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The effect of inhaling peppermint essence on some immune responses and cortisol follow one bout of exhausting exercise in female athlete.

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ABSTRACT

Previous research has indicated that odorant presentations can have both positive and negative effects on psychological perceptions of athletic task performance. Some of the scientists found that the presence of pleasant odor in a testing room enhanced performance on cognitive and physical tasks (push-ups and 400-m run, hand grip) and led to subjects indicating more motivation and interest in the task. For many years menthol has been used for treatment of respiratory disorders although, a bronchodilator effect of menthol has yet to be described, and any significant difference was not found in oxygen consumption. Inhaling peppermint is reported to be a stimulant for increased energy that would certainly benefit any athletic or non-athletic individual during an exercise bout.

The purpose of this research was to determine the effect of peppermint odor on VO_2max, minute ventilation, the respiratory Exchange Ratio (R), cortisol and salivary IgA. Subjects of this study were 36 female elite soccer players with mean age: 21.17 ± 3.15 years, height: 160.12 ± 6.24 cm, weight: 56.78 ± 8.76 kg and resting heart rate: 77.39 ± 7.88 bpm. They were divided into three groups randomly: control, inhalant peppermint essence and inhalant peppermint essence + alcohol. Kolmogorov-Smirnov Test showed that the distribution of data in groups were normal. Subjects' BMI were distinguished and ANOVA showed no significant difference in the mean of BMI of the groups ($p > 0.05$). According to Bruce recipe the subjects did exercise on treadmill till exhaustion. Heart rate during exercise, VO_2max, minute ventilation and R were measured by Gas Analyzer machine (k4b2). After the test subjects' salivary were collected to define salivary cortisol and IgA. Statistics analysis for independent groups was done by ANOVA ($p \leq 0.05$). It showed that inhalation of ambient odor (peppermint essence) doesn't have any significant effects on mentioned variables ($p > 0.05$), probably for intensity, time of exercise. So we suggest determining the effect of inhalation peppermint odor on exercise with longer time and lower intensity.

KEY WORDS: peppermint essence, VO_2max, the respiratory Exchange Ratio, cortisol, IgA and female elite soccer player

INTRODUCTION

Nowadays, utilization of ergogenic aids is spreading among athletes. Recent studies have indicated that consumption of unauthorized ergogenic aids endangers the athletes' health. In sporting competitions, the shortest time, the highest power, and increased time of exhaustion are among the most important factors that guarantee the athletes' success. In order to achieve this goal, consuming authorized supplements along with regular exercise and proper diet can improve the athletes' performance [14]. Available methods include using aromatherapy, carbohydrate and caffeine supplement to increase endurance performance and sodium bicarbonate loading due to its potential ergogenic effect on short-term events, creatine supplements, anabolic steroids, music, and so on [14, 15]. Consumption of carbohydrates before and during competition is permitted. Furthermore, consuming caffeine is allowed up to urinary concentration of 12 mcg/ml (US Olympic Committee) and 15 mcg/ml (National Institute of Student Athletes), and consuming higher doses before competition is forbidden [14]. Due to availability of enough literature on its effects on short- and long-term activities, creatine supplement is permitted without assigning any guidelines [14]. Replacing synthetic substances with natural compounds can improve the athletes' physical fitness and enhance vitality and health. Nowadays, plants are used for different purposes. Menthol oil (one of the effective compounds in peppermint oil) is a natural aromatic compound whose consumption may cause relaxation of muscles, an increase in power and physical performance, and decrease in fatigue [1, 6, 7].

Aromatherapy is an important ergogenic method that has been developed in recent years and attracted many physicians [14, 16]. Lately numerous studies have been conducted on the effect of aromatic compounds

