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Research Article

EFFECT OF HESPERIDIN ON CARDIOVASCULAR COMPLICATION IN STREPTOZOTOCIN-NICOTINAMIDE INDUCED TYPE 2 DIABETIC RATS

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ABSTRACT

Present study was designed to evaluate Hesperidin on cardiovascular complication in normal and Streptozotocin-Nicotinamide induced diabetic in rats. Hesperidin (100 mg/kg, p.o) was administered for 28 days in rats injected with single dose of Streptozotocin (65 mg/kg, i.p, STZ) and Nicotinamide (110 mg/kg, i.p, NIC) and after isoproterenol (200 mg/kg, s.c.) induced myocardial infarction in rats on 29th and 30th day. At the end of experimental period (i.e. on the day 31) blood samples were collected and animals were euthanized. A heart tissue sample of each rat was collected and glycogen and nitrite carried out for further estimations. Administration of STZ–NIC in rats showed a significant ($P<0.001$) increased in the levels of serum glucose, glycosylated heamoglobin (HbA1c), creatine kinase (CK), Glutamate oxaloacetate transferase (GOT), glycogen and nitrite whereas the levels of myocardial infarct size was found low to be significant ($p<0.05$). Treatment with Hesperidin significantly ($P<0.05$) decreased change HbA1c, glucose level, nitrite, CK and glycogen but significantly reduced GOT ($P<0.01$) in compared to diabetic control group. The myocardial infarction in diabetic rats also led to severe splaying of muscle fiber, heavy neutrophil infiltration and cellular edema than non diabetic rats. The HES treated diabetic rats exhibited reduction in necrosis with less fragmentation of fibres as compared to diabetic control groups, which reflects the cardio protective effect of HES. This study concluded that HES at 100 mg/kg may show reduce cardiovascular complication in type 2 diabetic rats.

Keywords: Hesperidin, cardioprotective, Type 2 diabetic, Histopathology.

INTRODUCTION

Three major metabolic abnormalities contribute to the development of hyperglycemia in Type 2 diabetes mellitus such as impaired insulin secretion in response to glucose, increased hepatic glucose production and decreased insulin-stimulated glucose uptake in peripheral tissues. The latter 2 abnormalities are primarily due to insulin resistance[1, 2]. Cardiovascular disease is one of the leading causes of death in the western world and diabetes mellitus has been identified as a primary risk factor[3], due to which there is alteration in vascular responsiveness to several vasoconstrictors and vasodilators[4]. Recently, a protective effect of Hesperidin against oxidative stress in liver and kidney of diabetic rabbits[5] has been reported.

Hesperidin (HES) is an abundant and inexpensive byproduct of Citrus cultivation and isolated from the ordinary orange Citrus aurantium and other species of the genus Citrus (family: Rutaceae). It is reported to have anti-allergic, radio protective, immunomodulator, anti-hypertensive and anti-oxidant properties. When Hesperidin is administered orally, it is hydrolyzed by intestinal micro flora to yield a major active metabolite Hesperidin.

So far the effect of Hesperidin on cardiovascular complication in type 2 diabetic rats has not been studied. Hence, the purpose of the present study was to instigate the effect of Hesperidin treatment on serum heart marker, heart tissue parameter and histopathological alteration in Isoproterenol Induced myocardial infarction in type 2 diabetic rats.

MATERIALS AND METHOD

Drugs and chemicals

Hesperidin was obtained from ACROS Lab, US. STZ and NIC were obtained from SIGMA, St. Louis, MO, USA. All other chemicals and reagents used in the study were of analytical grade.

Experimental animals

All experiments and protocols described in present study were approved by the Institutional Animal Ethics Committee (IAEC) of Dharmaj Degree Pharmacy College, Anand. Sprague Dawley rats (210 ± 15 g) were housed in-group of 3 animals per cage and maintained under standardized laboratory conditions (12- h light/dark cycle, 24°C) and provided free access to palleted CHAKKAN diet (Nav Maharashtra Oil Mills Pvt., Pune) and purified drinking water *ad libitium*.

