Original paper

A new type of polyphenols-containing dietary supplement for correction of lipids and inflammatory markers in patients with coronary artery disease

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Summary

Objective: To evaluate the impact of two original compositions of polyphenols-containing dietary supplement on lipid profile and level of C-reactive protein (CRP) in patients with angiographycally verified coronary artery disease (CAD).

Design and methods: 167 patients were selected during their scheduled post-event elective bicycle stress-test examination. All patients received standard CAD therapy and permanent statin therapy and had elevated total cholesterol (TC > 5.0 mmol/L) and/or CRP (>3.5 mg/L) levels. The study consisted of 2 days of polyphenol depletion followed by a 12-week supplementation period in a randomized, blinded, placebo-uncontrolled parallel design. Two different compositions SILVA 1 (Quercetine, linseed oil and Resveratrol), and SILVA 2 (Quercetine, linseed oil and Pycnogenol) were tested.

Results: All parameters changed compared baseline and 1 and 3 months in both groups. CRP decreased from 2.48 ± 1.62 mg/L at baseline to 1.97 ± 1.15 mg/L, high density cholesterol (HDL-C) increased from 1.18 ± 0.31 to 1.38 ± 0.34 mmol/L, also decrease of triglycerides (TG) from 1.5 to 1.29 mmol/L after 3 months treatment in SILVA I group was statistically significant ($p < 0.001$).

Changes of parameters between baseline and 1 or 3 months in SILVA II group were not statistically significant. Hovewer, decrease of CRP (from 2.6 ± 1.28 to 2.41 ± 1.68), decrease of low density cholesterol (LDL-C from 2.95 ± 1.2 to 2.88 ± 1.21), increase of HDL-C (from 1.25 ± 0.22 to 1.34 ± 0.23), decrease of TC (from 5.2 ± 1.3 to 5.1 ± 1.28) and decrease of TG (from 1.4 ± 0.41 to 1.3 ± 0.38) can be counted as tendency of changes.

Conclusion: This study reveals the superiority of treatment with statins in combination with composition containing Resveratrol for correction of lipid profile and inflamation marker CRP of patients with CAD.

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Introduction

More than 20 years ago it was shown that France and Finland, with similar intakes of cholesterol and saturated fat, consistently have had very different CAD mortality rates. Researchers explained this paradox as follows: given a high intake of cholesterol and saturated fat, the country in which people also consume more plant foods, including small amounts of liquid vegetable oils, and more vegetables (more antioxidants) had lower rates of CAD mortality [1]. Further epidemiologic studies have shown an association between a higher intake of polyphenols and a lower risk of cardiovascular diseases (CVD), diabetes mellitus, cancer and neurodegenerative disorders [2–4]. Polyphenols reduce the oxidation of low-density lipoproteins, enhance the vascular tone by molecular activation of endothelial nitric oxide, inhibit tissue grow factors, expression of adhesion molecules, reduce inflammation by reducing synthesis of inducible nitric oxide and

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inhibit cyclooxygenase-2. Furthermore, they inhibit cell apoptosis reducing necrotic tissue [4].

In the past few years identification of different polyphenols and development of phenolic compound extraction from fruits and vegetables has become a field of scientific programs, and many efforts have been made to provide rapid and accurate analytical methods for the identification of polyphenols. Plant phenolics include phenolics acids, flavonoids, tannins and the less common stilbenes and lignans.

So, polyphenols have received more and more attention in recent years due to a plethora of health promoting properties attributed to them. Particulary, resveratrol, quercetin and pycnogenol are in the focus from the cardiovascular health point of view.

