Effects of bread with *Nigella sativa* on hematologic factors in metabolic syndrome patients

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Summary. *Background: Nigella sativa (N. sativa)* has been used in traditional medicine and many studies have been performed in different communities in order to reveal the effects of it on medical disorders and chronic diseases. *Purpose:* The aim of this study was the investigation of effects of bread with *N. sativa* on hematologic factors in metabolic syndrome (MetS) patients. *Material and methods:* A randomized, double-blind, cross-over, clinical trial was conducted in 51 MetS patients of both sexes with age group of 20-65 years old in Chalus, north of Iran. Patients randomly divided in two groups. In phase 1, Intervention group (A, n =27) received daily a regular bread with *N. sativa* and wheat bran and control group (B, n=24) received the same bread without *N. sativa* for 2 month. After 2 weeks wash out period, phase 2 was started with replacement of two groups. Measuring of red blood cells (RBC), white blood cells (WBC), prothrombin time(Pt), hematocrite (Hct) and hemoglobin (Hb) was performed for patients in before and after of two phases. *Results:* In this study evaluated treatment, sequence and time effects of crossover intervention and revealed that consumption of bread with *N. sativa* has not significant treatment and sequence effects on RBC, WBC, PT, Hct and Hb (p>0.05). Time effect was only significant on RBC (P<0.05) and was not on other factors (p>0.05). *Conclusion:* Consumption of bread with *N. sativa* has not significant effect on hematologic factors in MetS patients.

Key words: hematocrite, hemoglobin, metabolic syndrome, *Nigella sativa*, prothrombin time

Introduction

The metabolic syndrome (MetS) is a cluster of metabolic risk factors that can lead to chronic diseases (1). Although the history of metabolic syndrome is back to many years ago, but the modern era of that have started with description by Reaven in 1980 (2). The National Cholesterol Education Panel-Adult Treatment Panel III (NCEP-ATPIII) define MetS by the presence of obesity, dyslipidemia, elevation of arterial blood pressure and glucose intolerance (3).

This syndrome can increase the risk of cardiovascular disease(CVD), diabetes mellitus, stroke and even cancers (4-6). Based on these reports, prevalence of MetS is variable in worldwide and Iranian community (7, 8) so is relatively high in different area of Iran (more than 25%) (9, 10). The etiology of MetS has not been revealed clearly, however, it is proposed that genetic, metabolic and environmental factors play important role in this (11, 12).

Although, the complementary therapies were existed many years ago around the world but the standard herbal medicine has started in recent decade and is considered as new approach to preventing and curing of diseases such as metabolic syndrome, diabetes mellitus and CVD (13). Nowadays there is much more

attention to use of plants as therapeutics, because of lower adverse effects. *Nigella sativa* (*N. sativa)* or black seed that belongs to family of Ranunculaceae has been used to improve health and cure diseases for centuries especially in the Middle East and southeast of Asia (14). A great focus is done on several traditional uses and therapeutic properties of *N. sativa* (15). The therapeutic properties of *N. sativa* is attributed to several component including proteins, amino acids, carbohydrates, fibers, oils (combination of fatty acids especially polyunsaturated fatty acids), volatile oil (frequently thymoquinone), mineral, alkaloids, flavonoids, saponins and etc (16).

Effects of supplementation of *N. sativa* is evaluated on clinical indices in several studies. But, there is few studies in case of effects on hematologic factors and most of those have done on animals model. In a study on animal model revealed that *N. sativa* could decreased count of leukocytes and platelets and increased hematocrit and hemoglobin levels significantly (17). Effects of *N. sativa* in another study on animal model showed as increasing in count of platelets and decreasing of white blood cells (WBC) while effects on red blood cells (RBC), hematocrite (Hct) and hemoglobin (Hb) were not significant (18) .

It is reported that *N. sativa* has inhibitory effect on arachidonic acid (AA)-induced platelet aggregation and blood coagulation in animal model (19).

Because, there is no human study in case of hematologic effects of *N. sativa* and the pharmacological form of *N. sativa* supplementation were existed in all of the studies, we decided to assess the effects of *N. sativa* as added to bread (as a regular food) on hematologic factors in metabolic syndrome patients.

