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Effect of eight weeks combined training and *Zataria multiflora* supplement on the levels of IL-1β and insulin resistance in overweight and obese men

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ABSTRACT

Introduction: The anti-inflammatory effects of exercise training and zataria multiflora have been proven. In the present study, researcher investigated the effect of eight weeks combined exercise training and *Zataria multiflora (Z. multiflora)* supplement on the levels of IL-1β and insulin resistance in overweight and obese men.

Materials and Methods: The 40 overweight and obese men with average age of 27.73 ± 2.15 years old and average body mass index (BMI) 28.41 ± 1.75 kg/m^2 were randomly divided into four groups including the placebo, *Z. multiflora*, combined training, and training + *Z. multiflora*. Combined exercise training program conducted for eight weeks and three sessions per week. *Z. multiflora* supplement was also consumed at 500 mg daily. Blood sampling was performed before and 48 hours after the eight weeks intervention. The levels of IL-1β and insulin were measured by ELISA method. Data were analyzed by analysis of covariance and Bonferroni post hoc test.

Results: The levels of IL-1β in training and training + *Z. multiflora* groups significantly decreased compared to placebo and *Z. multiflora* groups ($P < 0.001$). In addition, significant decrease of insulin resistance in training group compared to placebo ($P < 0.001$) and *Z. multiflora* ($P = 0.003$) groups, and also in training + *Z. multiflora* group compared to placebo *and Z. multiflora* groups were observed ($P < 0.001$).

Conclusion: Combined training alone or in combination with *Z. multiflora* supplementation can exert anti-inflammatory effects. Moreover, *Z. multiflora* supplementation cause the relative increase in exercise training effect for decrease in IL-1β and insulin resistance.

Keywords: Exercise Training, Zataria Multiflora, Inflammation, Cytokine

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Introduction

Obesity is a chronic disease which defined as extra adipose tissue expansion that remarkably increased in children and adults in developed and developing countries (1). Obesity result in increases the risk of metabolic diseases (such as type 2 diabetes and fatty liver), cardiovascular diseases (hypertension, myocardial infarction,

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stroke), musculoskeletal diseases (osteoarthritis), Alzheimer's, depression and some types of cancer (for example, breast, ovarian, prostate, liver, kidney, colon), and also leads to decreased quality of life, reduced productivity and social problems and is associated with an increased risk of secondary diseases (2).

In addition, adipose tissue plays an important role as an endocrine organ by secreting different types of adipokines including adipsin, resistin, visfatin, chemerin, and various types of inflammatory elements such as interleukin 1 beta (IL-1β), IL-6, IL-8, TNF-α, CRP, MCP-1, which are involved in regulating insulin resistance, controlling energy expenditure, and inflammatory processes (3) and accordingly, Obesity is considered an inflammatory condition (4).

Numerous studies have shown that IL-1 family cytokines, especially IL-1β, IL-1α and IL-8, play an important role in obesity-induced inflammation (5). IL-1β is a proinflammatory cytokine produced by activated macrophages and monocytes, which its function involves producing systemic and local responses to infection, injury, or immunological challenges by causing fever, activating lymphocytes and increased injection of leukocytes into the site of injury or infection (6). IL-1β stimulate a number of inflammatory mechanisms, which in turn affected the pathogenesis of chronic inflammatory diseases such as rheumatoid arthritis, type2 diabetes, Alzheimer's, stroke and Parkinson, but IL-1Ra as an antagonist reduces the destructive effects of IL-1β and IL-1a (7). In addition, IL-1β connected the obesity-induced inflammation with insulin resistance, and elevation the IL-1β levels is associated with inhibition of the insulin signaling pathway and decreased expression of IR-1 and GLUT-4, which may eventually lead to type 2 diabetes (8). Despite the pathological effects of obesity, exercise training known as a strong anti-inflammatory strategy, that can counteract the pathological effects of obesity through decreased levels of inflammatory mediators (9).

Based on the available evidence, reducing the levels of inflammatory factors following regular exercise training result in decrease in insulin resistance in obese individuals (10). In addition to exercise training, some researchers have reported the anti-inflammatory effects of some herbal plants including *Zataria multiflora* (*Z. multiflora*). *Z. multiflora* is a thyme-like plant is native to countries such as Iran, Afghanistan and Pakistan, which has various biological effects, the most well-known of which are anti-pain, anti-microbial and anti-inflammatory effects (11). Some researchers have also shown a greater effects of exercise training combined with *Z. multiflora* supplementation compared to exercise training or *Z. multiflora* ingestion alone. Taybi et al (2019) suggested that different intensities circuit resistance training along with *Z. multiflora* supplementation result in further decrease of inflammatory mediators' levels such as RBP4 and TNF-α compared to the exercise training or *Z. multiflora* groups in postmenopausal women (12). These findings represented a *Z. multiflora* importance for increasing the anti-inflammatory effects of exercise training. However, the simultaneous effect of *Z. multiflora* along with different exercise training on various inflammatory mediators is remarkably unknown. Therefore, the present study conducted aimed to investigate the effect of eight weeks combined training (aerobic-resistance) with *Z. multiflora* supplementation on the levels of IL-1β and insulin resistance in overweight and obese men.

