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Effect of Aquatic Extract of *Ferulago angulata* Boiss With Aerobic Exercises on Serum Levels of Interleukin-10 and C-Reactive Protein of Obese Males

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Abstract

Objectives: The aim of the present study was to evaluate the impacts of chavir aquatic extract (*Ferulago angulata* Boiss) along with aerobic exercises on serum levels of interleukin-10 (IL-10) and C-reactive protein (CRP) of obese males.

Materials and Methods: In this study, 40 males with body mass index (BMI) >30 kg/m² and average age of 33.63 ± 4.78 years were randomly categorized into 4 groups of 10 individuals as control, combination (using the aquatic extract of chavir along with aerobic exercises), consumption of chavir aquatic extract, and aerobic exercises. The aerobic exercises in both groups of combination and aerobic exercises consisted of running on treadmill for 20 minutes in 60%-70% maximum oxygen uptake (VO₂max). The aquatic extract consumption and the combination groups had to take 50 mg/mL/d of chavir aquatic extract every time. The control group received no intervention.

Results: Based on intragroup comparisons, body weight and BMI significantly decreased in the combination group; the content of body fat and waist-hip ratio (WHR) also reduced significantly in the aerobic, combination, and aquatic extract groups. In intergroup and intragroup comparison, CRP faced with a significant decrease in all groups (aerobic exercises, *Ferulago* aqueous extraction, and combination groups) and a considerable increase was also observed in the combination group regarding IL-10 variable.

Conclusions: Therefore, the effectiveness of the combination group regarding increasing IL-10 and decreasing CRP is more than other groups. As a result, using aqueous extract of *F. angulata* and doing aerobic exercise for 3 months reduced risk factors – cardiovascular, body composition, and increasing anti-inflammatory in obese men. Consequently, the effect of combination group to reduce the proinflammatory indexes and body factors of obese males was more compared to that of the other groups.

Keywords: Aerobic exercises, Chavir, IL-10, CRP, Obesity

Introduction

Obesity is a risk factor for different infections, inflammatory disorders, and cardiovascular diseases (1-4). There exists ample evidence regarding the relationship between obesity and increased inflammatory cytokines' levels. In fact, obesity is introduced as a low grade inflammation (5).

In addition, these cytokines can be a powerful risk factor for heart diseases including stroke and myocardial infraction (6). Obesity activates inflammatory pathways. Inflammatory is a systematic process in obesity which affects a large number of organs. However, probably one or several organs initiate this process. As getting calories and fat increase, the inflammatory pathways' activation in cells begins by nutrient perception and cytokine signaling (7,8).

Among the cytokines, interleukin-10 (IL-10) is secreted by monocytes, lymphocytes, and macrophages and is considered as a beneficial and protective cytokine in human metabolism (9). The IL-10 protects the excessive proinflammatory response that is more in the obese subjects (10). Since obesity is regarded as a proinflammatory state, serum multiple cytokine levels may differ based on the weight loss degree in obese patients. Due to the weight loss in obese patients along with improved metabolic disorders, the IL-10 levels rise (9). Recent research has demonstrated that the IL-10 is less prior to exercise, so regular exercise with moderate intensity reduces proinflammatory cytokines such as IL-6 and increases anti-inflammatory cytokines like IL-10 (10). Numerous studies indicated the inverse relationship between fitness and the amount of lymphocytes and inflammatory markers such

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as IL-6 and CRP, whereas it was indicated that IL-10 is positively correlated with fitness (11,12). For example, Calle et al stated that IL-10 increased after physical activity of reduced production of IL-6 and the reason of this decrease in some studies were due to its low level activity (13). In a study conducted by Hovanloo et al, it was found that there was no significant difference between the serum levels of IL-10 by the 6-session low-volume sprint interval training (SIT) and high-volume continuous endurance training (14). As a result, according to the contradiction in the research results, it seems that no general consensus exists on the role of exercise (and type of exercise) in the IL-10, yet.

