each parameter between STZ-rats and control group, and between STZ-rats and STZ-G rats ($p<0.05$).

Results

The injection of 65 mg/kg of BW STZ into 100-150gm Wistar-Furth rats resulted in polydipsia, polyuria, polyphagia, and stable hyperglycemia within 24 - 48 hours. The results (Table 1) indicated that the body weights of STZ rats were significantly decreased as compared to their controls at all three monitored time points (8, 12, 16 wk). However, body weights of STZ-G rats become significantly higher than STZ rats at 12 and 16 wk.

Table 1. Means ± SD of body weight (gm) of controls (CON), streptozotocin- induced diabetic rats (STZ-rats), and garlic-treated STZ-rats (STZ-G) at 8, 12, and 16 weeks of experiment.

	Body weight (gm)		
	8-wk	12-wk	16-wk
CON (n=6)	348.00 ± 24.26	423.83 ± 29.97	452.50 ± 22.53
STZ (n=6)	295.50 ± 40.99*	294.83 ± 40.40*	294.17 ± 33.31*
STZ-G (n=6)	301.50 ± 34.50^{ns}	379.83 ± 34.98**	393.50 ± 29.45**

* Statistical difference compared to controls ($p<0.05$).

** Statistical difference compared to STZ-rats ($p<0.05$).

ns = non significant difference compared to STZ-rats ($p<0.05$).

As shown in table 2, the ratios of heart weight per 100 gm body weight were calculated and summarized as means ± SD for each group of each monitored time point. At 8 wk, the ratio of heart weight per 100 gm body weight was significantly increased as compared to their age-matched controls. This elevation of the ratio trend persisted throughout the remainder of the study period (16 wk). These ratios indicated that STZ hearts tended to increase their sizes, and this is referred to as myocardial hypertrophy. Interestingly, at 12 and 16 weeks the ratios of heart weight per 100 gm body weight of the STZ-G group became significantly less than those of STZ groups.

Table 2. Means ± SD of ratios of heart weight per 100gm body weight of control, streptozotocin-induced diabetic rats (STZ-rats), and garlic-treated STZ-rats (STZ-G) at 8, 12, and 16 weeks of experiment.

	Ratio of heart weight per 100 gm body weight		
	8-wk	12-wk	16-wk
CON (n=6)	0.35 ± 0.02	0.35 ± 0.04	0.32 ± 0.05
STZ (n=6)	0.40 ± 0.04*	0.43 ± 0.05*	0.43 ± 0.03*
STZ-G(n=6)	0.40 ± 0.04^{ns}	0.32 ± 0.02**	0.31 ± 0.05**

* Statistical difference compared to controls ($p<0.05$).

** Statistical difference compared to STZ-rats ($p<0.05$).

ns = non significant difference compared to STZ-rats ($p<0.05$).

The results shown in figures 1A, 1B, 1C and 1D indicate that the levels of cholesterol and triglyceride in STZ-rats were significant higher than controls at all three monitored ages. There were significant decreases of HDL levels and increases of LDL levels as compared to controls at 16 wk after the STZ injection. Interestingly, the garlic extract seemed to attenuate these changes of HDL and LDL levels as showed in Fig. 1C and 1D.

