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# Barberry Treatment Reduces Serum Anti-Heat Shock Protein 27 and 60 Antibody Titres and High-sensitivity C-reactive Protein in Patients with Metabolic Syndrome: A Double-blind, Randomized Placebo-controlled Trial

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**Metabolic syndrome is an important risk factor for cardiovascular disease (CVD). The heat shock proteins (HSPs) are associated with risk factors for CVD. The aim of the present study was to survey the effect of barberry on antibody titres to HSPs and high-sensitivity C-reactive protein (hs-CRP) in patients with metabolic syndrome. In our study, subjects ($N = 106$, 79 women and 27 men, 18–65 years old) with metabolic syndrome were randomized into two groups: a group of patients who received three capsules of barberry and a control group who received three capsules of placebo for 6 weeks. Antibodies against HSPs 27, 60/65 and 70, hs-CRP and lipid profile were determined in patients before (week 0) and after (week 6) intervention. SPSS software (version 16.0; Inc, Chicago, IL) was used for data analysis. Results showed that barberry had no significant effect on serum level of anti-HSPs 65 and 70. But there was a significant decrease in anti-HSP 27 in both case and control groups ($p = 0.001$ and $p < 0.001$, respectively, in the case and control groups). Barberry decreased significantly anti-HSP 60 in the case group ($p = 0.03$). High-sensitivity CRP was decreased non-significantly ($p = 0.17$) in the case group and increased significantly ($p = 0.04$) in the control group. Barberry decreased significantly low-density lipoprotein and total cholesterol and increased significantly high-density cholesterol ($p < 0.05$). Results of the present study suggested that barberry supplementation in patients with metabolic syndrome decreased significantly anti-HSPs 27 and 60 and hs-CRP levels and improved lipid profile.**


*Keywords:* metabolic syndrome; anti-heat shock proteins; barberry.

## INTRODUCTION

Metabolic syndrome is a condition in which there are several cardiovascular risk factors that include central obesity, diabetes, obesity, dyslipidaemia and hypertension. It has many definitions according to different organizations (Cameron *et al*., 2004; Grundy, 2008), and studies show that people with the metabolic syndrome are at twice at risk of developing atherosclerotic cardiovascular disease (CVD) compared with those without the syndrome (Nesto, 2000; Gale, 2008). Metabolic syndrome has a high prevalence in developed countries. The high death rate from CVD highlights the essential need for effective methods to treat the many disorders classified as CVDs (Gale, 2008; Grundy, 2008). Thus, diagnosis and treatment of metabolic syndrome is important for prevention and even treatment of CVD. It is reported that the levels of several anti-heat shock protein (HSP) antibodies are predictors of risk of atherosclerosis (Xu, 2002). There is a strong relationship between HSP expression and the manifestations of atherosclerosis. Studies have shown an increase in HSP synthesis in atheromatous-plaque-rich regions (Berberian *et al*., 1990; Xu, 2002).

In traditional medicine, *Berberis vulgaris* has been used for the treatment of various heart diseases including hypertension and arrhythmia. Several studies have indicated the beneficial cardiovascular effects of berberine (active constituent of *B. vulgaris*) including preventing ischemia-induced ventricular tachyarrhythmia, improving cardiac contractility and lowering peripheral vascular resistance and blood pressure (Imanshahidi and Hosseinzadeh, 2008). Many studies have previously shown that many medical plants can decrease the signs of metabolic syndrome, including dyslipidaemia and hypertension. One of these medical plants is barberry (*B. vulgaris*). Berberine is an isoquinoline alkaloid found in an array of plants and has been used in Indian and Chinese medicines as antimicrobial, stomachic, and bitter tonic and in the treatment of oriental sores. Although pharmacological investigations of berberine have been reported by many in the past

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(Kulkarni and Dhir, 2010), Ghayour-Mobarhan (2005) has shown that several anti-HSPs as anti-HSPs 60 and 65 in subjects with dyslipidaemia are associated with metabolic syndrome (Ghayour-Mobarhan *et al.*, 2005). Because dyslipidaemia and hypertension are stimulators of the production of HSPs, and also because some studies have shown that barberry can improve lipid profile and hypertension, barberry can influence the level of anti-HSPs (Doggrell, 2005; Kermanshahi, 2006).

Farhadi and Gavadifar (2008) reported that barberry supplementation in a dose of 200 mg dried extract of barberry in patients with dyslipidaemia had an effect on triglyceride and total cholesterol; it decreased significantly these parameters (Farhadi and Gavadifar, 2008).

Anti-HSPs are novel risk factors of CVD, and metabolic syndrome also is a potent predictor of CVD; therefore, we measured the anti-HSP antibodies in patients with metabolic syndrome and surveyed the effect of barberry on these antibodies.