The C-reactive protein (CRP) was recognized as a risk factor for cardiovascular disease (12). The risk of heart attack in people with CRP concentration higher than 2.11 mg/L was reported three times higher than those with levels not higher than 55.0 g/L (15). Research results in this field can be inconsistent, as some studies indicated no change in CRP regarding the effect of aerobic exercise on obese subjects (16) and others have reported a decrease in CRP on the effect of such exercise as well (17). For instance, in the study by Zakavi et al, the reduced levels of CRP by exercise was reported (3). However, Kelley and Kelley found that 8-week aerobic exercise did not decrease CRP in obese children (18).

Given widespread effects of exercises on obesity inhibition and treatment and also its immunity disorders, it is assumed that these effects have an association with adipose tissue regulation (19). On the other hand, some studies have shown that excess fat can put safety at risk (20).

Ferulago angulata Boiss called chavir in Iran and belonging to the family Apiaceae, is a native plant which is grown in eastern regions of Iran. It is used as a sedative and digestive drug since ancient time and is consumed to treat intestinal worms, hemorrhoids with antimicrobial, and also antibacterial and antioxidant effects (21). Amirghofran et al, in an 8-week study on male mice reported that consuming 50 mg/mL of chavir significantly decreased NO (nitric oxide) production after 24 hours; moreover, the inflammatory cytokines' levels, such as IL-1 β , significantly decreased following the consumption of chavir extract after 48 hours. The researchers believed that chavir extract can reduce inflammations (22).

Considering the few studies regarding the effect of chavir on obesity, the aim of the present study was to evaluate the effect of aquatic extract of chavir along with aerobic exercise on serum levels of IL-10 and CRP in obese males.

Materials and Methods

The current quasi-experimental study was conducted in Baghmalek of Khuzestan in 2014. Individuals with body mass index (BMI) >30 were included in the study.

The inclusion criteria were: being healthy based on the health questionnaire, not smoking, receiving no medication, lack of cardiovascular, respiratory, renal, and

metabolic diseases, no participation in exercise program at least 2 months before enrolling in the current study, being on no nutritional diet (low-calories, low-fat, and high protein), and being in the age range of 30-40 years.

The exclusion criteria were: taking special drugs under the administration of physician, missing more than three sessions of the exercise classes, participating in regular exercise programs except for the program of the current study as well as following a weight-loss program and irregular consumption of chavir aquatic extract. Totally, 40 eligible volunteers were chosen and randomly classified into 4 groups of 10 as the control, aerobic exercise, using chavir aquatic extract, and combination (using chavir aquatic extract and doing aerobic exercise). The informed consent was gathered from all the subjects.

The VO₂max was determined using Bruce protocol (4). Three days after VO₂ testing, subjects started the exercise program. The heart bit rate/minute of the subjects was measured by treadmill (4). Then, to calculate VO₂max, the following equation was used (4, 23):

$$3 \text{ (time)} 0.12 - 2 \text{ (time)} 0.451 + \text{ (time)} 1.379 - 14.76 = \text{VO}_2\text{max}$$

To determine the level of heart rate severity, as a part of VO₂max, the maximum heart bit at the time of subjects' failure was measured using Caronen formula as follows (4, 23):

$$(\text{resting heart rate} + \text{severity of exercises}) * (\text{resting heart rate} - \text{maximum heart rate}) = \text{heart rate}$$

To identify the maximum heart rate, the formula (220-age) was used. It is noteworthy that in order to determine the maximum effort of the subjects, the required guidelines were given before testing and the subjects participated in the test on a competitive basis (23). The aerobic activities included general warm-up (10 minutes), aerobic exercise (20 minutes) as well as stretching exercise and cool-down (5 minutes). Aerobic exercise included running on treadmill for 20 minutes in 60%-70% of VO₂max. To evaluate the overweight of the subjects, their maximum heart rates were measured every four weeks and 60%-70% of VO₂max was adjusted accordingly (4, 23, 24). However, no intervention was conducted in the control group rather the follow-up.

