A pilot study of the effects of crocin on high-density lipoprotein cholesterol uptake capacity in patients with metabolic syndrome: a randomized clinical trial


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A Pilot Study of the Effects of Crocin on HDL Cholesterol Uptake Capacity in Patients with Metabolic Syndrome: A Randomized Clinical Trial

Authors

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The study protocol has been approved by Mashhad University of Medical Sciences (ID 960443).

**Acknowledgment**

The authors would like to thank the volunteers contributed in current study. We also thank to Mashhad University of Medical Sciences for financial supports. Dr. Maryam Saberi-Karimian is a post-doctoral fellow of Mashhad University of Medical Sciences (MUMS), which her fellowship being granted by MUMS, Iran.
Abstract

Trial design: A Randomized Clinical Trial

Introduction: HDL cholesterol uptake capacity (CUC) is reduced in patients with metabolic syndrome (MetS). We have assessed the effect of crocin supplementation on HDL CUC in patients with MetS.

Methods: 44 subjects with MetS were randomly allocated to one of two groups: one group received placebo and the other group received crocin at a dose of 30 mg (2 tablets of 15 mg per day) for 8 weeks. Serum biochemical parameters were measured using an AutoAnalyzer BT3000 (BioTechnica, Italy). The modified CUC method is a cell free, simple, and high-throughput assay that used to evaluate HDL CUC of serum samples. The decision tree analysis was undertaken using JMP Pro (SAS) version 13.

Results: The mean age of the crocin and placebo groups were 38.97±13.33 and 43.46±12.77 years, respectively. There was a significant increase in serum HDL CUC in the crocin group compared to the placebo group in patients with MetS (P-value<0.05). The decision tree analysis showed that serum HDL functionality was more important variable than HDL-C level in predicting patients with hypertension at baseline(P-value<0.05).

Conclusion: Crocin administration (30 mg for a period of 8 weeks) was found to improve serum HDL CUC in patients with MetS.

Keywords: Metabolic Syndrome; Crocin; HDL-C; HDL functionality

Trial registration: IRCT2013080514279N1
**Introduction**

The metabolic syndrome (MetS) is known also as syndrome X, Reaven syndrome, or insulin resistance syndrome (1). It has been reported that 20% of the adults have MetS in Western countries (2). The prevalence of MetS in the Middle East is reported to be 20.7-37.2% in males and 32.1-42.7% in females (3). A recent meta-analysis showed that the prevalence of MetS in adults in Iran was 30.4%, was more frequent in women than in men, and varied with age, and location (4).

A low-grade inflammation, an increased risk of thrombosis, and raised oxidative stress are associated with MetS (1).

Saffron or crocus Sativus L. is a plant from the Iridacea botanical family. Saffron has been used from ancient times for different purposes: as a dyestuff, and a food ingredient, and as a herbal drug with different effects. The stigma of the flower contains crocin that is a carotenoid pigment (5). It has been shown that saffron and crocin have anti-oxidative and anti-tumor properties (6); they have also been reported to have effects on learning behaviors and memory (7); and neuro-protective properties (8), and to have anti-depressant effects (9). Finally they have been reported to have some lipid lowering properties (10); reducing the level of serum triglyceride, cholesterol and low density lipoproteins, and raising the level of high density lipoproteins in animal models (11).

HDL is a lipoprotein with a wide range of different functions which are not related to HDL cholesterol (HDL-C) concentration (2). HDL is a heterogeneous particle varying in composition, size, and structure, which can be changed under various conditions including inflammation, senescence and certain diseases (13). Routine HDL-C level measurement provides some information around the HDL particle’s cholesterol content, but it cannot accurately assess HDL function. A simple assay is therefore required to assess HDL function for clinical applications (14). HDL function includes its ability to promote cholesterol efflux from macrophages, anti-inflammation, anti-oxidative and anti-thrombotic effects. It has been shown that in patients who suffer from MetS this effect of cholesterol efflux is impaired (15, 16).

