Original Article

Improvement of Endothelium-Dependent Relaxation in Aorta of Rat Models Type 1 and 2 Diabetes by Hespiridin

A Kouhpayeh[1], H Mirkhani[2], AA Nekooeian[3]

[1]Department of Pharmacology, Medical School; [2]Medicinal & Natural Products Chemistry Research Centre; [3]Cardiovascular Pharmacology Research Center, Department of Pharmacology, Shiraz University of Medical Sciences Shiraz, Iran

Background: Vascular disease is the principal cause of morbidity and mortality in patients with diabetes. A considerable body of evidence implicates oxidative stress as an important pathogenic factor of diabetic vasculopathies. In the present study, the effect of hesperidin, a flavanone glycoside with antioxidant activity, is studied in endothelium-dependent relaxation of the rat aorta in experimental diabetes mellitus type 1 (DM1) and type 2 (DM2).

Patients and Methods: Single dose intraperitoneal injection of streptozocin (60mg/kg) and subcutaneous daily injection of dexamethasone (10µg/kg for one month) were used to induce DM1 and DM2, respectively. Hesperidin (500mg/kg) was administered orally for two months in DM1 and one month in DM2. The effect of acetylcholine (Ach) on phenyl ephrine (PE) induced. PE contracted aorta was then studied and the EC50 and maximal relaxant effect of Ach were calculated and compared in the two groups.

Results: In the experimental DM1, hesperidin restored endothelium-dependent relaxation near to those of normal animals. Its effect on experimental DM2 consisted of a significant reduction of EC50 value of Ach compared to those of diabetic animals. It also showed a great but non-significant effect (P=0.07) on Ach-induced maximum relaxation compared to DM2 untreated animals.

Conclusion: These results show that hesperidin can improve vascular endothelial dysfunction in experimental diabetes mellitus.

Keywords: Experimental Diabetes Mellitus, Endothelium, Hesperidin, Aorta

Introduction

The incidence of diabetes mellitus is increasing globally. Vascular diseases are the principal causes of morbidity and mortality in patients with diabetes. The endothelium plays an important role in the regulation of vascular smooth muscle tone by producing vasodilator mediators. The loss of the modulatory role of the endothelium may be a critical initiating factor for diabetes-induced vascular diseases.[1,2]

Abnormal endothelial functions have been observed in vascular beds of diabetic animals.[3,4] The exact mechanisms, which lead to the development of endothelial dysfunction in diabetes, are still unknown.[4] However, it has been proposed that reduced nitric oxide synthase (NOS) activity, decreased availability of substrate or co-factor such as L-arginine and tetrahydrobiopterin for NO synthesis, enhanced inactivation of NO, impaired diffusion of NO to the underlying smooth muscle cells and decreased smooth muscle cell sensitivity to NO

Correspondence:
Hossein Mirkhani
P.O. Box 71345-1649, Shiraz, Iran
Telefax: +98-711-2307591
E-mail address: mirkhanh@sums.ac.ir

hypercholesterolemia, atherosclerosis, hypertension, and heart failure.[7,8] Oxidative stress is defined as an increase in the steady-state levels of reactive oxygen species (ROS). ROS are a family of molecules including molecular oxygen and its derivatives produced in all aerobic cells. Excessive production of ROS, outstripping endogenous antioxidant defense mechanisms, has been implicated in the oxidation of biological macromolecules such as DNA, proteins, carbohydrates, and lipids. This condition has commonly been referred to as oxidant stress.[8] These findings have prompted investigators to study the role of availability of antioxidant on vascular dysfunction.

Hesperidin is a flavonoid found mainly in citrus fruits. Flavonoids are polyphenolic compounds, which directly quench free radicals and inhibit enzymes of oxygen-reduction pathways [10]. Hesperidin was also shown to have a wide spectrum of pharmacological effects including anti-inflammatory, anticarcinogenic, antihypertensive and anti-atherogenic effects.[11,12]

Considering free radical quenching ability of hesperidin and the role of oxidative stress in vascular complications of diabetes, the present study was designed to examine the effects of hesperidin on endothelium-dependent relaxation of aorta of rats with type 1 and 2 diabetes.

Patients and Methods

The study was approved by Ethics Committee of the University.