Materials and Methods

Subjects

Present study subjects consist of overweight and obese men, age ranging 25-37 years old, among them 40 overweight and obese men chosen as a present study

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subject. All subjects voluntarily participated in the present study and all of them signed informed consent before conducting eight weeks intervention.

Study Design

The present study procedure approved by ethics committee of science and research branch, Islamic Azad University, Tehran, Iran, and all of study stages conducted according to ethical guidelines of the Helsinki Declaration. Following informing in the public places for recruiting the subjects, the 40 overweight and obese men with the body mass index (BMI) less than 35 kg/m^2 and further than 25 kg/m^2 chosen for take part in present study protocol. After determined the subjects, they were asked to laboratory following 12 weeks night fasting for blood sampling in the pre-test stage. The 7 ml blood was taken from the brachial vein from each subject. Moreover, the subject's height and weight were measured and after a few days, the present study intervention (exercise training, *Z. multiflora* supplementation or combination of exercise training and *Z. multiflora* supplementation) began. After eight weeks intervention, the subjects were again asked to the laboratory for post-test blood sampling similar to pre-test. After pre-test measurement, the subjects were randomly divided into four groups with 10 subjects in each group, including: Placebo, *Z. multiflora*, combined training, and combined training + *Z. multiflora* groups.

Inclusion criteria were the BMI less than 35 kg/m^2 and more than 25 kg/m^2, don't take part in the regular exercise training in the last year, no disease including type 2 diabetes, cardiovascular disease, hypertension, systemic diseases and lack of malignancies such as cancer, not consuming alcohol, not taking any medication or supplements during the or 1 month before the start intervention, no physical limitation for performing exercise training and accepting the all of study conditions. Exclusion criteria also included the following: don't regular taking part in the exercise training program sessions, allergy or sensitivity to *Z. multiflora* supplementation, forced to use the medication or nutritional supplements during 8 weeks intervention, presence of any disease other than obesity, injury during training program and inability to continue the exercise training program.

Z. multiflora Supplementation

Z. multiflora (Shirazi thyme) leaves were dried in the shade for 10 days. *Z. multiflora* leaves were then dried in an oven at 48 ° C for 48 hours and then powdered with Chinese pounder. 500 mg of dried and powdered *Z. multiflora* leaves was poured into a capsule and prepared for consumption. The subjects in the combined training + *Z. multiflora* group and the *Z. multiflora* consumed 500 mg of *Z. multiflora* daily after breakfast (as a 500 mg capsule) with 100 ml of water. The placebo group also consumed 100 ml of water daily with a placebo capsule (500 mg of wheat flour) after breakfast (12).

Combined Exercise Training

Combined training (aerobic-resistance) program performed three sessions per week for eight weeks. Combined training sessions included resistance and aerobic training. In each session, after warming up, firstly resistance training was performed and after five minutes rest, aerobic training was performed. Resistance training part consisted of 5 exercise including leg extension, leg curl, chest press, lat pulldown, and triceps curl. Subjects performed each resistance exercise in three sets and eight repetitions with an intensity of 75-80% 1RM and two-minute rest intervals between sets and three minutes rest between exercises. The aerobic training part consist of 10 minutes of continuous running with an increase in duration of 30 seconds per session with 70-75% of maximum heart rate, which was measured using a polar heart rate monitor (13).

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Generally, each combined training session lasted about 1 hour.

Blood Samples Assay

At the end of eight-week intervention and 48 hours after the last exercise session or *Z. multiflora* ingestion (in order to eliminate the acute effects of the last session of combined exercise or taking *Z. multiflora* supplement), the post-test blood samples collected. Blood samples were poured into a falcon tube and then centrifuged and obtained serum was stored at -70 ° C for measurement the serum levels of IL-1β. Blood glucose levels were measured using Pars Azmoun kit. Insulin levels were measured by ELISA (Demeditec company, catalog number: DE2935, sensitivity 1.76/IU/ml) method. In addition, serum levels of IL-1β were measured using ELISA (Biovendor, catalog number: RD194559200R, sensitivity 0.4 pg/ml) method. All measurements were performed according to instructions of the manufacturer of ELISA kits. In order to measure the percentage of body fat, a body composition analyzer made in South Korea (BOCA-X1) was used. In order to body composition analysis test, the subjects were asked to fully comply with the conditions considered for performing the body composition test (no metal objects, minimum coverage, at least six hours of fasting).

