

The Response of Some Indices of Delayed-Onset Muscle Soreness to One Session of Eccentric Exercise Following Short-term Rosemary Supplementation in Inactive Men

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Abstract

Introduction: One of the main problems associated with physical activity is muscle pain and soreness. Various strategies have been proposed to prevent or improve it quickly, one of which is the use of plants containing anti-inflammatory compounds. The aim of the present study was to investigate the effect of 14-day supplementation of rosemary on the response in concentration of lactate dehydrogenase, creatine kinase, lymphocyte and neutrophil counts, muscle swelling and muscle pain in inactive men after one session of eccentric activity.

Methods: In this semi-experimental study, 24 non-athlete male volunteers (mean age 26 ± 3 years; mean height 180.58 ± 8.11 cm; mean weight 81.6 ± 83.95 Kg and body mass index (BMI) 25.2 ± 13.82 kg/m²) were selected and randomly divided into 3 groups: (1) placebo (2) 0.5 ml of rosemary supplementation, and (3) 0.25 ml of rosemary supplementation. After 14 days of supplementation, the subjects performed eccentric activity on the leg press apparatus. Changes in serum levels of lactate dehydrogenase, creatine kinase and white blood cell count, and the amount of muscle pain and muscle swelling during the six stages (before and after supplementation as well as 4, 24, 48 and 72 hours after activity) were measured. For statistical analysis of the findings, one-way analysis of variance (ANOVA) and Bonferroni's post hoc test were used in SPSS software ($p \leq 0.05$).

Results: The results showed that rosemary supplementation significantly reduced the number of neutrophils in inactive men after 14 days of rosemary supplementation and 4 hours after eccentric contractions ($p \leq 0.05$), but had no significant effect on lymphocyte, creatine kinase, Lactate dehydrogenase and the amount of pain in inactive men ($p \geq 0.05$).

Conclusion: It seems that 14 days of rosemary supplementation has no significant effect on the reduction of muscle damage caused by eccentric contractions.

Keywords: Lactate Dehydrogenase, Creatine Kinase, Lymphocyte, Neutrophil, Rosemary, Exercise

Introduction

Eccentric exercise activities are a kind of sport activity that lead to muscle strength development. Although this kind of exercise is superior to exercises with isometric contractions, it is always associated with increased muscle problems (1). Muscle pain and soreness are common experiences after unusual and intense activity, especially after eccentric and resistance activities that occur in

the novice athletes and non-athletes, and so prevent them from doing physical activities (2, 3). Muscular soreness is divided into two types of acute muscle soreness and delayed onset muscular soreness (DOMS) (2). Muscle pains and cramps usually culminate within 24 to 48 hours of activity, and disappear at most 5 to 7 days later. Among the symptoms of delayed-onset muscle soreness (DOMS), we can point out muscle stiffness, inflammation and

swelling, decreased muscle amplitude, decreased muscle strength, increased reactive oxygen species, increased levels of creatine kinase (CPK) and lactate dehydrogenase (LDH) enzymes in serum and plasma as well as increased inflammatory responses (4, 5). In this vein, researchers have been investigating different approaches including the use of medicinal herbs and food supplements in order to cope with the adverse effects of delayed-onset muscle soreness due to eccentric and abnormal contractions, and to improve athletes' health and performance. One of these herbs with anti-inflammatory, analgesic and antioxidant effects that is available in our country is rosemary, which is scientifically called *Rosmarinus officinalis* (7, 8). Rosemary, in addition to its antioxidant effects, has significant anti-inflammatory properties. In the previous studies, chloroform and hexane extracts have anti-inflammatory properties at the level of indomethacin, and anti-inflammatory agents such as triterpene, oleic acid, and micrometric acid have been isolated (9). Regarding the ease of accessibility to this plant, especially in our country, and the scientific evidence of the anti-inflammatory, analgesic and anti-oxidant properties of rosemary (8 and 9), many attempts have been made to investigate delayed-onset muscle soreness (DOMS), yet the underlying cause of the incidence remains unknown. Nonetheless, the degree of damage depends on the intensity, duration and, most importantly, type of activity to be carried out (6). Also, due to the antioxidant effects of some medicinal plants, the importance of research in this subject is doubled, so the present study was conducted with the aim of investigating the effect of rosemary essence on some of the indicators of delayed onset muscle soreness in inactive men.