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on mental characteristics [9, 13, 17]. Raudenbush studied the effects of olfactory stimuli like peppermint oil, fragrance of jasmine, and dimethyl sulfide on the physiological performance of cardiovascular system among student athletes and concluded that they have no significant effect on heart rate and oxygen consumption [12]. Furthermore, recent research has indicated that inhalation of aromatic plants like peppermint positively affects the individuals' athletic capacity and aerobic and anaerobic power[3]. Peppermint contains menthol, methyl-ester, manthon, menthyl acetate and terpenes. Its untreated oil contains demethyl sulfide. Results of different studies have indicated that in the presence of menthol aroma, satisfaction about performance is higher and fatigue is lower. Researchers concluded that inhalation of menthol is effective in increasing motivation and temper and causes satisfaction with subjects' performance, energy, speed, watchfulness, and strength[2, 5]. The commonest application of menthol in the field of pharmaceutics is to alleviate typical symptoms of cold like cough and pain in the chest and sterilize respiratory tracts. Menthol has long been used for alleviation of respiratory disorders. There are also much medical evidence for beneficial effects of this substance on various intensities of cough and cold[1]. However, it effect on dilation of the bronchus is not clear [19]. On the other hand, it has been reported that athletes that are prone to infectious diseases especially infection of upper respiratory tracts are more prone to such disorders during exercise. Carrying out intense exercises causes reduction in resting levels of leukocytes, lymphocytes, cytotoxic activity, serum and secretory immunoglobulin concentration, neutrophils, and plasma glutamine concentration.

Reduction in the number of lymphocytes T after exercise and further decrease in the cells type 1 of T cells may be due to high level of plasma epinephrine. Relatively higher reduction in cells type 1 compared to cells type 2 of T cells in the recovery period might be because sensitivity to infections caused by viruses are observed after very intense exercise. The exercise-caused change in the level of salivary immunoglobulin A (S-IgA) that is the main agent in the mucosal immune system is directly related to occurrence of infection in upper respiratory tracts[4]. Conducting exercises with moderate intensity improves efficiency of the immune system [3] while with high intensity it inhibits some performances of the immune system [4]. In better words, intense physical activities have suppressive effects on the immune system [5]. In this regard, one can refer to reduction in the number of lymphocytes, number and activity of natural killer cells, and production of antibody [6]. In some cases, immunosuppression is so outstanding that the body status is likened to an open window in which clinical infections are highly expected [7]. Although IgA accounts for only 10-15% of the total serum immunoglobulin, the major part of immunoglobulin locates in mucus secretion and levels of IgA in mucus liquids relative to serum antibodies are related to the resistance of against upper respiratory infections.

Since S-IgA is the first barrier against pathogens in oral cavity and upper respiratory tract and inhibit bacterial adhesion, uptakes antigen across mucosal surfaces, and neutralizes toxins and bacteria [9]. Different studies have been conducted on the effects of exercise on acute and chronic response (resting levels) of the mucosal immune system. A review of the related literature indicates contradiction in the results and findings. Cross-country skiers were reported to have lower concentrations of S-IgA after competition. Reduction of S-IgA has been reported after swimming with high intensity, running, and running with increasing intensity on treadmill until exhaustion. On the other hand, it has been reported that submaximal exercise has no effect on S-IgA [19] and some studies have reported reduction of S-IgA as a result of one session exercise [11, 12, 13, 14]. However, other studies indicated increase [15, 16, 17, 18] or no change [19].

Cohen and Dressler (1982) studied the effect of aromatic vapors like menthol on the internal diameter of the airways in volunteers that suffered from severe cold by measuring forced expiratory volume, peak expiratory flow rate, and total airway resistance. They reported improvement in the internal diameter of the airways as a result of 20-60-minute breathing aromatic vapors [4]. Wright et al (1997) reported that menthol significantly decreases the increased resistance of airways caused by capsaicin injection [19]. With these properties, peppermint can be considered as one of the agents that helps the individual's mental and physical capacity increase [14]. It has been reported that inhalation of peppermint oil can be a motivator for increasing the athletes and non-athletes' energy during exercise [2]. However, there are few studies that reject or support this issue [13]. Therefore, according to the little and contradicting information on the effect of peppermint essence on physical and pulmonary performance, the present study was conducted in order to respond to this question, "What are the effects of inhalation of peppermint essence on the cellular performance and production of antibody and some cardiorespiratory variables?"