Male Spargue-Dawley rats (300-400 g) were obtained from Animal Breeding Center, Shiraz University of Medical Sciences, Shiraz, Iran. They were kept under alternate cycles, 12 hrs each, of light and dark, and temperature -controlled conditions (25 º C) with food and water given ad libithum. They were then assigned to type 1 or type 2 diabetic groups.

Experimental design and protocol for type 1 diabetes (DM1)

Animals were assigned to control (C1) and diabetic groups (6-7 in each group). The Diabetes was induced by intraperitoneal injections of streptozocin (STZ, 60 mg/kg). C1 group received equal volume of STZ vehicle (Dexamethasone). Rats from diabetic group with plasma glucose levels ≥250 mg/dl on day 5 after STZ administration were considered diabetic and used for the next studies

From the 6th day diabetic group was further divided into diabetic control (D1) and diabetic + hesperidin (D1H) groups, which were assigned to receive tap water and oral hesperidin 500 mg/kg/day, respectively. After 2 months, plasma glucose, triglyceride, total cholesterol, creatinine, urea and 24h urine protein levels were measured and compared with the levels obtained on day 5.

Experimental design and protocol for type 2 diabetes (DM2)

The studied animals were randomly allocated to two diabetic groups (6-7 in each group) of DM2, including D2 and hesperidin-fed D2H (500 mg/Kg/). A third group (C2) received dexamethasone and was used as control.

On day 0, the plasma glucose level of all groups was measured before and 50 minutes after an intraperitoneal injection of insulin (3 U/Kg, human regular dose). The same procedure was repeated for all groups 30 days after

dexamethasone injection. This is ambiguous, and needs clarification). Also, the plasma insulin level was measured on day 0 and 30 (before insulin regular injection). Reduced hypoglycemic effect of insulin and hyperinsulinemia were considered as insulin resistance.[13] After 1 month, plasma glucose, triglyceride, total cholesterol and creatinine levels were measured and compared with the levels obtained on day 0.

Isolated rat aorta

After two months in diabetes type 1 and 1 month in diabetes type 2 protocols, rats were weighed, anesthetized and killed by rapid decapitation. Their thoraxes were opened rapidly, and thoracic aortas were removed. The aortas were then freed of connective tissues, and divided into 4-mm rings. The rings were mounted on stainless steel triangles connected to a force transducer (K30, Hugo Saches Electronik) at a resting tension of 500 mg in individual organ chambers filled with physiological salt solution, pH=7.4, containing NaCl 118, KCl 4.8, $CaCl_2$ 2.5, KH_2PO_4 1.2, $MgSO_4$ 1.2, $NaHCO_3$ 25, Glucose 11 (mMs).and aerated with O_2 (95%) and CO_2 (5%) at 37 °C.

The tissue was allowed to equilibrate for 1 h. while the solutions of the chambers were changed every 15 min. After the equilibration period, contractions were made using phenylephrine (PE) at a concentration of 10^{-6} M which produced 70-80% maximum response) and the cumulative effect of acetylcholine (Ach) concentrations was recorded. The concentration-effect curves were plotted and the IC50 value and maximal relaxing effect of acetylcholine was calculated.

Chemicals

Phenylephrine hydrochloride and hesperidin were purchased from Sigma Chemical Company. Acetylcholine chloride and streptozocin (Zanosar®) were obtained from Merck and Pharmacia & Upjohn, respectively. Rat insulin ELISA kit was purchased from DRG international; glucose, triglycerides, cholesterol, creatinine, urea, and urine protein commercial kits from Pars Azmun, Iran.

Data analysis

All the data were expressed as mean ± standard error of the mean (SEM). Statistical analyses were performed using SPSS software (Ver. 11.5). Appropriate statistical tests (paired-t test, one way ANOVA with LSD post test) were performed on each set of data. $P < 0.05$ was considered significant.

Results

Laboratory measurements in DM1 animals

Success rate of DM1 induction was about 70% and the mortality rate among diabetics was about 20%.