Methods

In this semi-experimental study, 24 inactive men aged 18 to 28 years were selected as volunteer subjects. The criteria for the subject's inclusion in the present study were

physical health, absence of chronic diseases, including cardiovascular disease, diabetes, or any disease that prohibits subjects from engaging in this research activity, as well as being non-smoker. The criteria for exclusion of the subjects included lack of attention to the recommendations of the researchers and the subjects' announcement of the refusal to continue the work. After completing the questionnaire and the consent form to participate, subjects got prepared for a briefing session for this study. Subjects were then randomly divided into three groups: (1) placebo (2) 0.5 ml of rosemary supplementation, and (3) 0.25 ml of rosemary supplementation. The rosemary supplementation groups received it every eight hours three times daily for 14 days (10). To set out a delayed-onset muscle soreness (DOMS) protocol, subjects in groups 2 and 3 first warmed up for 10 minutes and then performed 5 sets of 20 kg weightlifting with 15 repetitions with one-minute interval rests between the sets. It should be noted that in case of the inability of the subjects to weightlift, 5 kilograms of weight dropped and they continued at a weight of 15 kg (11). During a briefing session, the subjects were asked not to do any exercise 48 hours prior to the first blood sampling, and not to use any nutritional or medication stimuli, and to record their dietary schedule one day prior to blood sampling. Before supplementation, blood samples were taken at 8:00 o'clock after 30 minutes of rest. After completing fourteen-day supplementation, the blood samples were taken on the fifteenth day with the same conditions as the first stage. Subjects then performed exercise for delayed-onset muscle soreness (DOMS) and after 4 hours of exercise, the third stage of blood sampling from the subjects was performed. Fourth blood sampling was performed 24 hours later, on the day after the training with 30 minutes of rest in the laboratory at 8:00 a.m.; the fifth blood sampling 48 hours after the training in the same conditions, and the sixth blood sampling

was conducted 72 hours after the training again in the same conditions as before. All blood samples were taken from the right brachial vein in sitting position. To measure lactate dehydrogenase, creatine kinase and lymphocyte and neutrophil counts, blood samples were transferred to the laboratory of Yasouj Shahid Beheshti Hospital by maintaining a cold chain. To measure the levels of the variables, blood samples were placed in an anticoagulant chamber and after transferring to the laboratory at standard conditions, blood samples were centrifuged at 4000 rpm for 15 minutes. Afterwards, to measure creatine kinase and lactate dehydrogenase, creatine kinase and lactate dehydrogenase kits at wavelength of 340 nm were used, and lymphocytes and monocytes were measured using Mindray BC- 5300-B which was a Chinese blood cell counting tool. Using the TALAG pain scale and visual analogue scale (VAS), the pain intensity test was scaled from no pain (0), to unimaginable/unspakable (10) scales. For analyzing the findings of the research and determining the difference between the levels of variables in the research groups, one-way ANOVA test with repeated measures and Bonferroni's post hoc test, and to determine the interaction of two effective factors (different doses and different times), combined analysis of variance test was used. Data were analyzed using SPSS software version 19 ($p \leq 0.05$).

Results

Demographic characteristics of subjects in research groups presented in Table 1. Also the mean and standard deviation of the variables of the research in different stages of measurement presented in Table 2. The results of one-way ANOVA with repeated measures showed that there was a significant difference at the creatine kinase levels of the subjects in different measurement times ($F = 13.20$, $p = 0.001$, and effect size = 0.38); however, the time interaction in the three groups of research

