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Original article

Retrospective analysis of the effects of a highly standardized mixture of *Berberis aristata, Silybum marianum*, and monacolins K and KA in diabetic patients with dyslipidemia

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Summary. *Background:* Berberine, an alkaloid with both glucose- and cholesterol-lowering action, is also characterized by an anti-diarrheal effect. Consequently, berberine-based therapies are recommended for diabetic patients with irritable bowel syndrome (IBS) or gut discomfort caused by metformin. *Aim:* As the anti-glycemic and cholesterol-lowering action of berberine is improved by co-administration with P-glycoprotein inhibitors and naturally derived statins, we have analyzed the effect of the food supplement Berberol®K (hereafter referred to as BSM) containing, berberine, silymarin, and a highly standardized red yeast rice containing monacolins K and KA in the ratio 1:1 but no secondary monacolins, dehydromonacolins, or citrinin (Monakopure™-K20). *Methods:* We retrospectively evaluated the effects of BSM in 59 diabetic patients with dyslipidemia and compared the results to those obtained in patients without treatment. Enrolled subjects had a diagnosis of IBS (and diarrhea), had diarrhea caused by metformin, or were statin intolerant. *Results:* After 6 months of BSM treatment, significant reductions of approximately 5%, 23%, 31%, and 20% were observed in glycated hemoglobin (HbA1c), total cholesterol (TC), low density lipoprotein-cholesterol (LDL), and triglyceride (TG) levels, respectively, and only five of the 31 treated subjects reported diarrhea compared with 22 of the 28 untreated patients. Regarding safety, treatment with BSM did not significant modify creatine phosphokinase (CPK), creatine, aspartate aminotransferase (AST) or alanine aminotransferase (ALT). *Conclusion:* BSM is a safe and effective food supplement likely useful as add-on therapy in diabetic subjects with dyslipidemia, especially if they are statin intolerant or with diarrhea caused by IBS or metformin. (www.actabiomedica.it)

Key words: berberine, Berberol®K, silymarin, P-glycoprotein, cholesterol, triglycerides, *Monascus purpureus*, Monakopure™-K20

Introduction

The prevalence of diabetes mellitus is increasing globally with almost 400 million individuals affected and approximately 5 million deaths reported annually. Type 2 diabetes mellitus (T2DM) is the most common form of the disease (1). Diet and physical exercise are initially used to treat T2DM, followed by oral hypoglycemic agents and then insulin injections (2). Despite their effectiveness, oral antidiabetic drugs have many limitations, with metformin, commonly used for T2DM, associated with a high incidence of gastrointestinal side effects, mainly diarrhea (3). Berberine, a natural isoquinoline alkaloid derivative endowed with both glucose- and cholesterol-lowering properties (4, 5), has a strong anti-diarrheal effect in both gut microbial infection (6) and irritable bowel syndrome (IBS) (7), and also reduces metformin gastrointestinal

side effects (8). Consequently, berberine-based therapies are suggested by physicians for diabetic patients with IBS or gut discomfort caused by metformin. The oral bioavailability of berberine is poor due to the inhibitory action of P-glycoprotein (P-gp) (9), but the pharmacological and clinical effects of berberine may be enhanced by the use of P-gp inhibitors (10) or by chemical modification of berberine allowing it to overcome P-gp antagonism (11). Indeed, the combined use of berberine and silymarin, a P-gp inhibitor (12), has been shown to be more effective than berberine alone in treating hypercholesterolemia and hyperglycemia (13-18). Moreover, the cholesterol-lowering properties of berberine are thought to be enhanced when it is associated with chemically or naturally derived statins, likely due to its opposite effect on proprotein convertase subtilisin/kexin type 9 (PCSK9) (19). As with synthetic statins, naturally derived statins, such as the monacolins found in *Monascus purpureus*-fermented rice, may also cause common side effects like myalgia or myopathy (20). However, due to the low dosage used in association with berberine, their tolerability profile should improve with few adverse events or impairment of liver transaminases or creatine phosphokinase (CPK) observed (21). Recently, a highly standardized food supplement containing berberine, silymarin, and monacolins K and KA from *Monascus* (with the other monacolins being below the detection limit) has been developed (22). We have therefore retrospectively analyzed its cholesterol-lowering and anti-hyperglycemic action in diabetic and dyslipidemic subjects with IBS, gastrointestinal discomfort due to metformin, or in statin-intolerance subjects with suboptimal lipid control despite the use of ezetimibe or fenofibrate.