Resveratrol is a trihydroxy trans form of stilbene found in some plants, fruits (as the mulberry), and seeds (as the peanut) and especially in the skin of grapes and certain grape-derived products (as red wine) and that has been linked to a reduced risk of coronary artery disease and cancer. It is becoming evident that resveratrol exerts cardioprotective benefits through the improvement of inflammatory markers, atherogenic profile, glucose metabolism and endothelial function [5]. Despite the effects observed, the poor bioavailability of resveratrol has been a classical drawback for this molecule and even a recurrent criticism used by some physicians or pharmacologists, i.e. '*resveratrol cannot exert benefits because it is rapidly metabolized and its presence in the bloodstream is negligible to justify any effect*'. However, the effects exist. More research should focus on identifying the actual metabolite(s) or signals or mediators responsible for these effects. In addition, and to overcome the poor bioavailabilty of resveratrol, intense research has been performed to enhance its bioavailability [6,7].

Quercetin is a plant polyphenol from the flavonoid group, found in many fruits, vegetables, leaves, and grains. Great interest is currently centered on the biologic activities of quercetin a polyphenol belonging to the class of flavonoids, natural products well known for their beneficial effects on health, long before their biochemical characterization [8]. In particular, quercetin is categorized as a flavonol, one of the five subclasses of flavonoid compounds with cardioprotective role by modulating cardiometabolic risk factors, improving the function of endothelial cells and vascular smooth muscle, which are responsible for governing blood pressure [9,10].

Most of **Pycnogenol's** benefits appear to be catered towards blood flow (with the common mechanism being related to Nitric Oxide being increased) and towards blood glucose control. Although there are many potential uses for Pycnogenol, the most well-studied use is for improving vascular health as a result of improved endothelial function and venous insufficiency. Controlled clinical trials have been published that demonstrate symptomatic improvement of blood circulation, blood pressure and platelet function normalization, and venous insufficiency. However, more studies with larger numbers of participants are needed to further establish these findings [11].

Taking into consideration the promissing data of multifactoral effect of above named polyfenol compounds on human health the thesis about significance of their combinations in secondary prevention of cardiovascular diseases, particulary CAD, can be raised. While statins are the treatment of choice for lowering LDL-C in the majority of patients, many patients retain a high CVD risk despite achieving the recommended LDL-C targets with statins. This 'residual risk' is mainly due to elevated TG and low HDL-C levels [12].

Following statin therapy optimisation additional therapy should be considered as part of a multifaceted approach to risk reduction.

So, the aim of our stydy was to evaluate the impact of 2 different original compositions of food supplement on lipid profile and level of CRP in patients with CAD.

Design and methods

Study subjects

The total study population consisted of 167 patients with clinically and angiographycally verified CAD. The inclusion criteria were male and female subjects in the age range 42–80 years. Patients with documented thyroid disease, malabsorbtion syndrome were excluded. Furthermore, subjects who were taking any medications known to influence the variables to be studied (excluding statins) were excluded. Body mass index (BMI) was calculated as weight in kg divided by height in meters squared; waist circumference was also measured. Blood pressure was measured from the left arm in seated patients with an automatic blood pressure monitor after a 10 min rest. Two measurements were taken at least 5 minutes apart, and the mean was used for analysis.

The investigation conforms with the principles outlined in the Declaration of Helsinki and approved by the institutional Ethics Committee. Informed consent was obtained from all subjects.

Patients were selected from outpatient clinic during their scheduled post-event elective bicycle stress-test examination. All patients received permanent statin therapy and had elevated choles-

Table 1.
Patient characteristics

Patients characteristics (n = 167)	
Gender	
Women	58.7%
Men	41.3%
Age (mean ± SD) years	66.4 ± 8.5
Arterial hypertension	64.5%
Diabetes mellitus	8.5%
Elevated TC with statin therapy (>5.0 mmol/L)	63.1%
Elevated CRP (>3.5 mg/L)	42.3%

Table 2.
Characteristics of study groups

	Patients (n = 167)	
	SILVA 1 (n = 84) M = 34, W = 50	SILVA 2 (n = 83) M = 35, W = 48
Age (mean) years	67.3 ± 8.8	65.7 ± 7.7
Arterial hypertension	67.8%	61.4%
Diabetes mellitus	9.5%	7.2%
Elevated TC with statin therapy (>5 mmol/L)	64.2%	61.4%
Elevated CRP (>5 mg/L)	42.8%	42.1%

terol (>5.0 mmol/L) and/or CRP (>3.5 mg/L) levels. The distribution of all patients according age, presence of arterial hypertension, diabetes mellitus and CRP is presented in Table 1.