On day 5, after STZ-treatment, animals were considered as diabetics if showed a significant hyperglycemia in comparison to controls (Table 1). Also, urine volume and 24h urine protein in DM1 animals increased significantly. On day 60, there were no significant differences among D1, DH1 and C1 groups in the plasma levels of creatinine (Table 1). On day 60, the plasma level of glucose, triglycerides, cholesterol, urine volume and 24 h urine protein of D1 and D1H groups showed significant increases as compared to that of C1 group, but there

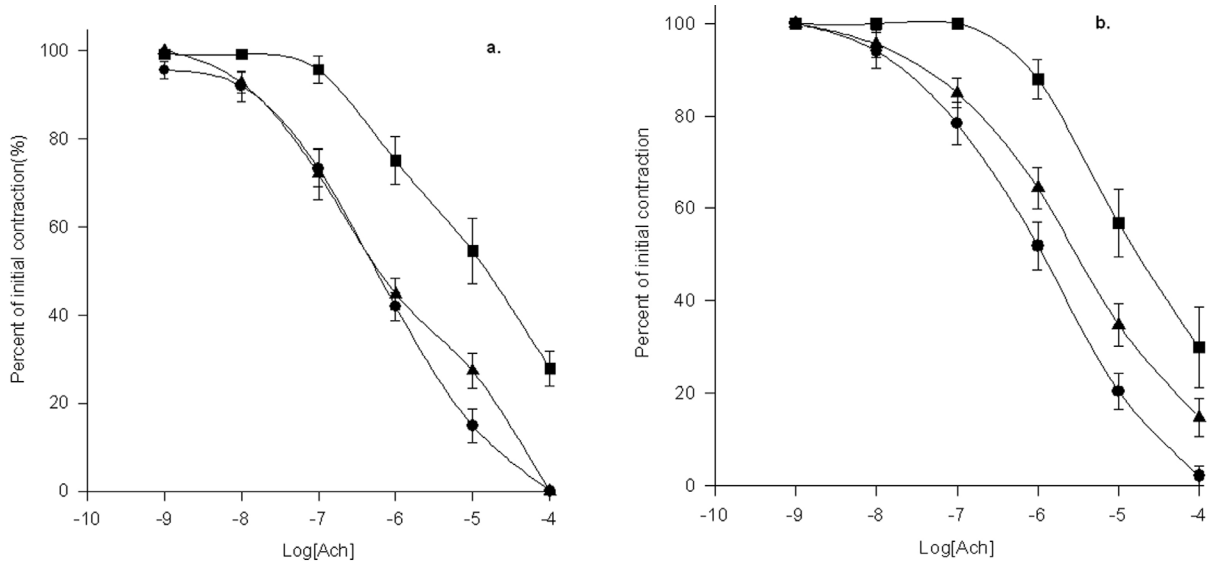

Figure 1: Vasorelaxant effect of cumulative concentrations of acetylcholine on PE-induced contraction (10^{-6} M) of isolated rat aorta in diabetic type 1 (a) and type 2 (b) studies. Each symbol represents 6-7 experiments. Standard errors of the means are indicated as vertical lines.
Control (●), diabetic (■), diabetic + hesperidine (▲).

were no significant differences between D1 and D1H groups in this regard (Table 1).While the plasma urea level of D1 and D1H groups was significantly higher than that of C1 group on day 60, this value was lower in D1H group compared to D1 (Table 1, P< 0.05).

Laboratory measurements in DM2 animals

There were no significant differences in biochemical parameters among the groups at baseline.

Thirty days injection of low doses of dexamethasone had no effect on plasma glucose levels in all groups. In contrast, on day30, the order of hypoglycemic effect of insulin was C2>D2H>D2 and the differences were significant (Table 2). Meanwhile, the plasma level of insulin showed the reverse order (P<0.05, Table 2). On day 30, the plasma level of triglycerides and cholesterol of D2 and D2H groups showed significant increments compared to that of C2 group, but there were no significant differences between the former groups in this regard (Table 2). Other biochemical parameters showed no differences among controls and treated animals (Table 2).

Isolated aorta

Maximal PE-induced (spell out PE) contractions in the aortic rings of C1, D1 and D1H rats were 309.1±52.6, 325.9±19.3 and 301.1±31.8 mg, respectively. In DM2 protocol, these values were 281.2±43.4, 334.3±33.3, and 217.7±22.1 mg, respectively for such groups. There were no significant differences in the maximum tension among the groups in each type of diabetes (Fig. 1).

