The effect of tapering and *Nigella sativa* on the histological structure of the lung after increasing interval exercise training

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Summary

Objectives: During maturation period in which the immune system of lung tissue is not fully developed, physical exercises may have a negative effect and cause inflammation. This study aimed to investigate the effects of tapering and *Nigella sativa* (*NS*) hydro-alcoholic extract on the reduction of lung tissue inflammation caused due to increasing interval exercise training (IIET) during maturation period by histological and stereological methods.
Methods: Nighty-five three weeks old rats after adaption were randomly divided into two control and exercise groups and 19 subgroups. The exercise group carried out a period of six weeks of undulating IIET followed by three weeks of load reduction performed by three models in two different times. Rats entered the taper phase were administrated by *NS* supplement in tapering and control groups. The lung tissue samples were processed by standard paraffin embedding, stained by H&E and examined by using point counting method through systematic random sampling in stereological study. The results were analyzed using by two-way ANOVA and LSD post hoc in α=0.05.
Results: The result showed that IIET caused severe inflammation in lung tissue and an increase in infiltration of inflammatory cells and lymphocytes into the connective tissues surrounding the respiratory air ways, vessels and interstitial lamellae. This severity of inflammation was considerably and similarly more in comparison to the basic and control groups (p=0.001). Stereological analysis in the taper exercise training groups with *NS* and without *NS* as well, reveled a significant decrease in the degree and intensity of lung tissue inflammation in the examined times in comparison to the IIET group (p=0.001).
Conclusion: Generally it can be concluded that performing *NS* and a three weeks period of tapering has a noticeable effect in the reduction of inflammation in lung tissue followed by interval exercise training.

Key words:
Increasing interval exercise training. Tapering. *Nigella sativa*. Lung. Inflammation.

El efecto del *tapering* y *Nigella sativa* sobre la estructura histológica del pulmón después de aumentar el entrenamiento de ejercicio de intervalo

Resumen

Objetivos: Durante el período de maduración en el que el sistema inmunitario del tejido pulmonar no está completamente desarrollado, el ejercicio físico puede tener un efecto negativo y causar inflamación. Este estudio tuvo como objetivo investigar los efectos del *tapering* y del extracto hidroalcohólico de *Nigella sativa* (*NS*) sobre la reducción de la inflamación del tejido pulmonar causada por el aumento de entrenamiento interválico (IIET) durante el período de maduración mediante métodos histológicos y estereológicos.
Métodos: Noventa y cinco ratas de tres semanas de edad, después de la adaptación, se dividieron aleatoriamente en dos grupos de control y ejercicio y 19 subgrupos. El grupo de ejercicio llevó a cabo un período de seis semanas de IIET ondulado seguido de tres semanas de tapering realizadas por tres modelos en dos momentos diferentes. Las ratas entraron en la fase de tapering y se les administró un suplemento de *NS* en ambos grupos. Las muestras de tejido pulmonar se procesaron mediante inclusión convencional de parafina, se tiñeron con H & E y se examinaron mediante el método de conteo puntual mediante muestreo aleatorio sistemático en un estudio estereológico. Los resultados se analizaron usando ANOVA de dos factores y LSD *post hoc* en α = 0,05
Resultados: Los resultados mostraron que el IIET causó inflamación severa en el tejido pulmonar y un aumento en la infiltración de células inflamatorias y linfocitos en los tejidos conectivos que rodean las vías respiratorias, los vasos y las lamelas intersticiales. Esta gravedad de la inflamación fue considerablemente mayor y similar en comparación con los grupos básico y de control (p = 0,001). El análisis estereológico en los grupos de *tapering* con *NS* y sin *NS* también, reveló una disminución significativa en el grado e intensidad de la inflamación del tejido pulmonar en las mediciones examinados en comparación con el grupo IIET (p = 0,001).
Conclusión: en general, se puede concluir que la realización de *NS* y un período de *tapering* de tres semanas tiene un efecto notable en la reducción de la inflamación en el tejido pulmonar seguida de entrenamiento de ejercicios a intervalos.

Palabras clave:
Aumento del entrenamiento de ejercicio interválico. *Tapering. Nigella sativa*. Pulmón. Inflamación.

