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Original article

The "Mohazell" herbal formula in combination with a calorie-restricted diet can improve systemic inflammation in obesity: a randomized double-blind, clinical trial

Eynollah Valizadeh[1], *Hossein Akbari*[2], *Faezeh Ghalichi*[3], *Alireza Ostadrahimi*[1]

[1]Nutrition Research Center, Faculty of Nutrition and Food Sciences, Tabriz University of Medical Sciences (TUOMS), Tabriz, Iran - E-mail: ostadrahimi@tbzmed.ac.ir; [2]Social Determinants of Health (SDH) Research Center, Kashan University of Medical Sciences, Kashan, Iran; [3]PhD candidate of Nutrition Sciences, Faculty of Nutrition and Food Sciences, Tabriz University of Medical Sciences (TUOMS), Tabriz, Iran

Summary. Inflammation is one of the primary mechanisms involved in the development of metabolic complications. The aim of the present study was to determine the effects of "Mohazell", a traditional herbal formula consisting of Origanum vulgare, Carumcarvi, Trachyspermum copticum and Ruta Graveolen in combination with a calorie-restricted diet on biomarkers of systemic inflammation in obese adults. In this double-blind placebo-controlled randomized clinical trial, 68 volunteer obese (Body mass index: 30–35 kg/m2) subjects aged 25–50 years were recruited. Participants were randomly divided into two groups, an intervention group (n=34) and a placebo group (n=34). Each group received either: (1) a low-calorie diet with 3 g/day of 'Mohazell' or (2) a low-calorie diet with 3 g/day placebo for 8 weeks. Patients weight was measured, their BMI was calculated and biochemical parameters such as high Sensitivity C-reactive protein (hs-CRP), tumor necrosis factor-alpha (TNF-α) and IL-6 were measured at baseline and after the intervention. No side effects were reported with the 'Mohazell' supplementation. 'Mohazell' decreased serum levels of TNF-α (p=0.001) and hs-CRP (p=0.04) in the treatment group. Also, IL-6 decreased insignificantly in both groups (p=0.78). Additionally, significant reductions were observed for weight, BMI, Energy and macronutrients (p<0.05). There were statistically significant differences for weight (0.023), BMI (0.046) and TNF-α (0.001) in between group analysis. The 'Mohazell' supplementation combined with a calorie-restricted diet may modulate systemic inflammatory biomarkers in obese adults. However, more studies are needed to clarify the efficacy of 'Mohazell' as an adjunct therapy to improve inflammatory parameters in obese subjects.

Key words: herbal medicine, obesity, caloric restriction, inflammation mediators, Mohazell

Introduction

The use of natural products for medicinal purposes has been documented for hundreds of years. The main reason for this popularity is the belief that most herbal medicines, due to their "natural" origin, are harmless and do not dispose side-effects. As a result, there is great interest in the use of plant based medicinal agents as alternative therapies(1). Herbs can play many safe and effective roles in obesity. According to Louise Tenney "herbs help the body adjust as well as supply vitamins and minerals. This combination acts as a general body cleanser, regulates metabolism, dissolves fat in the body, helps eliminate craving for food, stimulates glandular secretions, reduces water retention, boosts energy and helps in constipation"(2).

Obesity induces an inflammation state that is associated with several clinical complications, including

insulin resistance, diabetes, atherosclerosis and non-alcoholic fatty liver disease. Although the cause and the molecular participants of this process are not completely defined, adipose tissue has a central role in obesity (3). White adipose tissue is a primary site of chronic inflammation in obesity and is characterized by expression of pro-inflammatory cytokines interleukin-6 [IL-6], tumor necrosis factor-alpha [TNF-α], and high-sensitivity C-reactive protein [hs-CRP] and infiltration of a variety of immune cells, including macrophages, T lymphocytes, B lymphocytes, natural killer cells, and neutrophils which results in localized and systemic inflammation (4, 5) . TNF-a, is a cytokine that relays information from fat to brain and is increased in the adipose tissue of insulin-resistant obese individuals (4).