Addition of cumulative concentrations of Ach to aortic rings pre-contracted with PE induced a concentration-dependent relaxation in all the studied groups (Fig. 1). In D1 and D2 , the EC50 of Ach increased and its maximum relaxant effect reduced compared to C1 and

Table 1. Biochemical parameters of rats recruited in experimental diabetes type 1 study. Values are means ± SE. (n= 6-7)

	C1 (day 5)	D1(day 5)	D1H (day 5)	C1 (day 60)	D1(day 60)	D1H (day 60)
Glucose (mg/dl)	136.67± 0.10	532.12±26.35*	461.80±34.03*	126.33±6.25	541.86±23.29†	480.40±24.52†
T-Cholesterol (mg/dl)	32.17±3.57	41.71±2.92	43.80±6.02	45.83±3.71	62.42±4.42†	62.80±3.54†
Triglycerides (mg/dl)	57.67±8.15	50.71±13.06	83.80±17.98	59.67±7.98	187.57±42.55†	171.60±20.12†
Creatinine (mg/dl)	1.2±.013	1.3±0.014	1.53±0.32	0.97±0.17	1.21±0.040	1.06±0.13
Urea (mg/dl)	40.17±3.26	48.28±2.74	43.00±3.85	33.00±2.26	63.17±5.97†	46.20±4.01†,‡
Urine volume (ml)	8.83±1.01	51.14±6.41*	50.33±5.71*	8.17±1.40	77.43±5.80†	67.60±12.35†
Protein 24h (mg)	9.08±1.81	24.08±3.03*	20.07±1.44*	8.48±0.91	20.80±1.96†	25.01±1.06†

C1: Control, D1: Diabetic type1, D1H: Diabetic type 1 + hesperidin; Day 5= 5 days after streptozocin injection, Day 60= 60 days after streptozocin injection; * P< 0.05 compared with C1 on day 5, † P< 0.05 compared with C1 on day 60, ‡ P< 0.05 compared with D1 group on day 60.

C2 groups, respectively (P<0.001 for D1, P< 0.01 for D2; Fig 1 a, b; Table 3).

In D1H animals, these diabetes-induced impairments have were almost totally restored (P<0.001, Fig. 1a, Table 3). In D2H group, hesperidin induced a similar significant effect on the EC50 of Ach, though at a smaller level (P<0.01, Fig. 1b, Table 4). In this group, the maximum relaxant effect of Ach was also increased but this change did not show any statistical significance (P<0.07, Fig. 1b, Table 3).

Discussion

The effect of experimental type 1 and 2 diabetes on endothelium-dependent relaxation of the aorta was examined in the present study.

Experimental DM1 was induced by STZ which leads to rapid destruction of β cell, severe decline in insulin plasma level and profound hyperglycemia. In the present study, it induced severe hyperglycemia, elevation of triglycerides, cholesterol and urea plasma level, and increased 24h urine protein excretion (Table 1).

Table 2. Biochemical parameters of rats recruited in experimental diabetes type 2 study. Values are means± SE. (n= 6-7)

	C2 (day 0)	D2 (day 0)	D2H (day 0)	C2 (day 30)	D2 (day 30)	D2H (day 30)
Glucose (mg/dl)	133.8±11.84	126.00 ±8.48	126.5±5.72	124.8±7.42	122.67±11.84	125.67±8.44
Δ %Glucose (mg/dl)	62.11±1.50	60.93±1.89	57.48±2.20	58.69±3.10	32..93±1.56*	43.13±1.1*,†
Insulin (µg/L)	1.46±0.23	2.06±0.18	1.5±0.22	1.80±0.28	5.11±0.37*	4.13±0.25*,†
Triglycerides (mg/dl)	117.20±4.97	128.83±4.77	122.83±2.21	129.00±4.91	188.67±8.74*	180.50±5.48*
Total Cholesterol (mg/dl)	49.20±2.31	50.33±1.60	47.33±1.98	55.40±1.12	78.00±3.92*	75.50±4.09*
Creatinine (mg/dl)	1.26±0.13	1.10±.086	1.07±0.71	1.13±.016	1.1±0.05	0.98±0.01
Urea (mg/dl)	39.60±3.93	36.00±1.48	41.83±1.49	41.00±3.78	45.33.17±7.66	43.83±3.06

C2: Control, D2: Diabetic type2, D2H: Diabetic type 2 + hesperidin; Day 0= before dexamethasone injection, Day 30= 30 days after daily injections of dexamethasone (10 µg/Kg) ; Δ %Glucose: percent of blood glucose reduction 50 minutes after human regular insulin injection (3 U/Kg); * P< 0.05compared with C2 on day 30, † P< 0.05 compared with D2 group on day30.