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Introduction

Physical exercises with different intensity create different responses on the immune system function[1,2]. Increase in the volume and intensity of the exercise training along with a decrease in the recovery time may cause an overtraining in athletes and affect their immune system[3]. On the other hand, physical exercise can be a strong stimulus in the development of lung inflammation[4]. Findings of several studies shows that exercise training load beyond the normal range increases the risk of upper respiratory tract infection (URTI)[2,5] while moderate exercise not only decrease these risks but also reinforce the function of respiratory immune system[1,6]. Researches showed that the URTI symptoms in the most of the elite athletes are similar to general population[6], however, usual seasonal patterns of URTI are not seen in these athletes[7]. It is found out that short and long term physical exercise trainings with different grades and intensities affect the immunity and inflammatory factors in childhood and adulthood period[8]. There are still many unanswered questions on physical exercise mechanisms influencing inflammatory index before and during puberty[9].

Since mild inflammation in early stages is a helpful protective response against primary cellular damage factors which eliminates external invaders and necrotic tissue and also because too much inflammation causing severe damage to lung tissue can be life-threatening[10], it seems necessary to take some strategies in exercise training programs including taper to prevent overtraining and immune function decline[11,12]. Taper can be performed in the forms of frequency reduction, repetition and intensity of exercise training[3,13] in different time periods. One of the hardest challenges for sport science researchers and trainers is considered as to determine the most appropriate taper program[14]. Limited studies about intensity of exercise training during taper period showed athletes can make use of training intensity reduction programs in order to improve their performance in competition season[15]. Some reports reveled that time execution of swimmers[16], runners[17] and bike riders[18] improved due to taper programs. Previous studies suggest the favorable time period for taper is between 4 to 28 days or even more[8,13]. Though many studies have confirmed a two-week period taper, there have been some reports on the improvement of athletes' performance due to very short or very long period tapers[19]. Thomas *et al.*[3] concluded that the optimal time duration for taper depends on the training before the taper and no particular time limit can be specified for that.

Today, there are several methods other than taper such as medicinal plants using for reducing the symptoms of inflammation and boosting the immune system. Among these plants, *Nigella sativa L. (NS)* is a medicinal plant known in Iranian traditional medicine. This native plant has been used for the treatment of headaches, nasal congestion, asthma, and allergy as well as for boosting the immune system[20,21]. The biological properties of the seed of this plant include Anti-oxidative, anti-inflammatory, anti-tumor, anti-viral and anti-microbial effects as well as strengthening the immune system[22]. Previous studies indicated that *NS* has a protective effect in lung injury and pulmonary fibrosis[23,24]. Gholamzadeh *et al.*[25] revealed that *NS* has an anti-inflammatory effect and reduces pro/anti-inflammatory cytokine ratio in overtrained animals. They showed that this effect of *NS* is more pronounced in overtraining animals than control or moderate exercise animals. In fact, *NS* causes an immunoregulatory effect which somehow homogenizes immune state during different physiological status[25].

Since in the previous studies, the compatibilities of the high intensity trainings, the effects of taper and *NS* use during maturation period as to the inflammatory response of the lung did not come to a clear conclusion, this study was designed to investigate the effect of the performing different patterns of taper following a high-intensity interval training (HIIT) as well as influence of interactive effect of taper and *NS* on the microscopic properties of inflammation in lung tissue of male Wistar rats during maturation period.

Material and method

Animals

In this study, 95 male Wistar rats with an approximate age of 3 weeks and an average weight of 68±9 g were obtained from Pasteur Institute of Iran. In order to adaptation, animals (5 rats per cage) were maintained in transparent polycarbonate cages under controlled environment with a temperature of 23±2 ºC, humidity of 45-55 % and 12:12 hours light/dark cycle for two week. Throughout all stages of the study, ethics of working with laboratory animals such as free access to standard pellet diet and water *ad libitum*, euthanizing without pain, prevention of pain associated with surgery and sampling were taken into consideration according to the international recommendations about clinical and laboratory animals' researches, ratified in Helsinki and updated in 2008 by the American Physiology. After one weeks of familiarity with laboratory and manipulation, the rats were randomly divided into two control and exercise groups matched for their weight.