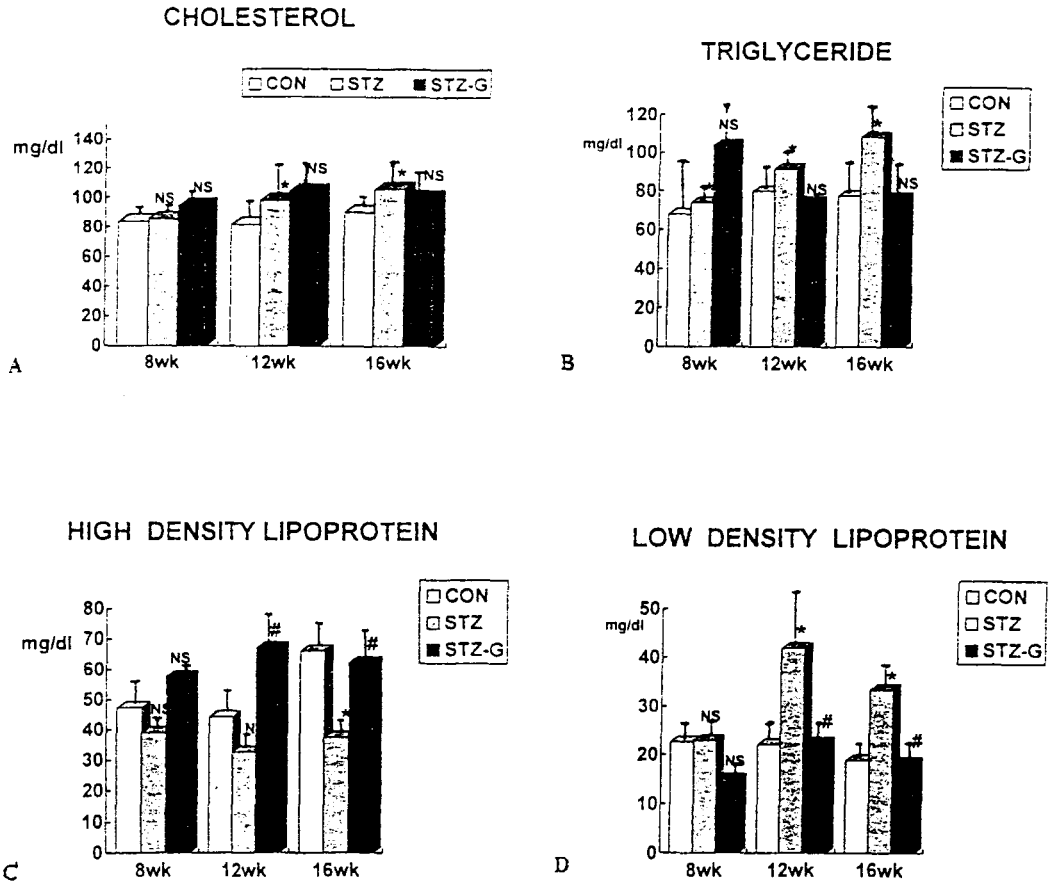

Figure 1. A-D respectively indicated means SD of plasma cholesterol, triglyceride, high density lipoprotein, and low density lipoprotein determined from controls (CON), STZ-induced diabetic rats, and garlic-treated STZ-rats (STZ-G) at 8, 12, and 16 weeks of experiment.
* Statistical difference compared to controls ($p<0.05$)
# Statistical difference compared to STZ ($p<0.05$)
NS = non significant difference compared to STZ-rats ($p<0.05$)

As shown in figure 2 and 3, the blood glucose and proteinuria values of STZ-rats were significantly increased as compared to the controls for all three monitored ages. Interestingly, the blood glucose and proteinuria values of STZ-G were significantly less than those of STZ rats for all three monitored ages.

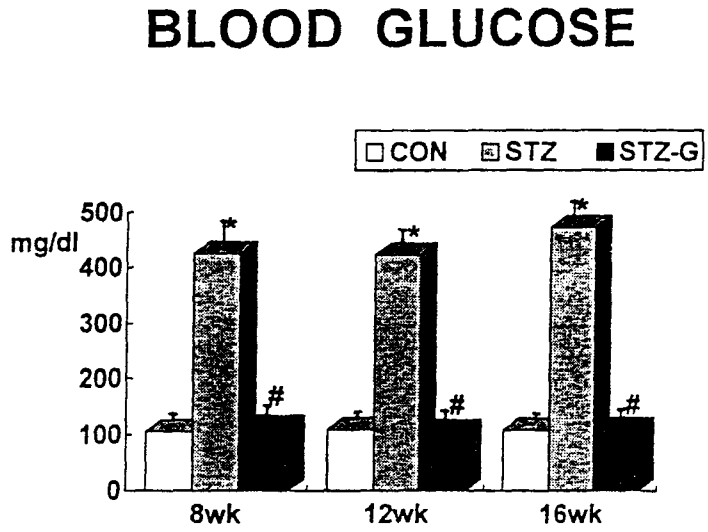

Figure 2. Means ± SD of plasma glucose (mg/dl) of controls (CON), streptozotocin-induced diabetic rats (STZ-rats), and garlic-treated STZ- rats (STZ-G) at 8, 12, 16 weeks of experiment.
* Statistical difference compared to controls ($p<0.05$)
# Statistical difference compared to STZ ($p<0.05$)