With regard to the significance of CVD, in the present study, we investigated the effect of barberry on anti-HSP antibodies, as the novel risk factors of CVD, high-sensitivity C-reactive protein (hs-CRP) and lipid profile in patients with metabolic syndrome.

## MATERIAL AND METHODS

**Study design.** This was a 6-week randomized, double-blind, placebo-controlled clinical trial. The investigation was conducted in the nutrition clinic of Qhaem Hospital, Mashhad, Iran. Flowchart of the study design is in Fig. 1. A total of 106 subjects with metabolic syndrome (defined by the International Diabetes Federation criteria, 2005), aged 18–65 years, with and without diabetes, who visited the nutrition clinic of Qhaem Hospital were randomly recruited. Participants were provided with information about the study by both verbal explanation and written information sheets. Inclusion criteria were an age of 18–65 years and criteria of metabolic syndrome according to the International Diabetic Federation criteria. Exclusion criteria included known systemic diseases, pregnant and lactating women and consumption of the lipid-lowering, antihypertensive and antidiabetic drugs. All patients provided written informed consent, and the protocol satisfied the Mashhad University of Medical Sciences Ethics Committee requirements.

The patients were randomly divided into two groups with 53 persons in each group by using a computer-generated code: the case group, receiving capsules of barberry 600 mg/day, and the control group, receiving a capsule of placebo. For a 6-week period, all participants were given dietary advice based on the American Heart Association guidelines. Compliance was monitored during a three-weekly visit, assessing compliance by counting capsules; subjects who did not take their capsules regularly or were intolerant to the medication were excluded from the study. Lipid profile and anti-HSPs 27, 60/65 and 70 and hs-CRP were determined in all patients at baseline and the end of study (after 6 weeks). Blood samples were collected in the morning after a 12-h fasting from each subject. Haemolysed samples were excluded from analysis. After separation, aliquots of serum were frozen at −80 °C until analysis. All patients were interviewed to collect information on their socio-demographic status, occupation, smoking behaviour, medical history and medication.

We used enzyme-linked immunosorbent assay (Stat Fax 2100; Awareness Technology, Inc. P. O. Box 1679, Palm City FL 34991, USA) for measuring anti-HSP antibodies, which is a validated method for measuring anti-HSP antibodies.

**Barberry capsule preparation.** Barberry juice was taken from Ghaen, Khorasan, Iran. It was formulated as a capsule containing 200 mg of dried barberry. Placebo capsules were matched for size, shape and volume of content and manufactured by the same company. The barberry capsules used in this study contained lactose,

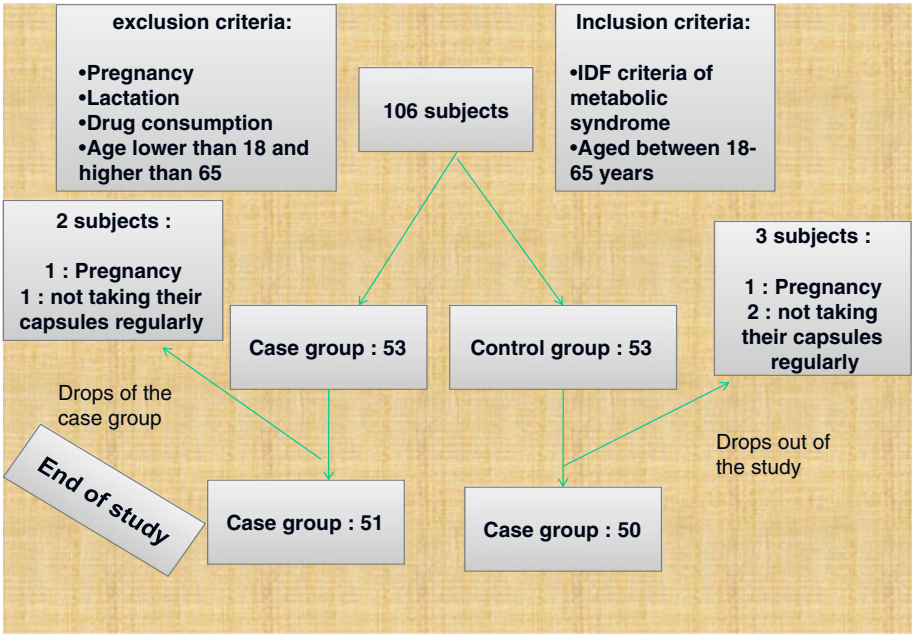


**Figure 1.** Flowchart of the study design. This figure is available in colour online at wileyonlinelibrary.com/journal/ptr.

starch and the aqueous and ethanol extract of barberry, and the placebo capsules contained lactose, starch and permitted food colour.