Exercise training program

The rats were divided into two control and exercise groups at first. Then, for getting acquainted with the treadmill, they performed the exercise training with the main pattern training including increasing interval training (IIT) but with a lower intensity for two weeks. After one week period of getting acquainted with the environment and the treadmill, they performed increasing interval undulating exercise training for six weeks and that was followed by 3 weeks of load reduction (taper) carried out by 3 models in two different times. Treatment and un-treatment with *NS* supplement was also observed during the tapering (Table 1). Finally, according to research design and sampling procedure after 6 weeks increasing interval exercise training, the first and third week of tapering, the animals were divided into 19 groups and each group included 5 rats.

The familiarity and compatibility phase included 4 sessions of interval exercise training per week with the speed of 10 to 25 meters per minute and the slope of zero percent lasting for 15 to 30 minutes. The increasing interval exercise training program was carried out in the form of 10 repetitions of 1 minute length and active rests of 2 minute length minutes in such a way that the total daily workout time for each rat was 30 minutes-long. The animals started the increasing interval exercise training at the speed of 25 m/min and ended it at the speed of 70 m/min[26]. Apart from the main activity, 5 minutes was estimated for warm-

Table 1. General specification of research protocol.

Weeks of training	Familiarization	1	2	3	4	5	6	7	8	9
Age (week)	4	5	6	7	8	9	10	11	12	13
Control	Control orientation	Control						taper control with *NS*		
								taper control without *NS*		
Interval training	Orientation	Training period						taper with *NS*		
								taper without *NS*		

Table 2. Increasing interval 6-weeks training program.

Week	Familiarization	First	Second	Third	Fourth	Fifth	Sixth
Training speed (m/min)	10-25	25-35	30-45	45-55	50-65	60-70	65-70
Rest speed (m/min)	10	10-20	15-25	25-30	25-35	30-35	30-35
Training duration (min)	1	1	1	1	1	1	1
Rest time between replications (min)	2	2	2	2	2	2	2
Set number	10	10	10	10	10	10	7
Session number per week	4	5	6	6	6	6	5

Table 3. Three weeks training program for reduced training load.

Groups	Last week pattern of increasing interval training All groups	Taper program		
		First taper (volume)	Second taper (frequency)	Third taper (intensity)
Training duration (min)	70	70	70	50
Rest duration (min)	25	25	25	25
Training duration (min)	2	2	2	2
Rest time duration (min)	10	7	10	10
Replication	6	6	4	6

ing up and 5 minutes for cooling down. This program was conducted in 6 weeks and each week included 6 sessions (Table 2). Following the increasing interval exercise trainings, the rats entered the taper phase in which *NS* supplement was used for the taper and control groups[27].

Preparation of hydro-alcoholic extract

Fifty five grams of *NS* powder was weighed with a scale of 0.001 precision and then was soaked in 30% distilled water mixed with 70% ethanol solution for 72 hours. During this period, the container of the solution was well sealed with paraffin and was kept at 20 to 25 ºC room temperature. The mixture was stirred with a glassy rod every six hours. After this period, the mixture was filtered through Whatman filter paper and its solvent was removed by mild temperature rotary (under 60 °C). Control and *NS* tapering groups was treated by extract via oral gavage at a dose of 500 mg/kg body weight.

Tissue sampling and histological studies

At the end of six week period of increasing interval exercise training, and at the end of the first and the third week of tapering (Table 3), the animals were euthanized with a mixture of ketamine hydrochloride (50 mg/kg) and xylazine (10 mg/kg), intraperitoneally and left lung was removed and fixed in a 10% buffered formalin solution. Lung tissue samples were dehydrated by passing through a graded series of ethanol and cleared by xylene and impregnated by paraffin. Tissue processing was done by histokinette 2000 (Lica, Germany) and samples were embedded in paraffin blocks. Then, 20 to 25 non-serial 5 µm sections from each block were obtained using rotary microtome and stained with hematoxylin-eosin (H&E). For quantitative and qualitative microscopic analysis of lung tissue, at least 10 microscopic fields from each section were examined at × 400 magnification using point counting and based on systematic uniform random strategy and unbiased stereological studies by a version 9 stereo-investigator system software (MBF Bioscience, Micro Bright Field, Inc., Germany). In each microscopic field, 0.016 mm^2 of lung tissue were analyzed. Inflammation index of lung tissue was evaluated using grading scale described by Braber *et al.*[28] based on the frequency and manner of the inflammatory cell presence. A value of 0 was assigned when no inflammation was detectable, a value of 1 was adjudged for occasional cuffing with inflammatory cells, a value of 2 when most bronchi or vessels were surrounded by a thin layer (one to

five cells thick) of inflammatory cells, and a value of 3 was given when most bronchi or vessels were surrounded by a thick layer (more than five cells thick) of inflammatory cells. Total lung inflammation was defined as the average of the peribronchial and perivascular inflammation scores[28]. All analyses were carried out by one evaluator who was blinded to the treatment groups.