Study protocol and blood collection

This study consisted of 2 days of polyphenol depletion followed by a 12-week supplementation period in a randomized, blinded, placebo-uncontrolled parallel design. During the polyphenol depletion period, all subjects were requested to restrict their consumption of polyphenols to avoid any influence on the study results. These food ingredients included onions, apples, red wine, tea, biological and freshly pressed fruit juices, berries, grapes, cherries, raisins, parsley, broccoli, cabbage, beans, and tomatoes.

Two different compositions SILVA 1 and SILVA 2 were tested. Both compositions were produced by Pharmaceutical Company HASCO-LEK S.A. (Poland) according the special order of Research Institute of Cardiology and regenerative medicine, University of Latvia. Composition SILVA 1 contains Quercetine, linseed oil and Resveratrol, the composition SILVA 2 consists of Quercetine, linseed oil and Pycnogenol. These compositions were added to patient standard therapy. Subsequently, the subjects were randomly assigned to either a SILVA1 group (n = 84) or a SILVA 2 group (n = 83). The distribution of patients of boath groups according age, presence of arterial hypertension, diabete mellitus and CRP is presented in Table 2. The subjects were instructed to take either 2 capsules of SILVA 1 or 2 capsules of SILVA 2 composition per day. The subjects were instructed to maintain their usual patterns of dietary intake during the study. Compliance was monitored through biweekly phone calls for capsule counts, and a nutritionist checked for changes in usual dietary patterns at the end of the study. Additionally, the subject's usual dietary intakes were assessed both at baseline and at 12 weeks after compositon supplementation using a 24-h recall method. All patients filled up the questionnaire from five questions (gender, education, salt consumption in daily food, cholesterol level, medicines usage). Venous blood samples were collected from the forearm in EDTA-treated and plain tubes after a fasting period, at baseline, at 4 and 12 weeks after the intervention.

Statistical analysis was done by SPSS program using Wilcoxon signed ranks and Mann Whitney U-tests.

Results

Data about dynamics of all examined parameters are summarized in Table 3.

Comparision of results on baseline and after 4-week use of one of the compositions shows no statistically significant changes. Comparing results on baseline and after 12 weeks statistically significant decrease of CRP and TG levels, increase of HDL-C in woman group ($p < 0.001$) were find.

Figure 1 shows that all investigated parameters have been changed from baseline to 1 and 3 months after the treatment. However, only CRP decrease from 2.48 ± 1.62 mg/L at baseline to 1.97 ± 1.15 mg/L, HDL increase from 1.18 ± 0.31 to 1.38 ± 0.34 mmol/L and decrease of TG from 1.5 ± 0.9 to 1.29 ± 0.8 mmol/L after 3 months treatment can be counted as statistically significant ($p < 0.001$). Nevertheless, the average decrease of TC from 5.12 to 5.03 mmol/L, the decrease of LDL-C from 2.91 to 2.81 mmol/L is necessary to note.