Data mining is a process in the field of computer science (17), that can be applied to clinical diagnostics (18). In this study we aimed to investigate the impact of crocin on HDL functionality in patients with Metabolic Syndrome. We have applied the decision tree algorithm to determine whether HDL-C level or HDL functionality is better in predicting the characteristic components of MetS.

**Methods**
**Trial design**
This study was approved by the Ethics Committee of the Mashhad University of Medical Sciences (ID number: 960443). It was a parallel, double-blind, placebo-controlled trial. All methods were carried out in accordance with Consolidated Standards of Reporting Trials 2010 guidelines and regulations (19). **The subjects were enrolled between the April and June 2014.**

**Participants**
The participants included 44 subjects with metabolic syndrome (MetS), who were reviewed in the Nutrition Clinic, Ghaem Hospital. Inclusion criteria were: 1- 18 to 70 years old, 2- Having Metabolic Syndrome (MetS) based on the National Cholesterol Education Program (NCEP) Adult Treatment Panel III (ATPIII) guideline: waist circumference > 102 cm in males and > 88 cm in females, impaired glucose tolerance and insulin resistance [fasting blood glucose (FBG)> 100 mg/dl], dyslipidaemia with augmented serum triglycerides (TG) > 150 mg/dl, and decreased serum high density lipoprotein cholesterol (HDL-C) < 40 mg/dl in males and HDL-C < 50 mg/dl in females), and a high blood pressure (> 130/85 mmHg), 3- If the patient was taking drug, he/she should maintain a steady dose and not change their treatment during the study.

Exclusion criteria were: pregnancy, breast feeding, suffering from systemic diseases with (for instance asthma, immunodeficiency syndrome, gout, rheumatoid arthritis and insulin treatment).

All subjects were provided with the same dietary advice based on the American Heart Association (AHA) guidelines. In addition, at baseline, dietary intakes were recorded and evaluated by a semi-quantitative FFQ.

**Interventions**
The control group taking a placebo, and 2-crocin group receiving 30 mg crocin (2 tablets of 15 mg per day) for a period of 8 weeks (Figure 1). Crocus sativus L. stigma was purchased from Novin Saffron Co. (Mashhad, Iran). The extraction process has been explained in details previously. The purity of the crocin crystals was determined using UV-visible spectrophotometery and HPLC compared to Fluka product and methanolic extract of saffron stigmas. Its purity was around 13 times higher than Fluka product (20). Crocin tablets were produced according to the method described by Nikbakht-Jam et al. (21); they contained 30±0.8 mg per tablet. The placebo tablets contained starch, and were matched with the crocin tablets in size, shape and color.

Compliance was determined using counting capsules every 2 weeks. If subjects did not have taken their tablets regularly up to 90% were omitted from the study.
Outcome measurements

Blood Sampling: 12-hour fast blood samples of every subject were taken into plain plastic tubes before and after the intervention. The serum was separated using centrifugation at 10,000 g for 15 min and then, aliquots of serum were frozen at -80°C.

HDL functionality: We used the cholesterol uptake capacity (CUC) method (22), a cell-free, simple, and high-throughput assay to evaluate one aspect of serum HDL functionality. The inter-assay and intra-assay CV were 13.07% and 6.65%, respectively. There are three major steps in this method. Firstly, for preparing the HDL fraction from human serum samples, apo B-containing lipoproteins were removed using polyethylene glycol (22). Secondly, the HDL fraction or apoB-depleted serum with fluorescent labelled (BODIPY) cholesterol were incubated. Thirdly, this mixture serum was added to microplate that coated by a monoclonal antibody against apolipoprotein A1 (ApoA1) to capture HDL. The amount of BODIPY-cholesterol uptake by HDL was measured followed by several washing. A parameter for CUC value was defined CUC %; percent of BODIPY-cholesterol uptake by apoB depleted sample that was normalized using HDL-C concentrations (Aghasizadeh et al., DOI: 10.1002/jcla.23770).