The leaves of chavir were made into fine powder by electric mill and unpowered parts were sifted. To prepare aquatic extract of chavir, 8 g of powdered leaves was wrapped in a double-layer tiffany, placed in a flask containing 100 mL of distilled sterile water and shaken for 24 hours at room temperature on a shaker. After providing the aquatic extract, the liquid was filtered under fume hood using 0.22 μ m Millipore filter and stored at 4°C up to the testing time (25). The consuming amount of chavir aqueous extract in the combination group was 50 mg/mL/session based on the previous similar studies (22).

The variables included age (year), height (centimeter), measured using stadiometer (SECA, Germany) to the nearest 1 mm, together with weight, body fat content,

BMI, and waist-hip ratio (WHR) (Inbody 3.0, Biospace, Seoul, South Korea). The ELISA method and Pasteur kit (made in USA) were used for measuring the IL-10 and the ELISA method and Bionic kit (made in Iran) were applied to measure the CRP.

Then, the subjects were asked to observe normal sleep and diet patterns prior to running the tests. Blood samples (5 mL) were obtained from the subjects' left hand anterior thoracic after 12 hours overnight fasting at 8:00 AM in a sitting position and resting condition, 48 hours before starting the first aerobic exercise session and consumption of chavir aquatic extract (pre-test), and 48 hours following the last exercise session consuming the aquatic extract (post-test at the 12th week). Blood samples were poured in the sterile tubes containing anticoagulant and ethylen diamine tetra acetate (EDTA). The samples were then exposed to centrifuge at 3000-3500 rpm for 10 minutes and separated serum samples were poured in 1 mL micro tubes and transferred to the laboratory for further tests and stored frozen at -70°C .

All post-test collected blood samples were tested based on the associated protocols. The variables of the study were measured again, three months after aerobic exercise and consumption of chavir aquatic extract.

Descriptive statistics were used for analyzing participants' characteristics and all of the variables including age, weight, height, BMI, percentage of body fat, WHR and serum levels of IL-0, and also CRP in four groups. After examining the normal distribution of data using the Kolmogorov-Smirnov test (K-S test), for intergroup comparing, paired *t* test were used. In addition, for intergroup comparisons, one-way analysis of variance (ANOVA) was used. Data were analyzed using SPSS (Statistical Package for the Social Sciences) software, version 20. The significance level was adjusted to $P < 0.050$.

Results

Demographic data, that is, information about participants' age, weight, height, and BMI are shown in Table 1. In Table 2, the results of the paired *t* test for the intragroup comparisons, and one-way ANOVA for the intergroup comparisons are presented. In the intragroup comparison using paired *t* test, it was found that the weight significantly decreased only in the combination group ($P < 0.05$) while no significant changes were found in the other groups. Moreover, based on ANOVA results, no significant difference was found in the intergroup

comparisons ($P \geq 0.05$) in terms of the weight between the groups. In the intragroup comparisons, a significant decrease in BMI was observed only in the combination group ($P < 0.05$); in other words, the effect of intervention on the variables such as weight and BMI was more in the combination group than those of the other groups; while in the intergroup comparisons, BMI did not illustrate any significant differences in the groups ($P \geq 0.05$). In the intragroup comparisons, the body fat content significantly decreased in the control, aerobic exercise, chavir aquatic extract consumption, and combination groups ($P \geq 0.05$). However, in the intergroup comparisons, no significant difference was found between the groups ($P \geq 0.05$). In addition, in the intragroup comparisons, the aerobic exercise, chavir aquatic extract consumption, and combination groups showed insignificant decreases in WHR ($P < 0.05$) while there was no significant difference in terms of WHR in the intergroup comparisons ($P \geq 0.05$).

In intergroup comparison, IL-10 considerably increased in the combination group ($P < 0.05$) and in intragroup comparison, a significant increase was also found in the combination group ($P < 0.05$) regarding IL-10 variable. Therefore, the effectiveness of the combination group on increasing IL-10 was more than that of the other groups.