In order to enhance performance, athletes are known to try a variety of aids to enhance performance and boost their chances of winning. Nowadays using ergogenic aids are common in athletes. New researches indicated using of these aids can enhance the risk of many illnesses. In sport competitions time of competitions, power of athletes and the time of recovery are the most important factors that can affect the athletes' success. So, by attention to the problem that the effects of inhaling peppermint odor on mucosal immune system isn't examined and insufficient and contradictory data is seen about the way of affecting on respiratory system, present study performed to examine the effect of inhaling peppermint odor on acute response of mucosal immune system and some of the respiratory system's indexes.

METHOD

Subjects

36 female soccer players of first and second team of Islamic republic of Iran super league have participate in this study. They divided into three groups (experimental and control) randomly. Then the aim of the study has explained for them and following an explanation of the risks and benefits of participation. Each subject signed a written consent statement. None of them had respiratory disorders, allergic history and didn't use any medicinal drugs. The individual properties are shown in table 1.

Table 1. The individual characteristics of subjects (M±SD)

	BMI	FAT%	LEAN Body Mass (Kg)	HR(bpm)	Weight (Kg)	Height (cm)	Age
Control	22.78	25.1	41.04	75.20 ± 6.75	59.96 ± 9.69	161.65 ± 6.94	**21.50 ± 1.65**
Peppermint Essence	21.9	24.47	42.35	77.08 ± 9.68	58.42 ± 8.02	161.90 ± 5.82	**21.85 ± 4.02**
Essence + ethanol	21.625	26.93	38.85	79.38 ± 6.70	53.29 ± 8.27	157.15 ± 5.36	**20.23 ± 3.03**
Total	**22.10**	**25.51**	**40.75**	**77.39 ± 7.88**	**56.78 ± 8.76**	**160.12 ± 6.24**	21.17 ± 3.15

Breath by breath pulmonary gas exchange

For measuring and evaluating breathing gases and VO_2max we used Gas Analyzer (model.K4b2, COSMED Company, Italy). After calibrating, a mask which was specified for collecting breathing gases, was set on subjects' face and ask them to do the test (Bruce Test). This mask remained on subjects' face during the test. The instrument collects and analyzes breathing gases automatically and recorded the results each two seconds.

Body Composition

For measuring subjects' fat mass and lean body mass we used Body Composition machine (In Body 3.0, Finland).

Peppermint essence Preparation

Peppermint plant is a part of herbal drugs which has antibacterial property that can use for treatment of cold, laryngitis, bronchitis, cough, whooping-cough [10]. It can also use for relaxation of active muscles. Inhaling the essence of this plant can be useful for asthma.

For producing the peppermint essence for this study, 100gr fresh leaves of peppermint plant (menthe piperita) was collected from Iranian National, Research and technology network for medicinal plants (NRTN-MP). At first it was analyzed with gas chromatograph machine (CG, model: shimadzu), then the essence was produced in an hour (Fig.1)

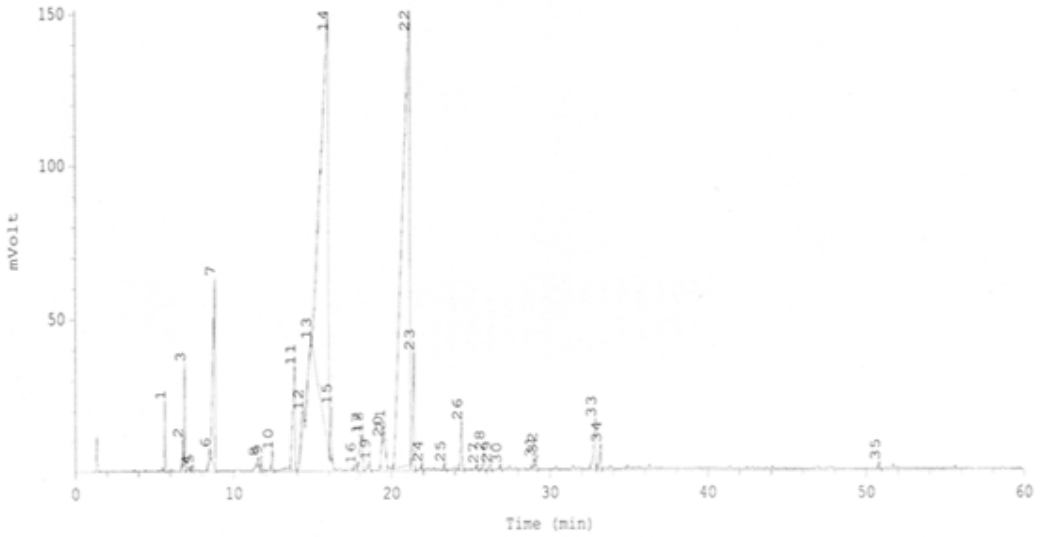

Fig1. The peppermint analyzation report with gas chromatograph machine.