Experimental induction of type 2 diabetes in rats

Type 2 diabetes was induced in rats by a single intraperitoneal (i.p) injection of Streptozotocin (65 mg/kg, STZ) in overnight fasting rats or mice followed by the i.p administration of Nicotinamide (110 mg/kg, NIC) after 15 minutes. STZ was dissolved in citrate buffer (pH 4.5) and NIC was dissolved in normal saline. After 7 days following STZ and NIC administration, blood was collected from retro-orbital puncture and serum samples were analyzed for blood glucose[6]. Animals showing fasting blood glucose higher than 210 mg/dL were considered as diabetic and used for the further study. Hesperidin (100 mg/kg, p.o) was administered for 28 days in diabetic rats and after isoproterenol induced myocardial infarction in rats on 29th and 30th day. At the end of experimental period (i.e. on the day 31) blood samples were collected and animals were euthanized. A heart tissue sample of each rat was collected and carried out for further estimations.

Experimental protocol

Animals were divided into following groups, each group containing 6 animals and the treatment period for whole study was 4 weeks.

Group 1: Non-diabetic control [0.5 % Sodium CMC (1 ml/kg/day, p.o) as vehicle for 4 weeks and (ND-CON)] and normal saline subcutaneously on 29th and 30th day.

Group 2: Non-diabetic control treated with HES (100 mg/kg/day, p.o) as a suspension [0.5 % Sodium CMC for 4 weeks (ND-HES)] and normal saline subcutaneously on 29th and 30th day.

Group 3: STZ-NIC diabetic control [0.5 % Sodium CMC (1 ml/kg/day, p.o) as vehicle for 4 weeks (D-CON)] and received ISO (200mg/kg, s.c.) on 29th and 30th day in normal saline.

Group 4: STZ-NIC diabetic rats treated with HES (100 mg/kg/day, p.o) as a suspension [0.5 % Sodium CMC for 4 weeks (D-HES)] and received ISO (200mg/kg, s.c.) on 29th and 30th day in normal saline.

Biochemical estimations

Characterization of type 2 diabetes model

Type 2 diabetes was confirmed by measuring fasting serum glucose using standard diagnostic kit (SPAN diagnostics Pvt., India) and the

degree of uncontrolled diabetic state was confirmed by measuring HbA1c (Ion Exchange Resin method). After 4 weeks, diabetes was confirmed by measuring glucose and HbA1c as mentioned above.

Estimation of serum markers

On 4th week blood samples were collected from retro-orbital plexus under light ether anesthesia and centrifuged at 2500 rpm for 20 minutes to separate serum. Glucose, HbA1c, CK and GOT were estimated using diagnostic kits (SPAN Diagnostics Pvt. India). *In vitro* quantitative determination of the activity of myocardial glycogen and myocardial nitrite[7] levels was performed.

Infarct size measurement

The suture was tired again. The myocardial infarct size was measured by injecting evans blue solutions (2 % in PBS) retrograde through aorta to area at risk (AAR). The heart was frozen and about 6-8 thin sections were cut (approx 1-1.5 mm) from apex to base. The sections were placed in triphenyl tetrazolium chloride (TTC) solution (1% in PBS, pH 7.4) and kept at 37 °C for 20 min. The sections were fixed in 10 % formal saline overnight; both sides of slides of slices were scanned with scanner for measurement of AAR and infarct size (IS) by image J Software (1.30v). The area free from blue staining was area at risk. The portion stained red colour was salvaged myocardium and stained whitish portion was infarct size. Each area i.e. total, AAR, IS of each image was measured five times and the measured five times and the mean of each area of every slide was calculated. This was done to ensure minimum error in measurement of each area. The complete area of all the sections in each slide was added to get total area. The zone free from blur stain of each section from the slide was added to get the AAR. It was calculated as percent of total area, while IS was calculated as percent of AAR.

Histological examination

After decapitation, the heart was rapidly dissected out and washed immediately with saline and fixed in 8% buffered formalin. Hearts which were stored in 8% formalin were embedded in paraffin, sections cut at 5 µm and were stained with haematoxyline and eosin. The sections of the heart were observed under microscope (Olympus BX8) for histological changes.

Statistical analysis

All of the data are expressed as mean ± SEM. Statistical significance between more than two groups was tested using one-way ANOVA followed by the Bonferroni multiple comparisons test or unpaired two-tailed student's t-test as appropriate using a computer-based fitting program (Prism, Graphpad 5). Differences were considered to be statistically significant when $p < 0.05$.