Table 3. pEC_{50} (-log EC50) and maximum relaxant effect of acetylcholine on PE-induced contraction of isolated rat aorta. Values are means ± SE (n=6-7).

	C1	D1	D1+H	C2	D2	D2+H
pEC_{50}	6.27±0.067	4.93±0.21*	6.13±0.13†	6.04±0.12	4.96±0.35‡	6.38±0.07†
Max relaxation (%)	100±0	72.12±4.05*	100±0†	97.96±2.04	70.13±8.68‡	85.71±4.65◘

C1: Control, D1: D type1, D1+H: Diabetic type1 + hesperidin, C2: Control, D2: Diabetic type2, D2H: Diabetic type 2 + hesperidin
* P<0.001 compared with C1group, † P< 0.001 compared with D1 group
‡ P< 0.01 compared with C2 group, ◘ P< 0.01 compared with D2 group

These effects were in concordance with complications arising in diabetic patients.

Low dose of dexamethasone (10 µg/kg/day) was applied to induce DM2. This method was used based on the suggestion of Severino et al., however it must be noted that in the present study the administered dose was higher than that used by them (2 µg/kg/day), which proved ineffective. The used model, which can be proposed as a convenient and inexpensive method to study early phases of diabetes type 2, induced insulin resistance in association with hypertriglyceridemia and hypercholesterolemia without alteration in blood glucose level. These changes were compatible with pre-diabetic phase in human.

Impaired endothelium-dependent relaxation of the aorta is the major vascular dysfunction in both experimental diabetes type 1 and type 2.[14,15] The effect of acetylcholine on PE-induced contraction of the rat aorta was therefore examined in regard to the effect of hesperidin on the diabetic vasculopathy. PE-induced- contractions did not show any significant differences among the studied groups either in DM1 or DM2. In other words experimental diabetes had no effect on α_1-adrenoceptor- mediated vascular contractions; however, opposite results has also been reported.[16]

Relaxant effect of Ach in both models of diabetes decreased significantly compared to control groups (Table 3, Fig 1). It is thus concluded that NO release from endothelial cells had been impaired in DM1 and DM2. The exact mechanism of decrease in NO bioactivity is not fully described but among the various observed metabolic disturbances in DM1, hyperglycemia appears to be the main cause of endothelial dysfunction. It has been shown that hyperglycemia generates reactive oxygen species (ROS) and the decrease in NO activity can be attributed to the overproduction of these species. Increased generation of ROS may also induce activation of different signaling pathway, as protein kinase C (PKC). PKC activation has been reported to cause additional generation of ROS.[9] Furthermore, the increase in TG and Cholesterol levels observed in the present study had also a significant role in endothelial dysfunction via enhanced NADPH oxidase-dependent O^-_2 generation and overproduction of ROS.[4]

In DM2 and in the context of insulin resistance, vascular dysfunction is detectable very early in the course of the disease even before overt hyperglycemia ensues, and it may play a key role in the vasculopathy associated with this disease. Data from animals and human studies have established insulin resistance as an important determinant of NO bioavailability.

Multiple mechanisms including oxidative stress and inflammation have been implicated in the development of endothelial dysfunction in the setting of insulin resistance. Vascular production of ROS, including superoxide and peroxynitrite, is increased in insulin resistance. These compounds scavenge free NO and reduce its bioavailability and also promote endothelial cell apoptosis.[17-19]

In the present study, hesperidin showed a remarkable inhibitory effect on the impaired aortic response to Ach, especially in DM1 (Fig. 1 and Table 3). The antioxidant effect of hesperidin and its ability to quench free radicals and inhibiting enzymes of oxygen-reduction pathways have been shown in several investigations.[10,20] Regarding the key role of ROS in the endothelial cell dysfunction in diabetes, this significant and favorable effect can be attributed to the antioxidant effect of hesperidin. It must be emphasized that in comparison to controls, hesperidin had no statistically significant effect on Ach-induced maximum aortic relaxation in type 2 diabetic animals (in contrast to the effect on EC50 of Ach, Fig 1, and Table 3). However, it must be noted that the corresponding P value had marginal significance (P=0.07) which might have also been affected by the limited number of animals used.