was not significant ($F = 1.77$, $p = 0.07$, and effect size = 0.14). The results of Bonferroni's post hoc test showed that creatine kinase levels after 14 days of rosemary supplementation were not significantly different with pre-test ($M = 1.48$, $p = 0.99$); but creatine kinase levels increased significantly 4 hours ($M = -8.20$, $p = 0.001$), 24 hours ($p = 0.001$, $M = -20.91$), 48 hours ($M = -29.95$, $p = 0.001$), and 72 hours ($M = -24.45$, $p = 0.001$) after exercise compared to the pre-test. The levels of creatine kinase increased significantly 48 hours ($M = -31.41$, $p = 0.003$), and 72 hours ($M = -25.91$, $p = 0.01$) after exercise compared to creatine kinase levels 14 days after creatine kinase consumption; creatine kinase levels did not differ significantly 4 hours after exercise and 24 hours after exercise ($M = -12.70$, $p = 0.09$), but creatine kinase levels 48 hours ($M = -21.75$, $p = 0.001$) and 72 hours ($M = -16.25$, $p = 0.01$) after exercise were significantly higher than 4 hours after exercise. Creatine kinase levels increased significantly during 48 hours after exercise compared to 24 hours after exercise ($M = -9.04$, $p = 0.001$); also levels of creatine kinase 72 hours after exercise was significantly increased compared to 48 hours after exercise ($M = -5.50$, $p = 0.001$). One-way analysis of variance test with repeated measures showed that time factor had a significant effect on neutrophil levels in research groups ($F_5 = 25.50$, $p = 0.001$ and effect size = 0.54); also, time interaction in different groups at neutrophil levels was significant ($F = 24.53$, $p = 0.001$ and effect size = 0.70). The results of Bonferroni's post hoc test showed that neutrophil levels of subjects after 14 days of rosemary supplementation ($M = 0.14$, $p = 0.01$) and 4 hours after exercise ($M = 0.13$, $p = 0.01$) were lower than the neutrophil levels in the pre-test; neutrophil levels in 4 hours were significantly less than 24 hours ($M = -40.27$, $p = 0.001$) and 48 hours ($M = -2.43$, $p = 0.001$) after exercise. However, neutrophil levels in subjects 24 hours after exercise were significantly higher than neutrophil levels at 48 hours ($M = 38.74$,

$p = 0.001$) and 72 hours ($M = 39.39$, $p = 0.001$) after exercise. However, neutrophil levels 48 hours after exercise were not significantly different from neutrophil levels at 72 hours after exercise ($M=1.55$, $p = 0.99$). Considering the significance of time interaction in three groups, the results of Bonferroni's post hoc test showed that the neutrophil levels of subjects with rosemary supplementation of 0.25 ml, 24 hours after exercise were significantly higher than placebo group ($M= 21.43$, $p=0.001$) and rosemary supplementation of 0.5 ml ($M=26.61$, $p=0.001$), however, there was no significant difference in neutrophil levels in placebo groups and Rosemary supplementation of 0.25 ml ($M= 5.17$, $p= 0.73$). The results of one-way analysis of variance test with repeated measures showed that there was a significant difference between the lymphocyte levels of subjects of the three groups at the time of measurement ($F= 17.87$, $p= 0.001$ and effect size =0.54), but the interaction of time and group in changes in lymphocyte levels was not significant ($F= 0.08$, $p= 0.99$ and effect size= 0.008). The results of Bonferroni's post hoc test showed that there was no significant difference between lymphocyte levels in the pre-test and after 14 days of rosemary supplementation ($M= 0.10$, $p = 0.99$); however, lymphocyte levels in the pre-test were significantly less than 4 hours ($M = -0.57$, $p = 0.001$), 24 hours ($M = -1.28$, $p = 0.001$), 48 hours ($M=-2.22$, $p = 0.001$) and 72 hours ($M = -1.87$, $p = 0.001$) after exercise. The levels of lymphocyte after 14 days of rosemary supplementation were significantly less than 48 hours after exercise ($M = -2.32$, $p= 0.009$); 4 hours after exercise, lymphocyte levels were significantly lower than 24 hours ($M = 0.70$, $p = 0.001$), 48 hours ($M = -1.64$, $p = 0.001$), and 72 hours ($M= -1.30$, $p= 0.001$) after exercise. 24 hours after exercise, lymphocyte levels were significantly lower than lymphocyte levels at 48 hours ($M = -0.93$, $p= 0.001$) and 72 hours ($M= -0.59$, $p = 0.001$) after exercise; however, there was no