Materials and methods

Study

Our study is a retrospective and controlled analysis of a 6-month supplementation period with a nutraceutical compound with possible cholesterol-lowering and anti-hyperglycemic properties, in diabetic subjects with a diagnosis of dyslipidemia. The trial and retrospective analysis were conducted in accordance with the principles stated in the Declaration of Helsinki and were consistent with Good Clinical Practice, as defined by the International Conference on Harmonization and in accordance with the ethical principles underlying European Union Directive 2001/20/EC and the United States Code of Federal Regulations, Title 21, Part 50 (21CFR50) (23). Data analysis, subject consent, and privacy forms were approved by the ethics boards before the study began. We analyzed food supplement use in patients attending a hospital (Bologna) in Italy between October 2015 and June 2016.

Patients

Potential patients, identified from a review of case notes and/or computerized clinic registers, were contacted by the investigators in person or by telephone. A total of 59 patients with T2DM and dyslipidemia were enrolled for this retrospective analysis. Of these, 28 served as untreated controls, while 31 received the food supplement.

Criteria

European subjects aged ≥18 years of both sexes and with T2DM were considered eligible for our retrospective analysis if they had a diagnosis of hypercholesterolemia and/or hypertriglyceridemia according to the ESC/ESH 2016 guidelines criteria (24). Subjects in the untreated group were considered eligible if their cholesterol and triglyceride levels were <240 mg/dL and <250 mg/dL, respectively. Of the 59 enrolled subjects with diabetes, 9 were statin intolerant, 10 had IBS, and 40 had metformin-related gastrointestinal discomfort. Patients with a diagnosis of statin intolerance were considered eligible for our study if, following correct statin use, they showed a CPK increase that was 3-10 times higher than the upper laboratory limit (ULL) and/or a rise in transaminase values 3-5 times higher than the ULL and/or asthenia or myalgia. All subjects included in our study were overweight or obese with a body mass index (BMI) of 25-40. Patients were excluded from our analysis if they had secondary dyslipidemia, impaired hepatic or renal function, an endocrine (except for diabetes) or

gastroenterological disorder, current or previous heart disease or stroke, malignancy or suspected malignancy, neurological or psychiatric disease, or a history of alcohol and/or drug abuse.

Product

We retrospectively analyzed the effect of the consumption of a finished food supplement, Berberol®K hereafter referred to as BSM, in form of a tablet and containing 500 mg/dose of berberine from *Berberis aristata* (extract tritation: 96% as berberine), 105 mg/dose of silymarin from *Silybum marianum* (extract tritation ≥60% as flavanolignans), and 50 mg/dose of Monakopure™-K20, hereafter referred to as MKP, from *M. purpureus* fermented rice extract (RYR; extract tritations: 20% monacolins K and KA in the ratio 1:1; secondary monacolins J, JA, M, MA, L, LA, X, and XA, plus dehydromonacolins DMK, DMJ, DMM, DML, and DMX <0.2% in total; and citrinin <50 ppb) (22). The finished product, BSM, was notified to the Italian Ministry of Health by Pharmextracta (Pontenure, PC, Italy) according to the provisions of law No. 169 of 2004, on May 2015 (notification number: 77055). BSM is a food supplement manufactured by Labomar (Istrana, TV, Italy) using food-grade active ingredients and excipients. The *B. aristata* and *S. marianum* extracts were provided by Labomar, and MKP by Labiotre (Tavarnelle Val di Pesa, FI, Italy). BSM was administered once a day after the main meal.

