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Barberry Administration and Pro-Oxidant–Antioxidant Balance in Patients With Metabolic Syndrome

Akram Mohammadi [1]; Amirhossein Sahebkar [2,3]; Tayyebeh Kermani [1]; Marzieh Zhilaee [1]; Shima Tavallaie [1]; Majid Ghayour Mobarhan [4,*]

[1]Biochemistry of Nutrition Research Center, Mashhad University of Medical Sciences, Mashhad, Iran
[2]Neurogenic Inflammation Research center, Mashhad University of Medical Sciences, Mashhad, Iran
[3]Biotechnology Research center, Mashhad University of Medical Sciences, Mashhad, Iran
[4]Cardiovascular Research center, Mashhad University of Medical Sciences, Mashhad, Iran

*Corresponding Author: Majid Ghayour Mobarhan, Cardiovascular Research center, Mashhad University of Medical Sciences, Mashhad, Iran. Tel: +98-5138421190, Fax: +98-5138823251, E-mail: ghayourm@mums.ac.ir

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Background: Metabolic syndrome is the constellation of several cardiometabolic risk factors, and is associated with a heightened risk of coronary heart disease (CHD). The pro-oxidant–antioxidant balance (PAB) is a measure of factors that promote and control oxidative stress. PAB may also be associated with the risk factors of CHD.

Objectives: This study aimed to explore the impact of supplementation with barberry, a fruit rich in antioxidants, on PAB in patients with metabolic syndrome.

Patients and Methods: A total of 106 patients diagnosed with metabolic syndrome were randomized in two groups: case and control. The case group received three capsules of barberry and the control group received three capsules of placebo for 6 weeks. Serum PAB was measured in all patients before and after the intervention.

Results: There was no significant difference between the groups regarding their baseline PAB values (P = 0.32). A significant decrease in PAB was observed in the barberry group (P = 0.022), whilst there was no significant change in the control group (P = 0.18). The magnitude of change in PAB during the study was significantly greater in the case group compared to the control group (P = 0.01).

Conclusions: Barberry supplementation reduces oxidative burden in patients with metabolic syndrome.

Keywords: Metabolic Cardiovascular Syndrome; Berberidaceae; Randomized Controlled Trial

1. Background

Metabolic syndrome (MS) is a clustering of several cardiometabolic risk factors, including central obesity, hypertension, hyperglycemia, and dyslipidaemia. MS is an important health concern due to its relation with increased risk of coronary heart disease (CHD). MS is defined in various by different health organizations (1). Its pathogenesis has two potential causative factors: central obesity and insulin resistance. However, other factors such as genetic predisposition, ageing, and physical inactivity are also implicated in its development (1, 2).

Developing societies experience an increasing trend in the prevalence of MS with rising affluence and ageing of the population. Because of the high CHD death rate, effective methods are warranted to treat MS-associated disorders (3, 4). This means that identification and treatment of the MS is important for prevention and even treatment of CHD.

Oxidative stress is the consequence of increased generation of pro-oxidant species accompanied by a depletion of biological antioxidants (5). Oxidative stress is important because it plays a crucial role in the pathophysiology of several disorders, including atherosclerosis and subsequent CHD as well as MS. Moreover, there is a strong correlation between PAB and atherosclerosis (6). Previous studies have shown that PAB values may serve as an indicator of the CHD risk and other oxidative stress-associated disorders (7).

In traditional medicine, *Berberis vulgaris* (Berberidaceae family) is recommended in the treatment of heart diseases, including hypertension and arrhythmia (8). Barberry is a bush with yellow timber and obovate leaves. The plant, which grows in Asia and Europe, has yellow flowers succeeded by oblong red colored fruits. Barberry extract contains ingredients such as berberine, berbamine, palmatine, oxyacanthine, malic acid, and berberubin (9). Many studies have previously shown that *Berberis vulgaris* possesses various medicinal properties such as antipyretic, antihyperglycaemic, anti-bacterial, anticancer, antihistaminic, antimicrobial, antioxidant, anticholinergic and hypolipidaemic activities (9, 10), that support the traditional use of this plant in Ayurveda and Chinese medicine. It is reported that berberine, an isoquinoline alkaloid (present in barberry) can be used as therapeutics against a number of diseases like hyperlipidemia, diabe-

tes, metabolic syndrome, obesity, and coronary artery disease. These compounds have antioxidant effects owing to their interaction with multiple molecular targets resulting in a synergistic and comprehensive pharmacological response (9, 11, 12).

2. Objectives

The present study aimed to investigate the effect of supplementation with barberry on PAB as a surrogate marker of systemic oxidative stress in patients with metabolic syndrome.