RESULTS

Characterization of type 2 diabetes

Single intraperitoneal (i.p) injection of Streptozotocin (65mg/kg) followed by i.p administration of Nicotinamide (110 mg/kg) to rats produced severe hyperglycemia and increased HbA1c in 70 to 80 % the animals (Figure 1).

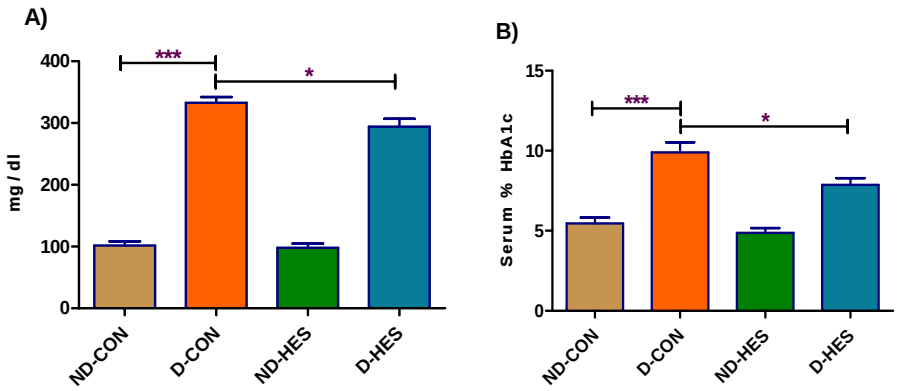


Fig. 1: Effect of Hesperidin (100 mg/kg/day, p.o) on changes in serum glucose and HbA1c level in normal and STZ-NIC induced diabetic rats.

Values are expressed as mean ± SEM for six animals in the group. ***P<0.001, *P<0.05 considered statistically significant as compared to respective control group.

Table 1: Effect of Hesperidin (100 mg/kg/day, p.o) on changes in body weight, heart weight and heart to body weight ratio after completion of myocardial infarction in normal and STZ-NIC induced diabetic rats.

Groups	Body weight (gm)		Heart weight (gm)	Heart to body weight ratio
	initial	Final		
ND-CON	240.6±12.5	261.6±15.4#	0.872±0.021	0.00333±0.00064
D-CON	249.2±17.4	224.4±16.1#	0.973±0.019*	0.00433±0.00027*
ND-HES	237.5±18.4	244.4±18.9	0.799±0.085	0.00326±0.00033
D-HES	239.1±16.8	254.2±17.9	0.943±0.066*	0.00370±0.00042

Values are expressed as mean ± SEM for six animals in the group. * P<0.05 compared to respective control group and # P<0.05 compared to initial weight.

Body weight and heart weight

Final body Weight of control animals was significant ($P < 0.05$) increased as compared to initial body weight. There was a significant reduction in final body weight as compared to initial body weight of D-CON diabetic group (Table 1). Hesperidin treatment had no significant effect on the body weight of D-CON group animals. There was a significant ($P < 0.05$) increased in heart weight of diabetic rats (D-CON). HES treatment could prevent increase in heart weight in diabetic rats (D-CON). Heart to body weight ratio of the entire group is show in (Table 1).

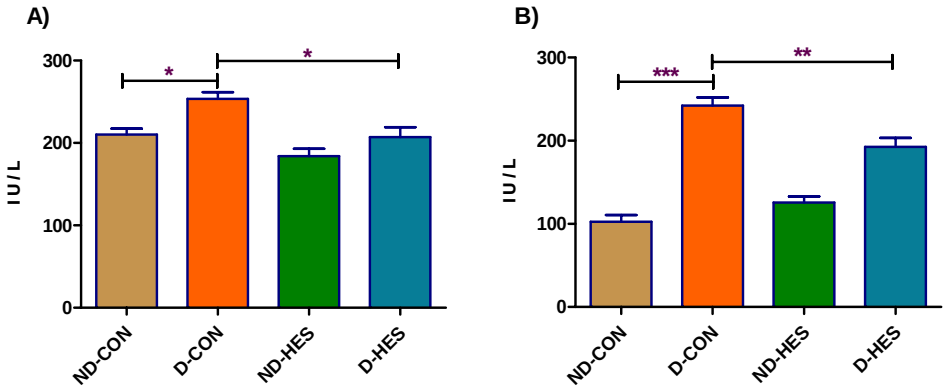


Fig. 2: Effect of Hesperidin (100 mg/kg/day, p.o) on changes in serum Creatine kinase (CK) and Glutamate oxalatoacetate transferase (GOT) level after completion of myocardial infarction in normal and STZ-NIC induced diabetic rats