Due to inhaling peppermint essence headache, vertigo and dizziness may be occurred. So for increasing evaporation ability we used alcohol 98%. To prevent the effect of alcohol on variables, we proposed a group to run with inhaling alcohol as placebo.

Measurements

Prior to data collection, subjects were familiarized with the laboratory setting and the mask that was used to collect expired gases during each trial. Oxygen consumption [VO2], maximum oxygen consumption [VO2max], minute ventilation [VE] and respiratory exchange ratio [RER] were obtained via breath by breath pulmonary gas exchange [Gas Analyzer]. HR was recorded using a Polar Heart Rate Monitor. A motorized treadmill [Technogym model: via G.perticaria,20,47035Gambettola(Forli), Italy] was used for the exercise mode. However the aroma was administered in a double blind cross over design.

After acclimation to the laboratory of national Olympic committee, subjects rested for 15 minutes for measuring resting heart rate then subjects' body composition was measured. Afterwards 2 drops (0.1ml) essence or the mixture of essence and alcohol or placebo (aquapura) set on the sterile cotton pad and set under the nose of each individual. Subjects inhaled the aroma with only nose breathing. When the special mask of Gas Analyzer was set on individual's face they were asked to begin the protocol of study that was running on treadmill according to Bruce protocol. Rate of perceived exertion (RPE) was obtained using the modified Borg scale. Heart rate was recorded per minute during the test. Salivary samples has been collected at rest, immediately and 15 minutes after the test.

For preventing effective factors all of the measurements were done in the same location and time and one technician who was not present for the trials. In Fig.2 all of the stages in collecting data were shown.

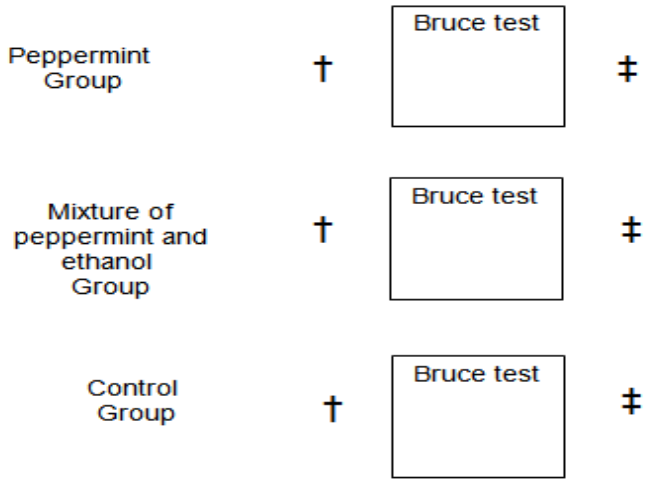

Fig 2.Time line of experimental design.† indicated measurement of descriptive characteristics. ‡ indicated measurement of time of running.

Statistic analyzing

Means and standard deviations were calculated for all variables. For comparing the effects of inhaling peppermint odor and the mixture of peppermint and ethanol on dependent variable independent one way ANOVA was used and when significant difference was seen, tukey test was done. Before these analyses were performed, the frequency distributions were tested for normality using the Kolmogrof-Smirnov test. The level of significance was set at ($P \leq 0.05$).All analyses were run by SPSS for Windows.

RESULTS

Cortisol

The first result of present study showed that a session of running until exhaustion with inhaling peppermint essence ($F_{2,18}=1.08, p=0.324, \mu^2=0.108$), mixture of peppermint and ethanol ($F_{2,20}=1.30, p=0.293, \mu^2=0.115$) and placebo ($F_{2,22}=1.11, p=0.345, \mu^2=0.092$) doesn't affect salivary cortisol.