Material and methods

Study design

A cross-over, double-blind, randomized clinical trial was conducted in 54 metabolic syndrome patients of both sexes with age group of 20-65 years old in Chalus, north of Iran. Our project is approved by medical ethics committee of Isfahan University of Medical Sciences. This clinical trial has been registered in Iranian Registry of Clinical Trials with registration number IRCT2015041821815N1. Subjects collected according to the data from several laboratories and general recalling. Our criteria for diagnosis of subjects according to the data from several laboratories and general recalling. Our criteria for diagnosis of subjects with metabolic syndrome was the definition of NCEP-ATPIII.

Inclusion criteria
1) Having at least 3 item of 5 item metabolic syndrome based on mentioned criteria:
   Waist circumference (W.C) >102 cm in male and >88 cm in female
   Blood pressure (B.P) ≥130/85 mmHg or medication therapy
   Fasting blood sugar (FBS) ≥100 mg/dl or medication therapy
   Serum triglyceride (TG) ≥150 mg/dl or medication therapy
   High density lipoprotein (HDL) <40 mg/dl for male and <50 mg/dl for female
2) Both sexes with age group 20-65 years old
3) Not to have chronic diseases(in liver, kidney, muscular and nervous system or others)
4) Not to have pregnancy and lactation (in women)
5) Not to have irregular diet

Exclusion criteria
1) Changing in medication or supplement consumption in study procedure
2) Pregnancy or lactating in study procedure
3) Having chronic diseases in study procedure
4) Not to adherence study protocol
5) Not to like to continue study procedure

Study procedure
After selection of subjects who had essential parameters to be included in the survey, a total of 54 persons with metabolic syndrome entered into the study and referred to School of Medical Science, Azad University of Chalus as performing center of study.

All of patients were informed about aims of survey and have accepted a letter of satisfaction from each theme for participating in the study.

Characteristics of subjects include age, gender, level of physical activity, telephone, address and other

information such as current diseases, medication and history of chronic diseases in family were recorded in a questionnaire.

For evaluating of unusual dietary intake, we assessed three dietary food records of subjects (a weekend and two week days). Patients who have been consumed medicinal plants or their energy intake had been below 1200 kcal or above 4000 kcal were excluded from the study.

It is served a sample of balanced diet to each of patients based on food guide pyramid and according to nutritional requirements in order to follow a standard diet. Evaluation of Daily energy requirement were based on formula suggested by the Institute of Medicine, Food and Nutrition Board (23). Also, it is devised to subjects not to change level of physical activity during the study.

Preparation of breads

The *N. sativa* (black seed) were purchased from local market. After several grinding and screening of seeds, the powder of black seed is delivered to a local bakery which was expert in producing of dietetic breads. It is considered two type of 100 gram massive bread for this study: a bread which include 3 gram powder of black seed and 3 gram wheat bran (for intervention group) and the other includes only 3 gram wheat bran and free of black seed (for control group). These breads were prepared by standard bakery materials and methods in hygiene situation and had been under control of researchers. After cooking of breads, those were became gradually cooled and put in package. Nutrient composition of two breads analyzed in food laboratory of School of Nutrition & Food Science, Isfahan University of Medical Sciences and summarized in Table 1. According to this table, there is a main difference in fat content of two bread that is due to high polyunsaturated fatty acids in *N. sativa*.

Randomization and Crossing over

54 patients who include in this study are divided by regular randomize method in two groups (group A, n=27 and group B, n=27).

According to cross-over study, in phase 1 one group (for example A) will be as intervention group and the other (B) as control group that after a washout

Table 1. Comparison of approximate nutrient composition of two breads

Nutrient (%)	Bread with *N. sativa*	Bread without *N. sativa*
Moisture	38.7	41.3
Ash	1.2	0.8
Carbohydrate	48.0	47.5
Protein	6.6	7.3
Fat	2.6	1.0
Fiber	2.9	2.1

period, in phase 2 the replacement of groups will be done.