It has been shown that oxidative stress is the cause and also the consequence of insulin resistance.[17] Hesperidin reduced insulin resistance and hyperinsulinemia in D2H animals (Table 2). This effect, which can also be due to its antioxidant action, may have an important implication in the prevention of diabetes because it is known that the insulin resistance is the early event and promoting factor in the natural history of diabetes type 2.[17,18]

In addition to antioxidant action of hesperidin, its anti-inflammatory effect must also be considered.[21] Interestingly, hyperglycemia, insulin resistance and increased TG plasma level lead to increased expression of interleukin IL-1β, IL-6 and tumor necrosis factor. These cytokines increase C-reactive protein (CRP) production in vascular endothelial cells. CRP reduces NO generation via reduction of eNOS expression and impairment of insulin-stimulated activation of eNOS in IRS-1/PI3-kinase signaling pathway. It also increases the expression of endothelial vascular cell adhesion molecule (VCAM)-1, intercellular adhesion molecule (ICAM)-1, and E-selectin which may play an additional role in endothelial dysfunction in diabetes.[18,22-24] It is quite possible that some of the observed effects of hesperidin in the present study have resulted from its additional anti-inflammatory effect. Hesperidin may thus improve diabetic vascular complications through its antioxidant/anti-inflammatory properties.

In DM1, hesperidin lowered the urea plasma level as compared to the diabetic controls but it did not significantly affect the increased levels of glucose, TG, cholesterol and proteinuria (Table 1). Its effect on urea plasma level can be attributed to the improvement of kidney homodynamic. However, a plausible explanation for discrepancy between hesperidin's effects on aortic relaxation and proteinuria (a marker of glomerular injury and diabetic nephropathy) is not available. This might be due to the different pathogenesis

of microvascular and macrovascular complications of diabetes or the rapid adverse effect of experimental diabetes on glomerular hemodynamics and microarchitecture. In the present model of inducing insulin resistance and diabetes type 2, there were no changes in plasma glucose level and excreted urine protein (Table 2). Hesperidin had no effect on the increased levels of TG and cholesterol in this group (Table 2). This is in contrast to the results of a study which pointed to its lowering effect on TG and cholesterol 25.

In conclusion, hesperidin preserves bioavailability of NO in aortic endothelial cells in both DM1 and DM2. This can be attributed to its antioxidants and anti-inflammatory effects and suggests its potential to improve some complications of diabetes.

Acknowledgements

The authors would like to thank A. Bagheri, R. Gholipoor, M. Mojahed, I. Arasteh and H. R. Zare for their technical assistance. This work was supported by grants 80-1314 and 84-2413 from Vice-chancellor of Research, Shiraz University of Medical Sciences. The authors declare that they have no Conflicts of Interest.

References

1 Schafer A, Flierl U, Vogt C, et al. Telmisartan improves vascular function and reduces platelet activation in rats with streptozotocin-induced diabetes mellitus. *Pharmacol Res* 2007; **56**:217-23.

2 Qian LB, Wang HP, Qiu WL, et al. Interleukin-2 protects against endothelial dysfunction induced by high glucose levels in rats. *Vasc Pharmacol* 2006; **45**:374-82.

3 Molnar J, Yu S, Mzhavia N, et al. Diabetes induces endothelial dysfunction but does not increase neointimal formation in high-fat diet fed C57Bl/6J mice. *Circ Res* 2005; **96**:1178-84.

4 Malakul W, Thirawarapan S, Suvitayavat W, et al. Type 1 diabetes and hypercholesterolemia reveal the contribution of endothelium-derived hyperpolarizing factor to endothelium-dependent relaxation of the rat aorta. *Clin Exp Pharmacol Physiol* 2008; **35**:192-200.

5 Ammar RF Jr, Gutterman DD, Brooks LA, et al. Free radicals mediate endothelial dysfunction of coronary arterioles in diabetes. *Cardiovasc Res* 2000; **47**:595-601.