3. Patients and Methods

3.1. Subjects

One hundred and six patients with metabolic syndrome (defined according to the International Diabetic Federation Criteria), aged 18-65 years old, and without diabetes were chosen sequentially from clinic and randomly recruited from those referring to the Nutrition Clinic of the Ghaem Hospital, Mashhad, Iran. The objectives and protocol of the study were explained to each participant prior to the study. Participants were provided with information about the study both by verbal explanation and written information sheets. Inclusion criteria were an age range of 18-65 years and having a metabolic syndrome according to IDF (International Diabetic Federation) criteria. Exclusion criteria included known systemic diseases; pregnancy; lactation; and consumption of the antidyslipidemic, antihypertensive and antidiabetic drugs. Written informed consent was obtained from all participants and the study protocol was approved by the Ethics Committee at the Mashhad University of Medical Sciences.

3.2. Study Design

The study was designed as a randomized double-blind and placebo-controlled trial. The research was conducted in the Nutrition Clinic of the Ghaem Hospital, Mashhad, Iran. Figure 1 shows the flowchart of the study design. Patients were randomly assigned to the treatment group receiving capsules of barberry (600 mg/day) for a period of 6 weeks, and control group receiving a placebo capsule for the same period. All participants were given dietary advice on the basis of the AHA (American Heart Association) guidelines during the study. Compliance was monitored during a three-weekly visit, assessing by counting capsules. Those subjects who refused to take their capsules regularly or were intolerant to the medication were excluded from the study. PAB were determined in all patients at baseline and at the end of the sixth week.

3.3. Blood Sampling

Twelve-hour fasting blood samples were collected from each subject at baseline as well as at the end of study (week 6). Hemolyzed samples were excluded from analysis. Separation of serum from blood samples was conducted by centrifugation at 10000g for 15 minutes.

3.4. Anthropometric Measurements

Anthropometric indices, including height, waist and hip circumference (cm) and weight (kg) were determined for each subject. Circumferences were measured with a plastic tape measure at the following levels: smallest waist circumference, umbilicus level, widest hip circumference, hips at the level of the anterior superior iliac spine of the iliac crest, and the highest thigh circumference.

3.5. Barberry Capsule Preparation

Barberry juice was provided in Ghaem, Khorasan Province, Iran. It was formulated as a capsule containing 200 mg of dried barberry. Barberry and placebo capsules were matched for size, shape, and color and manufactured by the same company (Khoosheh Sorkhe Shargh Agro Industrial Co., Tehran, Iran).

3.6. Pro-oxidant–antioxidant Assay

Measurement of PAB was performed using a previously described method (7). In brief, the standard solutions were prepared by mixing 250 μM hydrogen peroxide with uric acid (3 mM in 10 mM NaOH) in varying proportions (0-100%). In order to prepare TMB cation, 400 μL of TMB/DMSO solution (60 mg TMB in 10 mL DMSO) was added to 20 mL of acetate buffer (0.05M buffer, pH = 4.5), and then this solution was mixed with 70 μL of fresh chloramine T (100 mM), followed by incubation for 2 hours at room temperature in a dark place. Afterwards, 25 U of peroxidase enzyme solution was added to 20 mL of the TMB cation solution, dispensed in 1 mL and stored at -20°C. The working solution was prepared by mixing TMB cation solution (1 mL) with the TMB solution (10 mL),

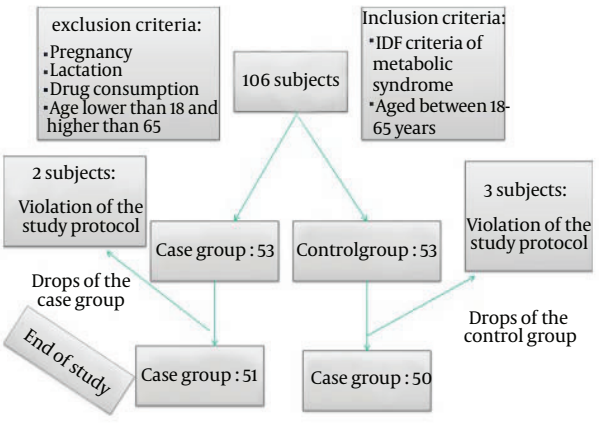

Figure 1. Flowchart of the Trial

incubated for 2 minutes at room temperature in a dark place and immediately used. Ten microliter of each sample, standard or blank (distilled water), was mixed with the working solution (200 μL), in each well of a 96 well plate, which was then incubated in a dark place at 37°C for 12 minutes. At the end of the incubation time, the colorimetric reaction was stopped by adding 100 μL of 2 M HCl to each well. Then, the optical density of plates were read at 450 nm using an ELISA reader with a reference wavelength of 620 or 570 nm. A standard curve was generated from the values relative to the standard samples. The values of the PAB were expressed in an arbitrary unit, which represents the percentage of hydrogen peroxide in the standard solution.