Therefore in phase 1, group A received daily a100 grams bread with *N. sativa* and group B received the same bread without *N. sativa* for 2 month. Delivery of breads for patients was performed weekly. After 2 month, wash out period was done for 2 weeks. Then, phase 2 is started with replacement of groups as group B could receive daily bread with *N. sativa* and group A could receive bread without that for 2 month.

Double-blinding

For performing of double-blind status in this study, a third person was recruited who informed about study groups and type of breads. Delivery of breads to patients was done by this person so researchers and subjects were not aware about type of breads. Although, two type of breads have almost the same shape.

Assessment of variables in study

Before the starting of phase 1, all of patients referred to laboratory for measuring of hematologic factors. Then, in the end of phase 1, beginning and end of phase 2 these assessments were continued.

Measuring of hematologic factors

For measuring of hematologic factors, the subjects were requested to go to laboratory. Samples of subjects were collected from venous blood and prepared for doing tests.

It is used a cell counter for measuring of RBC and WBC.

Hemoglobin determination performed by an automated cell counter from a tube of well-mixed EDTA-anticoagulated blood.

For measuring of hematocrit it is used tube containing anticoagulant and a microhematocrit centrifuge for 3 to 5 minutes at high speed. The hematocrit is a ratio of the packed cells to total volume.

Measuring of prothrombin time is done by quick test and based on torbidometry manner.

Statistical analysis

The statistical analysis was performed with using of software SPSS (version 22). It is assessed the comparison of effect of two types of breads (treatment effect), grouping of subjects (sequence effect) and before and after of intervention (time effect) on related parameters in this crossover study. The general linear model was used for evaluating of this effects. A p value of <0.05 was considered as significant.

Results

Of 54 metabolic syndrome patients who included in this study, three subjects exclude (2 men and 1 woman) in regards to the change of medication and therapy process (< 6% drop out). Therefore 51 patients completed the study. Biographic and clinical characteristics of patients summarized in Table 2. Number

Table 2. Biographic and clinical characteristics of participants

Variable	N
Number	51
Sex	
Male	30 (58.8)*
Female	21 (41.2)*
Age (year)	47±5.66**
BMI (Kg/m²)***	29.9±3.77**
History of Dyslipidemia (%)	62.7
History of Diabetes (%)	43.1
History of CVD (%)	41.2

*n (%)
**Parameters are expressed as Mean ± SD
*** Body mass index

of men and women were 30 and 21. Mean age was 47.5±5.7 years old. Mean BMI was 29.9±3.8 Kg/m². Besides, family history of diseases in patients represented in this table.

After dividing the patients in two groups (as crossover study), effects of treatment (two types bread), sequence (grouping of subjects) and time (before and after of intervention) of study were assessed. Mean and standard deviation (SD) of hematologic factors were mentioned based on type of the treatment (A or B), grouping of patients (AB or BA) and time of measuring of parameters (Pre or Post of intervention) (Table 3).

The results showed that consumption of bread with *N. sativa* has not significant treatment effect (as the most important effect) and sequence effect on red blood cells (RBC), white blood cells (WBC), prothrombin time (Pt), hematocrite (Hct) and hemoglobin (Hb) (p>0.05). Time effect was significant on RBC (P<0.05), marginal effect on Pt (p= 0.06) and was not significant on other factors (p>0.05) (Table 4).

Discussion

The aim of this study was to assess effects of bread with *N. sativa* on hematologic factors in metabolic syndrome patients in framework of crossover study. As it is mentioned above, these effects of study were not significant on most of these parameters. On the other hands, consumption of bread with *N. sativa* (in comparison of bread without that) does not have a significant effect on these parameters (treatment effect). There was no significant effect between grouping of patients (to be include in intervention and control group first or last) and this effect of study was not significant for all of parameters between two groups (sequence effect). Also, there was no significant effect between mean of changes of indices in beginning and end of study (time effect) except for RBC. These results were accordance to results of some of studies and were opposite with others which assessed effects of supplementation of *N. sativa* on these parameters. In study of Enamoto on rabbits revealed that the methanol soluble portion of *N. sativa* oil has inhibitory effects on arachidonic acid-induced platelet aggregation and blood coagulation (19).