Statistical analysis

All statistical analyses were performed using the SPSS software version 21. For the analysis of normal distribution of data, Kolmogorov-Smirnov test and for the comparison of the variables among groups, a two-way analysis of variance (ANOVA) followed by LSD post hoc test were used. Descriptive statistical data expressed as mean ± SD; differences of $p \leq 0.05$ was considered as significant and the rejection of null hypothesis.

Results

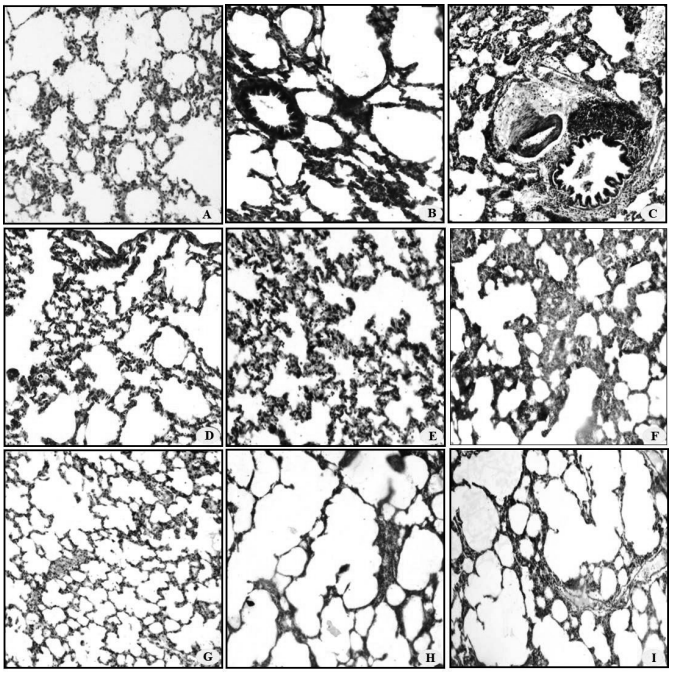
The study of histological structure of lung tissue has revealed that the lung parenchyma was normal in both control and basic groups (Figure 1-A and B). Structural integrity of lung tissue in taper groups treated by *NS* was greater and better than in the taper groups without *NS*. Furthermore, among the groups with *NS*, the third week taper groups had a better structure and a more integrated alveolus wall. The results of microscopic investigations showed that IIT had a significant effect on the lung tissue of rats during maturation period. These results indicated that the IIT caused severe inflammation in lung tissue and infiltration of inflammatory cells and lymphocytes into the connective tissue around the respiratory airway, vessels and interstitial lamellae (Figure 1-C). The taper exercises could decrease these damages in the lung tissue (Figure 1-D to 1-F). Although a small amount of emphysema, and mild interstitial inflammation was observed in some taper groups , this damage was less in the groups with *NS*, but the relative inhibitory effect of all three taper types were rather good (Figure 1-G to 1-I). Among these groups, the taper group treated with *NS* in the third week showed a better improvement in comparison to the other groups.

As it is shown in Figure 2, results indicated that the severity of lung tissue inflammation in control groups increased until the second week of tapering (age of 13 weeks) and then decreased. The severity of lung tissue inflammation in control groups without *NS* increased as similarly and significantly as in the basic and interval control groups (age of 11 weeks), (p=0.002). A similar and significant increase of inflammation has also been observed in the second week control animals with *NS* comparing with the basic and interval control groups (p=0.02). There was not a significant difference in the severity of lung tissue inflammation in the second week taper groups (age of 14 weeks) with and without *NS* comparing with the basic group (respectively p= 0.07 and p=0.30).

Figure 3 indicates that the implementation of the 6- week undulating and IIT (Table 2) during maturation period caused the most severe inflammation in lung tissue (grade 3) and the occurrence of this inflammation was similarly and significantly more frequent in comparison to the control and basic groups.