significant difference in the levels of lymphocytes in the three groups 48 hours and 72 hours after exercise ($M = 0.34$, $p = 0.16$). The results of one-way analysis of variance test with repeated measures showed that there was a significant difference in the levels of lactate dehydrogenase in subjects at different times ($F_5 = 64.10$, $p= 0.001$ and effect size= 0.75). However, time interaction in the three groups was not significant ($F= 2.36$, $p= 0.001$ and effect size= 0.18). The results of Bonferroni's post hoc test showed that the levels of lactate dehydrogenase in the pre-test were significantly higher than 14 days of rosemary supplementation ($M = 4.91$, $p = 0.001$), but the levels of lactate dehydrogenase in the pre-test were significantly lower than the levels of lactate dehydrogenase after 4 hours ($M = -8.91$, $p = 0.001$), 24 hours ($M = -24.66$, $p = 0.001$), 48 hours ($M = -35.34$, $p=0.001$) and 72 hours ($M = -26.54$, $p = 0.001$) after exercise. The levels of lactate dehydrogenase 14 days after rosemary supplementation were significantly lower than 4 hours ($M = -13.83$, $p = 0.001$), 24 hours ($M= -29.58$, $p = 0.001$), 48 hours ($M = -40.12$, $p = 0.001$), and 72 hours ($M = -31.45$, $p = 0.001$) after exercise. 4 hours after exercise, the levels of lactate dehydrogenase were significantly less than 24 hours ($M = -15.75$, $p = 0.001$), 48 hours ($M = -26.29$, $p = 0.001$) and 72 hours ($M= -17.62$, $p = 0.005$) after exercise. 24 hours after exercise, the levels of lactate dehydrogenase levels were significantly lower than 48 hours after exercise ($M = -10.54$, $p = 0.001$), but there was no significant difference in the levels of lactate dehydrogenase 24 hours and 72 hours after exercise ($M = -1.87$, $p = 0.99$); also, the levels of lactate dehydrogenase 48 hours after exercise were not significantly different from lactate dehydrogenase levels 72 hours after exercise ($M = 8.66$, $p = 0.36$); The results of one-way analysis of variance test with repeated measures showed that there was a significant difference in the time intervals of measuring the pain levels of subjects in three-

groups ($F= 67.79$, $p= 0.001$ and effect size $=0.92$); however, time interaction was not significant in the three groups ($F_5 = 0.44$, $p = 0.91$, and the effect size $=0.04$). The results of Bonferroni's post hoc test showed that there was no significant difference between pain levels in the pre-test and after 14 days of rosemary supplementation ($M = 0.001$, $p = 0.99$); the pre-test pain levels were significantly less than pain levels 4-hours ($M = -2.29$, $p = 0.001$), 24 hours ($M = -3.33$, $p = 0.001$), 48 hours ($M=-3.62$, $p = 0.001$) and 72 hours ($M = -3.29$, $p = 0.001$) after exercise; also, the levels of pain after 14 days of rosemary supplementation were significantly

lower than 4 hours ($M = -2.29$, $p = 0.001$), 24 hours ($M= -3.33$, $p = 0.001$), 48 hours ($M = -3.62$, $p = 0.001$) and 72 hours ($M = -3.29$, $p = 0.001$) after exercise. Pain levels 4 hours after exercise were significantly lower than 24 hours ($M = -1.04$, $p = 0.001$), 48 hours ($M = -1.33$, $p = 0.001$) and 72 hours ($M= -1.00$, $p = 0.001$) after exercise. Pain levels 24 hours after exercise were not significantly different from pain levels 48 hours ($M = -0.24$, $p = 0.99$) and 72 hours ($M = 0.04$, $p = 0.99$) after exercise; also, the pain levels 48 hours after exercise were not significantly different from the pain levels 72 hours after exercise ($M = 0.33$, $P = 0.055$).