Losing weight can modulate the inflammatory markers and prevent obesity-related complications(6). However, owing to difficulties in adhering to dietary recommendations for weight-loss, obese subjects often turn to anti-obesity medications and supplements(7). More recently, there has been an increased tendency to use medicinal plants to assist weight loss(8). Previous studies have provided evidence on the anti-obesity properties of some medicinal herbs such as green tea (9), pepper(10), Garcinia Extract(11), and Ni-Gella sativa(12). In this study, we used an herbal formulation consisting of four traditional plants including Origanum vulgare, Carumcarvi, Trachyspermum copticum and Ruta Graveolen. These traditional herbal plants have indicated many health improvement effects including antioxidant(13), anti-diabetic(14), anti-inflammatory (15) and anti-hyperlipidemic (16) effects in previous studies. Since regulation of glucose utilization(17), lipid mobilization(18) and inflammation mechanisms(19) can prevent obesity and weight gain, we designed a randomized double-blind, placebo-controlled clinical trial to evaluate the effect of these four herbal mixture supplement as a formula called "Mohazell" in combination with a calorie-restricted diet among obese adults.

Material and method

Participants

In this double-blind placebo-controlled randomized clinical trial, obese volunteers (n=68) referred to the Sheykholrais Medical Clinic of Tabriz, Iran, from April through July 2014 were recruited by simple random sampling. Afterwards, they were divided into two groups (n=34) based on age, sex, and body mass index (BMI). Inclusion criteria were as follows: subjects between the ages of 25–50 years with a BMI between 30-35 kg/m2, presence of visceral obesity assessed by waist circumference >88 cm in women and >102 cm in men. and those who were able to refer three times in the next two months for taking drugs and laboratory examination. Subjects were excluded if they had a history of cardiovascular, renal, hepatic, or pancreatic diseases, diabetes, recent, active or debilitating infectious diseases, or using antibiotics a month before the intervention. Also, if they were following a weight-loss diet or had been taking any anti-obesity medications in the previous 6 months, smoking, being pregnant or lactating, taking herbal drugs, medicinal herbs, alcohol, antioxidant supplements, aspirin, vitamin E, or any other anticoagulant medications. At the beginning of the trial, information was collected via comprehensive interviews on the general characteristics of the subjects, including age, family history of obesity, disease history, and current medications. Additionally, blood sample was obtained at the beginning and end of the trial for assessing hs-CRP, TNF-α and IL-6.

The primary outcome of the present study was the effect of traditional herbal formula with a low-calorie diet on serum levels of inflammatory parameters (IL-6, TNF-α, and hs-CRP) in obese subjects.

Ethical consideration

This trial was conducted according to the guidelines established in the Declaration of Helsinki and approved by the Ethics Committee of Tabriz University of Medical Sciences. Informed written consent was obtained from the participants and was registered on the Iranian registry of clinical trials (www.irct.ir/, IRCT201307272017N17).

Study Design

In this 8-week double-blind and randomized placebo-controlled clinical trial, 68 volunteer subjects were randomly divided into treatment group (n=34) and placebo group (n=34). Based on a study by Mansour et al (20) , by assuming a 90% power, 95%confidence

interval and considering a 20% dropout rate throughout study, 34 subjects were allocated in each group in order to have sufficient power to detect a significant interaction between groups. The treated group received 'Mohazell' supplement that each capsule contained 1g powdered dry plants consisting of Origanumvulgare, Carumcarvi, Trachyspermumcopticum, Ruta Graveolen and the placebo group received an identical appearance capsule containing 1g corn starch. "Mohazell" prepared from booali daroo company. Participants were blinded in regard to the type of capsules received. Three capsules a day (3 gram/day) were taken in both groups. For assessing dietary intake, a 24-hour dietary recall questionnaire was used over two weekdays and one weekend day at baseline and end of intervention. The nutrient composition program, Nutritionist4 was used to analyze macro- and micronutrient intakes. Daily caloric requirements were calculated by using the Mifflin equation. Total energy expenditure (TEE) were calculated with regard to physical activity and finally a reduction of 500 kcal/day were applied for all of estimated caloric requirements. The diet contained 55%, 15% and 30% of macronutrients including carbohydrate, protein and fat respectively(21). The weight loss diet was implied for all the subjects who participated in the study. For those who have agreed to participate in this study, educational sessions were implemented to reduce errors throughout the study and participants were given an informed consent form and were instructed to read it. Probable side effects were evaluated during the intervention.