Comparing these three groups at resting time showed a significant difference between salivary cortisol concentrations ($p=0.003$). Tukey test showed that this difference was between peppermint and mixture of peppermint and ethanol as cortisol concentration was higher in peppermint group. Also significant difference was seen between three groups immediately after the exercise ($p=0.003$) that this difference was between peppermint group and control, peppermint group and the mixture of peppermint and ethanol group. A significant

difference was seen in 15 minutes after the exercise (p=0.003) that this difference was between peppermint group and the mixture of peppermint and ethanol group (Fig 3, part A).

Immunoglobulin A (IgA)

Inhaling peppermint odor ($F_{2,18}$=0.168,p=0.846,μ^2 =0.018), the mixture of peppermint and ethanol ($F_{2,20}$=0.146,p=0.849,μ^2=0.014) and placebo ($F_{2,22}$=1.04,p=0.343,μ^2 =0.087) didn't affect salivary IgA at rest and immediately after the exercise. But 15 minutes after the exercise a significant difference was seen between control group and the mixture of peppermint and ethanol ($F_{2,30}$=4.68,p=0.017) (Fig 3, part B).

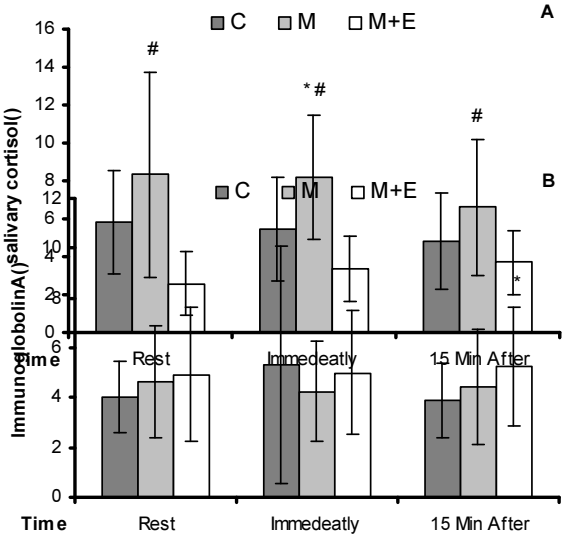

Fig 3. Changes in salivary cortisol (Part A) and IgA (Part B) in response to one session of running until exhaustion.

* Significant difference with control group
\# Significant difference with the mixture of peppermint and ethanol group

Other results are shown in table 2.

Table 2. Other results of subjects

	control	peppermint	Peppermint and ethanol	sig
Vo2max	29.25±3.03	31.54±5	31.20±5.19	0.404
Minute ventilation	92.39±10.43	92.07±9.01	86.30±15.10	0.371
Time of running(sec)	535.10±78.74	593±62.98	560.23±74.23	0.167
RER	1.87±0.21	1.72±0.23	1.82±0.18	0.865

DISCUSSION

Previous studied showed that peppermint odor can improve some aspects of physiological performance [12]. But present study didn't show any significant difference between groups. Quantity and quality of essence that used can affect the results. It is not clearly obvious that how much and how to use essence have the most effects on athletics' performance.

The results of present study about the effect of aromatherapy on exercise performance may be unpromising but by paying attention to intensity and duration of exercise it will be more understandable. By this method Simpson et al 2001 obtained same results [14].

By comparing Vo2max and minute ventilation among groups, significant differences weren't seen which is similar to the results of other researches[8, 10,14,16,18].

Researchers believe that many factors can affect the results of these studies that duration of exercise is one of them. A 12-14 minute exercise doesn't have sufficient stimulants for affecting physiological values in groups that inhaling peppermint odor. Although the mechanism that have main role in aromatherapy and metabolism is still unknown. When the duration of exercise is short scented combinations don't have significant affects[14]. By paying attention to this fact that the effects of menthol on human body isn't clearly obvious yet, Cohen and Dressler(1982) reported that inhaling menthol improved inner diameter of airways in volunteers who were suffering from cold[4]. Raudenbush (2000)believe that inhaling peppermint don't have significant effect on respiratory physiological evaluations and in some studies improved performance by increasing the