In intergroup comparison, CRP decreased significantly in the groups (aerobic exercises group, Ferulago aqueous extraction, and combination group) ($P < 0.05$). I Furthermore, it was found that in intragroup comparison, CRP decreased significantly in the groups (aerobic exercises group, Ferulago aqueous extraction, and combination group) ($P < 0.05$). The effectiveness of the combination group on decreasing CRP is more as compared to other groups.

Discussion

The results of the present study indicated that body contents significantly decreased based on intragroup comparisons, but there were no significant changes in body variables based on intergroup comparisons; however, there was only a significant decrease regarding WHR in the aerobic exercise, combination, and chavir aquatic extract consumption groups based on the intragroup comparisons.

Since the aerobic activity increased the lipolysis of adipose tissues, variables such as BMI, body fat content, and weight decreased accordingly (26). Zakavi et al reported that body content of juveniles with overweight and

Table 1. Descriptive Indices of Quantitative Variables in 4 Studied Groups

| Variable | Groups | | | |
|-------------|----------------------------|---------------------------|-------------------|-------------------|
| | Control | Ferulago Extraction Usage | Mixed | Aerobic Exercises |
| | Mean \pm SD ^a | Mean \pm SD | Mean \pm SD | Mean \pm SD |
| Age (y) | 31.08 \pm 4.14 | 34.42 \pm 3.45 | 33.45 \pm 5.44 | 34.45 \pm 5.42 |
| Height (cm) | 169.4 \pm 3.97 | 160.14 \pm 5.52 | 169.27 \pm 5.47 | 156.63 \pm 6.71 |

Mean \pm standard deviation of combination group (the group which used Ferulago aqueous extraction with aerobic exercises)

Table 2. Pre-test and Post-test Changes in the Study Variables

| Variable | Groups | Pre-test (Mean ± SD) | Post-test (Mean ± SD) | Intergroup (P Value) | Intergroup (P Value) |
|--------------------------|------------------------|-------------------------|--------------------------|-------------------------|-------------------------|
| Weight (kg) | Control | 82.61 ± 4.33 | 83.15 ± 4.92 | 0.311 | - |
| | Ferulago extract usage | 83.90 ± 6.85 | 83.51 ± 6.85 | 0.352 | 0.95 |
| | Aerobic exercises | 80.90 ± 5.61 | 80.58 ± 5.06 | 0.62 | 0.922 |
| | Mixed | 84.88 ± 13.86 | 82.06 ± 13.14 | 0.15 | 0.403 |
| BMI (kg/m ²) | Control | 32.37 ± 2.37 | 32.7 ± 2.01 | 0.293 | - |
| | Ferulago extract usage | 32.82 ± 2.47 | 32.65 ± 2.27 | 0.349 | 0.96 |
| | Aerobic exercises | 32.91 ± 2.55 | 32.77 ± 2.23 | 0.616 | 0.934 |
| | Mixed | 34.56 ± 4.94 | 33.38 ± 4.39 | 0.017* | 0.412 |
| Fat Percentage (%) | Control | 41.74 ± 3.11 | 42.77 ± 2.30 | 0.444 | - |
| | Ferulago extract usage | 41.22 ± 3.52 | 39.91 ± 3.64 | 0.018* | 0.417 |
| | Aerobic exercises | 41.9 ± 4.48 | 40.47 ± 4.78 | 0.008* | 0.247 |
| | Mixed | 34.56 ± 4.94 | 33.38 ± 4.39 | 0.017* | 0.405 |
| WHR | Control | 0.98 ± 0.03 | 0.98 ± 0.02 | 0.22 | - |
| | Ferulago extract usage | 0.99 ± 0.04 | 0.147 ± .280 | 0.004* | 0.97 |
| | Aerobic exercises | 0.99 ± 0.04 | 0.96 ± 0.04 | 0.003* | 0.69 |
| | Mixed | 1.01 ± 0.07 | 0.98 ± 0.06 | 0.001* | 0.219 |
| IL-10 (pg/mL) | Control | 35.06 ± 2.46 | 35.53 ± 2.40 | 0.671 | - |
| | Ferulago extract usage | 30.40 ± 3.19 | 30.47 ± 3.2 | 0.90 | 0.257 |
| | Aerobic exercises | 38.77 ± 7.81 | 38.76 ± 6.93 | 0.997 | 0.792 |
| | Mixed | 26.92 ± 4.62 | 30.08 ± 1.37 | 0.033* | 0.009** |
| CRP (mg/L) | Control | 0.1470 ± 0.028 | 0.1489 ± 0.020 | 0.625 | - |
| | Ferulago extract usage | 0.1420 ± 0.21 | 0.1176 ± 0.012 | 0.001* | 0.023** |
| | Aerobic exercises | 0.1476 ± 0.26 | 0.1214 ± 0.10 | 0.003* | 0.006** |
| | Mixed | 0.1487 ± 0.20 | 0.1102 ± 0.012 | 0.001* | 0.001** |