Diet and lifestyle

At the beginning of treatment all participants were instructed to follow a hypocaloric, low-glycemic-index diet. The controlled-energy diet (a daily caloric deficit of about 500-600 kcal) was based on NCEP-ATP III recommendations (24) with 50% of calories provided by carbohydrates, 30% by fat (<7% saturated, up to 10% polyunsaturated, and up to 20% monounsaturated fat), and 20% by protein, with a maximum cholesterol content of 300 mg/day, and 35 g/day of fiber. Participants were also encouraged to perform regular physical activity three or four times a week (riding a stationary bike for 20-30 minutes or walking briskly for 30 minutes).

Anti-diabetic therapies

The untreated and BSM-treated groups were prescribed a hypocaloric, low-glycemic-index diet, physical exercise, and hypoglycemic drugs. Eight of the untreated group were on metformin monotherapy, five were on sulphonylurea monotherapy and 15 were on combination therapy. Out of these 15, three were with metformin and sulphonylureas, two with metformin and dipeptidyl peptidase-4 (DPP-IV) inhibitors, two with metformin plus pioglitazone, one with metformin plus pioglitazone and acarbose, and seven with antidiabetic oral drugs plus injectable insulin. Two patients in the untreated group were also on ezetimibe (10 mg/day) monotherapy, while two were on fenofibrate (200 mg/day) monotherapy. Eight of the patients in the BSM-treated group were on metformin monotherapy, five were on sulphonylurea monotherapy, and 18 were on combination therapy. Out of these 18, four were with metformin and sulphonylurea, three with metformin and DPP-IV inhibitors, four with metformin and pioglitazone, one with metformin plus pioglitazone and acarbose, and six with oral antidiabetic drugs plus injectable insulin. Two patients in the BSM-treated group were also on ezetimibe (10 mg/day) monotherapy and three were on fenofibrate (200 mg/day) monotherapy.

Outcomes

The aim of our study was to retrospectively evaluate the following clinical outcomes in diabetic patients with dyslipidemia: BMI (calculated as weight in kilograms divided by the square of height in meters), fasting blood glucose (FG), glycated hemoglobin (HbA1c), total cholesterol (TC), low density lipoprotein-cholesterol (LDL), high density lipoprotein-cholesterol (HDL), triglycerides (TG), CPK, creatinine, aspartate aminotransferase (AST), and alanine aminotransferase (ALT). Treatment tolerability was assessed through patient interview and comparison of final clinical and laboratory values with baseline levels.

Statistical analysis

B-W-Subject Design Anova and Ancova were used for analysis depending on the variables being con-

sidered. A multiple comparison test (Tukey's HSD) was used to analyze possible differences between average values during the observation period. The α level was set at 0.05 and values were considered significant at $P<0.05$. NCSS 8 (NCSS, Kaysville, UT, USA) and JMP 10 (SAS Institute, Cary, NC, USA) software packages were used for analysis.

Results

Our study is a retrospective analysis of changes in clinical outcomes following 6-month treatment with BSM, a food supplement taken as a daily tablet and providing berberine (500 mg), silymarin (105 mg), and monacolins K and KA (10 mg) from RYR in the ratio 1:1, with all secondary monacolins and dehydromonacolins combined present at <0.2% (the HPLC detection limit). We analyzed 59 diabetic subjects with dyslipidemia, of whom 28 served as untreated controls, while 31 were treated with BSM. Nine subjects overall were statin intolerant and had been receiving treatment with ezetimibe (10 mg/day) or fenofibrate (200 mg/day) for 6 months or more. Table 1 reveals that the characteristics of subjects in the untreated and BSM-treated groups were similar. As regards glycemic parameters, Table 2 shows that a slight non-significant difference was observed in FG (-6.55% in the BSM-treated group versus -2.06% in the untreated group), while a significant difference was observed in HbA1c (-5.18% in the BSM-treated group versus +0.89% in the untreated group). As regards lipid profile (Table 2), the BSM-treated group demonstrated significant reductions in TC (about -23%), LDL (about -31%), and TG (about -20%) and a non-significant but considerable improvement in HDL (about +5%). No changes were observed in these parameters in the untreated group, but there was a slight non-significant reduction in TG of about 7%. As regards safety (Table 2), CPK, creatine, AST, and ALT, respectively, showed non-significant increases of about +16%, +3%, +9%, and +9% in the BSM-treated group, and non-significant changes of about -4%, +4%, +8%, and +3% in the untreated group. In terms of side effects (data not shown), no differences in gastric pain, gastric reflux, insomnia, headache, or skin rash were observed