6 Goirand F, Ovide-Bordeaux S, Renaud JF, et al. Effect of dietary docosahexaenoic acid on the endothelium-dependent vasorelaxation in diabetic rats. . *Clin Exp Pharmacol Physiol* 2005; **32**:184-90.

7 Ros RD, Assaloni R, Ceriello A. The preventive anti-oxidant action of thiazolidinediones: a new therapeutic prospect in diabetes and insulin resistance. *Diabetic Med* 2004; **21**:1249-52.

8 Tomasian D, Keaney JF, Vita JA. Antioxidants and the bioactivity of endothelium-derived nitric oxide. *Cardiovasc Res* 2000; **47**:426-35.

9 Reyes-Toso C, Linares LM, Witriw A, et al. Antioxidants restore aortic ring relaxation in pancreatectomized rats. *Life Sci* 2007; **81**:1467-72.

10 Balakrishnan A, Menon VP. Antioxidant properties of hesperidin in nicotine-induced lung toxicity. *Fund Clin Pharmacol* 2007; **21**:535-46.

11 Jin YR, Hanb XH, Zhang YH, et al. Antiplatelet activity of hesperetin, a bioflavonoid, is mainly mediated by inhibition of PLC-γ2 phosphorylation and cyclooxygenase-1 activity. *Atherosclerosis* 2007; **194**:144–52.

12 Serra H, Mendes T, Bronze MR, et al. Prediction of intestinal absorption and metabolism of pharmacologically active flavones and flavanones. *Bioorg Med Chem* 2008; **16**:4009-18.

13 Severino C, Brizzi P, Solinas A, et al. Low-dose dexamethasone in the rat: a model to study insulin resistance. *Am J Endocrinol Metab* 2002; **283**:E367-E373.

14 Bitar MS, Wahid S, Mustafa s, et al. Nitric oxide dynamics and endothelial dysfunction in type II model of genetic diabetes. *Eur J Pharmacol* 2005; **511**:53-64.

15 Abebe W. Effects of taurine on the reactivity of aorta from diabetic rats. *Life Sci* 2008; **82**:279-89.

16 Baluchnejadmojarad T, Roghani M, Imani A. Dose-dependent effect of captopril on aortic reactivity of streptozotocin-doabetic rats. *Clin Exp Pharmacol Physiol* 2004; **34**:342-47.

17 Calles-Escandon J, Cipolla M. Diabetes and endothelial dysfunction: A clinical perspective. *Endocr Rev* 2001; **22**:36-52.

18 Jansson PA. Endothelial dysfunction in insulin resistance and type 2 diabetes. *J Intern Med* 2007; **262**:173-83.

19 Cersosimo E, DeFronzo RA. Insulin resistance and endothelial dysfunction: the road map to cardiovascular diseases. *Diabetes-Metab Res Rev* 2006; **22**:423-36.

20 Tirkey N, Pilkhwal S, Kuhad A, et al. Hesperidin, a citrus bioflavonoid, decreases the oxidative stress produced by carbon tetrachloride in rat liver and kidney. *BMC Pharmacol* 2005; **5**:2.

21 Yeh CC, Kao SJ, Lin CC, et al. The immunomodulation of endotoxin-induced acute lung injury by hesperidin in vivo and in vitro. *Life Sci* 2007; **80**:1821-31.

22 Somfai GM, Knippel B, Ruzicska E, et al. Soluble semicarbazide-sensitive amine oxidase (SSAO) activity is related to oxidative stress and subchronic inflammation in streptozotocin-induced diabetic rats. *Neurochem Int* 2006; **48**:746-52.

23 Libby P. Fat Fuels the Flame: Triglyceride-Rich Lipoproteins and Arterial Inflammation. *Circ Res* 2007; **100**:299-301.

24 Kim F, Pham M, Luttrell I, et al. Toll-like receptor-4 mediates vascular inflammation and insulin resistance in diet-induced obesity. *Circ Res* 2007; **100**:1589-96.

25 Lee SH, Jeong TS, Park YB, et al. Hypocholesterolemic effect of hesperetin mediated by inhibition of 3-hydroxy-3-methylgultaryl coenzyme A reductase and acyl coenzyme A: cholesterol acyltransferase in rats fed high-cholesterol diet. *Nutr Res* 1999; **19:**1245-58.