Abbreviations: BMI, body mass index; WHR, waist-hip-ratio; IL-10, interleukin-10; CRP, C-reactive protein.

* The intragroup significance level based on paired *t* test was $P < 0.05$; ** The intergroup significance level was $P < 0.05$ based on ANOVA.

obesity improved after eight weeks of exercise (3), which is inconsistent with the findings of the current study. In another study, it was also indicated that a 12-week Pilates-based exercise significantly decreased body contents of obese elderly (4). This is compatible with the results of the present study. Therefore, decreasing body indices, such as BMI, body weight, and fat content, decreases the proinflammatory indexes. Generally, decreasing body indices play a key role in decreasing serum levels of CRP and the increase of anti-inflammatory (IL-10) indices is expected as well.

It seems that more exercise with moderate severity in each session decrease body mass, weight, and fat content; hence, according to the results of the current study, chavir aquatic extract dosage in the chavir extract consuming group was not sufficient to reduce body weight and BMI of the subjects. The results of the current study indicated that compared to the chavir extract consumption and aerobic exercise groups, the combination group reduced weight and BMI of the subjects more effectively. Therefore, the dosage of chavir aquatic extract along with the severity, time, and duration of the exercises in the combination group were effective enough to reduce body indices. Aerobic activity increased the lipolysis of adipose tissues (27); this result was inconsistent with that of the current study. In

the present study, the WHR significantly decreased after three-month consumption of chavir extract along with the aerobic exercise in the intragroup comparisons (28). In another study by Saremi et al, a 12-week aerobic exercise plan significantly decreased the body fat content in obese males (28). This is not in conformity with the results of the present study. The exercising protocol used by Saremi et al, was similar to that of the present study. A significant reduction in WHR was reported in both studies. The time of intervention in both studies was 12 weeks which may have been the cause for achieving the same results in both studies.

In intergroup and intragroup comparison, IL-10 faced with significant increase in the combination group ($P < 0.05$). Therefore, the effectiveness of the combination group on increasing anti-inflammatory index (IL-10) was more than that of the other groups.

Some studies (29,30) showed that the continuous physical activity had anti-inflammatory and therapeutic effects on a wide range of inflammation-associated diseases.

Before the exercise the IL-10 levels are less, so, a regular exercise with a moderate intensity reduces pro-inflammatory cytokines such as IL-6 and increases anti-inflammatory cytokines like IL-10 (10). Nunes