3.7. Statistical Analysis

All statistical analyses were performed using SPSS statistical software package. All data were presented as mean (SD) in each group. Data were assessed for normality by using the Kolmogorov-Smirnov test. Paired sample t-test and independent sample t-test were used for comparison of normally distributed data, whilst non-normally distributed data were compared using. A P value of < 0.05 was considered to be statistically significant.

4. Results

Table 1 presents the comparison of the baseline characteristics between 2 groups. They were comparable regarding their baseline characteristics, including age, gender distribution, BMI, smoking, and serum PAB levels ($P > 0.05$). Figure 2 shows the comparison of the PAB between 2 groups. PAB values were comparable between the 2 groups at baseline ($P = 0.324$). A significant reduction of PAB from baseline by the end of trial was observed in the barberry group ($P = 0.022$), whilst no significant change occurred in the control group ($P = 0.180$). The magnitude of changes in PAB levels was also significantly different between the 2 groups ($P = 0.01$; Figure 3).

Table 1. Comparison of the Baseline Characteristics Between Case and Control Groups (n = 53) [a, b, c]

Variable	Barberry Group	Control Group	P Value
Gender			0.44
Female	41	38	
Male	12	15	
Smokers	6 (11.3)	5 (9.4)	0.96
Age, y	38.96 ± 9.04	40.89 ± 9.61	0.30
BMI, kg/m^2	31.54 ± 3.92	32.37 ± 5.01	0.35

[a] Abbreviation: BMI, body mass index.
[b] Data are presented as or No. (%) or Mean ± SD.
[c] Mann-Whitney and independent sample t-test were used to compare non-parametric and parametric variables between two groups, respectively.

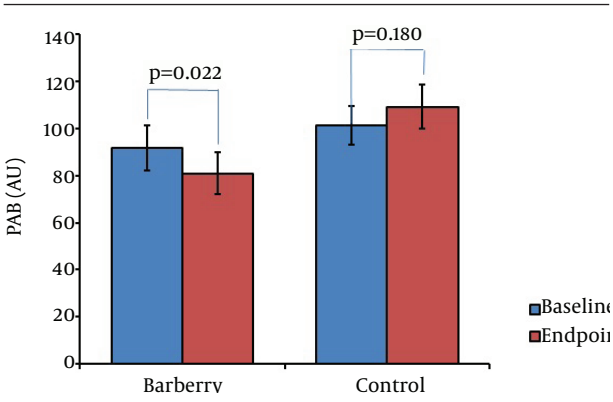

Figure 2. Comparison of Pretreatment vs. Posttreatment PAB Values in the Study Groups

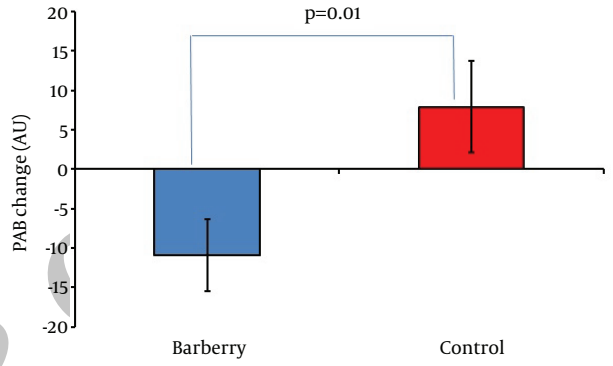

Figure 3. Comparison of PAB Changes in the Barberry and Control Group During the Course of Study

4.1. Precision and Storage

Intra-assay and inter-assay coefficients of variation (CV%) were determined for the PAB assay. The mean CV% of the intra-assay for 28 samples (analyzed in triplicate) was found to be 2.1%. The CV% of the inter-assay for 20 samples was between 4.1% and 8.5% with a mean of 6.1% (analyzed over 3 days).

5. Discussion

Results of the present study indicated that using barberry supplementation for six weeks in patients with metabolic syndrome decreased significantly PAB levels. To our knowledge, the present study is the first one to investigate the effect of barberry on PAB in patients with metabolic syndrome. Compelling evidence supports the antioxidant functions of barberry. Previous studies have shown that berberine prevents the ROS formation in several cell types like macrophages. Berberine has also the ability to chelate Fe^{2+} (13, 14). Moreover, berberine effectively scavenges different classes of free radicals, in particular O^{2-} and OH• (15). It also regulates the activity of colonic antioxidant enzymes, including glutathione

S-transferase, catalase, SOD, and glutathione peroxides in rats exposed to azoxymethane (16). Surveswaran et al. has reported that roots of *Berberis aristata* contain 3.59 mmol/100 g antioxidants and fruits of *Berberis vulgaris* contain a significantly higher amount of antioxidants compared with other fruits (17).