Data in Fig. 2 show no statistically significant changes of all parameters between baseline and 1 or 3 months follow up results. Hovewer, comparing the baseline and the results of the 3 months follow-up decrease of CRP (from 2.6 ± 1.28 to 2.41 ± 1.68 mg/L), decrease of LDL-C (from 2.95 ± 1.2 to 2.88 ± 1.21 mmol/l), increase of HDL-C (from 1.25 ± 0.52 to 1.34 ± 0.43 mmol/L), decrease of TC (from 5.2 ± 1.3 to 5.1 ± 1.28 mmol/L) and decrease of TG (from

Table 3.
Levels of CRP, TC, HDL-C, LDL-C, TG before treatment (B), after 4 week follow-up (4w) and 12 week follow-up in all examined patients (m ± SD)*

	CRP mg/L	TC mmol/L	HDL-C mmol/L			LDL-C mmol/L	TG mmol/L
			All	Man	Woman		
B	2.58 ± 1.28	5.18 ± 1.44	1.21 ± 0.39	1.11 ± 0.19	1.28 ± 0.21	2.93 ± 1.21	1.50 ± 0.35
4w	2.52 ± 1.79	5.15 ± 1.35	1.29 ± 0.31	1.12 ± 0.20	1.31 ± 0.23	2.90 ± 1.18	1.48 ± 0.32
12w	2.18 ± 1.08**	5.09 ± 1.28	1.36 ± 0.34	1.21 ± 0.22	1.43 ± 0.24**	2.86 ± 1.34	1.29 ± 0.28**

*167 pts were enroled (B) and participated at 4 week follow-up; 158 pts participated at 12 week follow-up examination.
**Statistically significant difference between B and 12w.

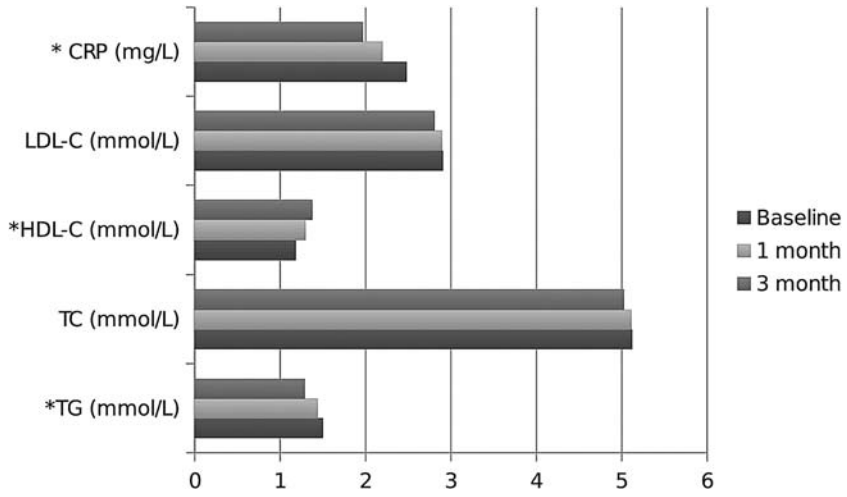

Figure 1. Data of investigated parameters in SILVA 1 group before and after 1 month ($n = 84$) and 3 months ($n = 77$) composition use.

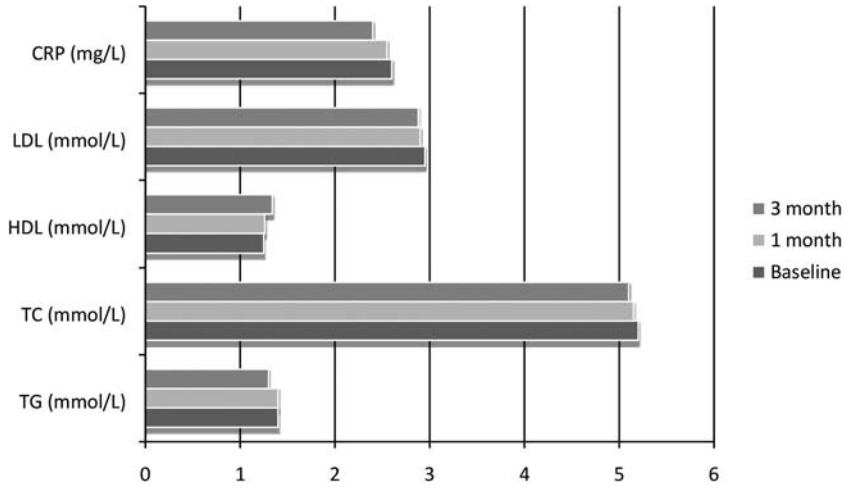

Figure 2. Data of investigated parameters in SILVA 2 group before treatment and after 1 month ($n = 83$) and 3 months ($n = 81$) treatment.