Biochemical factors: FBG, HDL-C, LDL-C, TG and cholesterol concentrations were measured in the Nutritional Science and Technology Group Laboratory. All biochemical factors were determined using an Auto Analyzer BT3000 (Bio Technica, Italy), (6).

Sample size determination

Regarding this was a pilot study, the power was calculated by 88% after data analysis using the follow below formula considering n=22, α=0.05, and changes in serum HDL functionality (0.19±0.30 a.u. and 0.10±0.40 a.u. in crocin and placebo groups, respectively):

\[
n = \frac{(z_{1-\frac{\alpha}{2}}^2 + z_{\frac{\alpha}{2}}^2)(s_1^2 + s_2^2)}{(\bar{x}_1 - \bar{x}_2)^2}
\]

Randomization

Subjects were allocated into one of two groups using random number tables so that, the subjects who entered the trial, chose a numbered sealed envelope containing the randomized allocation to the intervention or control group. The statistics generated the random allocation sequence, an employee was outside the research team blinded the drugs, care providers enrolled participants, and project’s physician assigned participants to interventions. All the subjects, care providers, physician and statistics were blinded on the interventions.

Statistical analysis
The normality of the data was assessed using the Kolmogorov–Smirnov test. Descriptive statistics were performed for all variables, including mean and standard deviation (mean ± SD) for normally distributed data and median and interquartile range (IQR) for non-normally distributed data. The Mann-Whitney and T student tests were used for non-normally and normally distributed data, respectively. General linear model and 95% intervals were used to estimate the association of Serum HDL-functionality and Crocin. Statistical analysis was analyzed using SPSS version 18.0 (SPSS, Chicago, IL). In addition, the decision tree method was employed using JMP Pro (SAS) version 13. The statistical analysis was intention-to-treat (ITT) basis. A P-value < 0.05 was considered significant.

**Ethical considerations**

This study was a sub-study of our previous project approved by the Ethics Committee of the Mashhad University of Medical Sciences (ID number: 960443; No. IRCT2013080514279N1; Date: 01.02.2015). Informed consent form was signed after explanation the study to all participants.
Results

A total of 44 subjects were categorized into 2 groups; crocin (n=22) and placebo (n=22) with a mean age of 38.97±13.33 and 43.46±2.77 years, respectively. Among these, 58.6% and 55.2% of women were included in the crocin and placebo groups respectively (Table 1). A subject in the crocin group was lost to follow-up because of potential allergic symptoms. There were no significant differences in baseline characteristics between crocin and placebo groups (p>0.05). Moreover, dietary intakes did not show any significant difference between 2 study groups at baseline (data was not shown). Accordingly, there was no significant difference in the clinical features from both groups at the end of the study (Table 2).

As summarized in Table 3, crocin was found to increase serum HDL CUC in subjects with MetS. The general linear model showed there was a significant difference in serum HDL-functionality between crocin and placebo groups (P-value<0.05), before adjusting for confounding factors (model 1). In addition, this result was statistically significant after adjusting for confounding factors such as age and sex (P-value<0.05, model 2).

The final decision tree with 2 layers is shown in Figure 2. This decision tree contained 2 input variables; serum HDL-C level and serum HDL functionality before the intervention. Serum HDL functionality was the first variable caused a break in the tree in patients with hypertension (P-value<0.05). Subjects with HDL functionality >0.89% did not have hypertension.

Discussion

This is the first clinical trial that assessed the effects of crocin on HDL functionality in patients with MetS. The results showed that crocin supplementation (30 mg for 8 weeks) increased the serum HDL CUC in patients with MetS. It was not associated with any significant adverse effect on these individuals.

Kermani et al. have evaluated the efficacy of crocin (100 mg/day crocin tablets for 6 weeks) on 48 patients with MetS. They have reported that this dose of crocin is well tolerated and has no side effect for a period of 6 weeks oral administration (23). We have previously reported that crocin at a dose of 30 mg/day for 8 weeks can significantly decrease serum prooxidant-antioxidant balance in individuals with MetS (24). While, we have shown that this dose of crocin did not improve the serum fasting blood glucose, serum lipids profile and cholesteryl ester transfer protein level in these subjects (25).