Table 1. Demographic characteristics of subjects in research groups

Variable	Group	Placebo	High Dose	Low Dose
Weight (kg)		79.96±8.81	81.12±8.02	80.75±7.32
Height (cm)		181.00±4.96	180.87±4.94	180.22±6.25
BMI (weight/height squared)		24.18±3.91	25.45±2.88	25.11±3.31

Table 2. The mean and standard deviation of the variables of the research in different stages of measurement

Level	Stage one	Stage two	Stage three	Stage Four	Stage Five	Stage six
Analog Pain	0.00	0.00	2.29	3.29	2.29	3.62
Lactate dehydrogenase	242.46	237.45	251.37	267.12	277.67	269.00
Creatine Kinase	138.87	142.04	147.08	159.79	168.83	163.33
Lymphocyte	21.2	21.3	21.97	22.68	33.62	23.27
Neutrophil	33.92	36.78	34.42	35.16	36.21	35.41

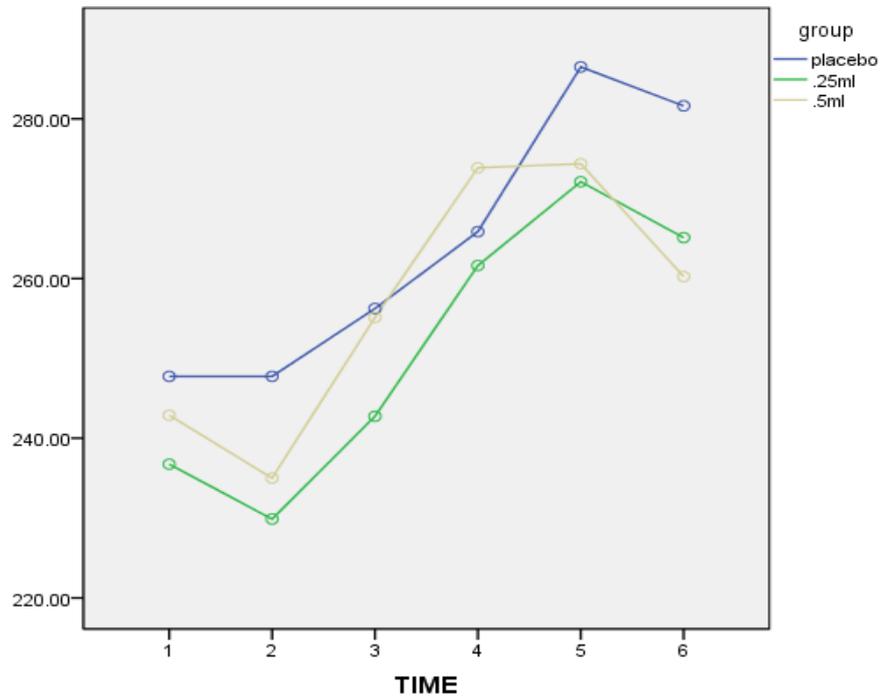


Figure 1. Lactate dehydrogenase levels of study groups (1) before rosemary supplementation, (2) after rosemary supplementation (3) 4 hours after exercise (4) 24 hours after exercise (5) 48 hours after exercise (6) 72 hours after exercise