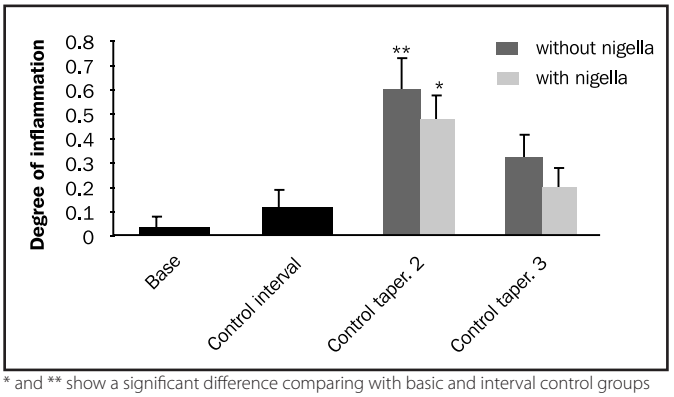
The results showed that the implementation of the three kinds of taper exercise training programs (frequency, repetition, intensity) following the IIT, could significantly decrease the amount and severity of lung tissue inflammation (p=0.001) in the studied times in comparison to interval training (Figure 4). After two weeks of frequency, repetition and intensity tapering, the degree of lung inflammation decreased 32, 49 and 52 percent respectively compared to the interval training and this decrease continued until the third week of the tapering (51, 52 and

Figure 1. Microscopic view of lung tissue in different groups (H&E, ×200).



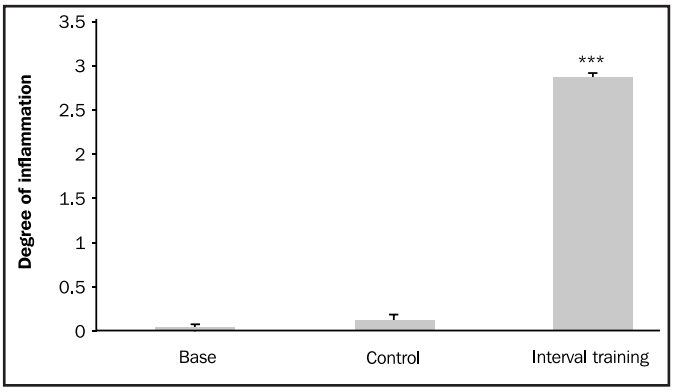
A) Normal lung tissue in the basic group; B) Lung tissue in the interval control group; C) IIT group with aggregation of lymphoid tissue and inflammatory cells around respiratory air way (solid arrow) and vessels (hollow arrow); D) two weeks control group without *NS*; E) two weeks frequency taper group without *NS*; F) two weeks repetition taper group without *NS*; G) three weeks frequency taper group with *NS*; H) three weeks repetition taper group with NS; I) three weeks intensity taper group with *NS*.

Figure 2. Comparison of lung tissue inflammation in control groups.



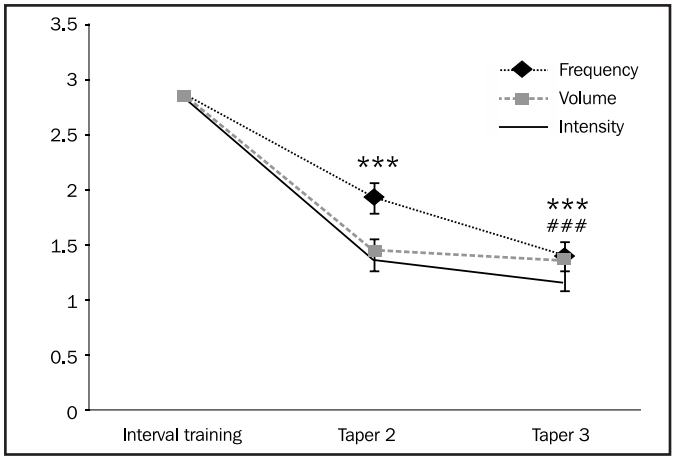
* and ** show a significant difference comparing with basic and interval control groups (respectively p<0.05 and p<0.01).

Figure 3. Comparison of lung tissue inflammation in the basic, control and increasing interval training groups.