**Statistical analysis.** All statistical analyses were performed using SPSS software (IBM Corporation, Armonk, NY, USA). All data were presented as mean ± SD and median and interquartile range in each group. Data were assessed for normality by using the Klomogrov–Smirnov test. Paired sample *T*-test and independent sample *T*-test for normally distributed data and Wilcoxon and Mann–Whitney tests for non-normally distributed data were used for data analysis. A $p$-value $< 0.05$ was considered as statistically significant.

## RESULTS

### Baseline characteristics of case and control groups

Table 1 shows the comparison of the baseline characteristics between groups. Data showed that there were no significant differences between the two groups with regard to baseline characteristics including age, gender distribution, body mass index, smoking, serum anti-HSPs and hs-CRP levels ($p > 0.05$, Table 1). Also, the serum levels of lipid profile had no significant difference between the two groups at baseline ($p > 0.05$)

### Effect of barberry on anti-HSP antibodies, hs-CRP and lipid profile

Table 2 shows the comparison of the anti-HSP antibodies, hs-CRP and lipid profile levels between groups. Anti-HSP 60 levels decreased significantly in the case (barberry) group ($p = 0.03$), but in the control group, this reduction was not significant ($p = 0.15$), and the results showed that there was no significant difference between the two groups with regard to anti-HSP 60 level ($p = 0.32$). With regard to anti-HSPs 65 and 70, there were no significant differences between before and after levels in both case and control groups and also between the two groups. With regard to anti-HSP 27, our results showed that there was a significant decrease in both case and control groups ($p = 0.001$ and $p < 0.001$, respectively), and also, there was a significant difference between the two groups ($p = 0.02$). With regard to hs-CRP, the results showed that in the case group, there was a non-significant decrease in its concentration during the study ($p = 0.17$) and there was a significant increase in the control group ($p = 0.04$); also, there was a significant difference between the two groups ($p = 0.04$). With regard to lipid profile, barberry decreased significantly low-density lipoprotein and total cholesterol and increased significantly high-density lipoprotein cholesterol ($p = 0.04$, 0.03 and 0.03, respectively), but there was a significant difference between groups only on total cholesterol ($p = 0.04$, Table 2).

## DISCUSSION

The results of the present study indicated that barberry supplementation for 6 weeks in patients with metabolic syndrome did not have any significant effect on anti-HSPs 65 and 70, but it decreased significantly anti-HSPs 27 and 60 and hs-CRP levels and also improved lipid profile. To our knowledge, there is no study on the effect of barberry on anti-HSP antibodies in human in worldwide level.

### Effect of barberry on anti-HSP antibodies, hs-CRP and lipid profile

Marinova *et al.* (2000) showed that berberine has an immunosuppressive effect in the tubulointerstitial nephritis model. In this study, results showed that berberine caused a decrease in the number of lymphocytes (Marinova *et al.*, 2000). In another study, berberine

**Table 1. Comparison of the baseline characteristics between case and control groups**

| | Case | Control | $p$-value |
|---|---|---|---|
| Number | 53 | 53 | — |
| Gender | | | |
| Female (*N*) | 41 | 38 | 0.44 |
| Male (*N*) | 12 | 15 | |
| Smokers | | | |
| (%) | 11.3 | 9.4 | 0.96 |
| Number | 6 | 5 | |
| Age (years) | 38.96 ± 9.04 (mean ± SD) | 40.89 ± 9.61 (mean ± SD) | 0.30 |
| BMI (kg/m$^2$) | 31.54 ± 3.92 (mean ± SD) | 32.37 ± 5.01 (mean ± SD) | 0.35 |
| Median anti-HSP 27 titre (IQR) | 0.47 (0.39–0.60) | 0.54 (0.37–0.61) | 0.55 |
| Median anti-HSP 60 titre (IQR) | 0.34 (0.28–0.40) | 0.32 (0.27–0.40) | 0.70 |
| Median anti-HSP 65 titre (IQR) | 0.47 (0.40–0.54) | 0.45 (0.39–0.54) | 0.38 |
| Median anti-HSP 70 titre (IQR) | 0.19 (0.14–0.54) | 0.19 (0.14–0.54) | 0.26 |
| Median hs-CRP concentration (IQR) | 3.66 (2.10–7.04) | 3.01 (2.32–5.17) | 0.77 |

BMI, body mass index; anti-HSP, anti-heat shock protein; IQR, interquartile range; CRP, C-reactive protein.

Values are expressed as mean ± SD, median ± IQR or number and percent. Mann–Whitney and independent sample *T*-test were used to compare non-parametric and parametric variables between the two groups, respectively. Data showed that there was no significant difference between the two groups with regard to baseline characteristics. None of our subjects had diabetes or consumed other prescription drugs.