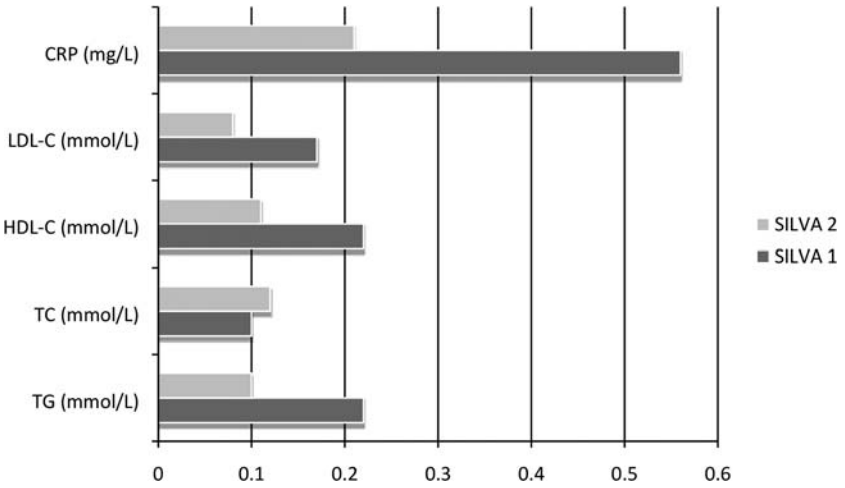

Figure 3. Comparision of changes of parameters (Δ) in SILVA 1 and SILVA 2 groups after 3 months follow-up.

1.4 ± 0.51 to 1.3 ± 0.48 mmol/L) exists, which can be counted as tendency of changes.

Comparision of parameter changes during the 3 months use of compounds represented in Fig. 3 clearly show the prevalence of SILVA I composition, i.e. the dynamics of parameters excluding TC is highly significantly more manifested using SILVA I composition than using SILVA II (p-value < 0.001). For example, CRP increase in SILVA I group is 0.56 ± 0.23 mg/L while in SILVA II group only 0.21 ± 0.16 mg/L; LDL-C decreased for 0.17 ± 0.08 mmol/L and 0.008 ± 0.004 mmol/L, HDL-C increased for 0.22 ± 0.15 mmol/L and 0.11 ± 0.08 mmol/L, TG decrease is for 0.22 ± 0.15 mmol/L and 0.1 ± 0.06 mmol/L respectively. Only TC decrease between groups isn't significantly different.

Discussion and conclusions

The use of statins to modulate levels of cholesterol and inflammation has led to remarkable reduction in CV endpoints in the frame of primary and secondary prevention of atherosclerotic CV disease. Cumulative evidence from clinical trials involving over 170,000 participants have demonstrated the beneficial effects of statins in the reduction of CV event rates [13].

However, studies in "real-world" populations and systematic reviews have shown that adherence to medication positively correlated with reduced CV risk, significantly improved health outcomes, a residual risk of about 69% persisted and this incomplete reduction of risk might also result in ongoing progression of disease [14].

So, despite the successes from the use of statins, analyses of clinical trial data reveal significant residual CV risk highlighting the need to retool our CV risk reduction algorithms. When patients do not show an adequate response to statin therapy, the guidelines recommend increasing the dosage of statins or to combining statins with another lipid-lowering drug. However, the evidence for statin combination therapy in improving CV outcomes remains inconclusive [14].