In addition, patients were weighed on a balance scales with 0.1-kg precision (Seca, Birmingham, UK) and height were measured to the nearest 0.1 cm using a stadiometer and BMI was calculated.

Blood sample and laboratory procedures

Blood for laboratory tests was drawn after 10-12 h overnight fasting of participants at baseline and the last visit (week eight). After centrifugation at 3000 rpm at 4°C for 10 minutes, the serum samples were stored in aliquots at -80°C. hs-CRP was measured by Immunoturbidimetry using Biosystems kites (manufactured in Spain).

TNF-α and IL-6 were measured by enzyme-linked immunosorbent assay (ELISA) technique and Orgenium biochemical kites (Finland).

Statistical Analysis

Data were analyzed by the SPSS version 20 software. Descriptive analysis was used for measuring frequency, mean and standard deviation. Quantitative data are expressed as mean±standard deviation and quantitative data are expressed as number and percent. Normality of the measures was controlled by the Kolmogorov–Smirnov test. Independent t test was used for comparison of the differences between groups. Comparison of before and after intervention was performed using paired t-test and Wilcoxon test. Also, Analysis of covariance (ANCOVA) was used for assessing variables mean changes. P values less than 0.05 were considered to be statistically significant.

Results

The flowchart of study design is presented in Figure 1. Of the 92 subjects that enrolled the study, 68 subjects were selected based on the inclusion and exclusion criteria. Three participants in the treatment group and one individual in placebo group were lost to follow-up during the study and were therefore not included in the statistical analysis. Baseline characteristics of subjects enrolled in the study are sum-

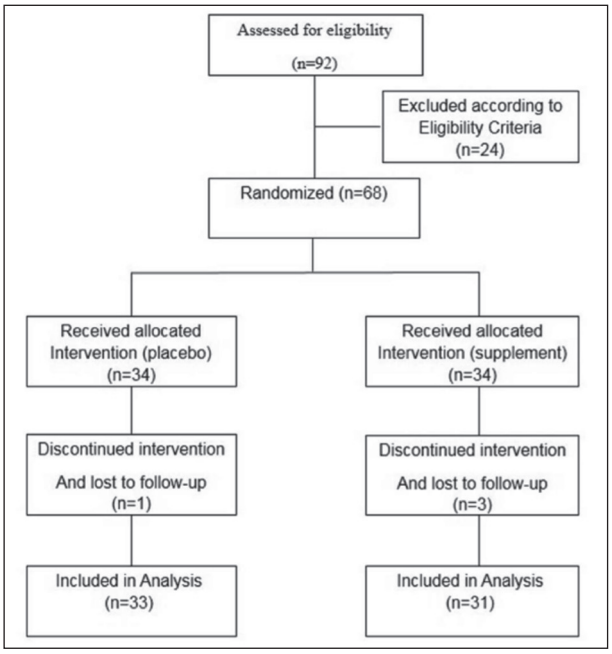

Figure 1. Flowchart of study design

marized in Table 1. Overall, the treatment group (n = 31) and placebo (n = 33) were similar respect to sex, age, level of education, marital status, body weight and BMI at baseline. In addition, there were no significant differences in dietary intake at baseline. According to table 2, participants weight and BMI declined after the intervention in both groups (p<0.001). Also, there were statistically significant differences for weight (p=0.023) and BMI (p=0.046) in between group comparison.

Inflammatory parameters before and after the intervention are listed in Table 3. As can be seen, there was a statistically significant decline for hs-CRP in the treatment group (p=0.04) and a statistically significant decrease for TNF-α in both groups before and after the intervention (p=0.001). However, IL-6 decreased insignificantly in both the treatment group (p=0.78) and control group (p=0.18). There was only statistically significant difference for TNF-α (p=0.001) in between group analysis.

The secondary outcome was the effect of traditional herbal formula with a low-calorie diet on energy, macronutrient, and fiber intake in obese subjects. According to table 4, Based on the 3-day food diaries, there was a significant reduction for energy and macronutrients in both groups (p>0.05).