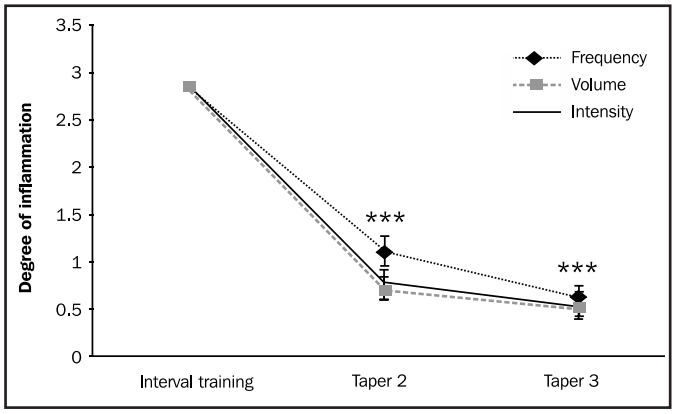
*** shows a significant difference (p<0.001) comparing with basic and interval control groups.

Figure 4. Comparison of lung tissue inflammation in interval and frequency, repetition and intensity taper groups without *N. sativa* over two and three weeks of tapering.



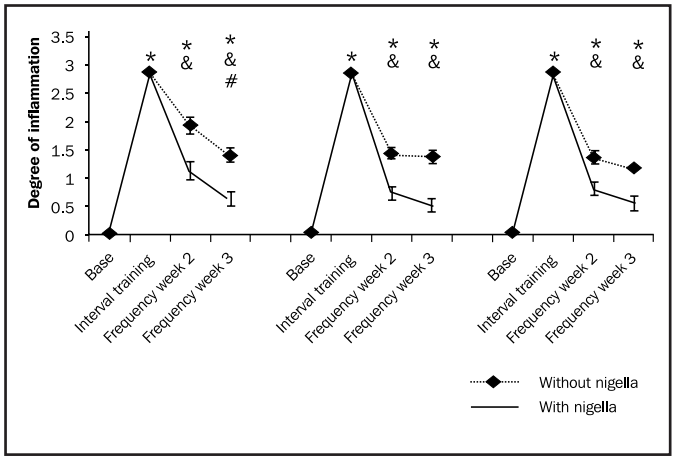
*** shows a significant difference (p<0.001) in three type of tapering comparing with increasing interval training group. ### shows a significant difference (p<0.001) in third week comparing with second week of frequency taper group.

Figure 5. Comparison of lung tissue inflammation in interval groups and frequency, repetition and intensity taper groups treated with *N. sativa* during the taper period.



*** shows a significant difference (p<0.001) in three type of tapering comparing with increasing interval training group.

Figure 6. Comparison of lung tissue inflammation in all groups of the study.



* shows a significant difference comparing with basic group. & shows a significant difference comparing with increasing interval training group. # shows a significant difference between 2nd and 3rd week of frequency taper group.

59 percent respectively in frequency, repetition and intensity taper). The results as summarized in Figure 4 also revealed that, compared to frequency and repetition taper groups, intensity decreasing taper group acted more effectively in the reduction of lung tissue inflammation.

The evaluation of the time effect on lung tissue inflammation showed that lung tissue inflammation was reduced 27, 6 and 15 percent respectively in the frequency, repetition and intensity taper groups of the third week compared to the second week (Figure 4). This reduction was significant only in frequency taper (p=0.001, p=0.60 and p=0.195 respectively in frequency, repetition, and intensity groups).

The evaluation of interactive effect of *NS* hydro-alcoholic extract use (as a supplement) and the implementation of different patterns of taper revealed the same amount of decrease in lung tissue inflammation as the groups without *NS* (Figure 5). The frequency, repetition and intensity groups, with a decrease of respectively 61%, 75% and 72% in the second week and a decrease of 77%, 82% and 80% in the third week, showed a similar and significant decrease in lung tissue inflammation comparing with interval exercise training group (p=0.001). In the survey of the time effect, a decrease of 43%, 28% and 30% in lung tissue inflammation was observed in the third week frequency, repetition and intensity taper groups compared to the second week taper groups. This change was significant only in frequency taper group (p=0.002, p=0.195, p=0.120 respectively in frequency, repetition, intensity groups).