**Table 2. Effect of barberry on anti-HSP antibodies and hs-CRP**

| | Groups | | | | | | |
|---|---|---|---|---|---|---|---|
| | Case | | | Control | | | |
| Parameters | Week 0 | Week 6 | *p*-value (within-group comparison) | Week 0 | Week 6 | *p*-value (within-group comparison) | Mann–Whitney test |
| Median anti-HSP 60 titre (IQR) | 0.34 (0.28–0.40) | 0.30 (0.18–0.34) | 0.03 | 0.32 (0.27–0.40) | 0.31 (0.20–0.40) | 0.15 | $p = 0.32$ |
| Median anti-HSP 65 titre (IQR) | 0.47 (0.40–0.54) | 0.42 (0.33–0.57) | 0.46 | 0.45 (0.39–0.54) | 0.44 (0.30–0.53) | 0.44 | $p = 0.07$ |
| Median anti-HSP 70 titre (IQR) | 0.19 (0.14–0.54) | 0.18 (0.13–0.26) | 0.88 | 0.19 (0.14–0.54) | 0.19 (0.14–0.54) | 0.29 | $p = 0.38$ |
| Median anti-HSP 27 titre (IQR) | 0.47 (0.39–0.60) | 0.36 (0.30–0.50) | 0.001 | 0.54 (0.37–0.61) | 0.45 (0.35–0.57) | $<0.001$ | $p = 0.02$ |
| Median hs-CRP concentration (IQR) | 3.66 (2.10–7.04) | 3.45 (2.04–5.36) | 0.17 | 3.01 (2.32–5.17) | 3.86 (1.91–8.01) | 0.04 | 0.04 |
| LDL-C (mean ± SD) | 114.41 ± 34.42 | 99.82 ± 32.98 | 0.04 | 118.38 ± 48.64 | 123.90 ± 37.34 | 0.47 | 0.38 |
| Total-C (mean ± SD) | 201.14 ± 46.14 | 175.25 ± 43.32 | 0.3 | 201.58 ± 49.16 | 2000.98 ± 45.42 | 0.62 | 0.04 |
| HDL-C (mean ± SD) | 49.23 ± 15.79 | 56.15 ± 8.87 | 0.3 | 47.42 ± 9.61 | 45.58 ± 9.30 | 0.15 | 0.40 |
| TG (mean ± SD) | 144.88 ± 83.73 | 145.45 ± 107.33 | 0.16 | 160.16 ± 99.33 | 178.72 ± 13.45 | 0.26 | 0.45 |

HSP, heat shock protein; CRP, C-reactive protein; LDL, low-density lipoprotein; HDL, high-density lipoprotein; TG, triglyceride; IQR, interquartile range.
Values are expressed as median ± IQR with regard to anti-HSPs and hs-CRP and mean ± SD with regard to lipid profile. Mann–Whitney and Wilcoxon tests were used to compare the anti-HSPs and hs-CRP between groups and before and after intervention in each group, respectively. Data showed that barberry significantly decreased anti-HSP 27 and 60 and hs-CRP concentrations increased significantly in the control group and also decreased anti-HSP 60 during intervention. Paired *T*-test and independent sample *T*-test were used to compare the lipid profile before and after study and between groups, respectively. Data showed that barberry decreased significantly LDL-C and total-C and increased significantly HDL-C and there was a significant difference between the two groups with regard to total-C.

was used in the immune modulation of adjuvant-induced arthritis in mice (Ivanovska and Philipov, 1996).

Our results showed that barberry supplementation decreased significantly anti-HSPs 27 and 60 antibodies, with no significant effect on anti-HSPs 65 and 70 levels. Part of this effect on anti-HSP may be due to the anti-immune-modulatory effect of barberry.

Kermanshahi (2006) surveyed the effect of dietary dried *B. vulgaris* fruit on some blood parameters of laying hens. They showed that barberry supplementation decreased significantly low-density lipoprotein cholesterol and increased significantly high-density lipoprotein cholesterol (Kermanshahi, 2010).

In conclusion, our results indicated that barberry supplementation decreased the anti-HSPs 27, 60/65 and 70, although only the reduction in anti-HSPs 27 and 60 were significant. There is need to study the effect of barberry on anti-HSP antibodies to answer the following question: why barberry had no significant effect on anti-HSPs 65 and 70 levels, despite a significant reduction on anti-HSPs 27 and 60 in patients with metabolic syndrome?

We suggested that it is better to determine the effect of barberry on the expression of the gen of anti-HSPs and hs-CRP in future works, instead of determining the serum level of these parameters (as a limitation in our study). But in a logical sense, it is better to do this pilot study first on serum levels to make sure that there is no technical error in the in-house enzyme-linked immunosorbent assay and after, determine the effect of barberry on the expression of the gen of anti-HSPs and hs-CRP.

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## Conflict of Interest

The authors have declared that there is no conflict of interest.

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