Oxidative stress is a main component of endothelial dysfunction (26), therefore can increase the risk of MetS(27). HDL dysfunction plays a key role in increasing systemic oxidative stress in MetS (28). It is well documented that small-dense HDL particles are
inactivated in subjects with MetS, and cannot efficiently exert their antioxidative activity (29). The impaired antioxidative activity is related to increased triglyceride and insulin levels, in that way antioxidative dysfunction of HDL particles in MetS can be prompted by abnormalities in both lipid and glucose metabolism (30). The antioxidative activity of small-dense HDL particles’ was reduced and plasma 8-isoprostanes elevated in subjects with MetS showing normal lipid levels, representing that these impairments are closely associated with a collection of several risk factors typical of MetS (30).

Cholesterol efflux capacity (CEC) is another method to determine the HDL functionality which is a cell based technique (31), whereas, CUC is a cell-free and simpler method. It has been shown that CUC relates to efflux capacity. The efficiency of HDL-induced cholesterol efflux from macrophages depends chiefly on the capacity of HDL to uptake cholesterol (22). Serum HDL CEC is known as a better and independent predictor of CVD risk than HDL-C. Though, it remains unclear whether therapies that increase CEC are cardioprotective or not (32). The CEC is the HDL’s ability to promote cholesterol efflux from cultured macrophages with samples derived from serum (33). It has been shown that CEC is reduced in patients with MetS, diabetes mellitus, chronic kidney disease and autoimmune disorders (34). It has also been shown that male gender and current smoking are related to decreased CEC (35, 36). As well, factors such as duration of diabetes mellitus and degree of proteinuria and insulin resistance can effect on associations with CEC (37).

In the current study, the results of decision tree analysis showed that subjects with HDL functionality >0.89% did not have hypertension. It is possible that a high HDL functionality protects the endothelium from dysfunction that needs to be evaluated in future studies. Previous studies had reported the associated risk factors with hypertension (38, 39). Chang et al. have mentioned the associated risk factors of hypertension including systolic/diastolic blood pressure, triglyceride, glutamate pyturate transaminase, creatinine, age and uric acid (38). Ture et al. have shown that age, gender, family history of hypertension, lipoprotein(a), triglyceride (TG), uric acid, total cholesterol (TC), body mass index (BMI) and smoking habits were reliable indicators for predicting the hypertension (39). We also have previously reported that age, BMI and gender were the most important associated factors with hypertension using a decision-tree algorithm (40). It is well documented that hypertension is related to altered endothelium-dependent relaxation (41). Moreover, the endothelial cells have receptors to import the ApoA1 and HDLs particles that mediate intracellular signaling (42). Further studies are needed to determine the molecular mechanisms of the protective effects of high HDL functionality on endothelium.

**Study limitations**
The effects of different doses of crocin should be evaluated in future studies to assess its dose-dependent effects. Other study limitations were the small sample size to make a decision tree, the lack of complete matching, as well as the ages whilst not significant are quite different. In addition, the physical activity level was not evaluated in this study.

**Conclusions**
The crocin administration (30 mg for a period of 8 weeks) improved the serum HDL functionality in patients with MetS, as well, no side effects were observed.
Other information

Registration: IRCT2013080514279N1

Protocol: The full trial protocol can be accessed in [https://en.irct.ir/trial/13904](https://en.irct.ir/trial/13904), as well, a detailed version of CUC measurement protocol will be available in our new accepted manuscript in JCLA (Aghasizadeh et al., DOI: 10.1002/jcla.23770).

Funding:

The study has been supported financially by Mashhad University of Medical Sciences (ID 960443).
References

# CONSORT 2010 checklist of information to include when reporting a randomised trial*

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