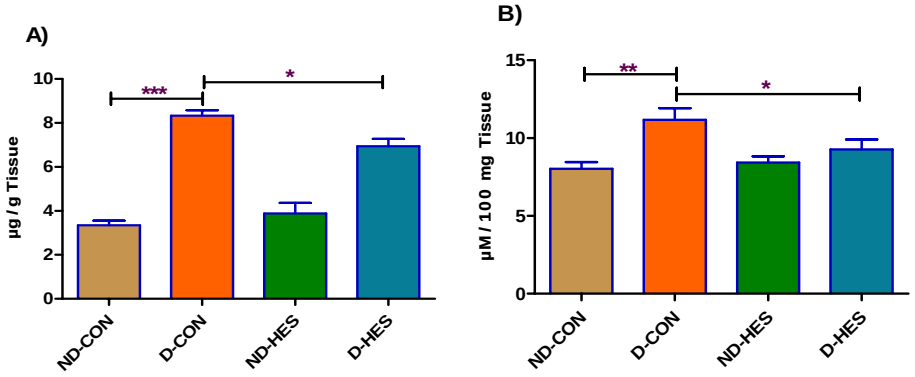
Values are expressed as mean ± SEM (n=6). *P<0.05, **P<0.01, ***P<0.001 considered statistically significant as compared to respective control group.

Effect of HES on serum enzymes

There was a significant (P<0.05) increase in serum CK and GOT (P< 0.001) level after myocardial infarction in D-CON group as compared to ND-CON group (Fig. 1). Treatment of HES in STZ-NIC diabetic rats (D-HES) as well as in non diabetic rats (ND-HES) could reduce (P< 0.001) elevated levels of serum CK and GOT as compared to D-CON group and respectively (Figure 2).

Myocardial infarct size

There was a significant (P < 0.05) increase in infarct size after myocardial infarction in diabetic rats (D-CON) as compared to ND-CON. HES treatment significantly (P < 0.05) reduced infarct size in D-HES group as compared to D-CON group (Figure 4, 5). However, treatment with HES could not reduce infarct size in non diabetic rats (ND-HES) as compared to ND-CON group (Figure 4, 5).



Values are expressed as mean ± SEM (n=6). *P<0.05, **P<0.01, ***P<0.001 considered statistically significant as compared to respective Control group.

Fig. 3: Effect of Hesperidin (100 mg/kg/day, p.o) on myocardial changes in Glycogen (A) and Nitrite (B) level after completion of myocardial infarction in normal and STZ-NIC induced diabetic rats.

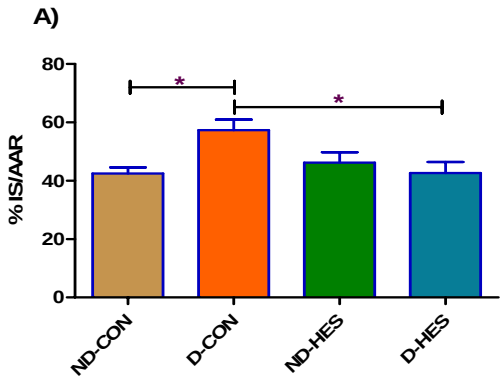


Fig. 4: Effect of Hesperidin (100 mg/kg/day, p.o) on myocardial infact size changes after completion of myocardial infarction in normal and STZ-NIC induced diabetic rats.

Values are expressed as mean ± SEM (n=6). *P<0.05, **P<0.01 considered statistically significant as compared to respective control group.

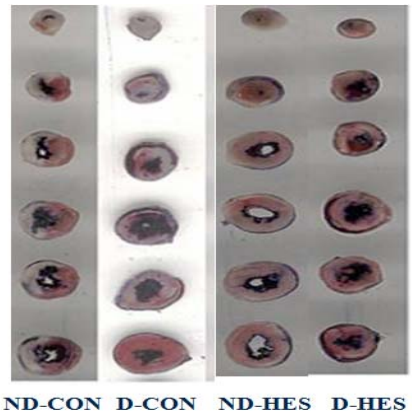


Fig. 5: Effect of Hesperidin (100 mg/kg/day, p.o) on TTC stained myocardial sections changes after completion of myocardial infarction in normal and STZ-NIC induced diabetic rats.