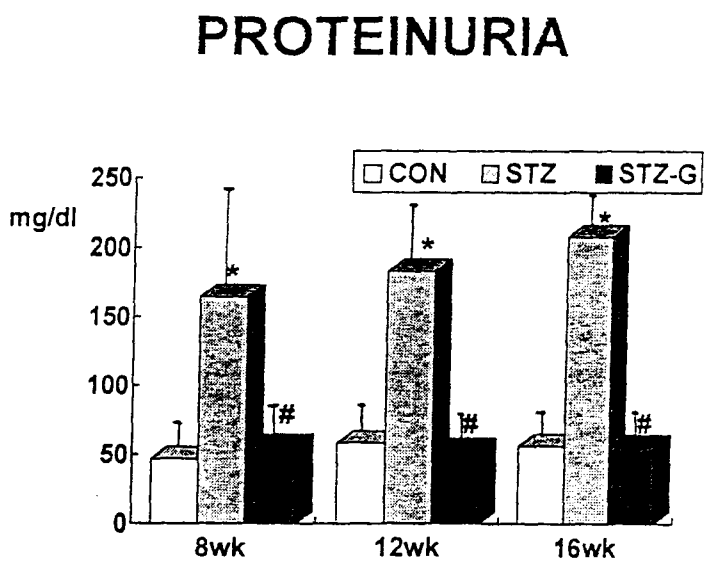

Figure 3. Means ± SD of proteinuria (mg/dl) determined from controls (CON), streptozotocin-induced diabetic rats (STZ-rats), and STZ-rats treated with garlic extract (STZ-G) at 8, 12, and 16 weeks after the streptozotocin injections.
* Statistical difference compared to controls ($p<0.05$)
# Statistical difference compared to STZ ($p<0.05$)

The CAP values are shown in figure 4. The results indicate that the CAP values of STZ-rats were significantly increased over those of controls for all three monitored ages. However, the CAP values of STZ-G rats were significantly less than those of STZ rats for all three monitored time points.

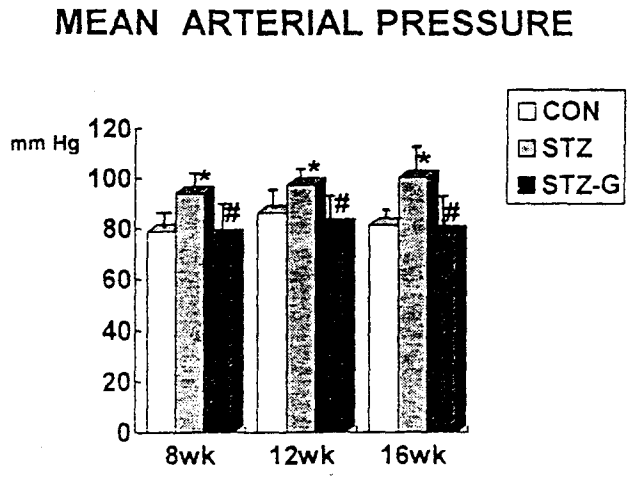

Figure 4. Means ± SD of carotid arterial pressure (CAP; mmHg) determined from controls (CON), streptozotocin-induced diabetic rats (STZ-rats), and STZ-rats treated with garlic extract (STZ-G) at 8, 12, and 16 weeks after the streptozotocin injections.
* Statistical difference compared to controls ($p<0.05$)
# Statistical difference compared to STZ ($p<0.05$)

In figure 5, the AFR values of STZ rats show significant decreases over those of controls. Interestingly, the AFR values of STZ-G were significantly increased over those of STZ rats for all three monitored ages.

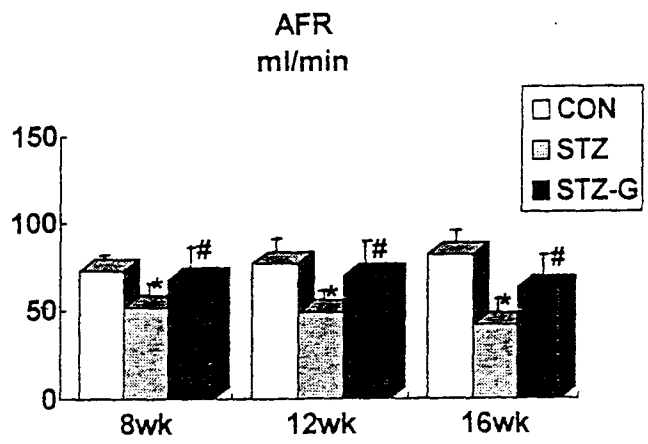

Figure 5. Means ± SD of aortic flow rate (AFR, ml/min) determined from controls (CON), streptozotocin-induced diabetic rats (STZ-rats), and STZ-rats treated with garlic extract (STZ-G) at 8, 12, 16 weeks after the streptozotocin injections.
* Statistical difference compared to controls ($p<0.05$)
# Statistical difference compared to STZ ($p<0.05$)

As shown in figure 6, the LVIC values of STZ rats, which were demonstrated as percent of heart weights, were significantly decreased over those of controls at 12 and 16 weeks. However, the LVIC values of STZ-G rats were significantly increased over those of STZ rats at 16 weeks.