The results of the present study showed that the degree of lung tissue inflammation in all taper groups was significantly higher comparing to their counterpart (p=0.04) and basic (p=0.001) groups. As it is shown in Figure 6, the use of hydro-alcoholic extract of *NS* in the taper groups could decrease the amount of lung tissue inflammation caused by interval exercise. Lung tissue inflammation in the frequency, repetition and intensity groups treated with *NS* in the second week decreased

respectively 42%, 54%, and 50% comparing with the groups untreated with *NS* and this decrease was respectively 62%, 41% and 52% in the third week. In short, it can be concluded that the increasing interval exercise along with the implementation of intensity and repetition tapers and using the hydro-alcoholic extract of *NS* in a time period of three weeks had the most decrease in lung tissue inflammation.

Discussion

This study analyzed the microscopical changes and structural remodeling of lung tissue caused by inflammation in it through implementation of a period of increasing interval exercise followed by different patterns of tapering during maturation period. Since changes in the control groups can indicate changes in the lung tissue during maturation period, this study was designed to analyze the maturation process as well. The age range for the research protocol was 5-14 weeks. According to Sengupta[14] this age range in rats is equivalent to the age range of 6-16 years old in humans. Therefore the male rats in the control groups, treated and untreated with *NS*, were in their maturation period in the second week of tapering. It is assumed that the significant increase of lung tissue inflammation in these groups is due to being in the critical period of maturation which is related to developmental changes of immune system brought about by maturation and increasing efficiency of antigenic system[9].

Our results showed that the implementation of increasing interval exercise during maturation period causes severe lung tissue inflammation and leads the inflammatory and lymphoid cells into it. Although the amount of lung tissue inflammation in different periods and different patterns has decreased in animals both treated and untreated with *NS* hydro-alcoholic extract, but it is still significantly higher in comparison to the basic and control groups. It is concluded that this is because of the downfall of immune system which occurs following a high-intensity exercise over a long period of time[5].

Although several different studies have been done about the influence of high intensity exercise on the immune system and the inflammatory factors in the body, but this study is unique for the microscopical analysis it makes on the influence of high intensity exercise on the immune system of lung tissue and occurrence of inflammation in it, using histological and stereological methods. A few studies have been done about the effects of high intensity exercise on immune system function The presence of pulmonary macrophages is very essential in adjusting the acute and chronic inflammatory responses and the call of into the spreadable site of inflammation in the lung[29]. Michna *et al*.[30] observed that, after a period of intense training, the immigrant peritoneal macrophages of humans and mice had a better performance in responding to the chemotactic factors. The previous studies showed that acute and chronic exercise training has an effective catalytic role in many macrophage functions. On the other hand, the increasing rate of macrophage function varies according to the intensity and duration of exercise[31].

Previous study by Sobhani *et al*.[32] showed that HIIT in maturing rats cause in airway narrowing of the lung parenchyma. Yadegari *et al*.[33] also indicated that HIIT leads to parenchymal remodeling in lung tissue by induction of inflammation. Our recent research revealed that six weeks HIIT significantly increase number of alveolar macrophage in lung tissue[34]. Review of Ramel *et al*.[35], Murphy *et al*.[36] and Yamamoto *et al*.[37] studies suggested that high intensity physical activities increases the number of neutrophils, while this increase has not been observed in low intensity physical activities.

Some studies showed that, the function of lymphocytes, in long periods, is sensitive to the increase of exercise intensity in endurance activities[38,39]. It can be concluded that, high intensity exercises decrease the function of lymphocytes and macrophages due to an increase in the circulation of stress hormones, especially cortisol, and a change in the balance of pro-inflammatory or anti-inflammatory cytokines when responding to the exercise training[38]. In a study conducted on 18 swimmers on the national level and 11 healthy untrained volunteers, it was found out that the number of monocytes, neutrophils and dendritic cell subsets as well as the amount of IL-1β, IL-6, and IL-12 decreases in these athletes during the training season. The results of this study supported the idea that long-term high intensity exercise may affect the innate immune cells function, reduce their capacity in responding to acute challenges, and increase the risk of URTI[40].

Previous studies have shown that daily repeated physical activity during long periods, in athletes and especially endurance athletes, induces damage to the epithelium cells and increases the inflammation in their respiratory mucosa[41]. Thus, it can be concluded that despite of multiple mechanisms of innate and adaptive immunity being there, the implementation of high intensity exercise training, weakens the immune system of the lung tissue. The reported severity of inflammation in the lung tissue of IIT animals in this study also confirms this possibility.