Statistical Analysis

All data analysis was performed using SPSS-24 software. First, the distribution of data was examined. Since the results of Shapiro-Vilk test showed that the data have a normal distribution ($P > 0.05$), analysis of covariance test was used to compare between groups changes and if the between groups difference was significant statistically, Bonferroni post-hoc test was used to compare different groups together. Moreover, within group difference were determined by paired t test. For all tests, the significance was considered at $p<0.05$ and if p value was less than 0.05, the changes were considered significant statistically.

Results

Subjects' characteristics including age, height, body weight and BMI in the placebo, *Z. multiflora*, training and training + *Z. multiflora* groups were presented as a Mean ± SD in Table 1.

The levels of glucose, insulin, insulin resistance (HOMA-IR), percent body fat and BMI before and after eight weeks intervention (combined training, *Z. multiflora* or their combination) for placebo, Z. multiflora, training and training + *Z. multiflora* groups reported in Table 2.

According to analysis of covariance test findings for HOMA-IR, there was a significant difference between groups ($P < 0.001$). Bonferroni post-hoc test indicated that decreased in HOMA-IR in T and ST groups compared to P and S groups was significant statistically ($P < 0.001$). Moreover, paired t test indicated that HOMA-IR significantly decreased in T ($P < 0.001$) and ST ($P < 0.001$) groups, but no significant changes were observed for HOMA-IR in P ($P = 0.186$) and S ($P = 0.068$) groups. In addition, percent body fat and BMI significantly decreased in T and ST groups compared to P and S groups ($P < 0.001$).

Table 1. Subjects' characteristics at the baseline in the different groups.

Characteristic	P (n=10)	S (n=10)	T (n=10)	ST (n=10)	P value
Age (years)	29.12 ± 2.98	27.64 ± 1.71	26.75 ± 1.48	27.33 ± 1.64	0.081
Height (cm)	174.41 ± 4.34	173.13 ± 3.14	175.30 ± 4.62	174.52 ± 3.70	0.669
Weight (kg)	84.93 ± 4.76	85.72 ± 5.13	86.6 2± 6.85	88.11 ± 6.71	0.659
BMI (kg/m^2)	27.92 ± 1.65	28.61 ± 1.93	28.13 ± 1.94	28.9 0± 1.57	0.635

Data are shown as Mean ± SD. P: Placebo, S: *Z. multiflora*, T: Training. ST: Training + *Z. multiflora*.

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Table 2. The levels of variables in the pre and post-test of different groups under study.

Variables	Stage	P	S	T	ST	Between groups P
Glucose	Pre test	93.91 ± 7.07	104.35 ± 10.42	90.35 ± 5.51	96.51 ± 8.87	0.034
	Post test	95.32 ± 8.16	101.63 ± 9.21	88.12 ± 5.17	92.44 ± 5.77	
Paired t test		0.346	0.092	0.051	0.010	-
Insulin	Pre test	7.213 ± 1.10	7.742 ± 1.12	6.911 ± 0.70	7.02 ± 1.19	<0.001
	Post test	7.32 ± 1.34	7.55 ± 0.99	6.05 ± 0.57	5.94 ± 0.83	
Paired t test		0.343	0.187	0.001	0.001	-
HOMA-IR	Pre test	1.66 ± 0.31	1.99 ± 0.35	1.53 ± 0.20	1.66 ± 0.33	<0.001
	Post test	1.71 ± 0.32	1.89 ± 0.32	1.31 ± 0.16	1.35 ± 0.21	
Paired t test		0.186	0.068	<0.001	<0.001	-
Percent body fat (%)	Pre test	28.30 ± 2.19	29.11 ± 1.50	27.73 ± 2.45	29.91 ± 2.78	<0.001
	Post test	28.42 ± 2.30	29.34 ± 1.62	26.53 ± 2.14	28.81 ± 2.73	
Paired t test		0.331	0.153	<0.001	0.001	-
BMI (kg/m^2)	Pre test	27.96 ± 1.65	28.62 ± 1.93	28.17 ± 1.94	28.90 ± 1.57	<0.001
	Post test	28.01 ± 1.68	28.68 ± 1.98	27.64 ± 1.86	28.47 ± 1.54	
Paired t test		0.341	0.260	<0.001	<0.001	-

Data are shown as Mean ± SD. P: Placebo, S: *Z. multiflora*, T: Training. ST: Training + *Z. multiflora*.