Table 1. Demographic data and baseline characteristics of the study subjects

Variable	Intervention	Control	P-value
Age(y)(m±SD)	38.77	39.33	0.632
Weight(kg)(m±SD)	101.2±16.9	100.5±9.6	0.525
BMI (Kg/m2)(m±SD)	39.9±9.6	39.8±5.3	0.247
Gender, N(%)			0.866
Females	21(67.7)	23(69.7)	
Males	10(32.3)	10(30.3)	
Education, N(%)			0.949
Less than diploma	11(35.5)	13(39.4)	
Diploma	12(38.7)	12(36.4)	
BS or upper	8(25.8)	8(24.2)	
Marital, N(%)			0.777
Single	13(41.9)	15(45.5)	
Married	18(58.1)	18(54.5)	

Descriptive statistics

Table 2. Comparing participant weigh and BMI before and after the intervention

variable	Treatment group		Control group		p-value**
	Before mean ±SD	After mean ±SD	Before mean ±SD	After mean ±SD	
Weight (kg)	101.2±16.9	96.1±16.96	100.5±9.6	97.6±9.5	0/023
p-value*	<0/001		<0/001		
BMI (Kg/m2)	39.9±9.6	36±9.9	39.8±5.3	38.3±4.45	0/046
p-value	<0/001		<0/001		

***P-value assessed by Paired t-test*
**P-value assessed by Independent t-test*

Table 3. Comparing inflammatory markers before and after the intervention

variable	Treatment group		p-value*	Control group		p-value*
	Before mean ±SD	After mean ±SD		Before mean ±SD	After mean ±SD	
hs-CRP (mg/l)	4.04±5.88	2.79±3.28		7.40±5.41	6.04±3.72	0.921
P-value**	0.04			0.15		
TNF-α(pg/ml)	51.25±22.64	23.82±9.68		28.95±22.46	20.35±17.24	0.001
P-value**	0.001			0.001		
IL-6 (pg/ml)	18.24±17.20	11.14±10.45		17.84±20.70	13.92±9.18	0.293
P-value**	0.78			0.18		

***P-value assessed by Paired t-test*
P-value ANCOVA test after intervention*

Table 4. Comparing participant's energy and macronutrient consumption before and after the intervention

variable	Treatment group		p-value*	Control group		p-value*
	Before mean ±SD	After mean ±SD		Before mean ±SD	After mean ±SD	
Energy (Kcal/day)	2870±226	105±2218	04/0	219±2503	202 ± 2000	03/0
Carbohydrates (g/day)	394±31	282±17	0/03	393±33	321±35	02/0
Energy consumed from Carbohydrates (%)	55±1	51±1	0/21	57±1	59±1	0/95
Protein (g/day)	105±7	89±8	0/02	101±7	86±7	0/04
Energy consumed from Protein (%)	14±9/0	16±7/0	0/16	14±1	15±1	0/22
Fat (g/day)	94±9	79±6	<0/001	86±8	70±7	<0/001
Energy consumed from Fat (%)	29±1	32±1	0/51	28±1	26±1	0/45

**P-value assessed by Paired t-test*

Adverse events

The supplement was generally well tolerated and no remarkable adverse events were reported in the treated group.

Discussion

In the present study, consumption of 3 g/day 'Mohazell' concurrent with a calorie-restricted diet decreased body weight and serum levels of TNF-α and hs-CRP in the study subjects after 8 weeks. In addition, an insignificant decline was observed for IL-6 in both groups. There was only statistically significant difference for TNF-α (p=0.001) in between group analysis. Thus, it seems that traditional herbal formula may help manage weight and inflammatory status in obese individuals. To the best of our knowledge, no clinical trials have evaluated the effects of a 'Mohazell' supplement consisting of Origanumvulgare, Carumcarvi, Trachyspermumcopticum, Ruta Graveolen with calorie restriction on inflammatory parameters in obese subjects, and clinical trials on the anti-inflammatory effects of traditional herbal medicines are limited (22).