motivation[12].However some researchers believe the effect of odor combinations on physiological systems in human, but it is reported that if athlete's performance is in maximum intensity this effect will be at minimum size.Peppermint essence has a tranquillizing effect on smooth muscles in vitro but it isn't still distinguished that under which conditions peppermint has this effect completely in human's body. Peppermint essence causes diminishing of action potential's dispersion and calcium flowing and this is the effect of menthol. Researchers suggest that menthol has the same mechanism of smooth iliac muscles on bronchial smooth muscles and prevents calcium flow. On the one hand, menthol affects smooth muscles and sensory nerves; therefore, it has a bilateral function. However, there is no evidence for direct effect of menthol on bronchial smooth muscles. Therefore, it may be better to utilize aromatic compounds during physical activities in order to come up with further effect; therefore, in the long run this compound can be used as a supplement to exercise sessions in order to push the athletes to potential VO2man.

In regard with the effect of peppermint oil on running time, the results of the present study are in agreement with those of Volker et al, Kell et al, Gubalt et al who investigated the effect of inhaled salbutamol on running time among 12 healthy professional non-smoking cyclists, and Radenboush et al [2, 8, 12, 18]. Researchers have reported no significant effect of salbutamol on plasma concentrations of glucose and lipids and the activity of central nervous system. Moreover, it has been concluded that salbutamol and salmeterol has no ergogenic effect. Other researchers believe that pleasant odors cause temper to improve; therefore, when temper and motivation are closely related, increase in temper may cause motivation. In sports skills; however, if an athlete does not have necessary skill to carry out a technique, increasing his/her motivation will have no effect on his/her performance.

The results of the present study indicate that there was no significant difference between the experimental and control groups in terms of the respiratory exchange ratio following inhalation of peppermint oil, its compounds, and alcohol. In a physical activity that lasts less than 15 minutes, the body's fat cannot be considered as an energy source according to the observed respiratory exchange during the exercise. When the observed respiratory exchange during the exercise is 0.90%, only 0.33% of the lipids participate in the energy production process while in the respiratory exchange ratio of 0.95 percent, 17% of lipids are involved with metabolism. Aromatic compounds might have beneficial effects when lipids are the main path of energy production. Exercise time may also influence the results. Since Volker et al did not find any significant effect of aromatherapy on athletic performance, Simpson suggested that duration of each session should be increased up to 120 minutes and exercise intensity should be reduced, in this case it is possible that the effects of aromatherapy on athletes appear[14]. Researchers believe that the type of sports affects salivary cortisol[12,14]. Exercise intensity, homodynamic conditions, thermal stress, body posture (exercising in vertical and horizontal postures), and the level of the exercise can be effective in occurrence of these discrepancies[3]. According to the results of previous studies, suppression of the mucosal immune system is the result of increased intensity and exercise duration, and differences among the results may be due to secretion of stress hormones[4]. One of the probable mechanisms for reduction in concentration of S-IgA may be a remarkable increase in salivary cortisol level whose suppressive effect on the immune system has been proved. In the present study, there was no significant difference between cortisol concentrations. Another reason for absence of significant difference in S-IgA concentrations in testing conditions may be related to consumption of menthol-contained oil. Another reason is the method of consuming the oil. That the athlete inhales the oil during the exercise or there are different times before starting the exercise may cause different effects. If the subject inhales the oil some time before the exercise, the olfactory stimuli may takes longer to influence physiological agents. However, it is not yet clear which method has an optimal effect on conducting physical activity[5]. Therefore, it is recommended that the effect of inhaling peppermint oil on exercise of longer time and lower intensity be investigated in the future.

Conclusion

The results of the present study indicate that inhalation of peppermint oil, its compounds, or alcohol does not have any significant effects on athletes' physiological performance, which may be due to factors like duration and intensity of exercise or the amount of the oil used in the present study. One of the factors that might have influenced the results of the present study was high intensity and short duration of conducting the test. Therefore, it is suggested that different duration, intensity, and amount of oil be utilized in future studies in order to make sure of the effect of aromatherapy on athletes' physiological performance. Because nowadays hundredths of second determines an athlete's success or defeat, athletes should figure out methods to improve their performance.

Therefore, according to the results of the present study, it is recommended that inhalation of peppermint oil (menthol) while running up to exhaustion has no effect on running time, S-IgA, or cortisol.

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