Table 1. Numbers and characteristics of enrolled subjects

Parameter	Untreated (N=28)	BSM-treated (N=31)	P value
Sex (M/F)	12/16	13/18	NS
Age (years)	60.9±6.3	62.7±8.2	NS
Diabetes (years)*	7.3±4.5	7.1±5.1	NS
Statin intolerant	4	5	NS
IBS diagnosed	5	5	NS
Metformin-induced diarrhea	20	21	NS
Anti-diabetic treatment			
Metformin	8	8	NS
Sulphonylurea	5	5	NS
Metformin+sulphonylurea	3	4	NS
Metformin+DPP-4 inhibitors	2	3	NS
Metformin+pioglitazone	2	4	NS
Metformin+pioglitazone+acarbose	1	1	NS
Oral drugs+insulin	7	6	NS
Cholesterol-lowering treatment			
Ezetimibe	2	2	NS
Fenofibrate	2	3	NS

Notes: BSM is a supplement containing berberine, silymarin, and monacolins K and KA. *Years from diagnosis. All values are expressed as median ± standard deviation.
Abbreviations: F, females; M, males; N, number of subjects; NS, not significant.

Table 2. Clinical outcomes in patients with diabetes and dyslipidemia at T=0 and after 6 months

Parameter	Untreated (T=0) (N=28)	Untreated (T=6) (N=28)	Δ%	P value	BSM-treated (T=0) (N=31)	BSM-treated (T=6) (N=31)	Δ%	P value
BMI (kg/m²)	31.2±6.8	31.3±6.7	+0.32	NS	30.9±7.5	30.8±6.9	-0.32	NS
FG (mg/dL)	126.3±12.5	123.7±12.8	-2.06	NS	129.7±9.3	121.2±9.6	-6.55	NS
HbA1c (%)	6.73±0.41	6.79±0.38	+0.89	NS	6.75±0.29	6.40±0.32	-5.18	<0.05
TC (mg/dL)	223.1±17.5	221.8±18.4	-0.58	NS	249.5±22.7	191.3±21.6	- 23.3	<0.01
HDL (mg/dL)	45.5±9.7	44.2±10.4	-2.86	NS	43.8.2±11.8	45.9±12.2	+4.79	NS
LDL (mg/dL)	129.4±23.1	131.3±19.8	+1.47	NS	175.5±26.5	121.5±22.5	-30.77	<0.01
TG (mg/dL)	175.3±42.7	162.7±49.4	-7.19	NS	166.7±43.9	135.4±42.6	-18.77	<0.05
CPK (U/L)	110.5±82.7	106.3±79.9	-3.81	NS	102.5±84.4	119.2±79.3	+16.29	NS
Cr (mg/dL)	0.77±0.2	0.80±0.5	+3.90	NS	0.88±0.8	0.91±0.9	+3.41	NS
AST (U/L)	28.8±13.5	31.1±15.3	+7.99	NS	26.9±14.2	29.3±15.8	+8.92	NS
ALT (U/L)	36.9±21.6	38.1±23.5	+3.25	NS	34.2±19.6	37.4±21.5	+9.36	NS

Note: All values are expressed as median ± standard deviation.
Abbreviations: ALT, alanine transferase; AST, aspartate transferase; BMI, body mass index; CPK, creatine phosphokinase; Cr, creatine; FG, fasting glucose; HbA1c, glycated hemoglobin; HDL, high density lipoprotein; LDL, low density lipoprotein; N, number of subjects; NS, not significant; TC, total cholesterol; TG, triglycerides.

between the two groups. However, a considerable difference was observed regarding bowel discomfort: moderate constipation, meteorism, and flatulence and diarrhea, respectively, were reported by six, eight and five subjects in the BSM-treated group and by two, 10 and 22 subjects in the untreated group. Our analysis revealed good compliance in all groups and no significant drop-out (data not shown).