According to paired t test findings, observed decrease in percent body fat and BMI in T and ST group was significant, but there was no significant difference for percent body fat and BMI in P and S groups (P > 0.05) (Table 2).

IL-1β data analysis by means of analysis of covariance test indicated that between groups difference was significant statistically (P < 0.001) and Bonferroni post-hoc test indicated that IL-1β decreased in T and ST groups significantly compared to P and S groups (P < 0.001). Intragroup analysis by paired t test represented that IL-1β levels in T and ST groups significantly decreased (P < 0.001), although the observed changes in P (P = 0.382) and S (P = 0.1388) groups wasn't significant (Figure 1).

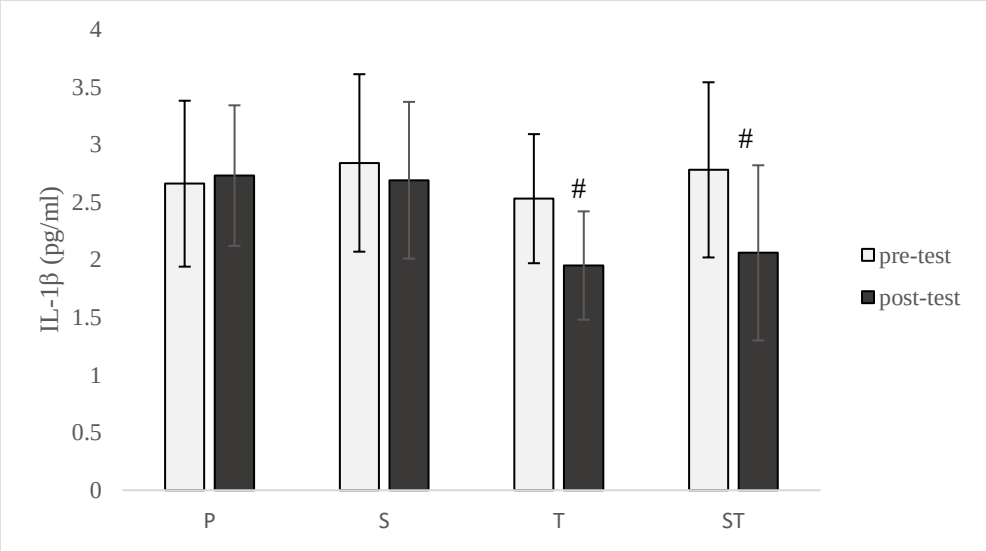


Figure 1. IL-1β levels in the pre and post-test groups under study. # Indicates significant decrease compared to P and S groups. P: Placebo, S: *Z. multiflora*, T: Training. ST: Training + *Z. multiflora*.

Discussion

The present study main finding was that eight weeks combined training and combined training along with *Z. multiflora* leads to a significant decrease in serum levels of IL-1β compared to the placebo and *Z. multiflora* groups. The present study findings showed that *Z. multiflora* supplementation can increase the anti-inflammatory effects of exercise training

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non-significantly. Consistent with the present study findings, Salamat et al (2016) (14) showed that aerobic and combined training (resistance-endurance) leads to a significant decrease in serum levels of IL-1β and IL-6. However, no significant change in serum IL-1β and IL-6 levels was observed in the resistance training group. The researchers suggested that aerobic training was more effective in reducing inflammatory mediators compared to other type of exercise training (14). Based on these statements, observed decrease in inflammatory cytokines in the present study following combined training can be attributed to the effectiveness of the aerobic part of combined training.

In another study, Farinha et al (2015) (15) reported that 12 weeks aerobic training in women with metabolic syndrome result in decrease in IL-1β levels, which decreased this inflammatory mediator was associated with a significant increase in IL-10 levels as an anti-inflammatory cytokine, and consistent with our findings, decreased levels of IL-1β was associated with decreased body fat percentage, insulin resistance and increased VO2max. In addition, decrease in oxidative stress and increase the antioxidant capacity were also observed in the trained group (15). In support of these findings regarding the relationship between oxidative stress and inflammation with obesity, it has been suggested that obesity cause to decrease in antioxidant enzymes activity and increasing the oxidative stress activity (16). In fact, adipose tissue increases the secretion of adipokines, which in turn cause the production of ROS, and therefore, adipose tissue is considered as an independent factor in systemic oxidative stress (17), oxidative stress in turn has a direct relationship and interaction with inflammation (18). Unfortunately, in the present study, the changes in the oxidative and antioxidant enzymes have not been investigated. Consistent with the present findings, previous studies have considered the combined training (aerobic-resistance) as an effective strategy to decreased the inflammatory mediators' levels, which the anti-inflammatory effect of combined training is remarkably exerted by decrease in visceral adipose tissue (19). According to these statements, another finding of the present study was that eight weeks combined training leads to a significant reduction in body fat percentage. Exercise training has an effective role in combating obesity and its related disorders, and exercise training play an important role in obesity management through reducing body fat and increasing lean body mass, which is associated with the least side effects (20). Generally, its reported that anti-inflammatory effects of exercise training are exerted by different mechanisms, including the decrease in visceral adipose tissue, secretion of different anti-inflammatory cytokines from contractile skeletal muscle (myokines), decrease in the expression of Toll-Like receptors (TLRs) on monocytes and macrophages (with subsequent inhibition in downstream pathways such as the production of proinflammatory cytokines and the expression of MHC and co-stimulatory molecules), inhibiting the infiltration of monocytes and macrophages into adipose tissue and changing the macrophages phenotype within adipose tissue (21).