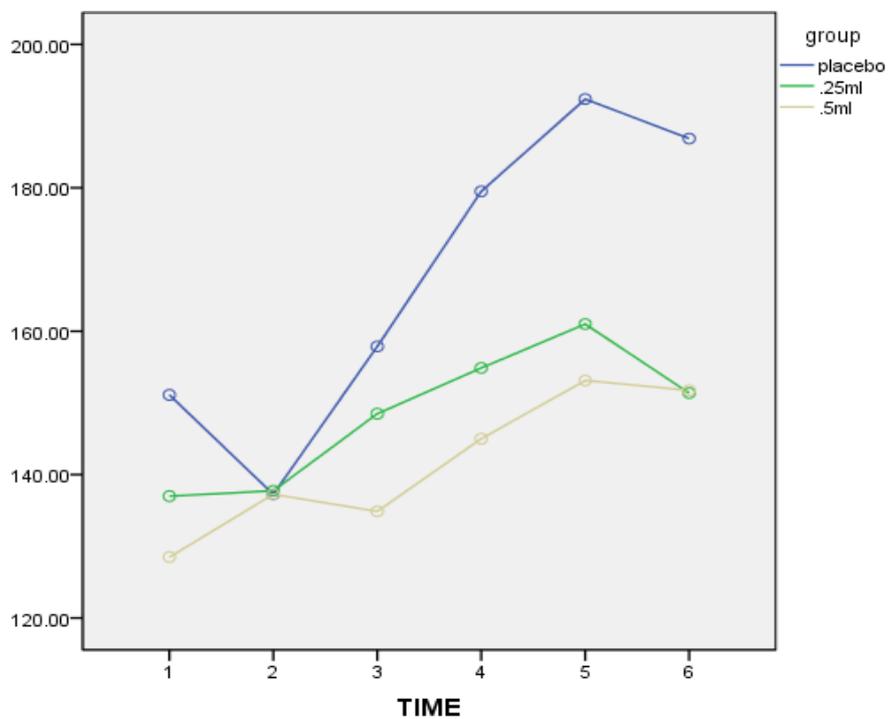


Figure 2. Creatine kinase levels of the study groups (1) before rosemary supplementation (2) after rosemary supplementation (3) 4 hours after exercise (4) 24 hours after exercise (5) 48 hours after exercise (6) 72 hours after exercise

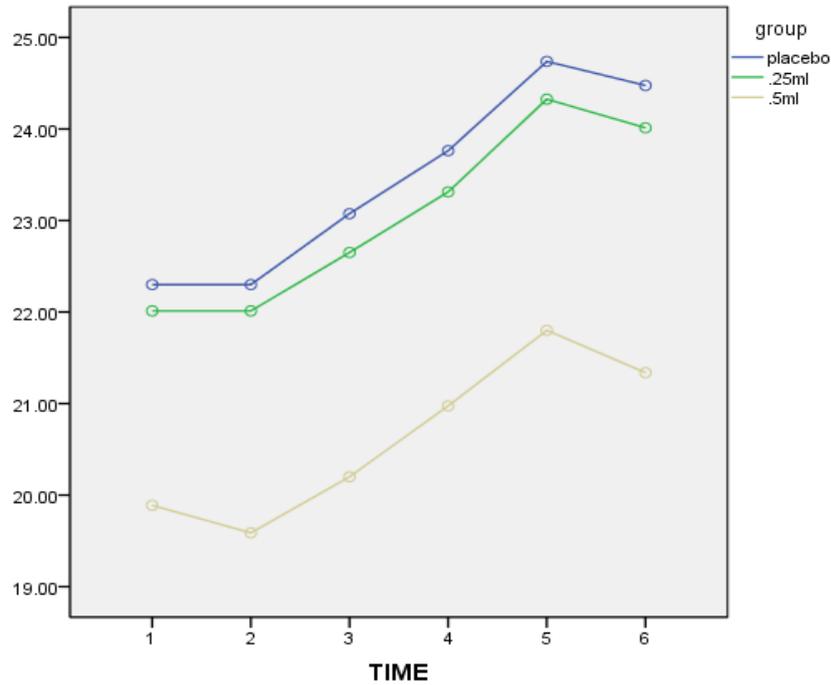


Figure 3. Lymphocyte levels of the study groups (1) before rosemary supplementation (2) after rosemary supplementation (3) 4 hours after exercise (4) 24 hours after exercise (5) 48 hours after exercise (6) 72 hours after exercise