Discussion

We have conducted a retrospective analysis in diabetic subjects with dyslipidemia, comparing the role played by a controlled diet plus physical exercise (lifestyle intervention) with a lifestyle intervention plus a food supplement containing berberine, silymarin, and monacolins K and KA from RYR. The results of our analysis indicate the poor efficacy of the proposed lifestyle intervention in contrast to the safety and efficacy of BSM in significantly reducing the lipid profile of patients. Modest CPK global increases were observed, so our results also suggest the possibility of using BSM as add-on therapy in statin-intolerant subjects. Our CPK finding is likely due to the low dose of monacolins administered (10 mg/dose/day), so we presume that higher dosages would have likely generated a larger CPK increase. Treatment with BSM also improved the lipid profile of the statin-intolerant subjects. Although the very small number of subjects did not allow us to perform statistical analysis, our results (data not shown) demonstrate that in the BSM-treated group, BSM administered as add-on therapy to ezetimibe or fenofibrate resulted in approximately 15%, 22%, and 25% greater reductions in TC, LDL, and TG, respectively, than in the untreated group, while HDL increased by approximately 2.5% more than in the untreated group.

We suggest that there may be three pharmacological reasons for these interesting results. First, berberine up-regulates LDL-receptor (LDL-r) expression independently of sterol regulatory element-binding proteins, but is dependent on extracellular signal-regulated kinases (ERK) and c-Jun N-terminal kinase (JNK) activation, which results in a reduction in TC and LDL. This up-modulation occurs through a post-transcriptional mechanism that stabilizes the mRNA and makes berberine a cholesterol-lowering compound endowed with a mechanism of action different from that of statins (25). Second, monacolins K and KA have well-known inhibiting effects on 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase (26). Third, berberine and monacolins exert a synergist effect on PCSK9. This protein lowers LDL-r, preventing it from internalizing LDL particles. It works post-transcriptionally, downregulat-

ing the LDL-r by binding to the receptor's epidermal growth factor-like repeat A on the cell surface and shuttling the LDL-r to the lysosomes for degradation. Both chemically and naturally derived statins increase the plasma level of PCSK9. In contrast, berberine decreases PCSK9 mRNA and protein levels in a time- and dose-dependent manner. This is not due to increased degradation of PCSK9 mRNA but most likely to decreased transcription of the PCSK9 gene (19). Our analysis suggests that treatment with BSM also has a favorable effect on glycemic control as FG and HbA1c both improved, the latter significantly so. These observed effects are due to the content of berberine plus silymarin, while the lack of a significant result for FG is probably due to the low dosages used (500 mg/dose and 105 mg/dose, respectively). Indeed, other researchers have reported significant reductions in FG after administration of 1500 mg of berberine alone (27) or 1000 mg of berberine plus 210 mg of silymarin (17).

Although it was not an aim of our retrospective analysis to demonstrate that add-on therapy with BSM can reduce metformin-induced or IBS-related diarrhea, we did observe a global reduction in the number of diarrhea-affected subjects, which finding supports the results of other studies (6-8). An important aspect, that we have been not able to properly evaluate, is the insulin-saving action of berberine plus silymarin administration in patients using injectable insulin, which was previously reported by Derosa et al (28).

Our analysis has some limitations that could affect the results. It was not a prospective study with endpoints declared before the study started, supplements were not administered under blind conditions, subjects were not randomized, the overall number of participants was small, and the add-on effect of BSM to ezetimibe or fenofibrate was evaluated in only five subjects (compared to four in the control group).