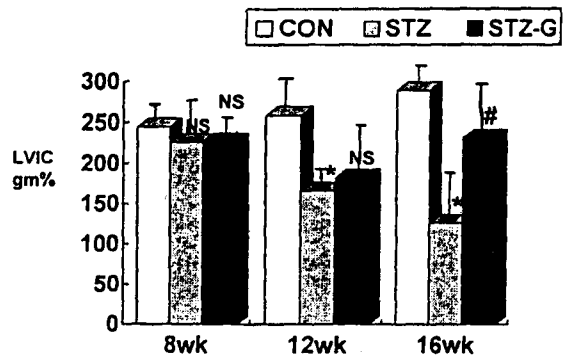

Figure 6. Means ± SD of left ventricular isotonic contraction (LVIC) determined from controls (CON), streptozotocin-induced diabetic rats (STZ-rats), and STZ-rats treated with garlic extract (STZ-G) at 8, 12, 16 weeks after the streptozotocin injections.
* Statistical difference compared to controls ($p<0.05$)
# Statistical difference compared to STZ ($p<0.05$)
NS = non significant difference compared to control or STZ-rats ($p<0.05$)

The CR values are shown in figure 7. The results indicate that the CR values of STZ rats were significantly higher than those of controls for all three monitored ages. Interestingly, the CR values of STZ-G were significantly less than those of STZ-rats at 12 and 16 weeks.

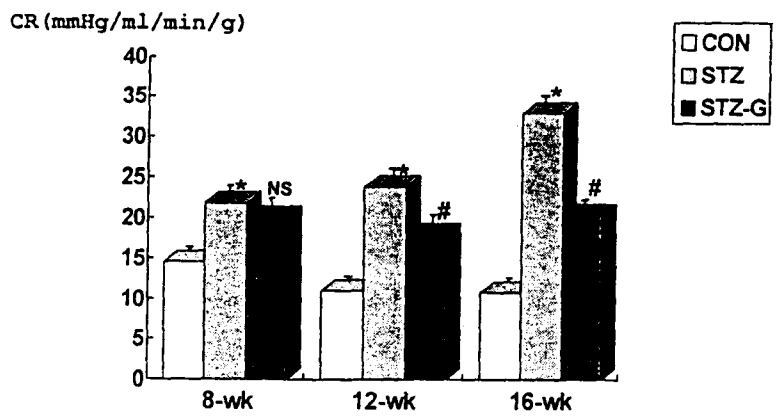

Figure 7. Means ± SD of coronary vascular resistance (mmHg/ml/min/g) determined from controls (CON), streptozotocin-induced diabetic rats (STZ-rats), and STZ-rats treated with garlic extract (STZ-G) at 8, 12, 16 weeks after the streptozotocin injections.
* Statistical difference compared to controls ($p<0.05$)
# Statistical difference compared to STZ ($p<0.05$)
NS = non significant difference compared to STZ-rats ($p<0.05$)

Discussion

In the present study, it appears that the daily oral feeding of crude garlic extract with doses of 100 mg/kg of BW could attenuate and prevent abnormalities of cardiovascular functions observed in STZ-induced diabetic rats. Those such: the increase of AFR, and LVIC and the decrease of CR and CAP. The results also indicated that the cardiovascular protective effects of garlic extract were concomitant with the abilities of normalizing dyslipidemia, lowering blood glucose and proteinuria.

The possible hypothesis of these effects of garlic extract might involve various reported actions. The first is the action of garlic on reducing plasma LDL and increasing HDL levels. It has been suggested that garlic could normalize dyslipidemia by inactivating coenzyme A and HMG coenzyme A reductase in the lipid anabolic pathway.[15] Second is the hypoglycemic effect of garlic. In 1975, Jain and his co-workers[16] showed that garlic extract could lower blood glucose in alloxan-induced diabetic rabbits. And they proposed that this hypoglycemic action might be a direct one by increasing either the pancreatic secretion of insulin from the beta cells or its release from bound insulin. Third is the hypotensive effect of garlic. It has been suggested that garlic could lower blood pressure through its prostaglandin-like mechanism.[17]

We believe that these three major actions might be the reason why garlic can delay or prevent those diabetic cardiovascular complications as we observed in the STZ-rats in our present studies. As garlic can normalize dyslipidemia, hyperglycemia, and hypertension, it could therefore, offset the risks of cardiovascular abnormalities and myocardial and endothelial cells are then able to perform their normal physiological functions.

It is now well established that diabetic nephropathy is characterized by basement membrane thickening and mesangial expansion. Interestingly, high blood glucose was believed to be the major cause of this pathology.[18-20] Therefore, we believe that the garlic extract lowered proteinuria in the STZ-G rats probably due to this hypoglycemic action also.

Thus, on the basis of our findings, we believe that garlic could be a great therapeutic tool in preventing diabetic complications, especially in prolonging the patients life. However, further investigations are needed to confirm the mechanisms and also to define the active ingredients of garlic, the smart traditional medicine.

Acknowledgments

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