ND-CON = Non-diabetic control, D-CON = STZ-NIC diabetic control, ND-HES = Non-diabetic control treated with HES, D-HES = STZ-NIC diabetic rats treated with HES.

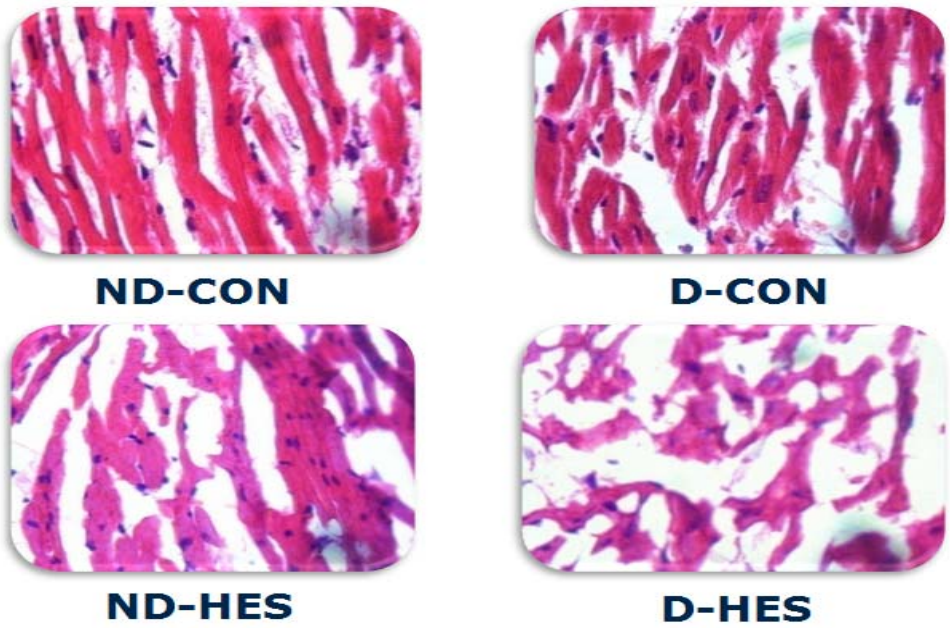


Fig. 6: Effect of Hesperidin (100 mg/kg/day, p.o) on light micrographs of histopathological section of heart changes after completion of myocardial infarction in normal and STZ-NIC induced diabetic rats.

ND-CON = Non-diabetic control, ND-HES = Non-diabetic control treated with HES, D-CON = STZ-NIC diabetic control, D-HES = STZ-NIC diabetic rats treated with HES.

Effect of HES on myocardial tissue parameter

There was a significant ($P < 0.001$) increase in myocardial glycogen level in D-CON group as compared to ND-CON group after myocardial infarction. HES treatment significantly ($P < 0.05$) decreased glycogen deposition in diabetic animal (D-HES) as compared to diabetic control group (ND-HES) (Figure 3). There was a significant ($P < 0.01$) increase in myocardial nitrite level in D-CON group as compared to ND-CON group after myocardial infarction. HES treatment in diabetic rats (D-CON) and non diabetic rats (ND-CON) significantly ($P < 0.05$) reduced nitrite level in heart in diabetic animal (D-HES) as compared to diabetic control group (D-CON) and non diabetic rats (ND-HES) respectively (Figure 3B).

Histopathology of heart

The photomicrographs revealed that induction of myocardial infarction caused more necrotic damage along with focal loss and fragmentation of muscle fibres of myocardial in diabetic rats (D-CON) than non diabetic rats (ND-CON) (Fig. 6).

The myocardial infarction in diabetic rats (D-CON) also led to severe splaying of muscle fiber, heavy neutrophil infiltration and cellular edema than non diabetic rats (ND-CON). The HES treated diabetic rats (D-HES) exhibited reduction in necrosis with less fragmentation of fibres as compared to D-CON groups, which reflects the cardio protective effect of HES (Fig. 6). However, HES treatment could protect myocardial infarction against in non diabetic rats (ND-HES).