Despite this, to our knowledge this analysis is the first concerning the use of a food supplement containing a highly modified and standardized RYR product in diabetic subjects with dyslipidemia. RYR is fermented rice on which red yeast (*M. purpureus*) has been grown. The extract mainly contains monacolin K (the natural equivalent of lovastatin), and its acid form, KA. While monacolin KA inhibits HMG-CoA reductase, monacolin K is a pro-drug able to reduce plasma cholesterol levels only after liver metabolism has transformed it into KA. In addition to monacolins K and KA, RYR extract also contains secondary monacolins J, JA, L, LA, X, XA, M, and MA and other degradation products, known as dehydromonacolins, produced during fermentation by the yeast. Some of the secondary monacolins have low activity, while the degradation products are thought to be inactive and likely toxic to human cells (29-31). Although MK and MKA are normally the most effective and abundant monacolins in RYR extracts, their content, as well as that of one of the other monacolins, is not standardized and can vary considerably among extracts. Due to this variability, it is difficult to evaluate the biological effects of fermented red yeast extracts (32). Although RYR products are not standardized, physicians often recommend them as an alternative treatment for hyperlipidemia even though they contain statins which, even if of natural origin, can cause adverse side effects (33-36).

The citrinin content of RYR is also of concern. Citrinin is a secondary toxic metabolite, produced during rice fermentation and known to be nephrotoxic, hepatotoxic, and possibly carcinogenic in humans and animals. The mechanism of citrinin toxicity is not fully understood, particularly whether its toxicity and genotoxicity are the consequence of oxidative stress or of the increased permeability of mitochondrial membranes. It has been also suggested that citrinin requires complex cellular biotransformation to exert its damaging effects (37-38). Although the level of citrinin is regulated and set at 2 ppm in food supplements (39), it is thought that at least 30% of *Monascus* extracts are heavily contaminated with citrinin (30). Similarly, we recently demonstrated a considerable citrinin presence in four of 32 Chinese red yeast-fermented dried extracts (22). However, BSM contains less than 50 ppb citrinin so as to avoid any toxicity, as well as only monacolins K and KA in a ratio of 1:1 and less than 0.2% combined secondary monacolins and dehydromonacolins. This means that BSM, in contrast to other food supplements clinically tested so far in diabetic subjects with dyslipidemia and claimed to contain RYR, contains only 5 mg/dose of monacolin K (chemically lovastatin) and 5 mg/dose of monacolin KA (chemically

the acid form of lovastatin), guaranteeing high standardization.

In conclusion, our retrospective analysis suggests that BSM, a product containing food-grade berberine, silymarin, and monacolins K and KA, is a safe and effective cholesterol-lowering food supplement for use (1) in diabetic subjects with dyslipidemia; (2) as add-on therapy in patients who are not severely statin intolerant; and (3) in subjects with a negative perception of statins and mild dyslipidemia, who prefer a treatment seen as natural.

Competing interests

FDP is a member of the Scientific Council of Pharmextracta, the company marketing BSM. The other authors report no conflict of interest.