et al reported that endurance safety impacts cause a considerable increase in serum IL-10 concentrations in blood (31). In their study Hirose et al stated that resistive eccentric activities cause a significant increase in serum IL-10 (32). Moreover, Markovitch et al and Wilund explained that by increasing physical fitness, concentrations of IL-10 increase; thus, IL-10 has a direct relationship with the fitness (12,33). Kasapis and Thompson in their study also indicated that some beneficial effects of physical activity increase the IL-10 by stimulating anti-inflammatory actions. The reason for increased IL-10 in these studies depended on intensity of physical activity and fitness levels of people (34). However, in a study by Havanlu et al, no significant difference was found in the serum levels of IL-10 by the 6-session low-volume SIT and high-volume continuous endurance training (14). Uchida et al reported that there was no change in IL-10 in a comparison between the effects of different intensities of the bench press (35). This result is in line with the results of the current study. Calle et al., pointed out that an increase in IL-10 after exercise was induced by increased production of IL-6 and the reason for a failure of the increase in some studies was reported to be the low volume of activity (13). Moreover, Neubauer et al suggested that intensity of exercise is the factor of increased IL-10 (36). It is the reason for consistency or inconsistency of the results of this study with those of other studies.

Longtime activity can change the regulation of inflammation (37). In this regard, the first mechanism is that the aerobic exercise can reduce gene expression and serum level of leukocytes, inhibit endothelial monocytes reactions, and finally lead to decreased cytokines (21). Besides, the antioxidant effects of exercises are different mechanisms which reduce the inflammation (38). Hence, considering the antioxidant and anti-inflammatory effects of chavir (22,39), it can be concluded that combination of aerobic exercises and consumption of chavir extract can affect reducing the inflammation.

Several studies examined the influence of aerobic exercise on the level of serum CRP and mainly the reduction of serum concentrations of CRP. In intergroup and intragroup comparison, CRP faced with significant decreased in the groups (aerobic exercises group, Ferulago aqueous extraction, and combination group) ($P < 0.05$). The effectiveness of the combination group is more on decreasing CRP as compared to other groups. The findings are consistent with those of some studies (17,40-42). For example, Martins et al in their study showed that aerobic and resistance exercises significantly decrease CRP values (43). Tchern et al stated that weight loss alone, without changing physical activity, lowers CRP significantly (41). Furthermore, Olson et al reported the reduced CRP levels after one year of resistance exercise in the obese women (42). Lakka et al found the reduced levels of CRP by exercise, as well (17). However, the findings of this research are inconsistent with the results obtained by some

other studies (16,18,21,26). It was reported by Kelley and Kelley that 8 weeks of aerobic exercise in obese children did not result in decreased CRP (18). Besides, Hammett et al and Nassis et al in their study reported no difference in CRP levels in obese individuals (16,44). The length and intensity of the training period are important determining factors of change in CRP by the exercise. Investigations have shown that in both genders of obese individuals, CRP level was higher than ordinary people. Obesity created an inflammatory condition in the body that was associated with increased CRP (45). Interleukin secreted from adipose tissue (IL-6 and TNF- α) contributed to an increase observed in the obesity (46). The amounts of CRP in the extreme and heavy sports considerably increased; although it reduced in the regular moderate intensity exercises (43).

Immune modulating effects of medical plants such as chavir were evaluated in the previous studies (22,42). For example, Amirghofran et al studied 8-week male mice and reported that consumption of 50 mg/mL chavir extract significantly decreased NO production following 24 hours in the cases; besides, taking the same dose after 48 hours significantly reduced the level of inflammatory cytokines such as IL-1 β in the studied mice. According to the results of these studies, chavir extract had anti-inflammatory effects (22); although using chavir extract was more effective in the combination group.

Limitations of the Study

Personal differences among the subjects of the current study such as mental factors and socio-economic situations can be considered as the study limitation.

Conclusions

The combination group effectiveness of the study on increasing IL-10 and decreasing CRP is more as compared to other groups. Therefore, using aqueous extract of *F. angulata* and doing aerobic exercise for 3 months reduced risk factors including cardiovascular, body composition, and increasing anti-inflammatory in obese men. As a result, the effect of combination group to reduce the proinflammatory indexes and body factors of obese males was more than that of the other groups.

Conflict of Interests

Authors have no conflict of interests.

Ethical Issues

The current study was a research project approved in the Ethics Committee of Abadan University of Medical Sciences (registration code: 93U-044) in 2014.

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