In this context our methodological approach to add resveratrol and pygnogenol containing compounds to statin therapy seems to be promising. Particularly, our findings show the significant decrease of TG and CRP as well as the increase of HDL-C (in woman) in common patient group after 3 months of compound use (Table 3). However, the analysis of indicator changes inside SILVA I and SILVA II shows the significant differences of groups average data. Firstly, there are not differences between background and 4-week data in both groups. Only after 3 months period of compound use we can find and analyse the significant dynamics of parameters. It was shown that only in SILVA I (resveretrol group) the changes of CRP, HDL-C and TG from background levels to 3 months use levels were statistically significant while data of SILVA II group patients showed only the tendency of changes. The average decrease of CRP in SILVA I group was 20.6%, in SILVA II group only 7.3%; the increase of HDL-C in SILVA I group was 14.5% while in SILVA II group 6.7%; the decrease of TG in SILVA I was for 14.0% and in SILVA II for 7.1%. It's necessary to stress that boath: resveratrol and pygnogenol countaining compounds did not have the sufficient influence on TC and LDL-C data.

As mentioned above the 'residual risk' is mainly due to elevated triglyceride and low high-density lipoprotein cholesterol levels [12]. Following statin therapy optimisation additional pharmacotherapy should be considered as part of a multifaceted approach to risk reduction. In this context our results of influence of SILVA I compo-

sition on TG decrease and HDL-C level increase deserve a special attention. As demonstrated in the Fig. 1 beside the decrease of CRP the statistically significant decrease of TG and increase of HDL-C was find using the SILVA I composition.

Our data of use of compound containing quercetine, linseed oil and resveratrol have to be compared with results of existing similar clinical trials of resveratrol influence on lipid and inflamatory markers. Results of randomized, double-blinded, active-controlled trial of use of combination of resveratrol and calcium fructoborate in 29 patients with stable angina pectoris showed the significant hsCRP decrease (30.3% at 60 days). Lipid markers showed slight changes from baseline in all groups [15].

Controversal data concerning the dynamics of CRP were demonstrated in three parallel arms, randomized, triple-blind, dose-response, placebo-controlled trial [16] of 25 patients with stable CAD who used resveratrol-containing grape extract. A non-statistically significant dose-dependent decrease of 8% ($p = 0.09$) and 18% ($p = 0.17$) was observed for high-sensitivity C-reactive protein (hsCRP) after 6 and 12 months, respectively. Even though hsCRP decreased 38% versus placebo after 12 months, no significant intergroup differences were found.

In a prospective, open-label, randomized, controlled trial 62 pts with diabetes mellitus (T2DM) randomized into control and intervention groups, results revealed that supplementation of resveratrol for 3 months significantly improves the systolic blood pressure (mean ± SD, 139.71 ± 16.10 vs 127.92 ± 15.37; $p < 0.05$) and total cholesterol (mean ± SD, 4.70 ± 0.90 vs 4.33 ± 0.76; $p < 0.05$). No significant changes in high-density lipoprotein and low-density lipoprotein cholesterols were observed [17].

Our results and numerous examples of results from similar studies demonstrate that resveratrol exerts cardioprotective benefits through the improvement of inflammatory markers and atherogenic profile [5]. These effects have been observed in healthy volunteers and medicated patients. However, the specific mechanisms of polyphenol impact are not yet clear. In these circumstances 3 suggestions are topical: 1) Since the origin of residual risk is multifactorial, the adoption of individual patient management should be considered as a serious option to reach therapeutic goals. 2) The 'residual risk' is mainly due to elevated triglyceride (TG) and low high-density lipoprotein cholesterol (HDL-C) levels. Following statin therapy optimisation additional pharmacotherapy should be considered as part of a multifaceted approach to risk reduction. 3) Despite the available studies and accumulated data about polyphenols, more detailed clinical trials are needed to find most beneficial combinations of biologically active compounds/bioavailability enhancers in order to provide formulations for food supplement and herbal medicine industry.