Table 3. Descriptive statistics of hematologic factors in treatments, groups and times of study

Effects of study Treatment	Sequence (Group)	Time	RBC Mean±SD	WBC Mean±SD	pt Mean±SD	Hct Mean±SD	Hb Mean±SD
A	AB	PRE	492.78±48.9	62.74±13.3	12.89±1.3	41.56±3.2	13.85±1.0
		POST	463.15±54.0	63.37±13.6	12.15±0.8	40.78±2.6	13.58±0.8
	BA	PRE	451.46±42.6	72.08±22.7	11.38±0.7	40.75±3.4	13.58±1.1
		POST	444.38±42.6	68.21±14.8	11.75±0.8	41.25±3.0	13.75±1.0
B	AB	PRE	469.63±61.2	67.30±13.7	11.67±0.7	41.74±3.1	13.92±1.0
		POST	456.11±41.0	67.44±16.1	11.78 ±0.8	41.04±2.9	13.70±0.9
	BA	PRE	478.33±66.7	65.46±17.0	12.71±0.8	41.12±3.1	13.74±1.0
		POST	466.46±58.8	69.00±15.0	11.96±0.9	41.46±2.9	13.85±0.9

A: Bread with N. sativa   B: Bread without N. sativa
AB: The group who consumed firstly bread with N. sativa and after bread without it
BA: The group who consumed firstly bread without N. sativa and after bread with it
PRE: The beginning of phase   POST: The end of phase

Table 4. Effects of treatment, sequence and time of study on hematologic factors

Parameter	Treatment			Sequence			Time		
	Mean square	F	P value	Mean square	F	P value	Mean square	F	P value
RBC	1119.487	.403	**.526**	5350.506	1.926	.167	12252.330	4.410	.037
WBC	24.838	.097	**.755**	613.562	2.405	.123	.627	.002	.960
PT	.008	.009	**.924**	1.512	1.662	.199	3.206	3.524	.062
HCT	3.355	.356	**.551**	.885	.094	.760	1.334	.142	.707
HB	.638	.611	**.436**	.053	.050	.823	.150	.144	.705

Zaoui in his study on rats reported that *N. sativa* could decreased count of leukocytes and platelets and increased hematocrit and hemoglobin levels significantly (17). In study of Asgary on rabbits is showed that using of *N. sativa* significantly increased platelet count and decreased WBC counts but did not have any effects on other hematologic factors including RBC, Hct, Hb (18). Haq in his study as in vitro in human revealed that *N. sativa* has stimulatory effect on activity of macrophages (20) .

As it is mentioned before, there is few studies about effects of *N. sativa* on hematologic factors and all of them were in animal models (or in vitro) and in form non nutritional. Because, there is no human study in this field and results in regards to animal models is not comparable to human overall, conclusion in this is not easy.

Several reasons could propose for no effect of bread with *N. sativa* on hematologic factors:
   * Effect of cooking on quality and characteristic of *N. sativa*
   * Not consumption of breads totally by patients
   * Irregular consumption of breads by patients
   * Different characteristic of patients in study (21)
Nevertheless, we could not show significant effect of bread with *N. sativa* on hematologic factors in our study. Significant time effect of this bread on RBC may induced from spending of time in study or interaction of drug in patients or other factors.

Conclusion

We can conclude from this crossover study that in spite of significant time effect of bread with *N. sa-*

tiva on RBC, treatment (as main effect) and sequence effect of this study did not change significantly hematologic factors in metabolic syndrome patients.

As this study is one of the most newest trial in the field of food form of *N. sativa* supplementation, it is proposed that the performance of the other studies in this field and on other food have revealed clearly the therapeutic effects of this useful seed.

Authors Contribution

AM contributed in the preparation of the work and conducting the study, BM contributed in the analyzing of results and counseling for statistical approach, LA contributed in the revising of the draft and counseling for trial and MHE (as Corresponding author) contributed in the revising of the draft, agreed for all aspects of the work and approval of the final version of manuscript.

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