Berberine hydrochloride has large reductive ability and can scavenge different radicals, especially ABTS radicals, hydroxyl radicals, DPPH (2, 2-diphenyl 1-picrylhydrazyl) radicals, and superoxide anion (18). The antioxidant content of *Berberis vulgaris* (Crni Lug origin) fruit has been determined by Zovko Koncic et al. using DPPH assay. The results indicated that the antioxidant contents of the roots, branches, and leaves were 1292.86 μg/mL, 691.57 μg/mL, and 65.09 μg/mL, respectively (19). PAB values have been previously shown to be elevated in patients with angiographically documented CAD and ACS (7). Alamdari et al. indicated that PAB may be a potential cardiovascular risk predictor (7). A number of trials have also demonstrated that PAB values may serve as an indicator of oxidative stress (20-23).

Ji-Yin Zhou et al. (2011) investigated protective effect of berberine on antioxidant enzymes in diabetic rat liver. Berberine reduced malondialdehyde concentrations and enhanced superoxide dismutase, catalase, glutathione peroxidase, and glutathione activities in liver tissue and serum of diabetic rats (24). Zhou has used purified berberine, which its content is higher compared to the berberine in barberry of our study. This study was conducted on animals but ours on MS patients. In a clinical trial, Cheng et al. (25) have shown that berberine improves endothelial function in humans via inhibiting endothelial microparticles-mediated oxidative stress in vascular endothelium. In their study, 12 healthy subjects consumed a 1-month berberine therapy and 11 healthy subjects were selected as control. Serum malondialdehyde (MDA) and circulating endothelial microparticles were measured before and after therapy. Circulating CD^{31+}/CD^{42-} MPs and the levels of serum MDA are significantly decreased in the berberine group compared to the control group. Berberine also caused a remarkable decline in MDA levels suggesting the efficacy of this phytochemical in reducing lipid peroxidation (25).

In one study, berberine was shown to ameliorate renal injury in streptozotocin-induced diabetic rats by attenuating oxidative stress and down-regulating the activity of aldose reductase. In the aforementioned study, berberine was orally administered at a dose of 200 mg/kg/day and was shown to significantly increase serum SOD activity and decrease the content of MDA compared to diabetic model group ($P < 0.05$) (26). This study also used purified berberine. The present trial had a number of limitations. For example, the trial recruited a relatively small number of patients and its results should be considered as pilot. In addition, although PAB is an overall oxidative stress biomarker, it would be useful to measure other measures of oxidative stress to confirm the pattern of PAB changes found in this study.

Overall, although PAB assay still needs to be further explored in terms of sensitivity and specificity for the assessment of oxidative burden in diseases, the current body of evidence clearly indicates that PAB increases in oxidative stress-related disorders and antioxidant can effectively reduce this measure of oxidation (21-30). PAB values are also known to be significantly and linearly associated with the concentration of pro-oxidant and antioxidant species, and correlate with standard carbonyl assay, advanced glycation end products assay and advanced oxidative protein products assay as standard measures for oxidative stress.

The findings of the present study indicated that supplementation with barberry (600 mg/day for 6 weeks) is associated with the suppression of systemic oxidative stress (as assessed by PAB). Current available data suggests that modulation of systemic oxidative stress is a plausible mechanism that can explain, at least in part, the well-known cardioprotective effects of barberry. Nevertheless, future large-scale trials with a longer duration of follow-up are warranted to confirm the present findings, and also compare the antioxidant effects of barberry with those of standard compounds e.g. α-tocopherol, ascorbic acid, and N-acetyl cysteine. Moreover, the present findings need to be validated by measuring additional standard pro-oxidant/antioxidant biomarkers including F2-isoprostanes, MDA and oxidized low-density lipoprotein.

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Authors' Contributions

Majid Ghayour Mobarhan and Tayyebeh Kermani developed the original idea; Majid Ghayour Mobarhan and Marzieh Zhilaee conducted the clinical evaluations; Akram Mohammadi and Shima Tavallaie did the experimental work; Amirhossein Sahebkar and Akram Mohammadi interpreted the data and performed statistical analysis; Akram Mohammadi and Amirhossein Sahebkar prepared and submitted the manuscript.

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