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Conflict of interest

The authors report no conflicts of interest in this work.

References

[1] Artaud-Wild SM, Connor SL, Sexton G, Connor WE. Differences in coronary mortality can be explained by differences in cholesterol and saturated fat intakes in 40 countries but not in France and Finland. A paradox. Circulation 1993;88:2771–27.
[2] Hollman PC, Geelen A, Kromhout D. Dietary flavonol intake may lower stroke risk in men and women. J Nutr 2010;140:600–4;
[3] Pandey KB, Rizvi SI. Plant polyphenols as dietary antioxidants in human health and disease. Oxid Med Cell Longev 2009; 2:270–8.
[4] Curin Y, Andriantsitohaina R. Polyphenols as potential therapeutical agents against cardiovascular diseases. Pharmacol Rep 2005;57(Suppl):97–107.
[5] Tomé-Carneiro J1, Larrosa M, González-Sarrías A, Tomás-Barberán FA, García-Conesa MT, Espín JC. Resveratrol and clinical trials: the crossroad from in vitro studies to human evidence. Curr Pharm Des 2013;19:6064–93.
[6] Santos AC, Veiga F, Ribeiro AJ. New delivery systems to improve the bioavailability of resveratrol. Expert Opin Drug Deliv 2011;8:973–90.
[7] James M, Smoliga JM, Blanchard O. Enhancing the delivery of resveratrol in humans: if low bioavailability is the problem, what is the solution? Molecules 2014;19:17154–72.
[8] D'Andrea G. Quercetin: A flavonol with multifaceted therapeutic applications? Fitoterapia 2015;106:256–71.
[9] Lee KH, Park E, Lee HJ, Kim MO, Cha YJ, Kim J, et al. Effects of daily quercetin-rich supplementation on cardiometabolic risks in male smokers. Nutr Res Pract 2011;5:28–33.
[10] Edwards RL, Lyon T, Litwin SE, Rabovsky A, Symons JD, Jalili T. Quercetin reduces blood pressure in hypertensive subjects. J Nutr 2007;137:2405–11.
[11] PYCNOGENOL®. Clinical overview. http://abc.herbalgram.org/site/DocServer/Pycnog_CO.pdf?docID=1762.
[12] Reiner Z Managing the residual cardiovascular disease risk associated with HDL-cholesterol and triglycerides in statin-treated patients: a clinical update. Nutr Metab Cardiovasc Dis 2013;23:799–807.
[13] Sampson UK, Fazio S, Linton MF. Residual cardiovascular risk despite optimal LDL cholesterol reduction with statins: the evidence, etiology, and therapeutic challenges. Curr Atheroscler Rep 2012;14:1–10.

[14] Perrone V, Sangiorgi D, Buda S, Degli Esposti L. Residual cardiovascular risk in patients who received lipid-lowering treatment in a real-life setting: retrospective study. Clinicoecon Outcomes Res 2016;8:649–55.
[15] Militaru C, Donoiu I, Craciun A, Scorei ID, Bulearca AM, Scorei RI. Oral resveratrol and calcium fructoborate supplementation in subjects with stable angina pectoris.Effects on lipid profiles inflammation markers and quality of life. Nutrition 2013;29:178–83.
[16] Tomé-Carneiro J, Gonzálvez M, Larrosa M, Yáñez-Gascón MJ, García-Almagro FJ, Ruiz-Ros JA, et al. Grape resveratrol increases serum adiponectin and downregulates inflammatory genes in peripheral blood mononuclear cells: a triple-blind, placebo-controlled, one-year clinical trial in patients with stable coronary artery disease. Cardiovasc Drugs Ther 2013;27:37–48.
[17] Bhatt JK, Thomas S, Nanjan MJ. Resveratrol supplementation improves glycemic control in type 2 diabetes mellitus. Nutr Res 2012;32:537–41.