Another finding of present study was that ingestion of *Z. multiflora* alone don't have a significant effect on the levels of IL-1β and insulin resistance. IL-1β levels in *Z. multiflora*, training and training + *Z. multiflora* groups decreased 5.28%, 22.92% and 25.89% respectively, which indicates a further decrease of IL-1β levels in the exercise training + *Z. multiflora* group, although was not statistically significant. It has been reported that *Z. multiflora* can exert the anti-oxidant and anti-inflammatory effects and therefore can be considered as a therapeutic target (22). Unfortunately, limited studies have been conducted about the simultaneous effect of exercise training and *Z. multiflora* on the

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levels of inflammatory mediators. Taybi et al (2019) reported that different intensities circuit resistance training (35 and 55% 1RM) in postmenopausal women leads to a significant decrease in RBP4 levels as an inflammatory factor, the highest RBP-4 decrease was observed in the 55% group along with *Z. multiflora* supplementation (12). Moreover, TNF-α levels don't change significantly in the trained groups (35 and 55% 1RM), but a significant decrease in TNF-α levels were observed in both trained groups (35 and 55% 1RM) along with *Z. multiflora* supplementation, and decrease in TNF-α levels following circuit resistance training with 55% 1RM combined with *Z. multiflora* ingestion was significant compared to circuit resistance training with 35% 1RM and *Z. multiflora* supplementation, finally consumption of *Z. multiflora* alone don't change the circulation levels of TNF-α (12). Khazdair et al (2019) indicated that *Z. multiflora* can decreased the inflammatory cytokines (such as IL-6 and IL-8) levels simultaneously with the increasing anti-inflammatory mediators (such as IL-10) (23). In addition, animal researches indicated that *Z. multiflora* reduce the expression of proinflammatory cytokines (IL-4, TGF-β and IL-17) and increase the expression of anti-inflammatory cytokines (IFN-γ) (24). Another finding of present study was that combined training alone or along with *Z. multiflora* significantly decreased insulin resistance, and observed decrease in insulin resistance for training and training + *Z. multiflora* groups was 14.37% and 18.67% respectively. Despite decrease in insulin resistance in the *Z. multiflora* group (-5.28%), the observed changes weren't significant statistically. The exact mechanism involved in obesity-induced inflammation and development of insulin resistance are not yet fully understood. However, it has been reported that increased infiltration of Th1, $CD4^+$ and $CD8^+$ T cells in obesity and decrease in the levels of Th2 and Tregs cell, significantly affected the human health by amplification inflammation and insulin resistance (25). Ghanbari-Niaki et al. (2019) reported that eight weeks circuit resistance training with different intensities (35, 55 and 85% 1RM) alone or combined with *Z. multiflora* supplementation, decrease the insulin resistance, which observed decrease in trained groups along with *Z. multiflora* consumption was higher compared to trained groups alone (26). Overall, the present findings showed that *Z. multiflora* supplementation along with eight weeks combined training relatively increased the anti-inflammatory effects of combined training and its effect in decreasing insulin resistance. Therefore, it seems that adding *Z. multiflora* to combined exercise training amplify the positive effects of combined training.

Conclusion

In conclusion, the present study indicated that eight weeks combined training alone and in combination with *Z. multiflora* supplementation reduces systemic inflammation and insulin resistance, which observed decrease in IL-1β and insulin resistance was further in the training+ *Z. multiflora* group compared to training group, but wasn't significant statistically. It seems that further duration of combined training and *Z. multiflora* supplementation can result in further and significant decrease in inflammatory mediators and insulin resistance.

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Conflicts of Interest

The authors declare that there is no conflict of interest.

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