References

1. Ho J, Leung AKC, Rabi D. Hypoglycemic agents in the management of type 2 diabetes mellitus. Recent Pat Endocr Metab Immune Drug Discov 2011; 5(1): 66-73.
2. El-Kaissi S, Sherbeeni S. Pharmacological management of type 2 diabetes mellitus: an update. Curr Diabetes Rev 2011; 7(6): 392-405.
3. Hoffmann IS, Roa M, Torrico F, Cubeddu LX. Ondansetron and metformin-induced gastrointestinal side effects. Am J Ther 2003; 10(6): 447-451.
4. Dong H, Zhao Y, Zhao L, Lu F. The effects of berberine on blood lipids: a systemic review and meta-analysis of randomized controlled trials. Planta Med 2013; 79: 437-446.
5. Dong H, Wang N, Zhao L, Lu F. Berberine in the treatment of type 2 diabetes mellitus: a systemic review and meta-analysis. Evid Based Complement Alternat Med 2012; 2012: 591654.
6. Chen C, Yu Z, Li Y, Fichna J, Storr M. Effects of berberine in the gastrointestinal tract - a review of actions and therapeutic implications. Am J Chin Med 2014; 42(5): 1053-1070.
7. Chen C, Tao C, Liu Z, Lu M, Pan Q, Zheng L, et al. A randomized clinical trial of berberine hydrochloride in patients with diarrhea-predominant irritable bowel syndrome. Phytother Res 2015; 29(11): 1822-1827.
8. Gan JR, Liu XL. Therapeutic efficacy of berberine in treatment of diarrhea caused by metformin hydrochloride: a report of 19 cases. Hunan J Trad Chin Med 2012; 6: 002.
9. Chen W, Miao YQ, Fan DJ, Yang SS, Lin X, Meng LK, et al. Bioavailability study of berberine and the enhancing effects of TPGS on intestinal absorption in rats. AAPS PharmSciTech 2011; 12(2): 705-711.
10. Pan GY, Wang GJ, Liu XD, Fawcett JP, Xie YY. The involvement of P-glycoprotein in berberine absorption. Pharmacol Toxicol 2002; 91(4): 193-197.
11. Shan YQ, Ren G, Wang YX, Pang J, Zhao ZY, Yao J, et al. Berberine analogue IMB-Y53 improves glucose-lowering efficacy by averting cellular efflux especially P-glycoprotein efflux. Metabolism 2013; 62(3): 446-456.
12. Zhou S, Lim LY, Chowbay B. Herbal modulation of P-glycoprotein. Drug Metab Rev 2004; 36(1): 57-104.
13. Di Pierro F, Putignano P, Villanova N, Montesi L, Moscatiello S, Marchesini G. Preliminary study about the possible glycemic clinical advantage in using a fixed combination of *Berberis aristata* and *Silybum marianum* standardized extracts versus only *Berberis aristata* in patients with type 2 diabetes. Clin Pharmacol 2013; 5: 167-174.
14. Derosa G, Bonaventura A, Bianchi L, Romano D, D'Angelo A, Fogari E, et al. *Berberis aristata/Silybum marianum* fixed combination on lipid profile and insulin secretion in dyslipidemic patients. Expert Opin Biol Ther 2013; 13(11): 1495-1506.
15. Derosa G, Bonaventura A, Bianchi L, Romano D, D'Angelo A, Fogari E, et al. Effects of *Berberis aristata/Silybum marianum* association on metabolic parameters and adipocytokines in overweight dyslipidemic patients. J Biol Regul Homeost Agents 2013; 27(3): 717-728.
16. Derosa G, Romano D, D'Angelo A, Maffioli P. *Berberis aristata* combined with *Silybum marianum* on lipid profile in patients not tolerating statins at high doses. Atherosclerosis 2015; 239(1): 87-92.
17. Di Pierro F, Villanova N, Agostini F, Marzocchi R, Soverini V, Marchesini G. Pilot study on the additive effects of berberine and oral type 2 diabetes agents for patients with suboptimal glycemic control. Diabetes Metab Syndr Obes 2012; 5: 213-217.
18. Di Pierro F, Bellone I, Rapacioli G, Putignano P. Clinical role of a fixed combination of standardized *Berberis aristata* and *Silybum marianum* extracts in diabetic and hypercholesterolemic patients intolerant to statins. Diabetes Metab Syndr Obes 2015; 8: 89-96.
19. Cameron J, Ranheim T, Kulseth MA, Leren TP, Berge KE. Berberine decreases PCSK9 expression in HepG2 cells. Atherosclerosis 2008; 201(2): 266-273.
20. Halbert SC, French B, Gordon RY, Farrar JT, Schmitz K, Morris PB, et al. Tolerability of red yeast rice (2,400 mg twice daily) versus pravastatin (20 mg twice daily) in patients with previous statin intolerance. Am J Cardiol 2010; 105(2): 198-204.
21. Cicero AF, Rovati LC, Setnikar I. Eulipidemic effects of berberine administered alone or in combination with other natural cholesterol-lowering agents. A single-blind clinical investigation. Arzneimittelforschung 2007; 57(1): 26-30.
22. Nannoni G, Alì A, Di Pierro F. Development of a new highly standardized and granulated extract from *Monascus purpureus* with a high content of monacolin K and KA and free of inactive secondary monacolins and citrinin. Nutrafoods 2015; 14(4): 1-9.