In the present study, a weight-loss diet was recommended for all the participants, and thus, a reduction in body weight and BMI was observed in both groups. Additionally, based on the 3-day food diaries, there was a significant reduction for energy and macronutrients in both groups (p>0.05). In obesity, hypoxia disturbs the balance between pro-inflammatory and anti-inflammatory activities in adipose tissue, and activates nuclear factor-kappa B (NF-kB) in adipocytes and macrophages. Losing weight may decrease the level of adiponectin, an anti-inflammatory and anti-atherosclerotic hormone, which can modulate the inflammation profile(23). In the present study, hs-CRP decreased significantly in the treatment group and TNF- α significantly decreased in both groups without any decline in the IL-6 status.

In Ocaña-Fuentes and colleagues study, the anti-inflammatory effects of oregano (Origanum vulgare) was assessed. The results showed a decrease in pro-inflammatory TNF- α and IL-6 cytokines synthesis, as well as an increase in the production of anti-inflammatory cytokine IL-10 (16). In another study, the Anti-inflammatory effect of quinoline alkaloid skimmianine (SKM) isolated from *Ruta graveolens* L was assessed. A 5.0 mg/kg body weight dose of SKM resulted in a decrease in the mRNA levels of TNF-α and IL-6(24).

Differences in prescribed dosages of 'Mohazell', baseline inflammatory parameters, disease background, duration of intervention, dietary intake, adipose tissue distribution, lifestyle and physical activity may lead to differences in obtained results. Moreover, in the previous studies, the participants did not receive a low-calorie diet in the previous studies, and only the effects of supplementation were evaluated.

The present study had few limitations as follows: (1) the intervention was limited to 8 weeks, and its ef-

fect over longer periods of time is not clear; and (2) the pure effect of 'Mohazell' (without a low-calorie diet) was not evaluated. The strengths of this study were that double-blinded and biochemical parameters were adjusted for some of the known confounding factors. Also, the combination of 4 herbs in one single formula can increase the efficiency of this herbal medicine. In this field further studies are recommended (1) using a cross-over design to determine the efficacy of the 'Mohazell' against a placebo; (2) evaluating the effect of 'Mohazell' in different dosages and forms (extract, oil, and powder) with and without a low-calorie diet.

To Conclude, 'Mohazell' in combination with a calorie-restricted diet is more efficient than a calorie-restricted diet alone in reducing systemic inflammation in obese adults. However, more studies are suggested clarifying the efficacy of 'Mohazell' as a complementary therapy for modulating inflammatory parameters in obese individuals along with weight restriction diets.