For a reduction of disorders in immune system, physiological capacity and mood state profiles of athletes following a long-term and intensive exercise, performing a taper with a gradual reduction in the load of exercise can be recommended by the sport trainers to the athletes as an appropriate approach[13]. The results of this study showed that the implementation of taper patterns (frequency, repetition and intensity) after a period of IIT, could significantly decrease the lung tissue inflammation caused by intensive exercise training but the inflammation was still significantly higher in comparison to the control group after 3 weeks of taper. Regarding the time effect of the taper, the implementation of a three-week repetition and intensity taper was more effective in enhancing the immune system and reducing the lung tissue inflammation subsequent to intensive exercise than a two-week taper. The results of this study were in line with previous studies[3,42].

Mujica *et al*.[42], having observed the trained athletes during 1-3 weeks of taper, reported enhanced performance often accompanied by increased anabolic activity, reduced physiological stress and restoration of mucosal immunity and immune function. It has also been shown that a 6-day taper in the middle-distance runners improved the performance in 800 meter runners[16]. Two weeks taper in triathletes[43], one week taper in rugby league players[44,45] and two weeks taper in judo athletes[11] resulted in increased T/C ratio and improved performance. It can be concluded that the recovery or the enhancement of immune system function during taper is dependent on the amount of immune system diminution during intensive exercises[3].

On the other hand, by the increase of volume and intensity of training during the pre-competitive season, sport trainers will also concern

about serious matters other than increased risk of sport injuries and URTI development. One of these concerns is the increasing tendency of athletes to take sport medicines and chemical supplements, some of which are completely ineffective in long term use[3]. Herbal drugs and supplements as natural treatment (complementary treatment) with fewer side effects and multiple properties can be the best alternative for athletes[46].

In the last three decades, extensive research has been done on the biological effects of *NS* seeds. In numerous scientific articles, the antioxidant, anti-inflammatory, immune booster and antihistamines properties of numerous compounds in *NS* hydro-alcoholic extract have been pointed out[47]. One of the special features of *NS* is its role in regulating immune function in treadmill exercised rat[25]. The effect of *NS* hydro-alcoholic extracts use on the reduction of lung tissue inflammation[23,24] specially induced by intensive exercise training has also been observed in this study. While the implementation of different patterns of taper reduced the lung tissue inflammation, but *NS* hydro-alcoholic extracts use enhanced this reduction.

Although the amount of lung tissue inflammation in the animals significantly decreased in all three types of taper treated with *NS*, in comparison to their counterparts, but the implementation of three weeks of intensity and repetition taper accompanied by *NS* hydro-alcoholic extracts use, was more effective in the reduction of lung tissue inflammation induced by intensive exercise. Previous studies have indicated that some of *NS* compounds have the effect of reinforcing the cellular immunity[48]. Thymoquinone's anti-inflammatory properties, the major compound of *NS* extract, works through the suppression of inflammatory mediators such as prostaglandins and leukotrienes[49,50].

This study is among the few studies that examine the effect of taper on lung tissue safety mechanism during maturation period. The results of this study showed that although increase of interval training intensity has been undulating and gradual but the immune system of the lung tissue is not able to cope with that and it causes severe inflammation in the lung tissue. This problem during pre-maturation period may have a negative effect on the performance of the athletes and the results of the competition or even negative effects on the future of their sport. Our results indicated that the implementation of three types of taper decreases the lung tissue inflammation induced by IIT. It can be concluded that a reduction in the load of exercise alone can compensate for the induced weakness, or enhance the immune function so that the lung tissue inflammation decreases. But since the interactive effect of *NS* hydro-alcoholic extract use and taper was more effective in the reduction of lung tissue inflammation, we can conclude that reducing the exercise load accompanied by *NS* hydro-alcoholic extract use associated with particular anti- inflammatory properties, has a more prominent role in safety mechanism of lung tissue. Thus the implementation of taper along with *NS* hydro-alcoholic extract use will enhance the immune system of the lung tissue during maturation period and subsequently reduces the induced damages. It may also make the sport life span longer. Decrease of the intensity or frequency of the exercise load is a good pattern for taper program. However the results of the study revealed that the implementation of 3 weeks taper is more effective in the reduction of lung tissue inflammation than 2 weeks taper. It is suggested in the future studies, Changes in various factors of innate and adaptive immunity of lung tissue in pre-maturation period induced by increasing interval exercise or taper as well as the optimal taper duration in human models should be studied.

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Conflict of interest

The authors do not declare a conflict of interest.

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