23. World Medical Association. World Medical Association Declaration of Helsinki: ethical principles for medical research involving human subjects. J Postgrad Med 2002; 48(3): 206-208.
24. 2016 ESC/EAS Guidelines for the Management of Dyslipidaemias. European Heart Journal doi:10.1093/eurheartj/ehw272
25. Kong W, Wei J, Abidi P, Lin M, Inaba S, Li C, et al. Berberine is a novel cholesterol-lowering drug working through a unique mechanism distinct from statins. Nat Med 2004; 10(12): 1344-1351.
26. Kuncl RW. Agents and mechanisms of toxic myopathy. Curr Opin Neurol 2009; 22(5): 506-15.
27. Yin J, Xing H, Ye J. Efficacy of berberine in patients with type 2 diabetes mellitus. Metabolism 2008; 57(5): 712-717.
28. Derosa G, D'Angelo A, Maffioli P. The role of a fixed *Berberis aristata/Silybum marianum* combination in the treatment of type 1 diabetes mellitus. Clin Nutr 2015 Sep 2. pii: S0261-5614(15)00225-3.
29. Huang HN, Hua YY, Bao GR, Xie LH. The quantification of monacolin K in some red yeast rice from Fujian province and the comparison of the other product. Chem Pharm Bull (Tokyo) 2006; 54(5): 687-689.
30. Gordon RY, Cooperman T, Obermeyer W, Becker DJ. Marked variability of monacolin levels in commercial red yeast rice products: buyer beware! Arch Intern Med 2010; 170(19): 1722-1727.
31. Zhu L, Yau LF, Lu JG, Zhu GY, Wang JR, Han QB, et al. Cytotoxic dehydromonacolins from red yeast rice. J Agric Food Chem 2012; 60(4): 934-939.
32. Gordon RY, Becker DJ. The role of red yeast rice for the physician. Curr Atheroscler Rep 2011; 13(1): 73-80.
33. Prasad GV, Wong T, Meliton G, Bhaloo S. Rhabdomyolysis due to red yeast rice (*Monascus purpureus*) in a renal transplant recipient. Transplantation 2002; 74(8): 1200-1201.
34. Halbert SC, French B, Gordon RY, Farrar JT, Schmitz K, Morris PB, et al. Tolerability of red yeast rice (2,400 mg twice daily) versus pravastatin (20 mg twice daily) in patients with previous statin intolerance. Am J Cardiol 2010; 105(2): 198-204.
35. Kuncl RW. Agents and mechanisms of toxic myopathy. Curr Opin Neurol 2009; 22(5): 506-515.
36. Klimek M, Wang S, Ogunkanmi A. Safety and efficacy of red yeast rice (*Monascus purpureus*) as an alternative therapy for hyperlipidemia. P T. 2009; 34(6): 313-327.
37. Lin YL, Wang TH, Lee MH, Su NW. Biologically active components and nutraceuticals in the Monascus-fermented rice: a review. Appl Microbiol Biotechnol 2008; 77(5): 965-973.
38. Flajs D, Peraica M. Toxicological properties of citrinin. Arh Hig Rada Toksikol 2009; 60(4): 457-464.
39. European Union Guideline No. 212/2014. Commission held in March 6th, 2014. Official Report (EU): L 67/3IT. L 67/3-4.

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