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References

1. Ignjatovic V, Ogru E, Heffernan M, Libinaki R, Lim Y, Ng F. Studies on the use of "slimax", a chinese herbal mixture, in the treatment of human obesity. Pharmaceutical biology. 2000;38(1):30-5.
2. Moreno DA, Ilic N, Poulev A, Brasaemle DL, Fried SK, Raskin I. Inhibitory effects of grape seed extract on lipases. Nutrition. 2003;19(10):876-9.
3. Ferrante AW, Jr. Obesity-induced inflammation: a metabolic dialogue in the language of inflammation. Journal of internal medicine. 2007;262(4):408-14.
4. Ye J, McGuinness OP. Inflammation during obesity is not all bad: evidence from animal and human studies. American journal of physiology Endocrinology and metabolism. 2013;304(5):E466-77.
5. Ellulu MS, Patimah I, Khaza'ai H, Rahmat A, Abed Y. Obesity and inflammation: the linking mechanism and the complications. 2016.
6. Savini I, Catani MV, Evangelista D, Gasperi V, Avigliano L. Obesity-associated oxidative stress: strategies finalized to improve redox state. International journal of molecular sciences. 2013;14(5):10497-538.
7. Pillitteri JL, Shiffman S, Rohay JM, Harkins AM, Burton SL, Wadden TA. Use of dietary supplements for weight loss in the United States: results of a national survey. Obesity (Silver Spring, Md). 2008;16(4):790-6.
8. Kazemipoor M, Mohamed CWJW, Cordell GA, Yaze I. Safety, efficacy and metabolism of traditional medicinal plants in the management of obesity: a review. International Journal of Chemical Engineering and Applications. 2012;3(4):288.
9. Hsu C-H, Tsai T-H, Kao Y-H, Hwang K-C, Tseng T-Y, Chou P. Effect of green tea extract on obese women: a randomized, double-blind, placebo-controlled clinical trial. Clinical Nutrition. 2008;27(3):363-70.
10. Ahn IS, Do MS, Kim SO, Jung HS, Kim YI, Kim HJ, et al. Antiobesity effect of Kochujang (Korean fermented red pepper paste) extract in 3T3-L1 adipocytes. Journal of medicinal food. 2006;9(1):15-21.
11. Onakpoya I, Hung SK, Perry R, Wider B, Ernst E. The Use of Garcinia Extract (Hydroxycitric Acid) as a Weight loss Supplement: A Systematic Review and Meta-Analysis of Randomised Clinical Trials. Journal of obesity. 2011;2011:509038.
12. Heshmati J, Namazi N, Memarzadeh M-R, Taghizadeh M, Kolahdooz F. Nigella sativa oil affects glucose metabolism and lipid concentrations in patients with type 2 diabetes: a randomized, double-blind, placebo-controlled trial. Food Research International. 2015;70:87-93.
13. Chun S-S, Vattem DA, Lin Y-T, Shetty K. Phenolic antioxidants from clonal oregano (Origanum vulgare) with antimicrobial activity against Helicobacter pylori. Process Biochemistry. 2005;40(2):809-16.
14. Ene A, Nwankwo E, Samdi L. Alloxan-induced diabetes in rats and the effects of black caraway (Carum carvi L.) oil on their body weight. Res J Med Med Sci. 2007;2(2):48-52.
15. Ratheesh M, Shyni G, Sindhu G, Helen A. Protective effects of isolated polyphenolic and alkaloid fractions of Ruta graveolens L. on acute and chronic models of inflammation. Inflammation. 2010;33(1):18-24.
16. Haidari F, Seyed-Sadjadi N, Taha-Jalali M, Mohammed-Shahi M. The effect of oral administration of Carum carvi on weight, serum glucose, and lipid profile in streptozotocin-induced diabetic rats. Saudi medical journal. 2011;32(7):695-700.
17. Sadasivan SK, Vasamsetti B, Singh J, Marikunte VV, Oommen AM, Jagannath M, et al. Exogenous administration of spermine improves glucose utilization and decreases

bodyweight in mice. European journal of pharmacology. 2014;729:94-9.
18. Cho HM, Kang YH, Yoo H, Yoon SY, Kang SW, Chang EJ, et al. Panax red ginseng extract regulates energy expenditures by modulating PKA dependent lipid mobilization in adipose tissue. Biochemical and biophysical research communications. 2014;447(4):644-8.
19. Singh P, Sharma P, Sahakyan KR, Davison DE, Sert-Kuniyoshi FH, Romero-Corral A, et al. Differential effects of leptin on adiponectin expression with weight gain versus obesity. International Journal of Obesity. 2015.
20. Mansour MS, Ni YM, Roberts AL, Kelleman M, Roychoudhury A, St-Onge MP. Ginger consumption enhances the thermic effect of food and promotes feelings of satiety without affecting metabolic and hormonal parameters in overweight men: a pilot study. Metabolism: clinical and experimental. 2012;61(10):1347-52.
21. Mahan LK, Raymond JL. Krause's food & the nutrition care process: Elsevier Health Sciences; 2016.
22. E V, H K, A O. Efficiency of four traditional herbal mixtures as an anti-obesity supplement in obese adult subjects: A randomized controlled trial. AJPRHC 2016.
23. Ouchi N, Walsh K. Adiponectin as an anti-inflammatory factor. Clinica chimica acta. 2007;380(1):24-30.
24. Ratheesh M, Sindhu G, Helen A. Anti-inflammatory effect of quinoline alkaloid skimmianine isolated from Ruta graveolens L. Inflammation research : official journal of the European Histamine Research Society [et al]. 2013;62(4):367-76.

Correspondence:
Alireza Ostadrahimi
Nutrition Research Center, Department of Nutrition and Food Sciences, Tabriz University of Medical Sciences (TUOMS), Tabriz, Iran
Tel: +989144018952
E-mail: ostadrahimi@tbzmed.ac.ir