DISCUSSION

The present study was under taken with the objective of exploring the Hesperidin on cardiovascular Complication in normal and STZ-NIC induced diabetic rats. Recent studies have suggested that prevalence of type 2 diabetes is rapidly increasing. Heart failure of myocardial infarction or ischemic origin is more frequent and severe in patients with diabetes. Diabetes is an independent risk factor for cardiac failure[8], although its detrimental impact on the myocardium remains to be identified. The significant amount of myocytes loss in this model of non insulin dependent diabetes mellitus is consistent with a greater vulnerability of the diabetic heart to cardiac processes.

In the present study, an increase in the levels of serum glucose and HbA1c in STZ-NIC treated rats confirmed the induction of diabetes mellitus. No significant was observed in the glucose and HbA1c level in diabetic rats after treatment with HES (100 mg/kg) when compared with D-CON rats at the end of experimental period. There was a significant increase in heart weight in STZ-NIC diabetic rats which may be due to cardiomyopathy associated with diabetes. It was reflected by increase in serum CK and GOT levels along with heart weight to body weight ratio. Hesperidin could protect the heart from cardiomyopathy associated with STZ-NIC diabetes. This may be the reason for decreased serum CK and GOT level in D-HES group.

Myocardial infarction causes further reduction in nitric oxide due to endothelial dysfunction. Hesperidin reduced myocardial infarct size in STZ-NIC diabetic rats. The glycogen deposition in heart is increased in STZ-NIC diabetic rats which may be due to reduction in glucose utilization. HES reduced cardiac glycogen content in STZ-NIC diabetic rats (D-HES) by increasing glucose utilization after myocardial infarction. Therefore, another possibility for cardioprotection by HES may be shifting of energy substrate metabolism from fatty acid to glucose.

The serum CK, GOT levels, cardiac nitrite level along with histopathological studies suggest cardioprotective role of Hesperidin against myocardial infarction in diabetic and non diabetic rats. The cardioprotective mechanism may be one or more from inhibition of angiotensive – II mediated detrimental effects, reduction in NO destruction, direct coronary vasodilation and thus improvement in oxygen supply to the myocardium.

There may be several mechanisms for cardioprotective by HES against myocardial infarction. It may be due to improvement in NO availability in STZ-NIC diabetic rats. Administration of STZ caused increase in serum CK, GOT and Hesperidin (100 mg/kg, p.o) could reduce them. This study concluded that HES at 100 mg/kg may show reduced on serum heart marker CK and GOT, heart tissue parameter glycogen and nitrite and histopathological alteration in diabetic rats.

REFERENCES

1. Kahn SE, Porte DJ. The pathophysiology of type II diabetes mellitus: Implications for treatment. Diabetes Mellitus: Theory and Practice. New York: Elsevier Science, 1990; 436-456.
2. Leibowitz HE. Oral hypoglycemic agents. Diabetes Mellitus: Theory and Practice. New York: Elsevier Science, 1990; 554-574.
3. Uemura S, Matsushita H, Li W, Glassford AJ, Asagami T, Lee KH, et al. Diabetes mellitus enhances vascular matrix metalloproteinase activity: role of oxidative stress. Circ Res 2001: 88; 1291– 1298.
4. Senses V, Ozyazgan S, Ince E, Tuncdemir M, Kaya F, Ozturk M, et al. Effect of 5-aminoimidazole-4-carboxamide riboside (AICA-r) on isolated thoracic aorta responses in streptozotocin-diabetic rats. J Basic Clin Physiol Pharmacol 2001; 12:227– 248.
5. Gumieniczek A. Effect of the new thiazolidinedione-Hesperidin on the development of oxidative stress in liver and kidney of diabetic rabbits. Life Sci 2003; 74:553–562.
6. Masiello P, Broca C, Gross R, Roye M, Manteghetti M, Hillaire-Buys D, et al. Experimental NIDDM: development of a new model in adult rats administered Streptozotocin and Nicotinamide. Diabetes 1998; 47: 224–229.
7. Guevara S M, Iwanejko J, Dembinaka-Kiec A, Pankiewicz J, Wanat A, Anna P, et al. Determination of nitrite/nitrate in human biological material by the simple Griess reaction. Clin Chim Acta. 1998; 274: 177-88.
8. Kannel W B, Mcgee D L, Diabetes and cardiovascular disease. The Framingham study. JAMA 1979; 241:2035-2038.