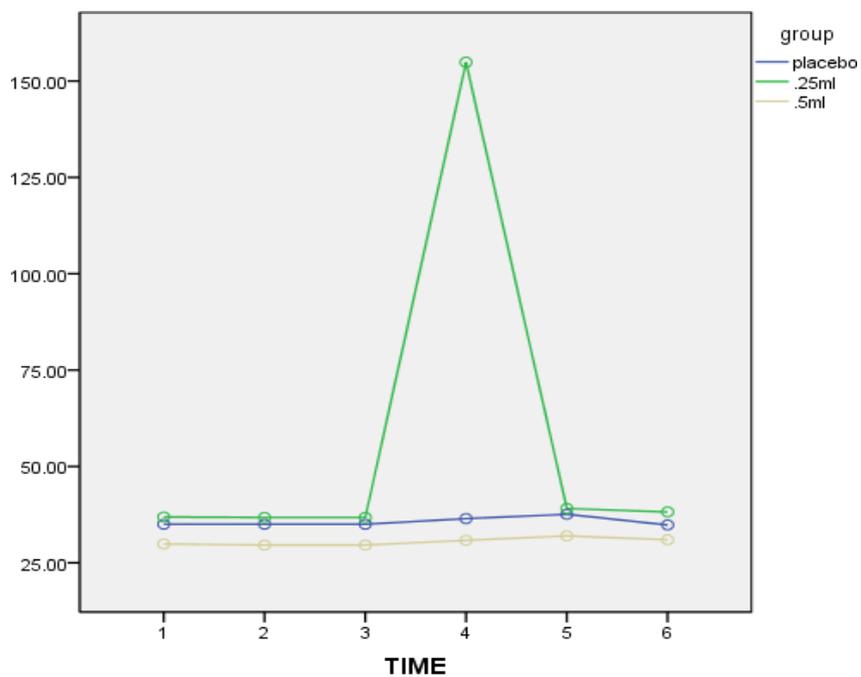


Figure 4. Neutrophil levels of the study groups (1) before rosemary supplementation, (2) after rosemary supplementation (3) 4 hours after exercise (4) 24 hours after exercise (5) 48 hours after exercise (6) 72 hours after exercise

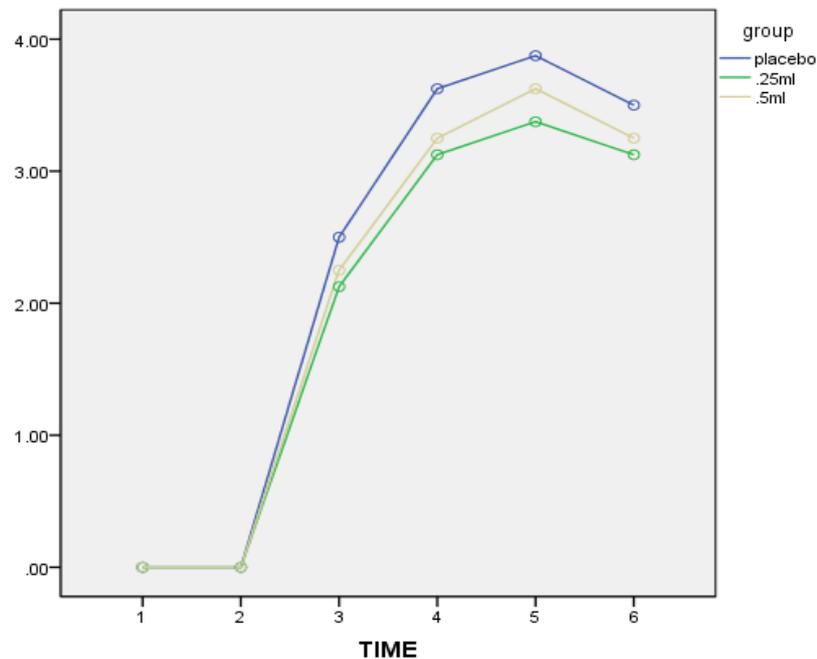


Figure 5. Analog pain levels of the study groups (1) before rosemary supplementation, (2) after rosemary supplementation (3) 4 hours after exercise (4) 24 hours after exercise (5) 48 hours after exercise (6) 72 hours after exercise

Discussion

The results showed that the levels of creatine kinase increased significantly 4 hours after exercise to 72 hours after exercise by degrees. In addition, neutrophil levels remained low for 4 hours after exercise. Neutrophil levels, however, increased significantly 24 hours after exercise, and continued to persist 72 hours after exercise still more than 4 hours after exercise. Lymphocyte levels increased gradually from 4 hours after exercise to 48 hours after exercise, but remained constant at 48 and 72 hours. Lactate levels of inactive men rose gradually during 4 hours after exercise until 72 hours after exercise, however, remained constant at 24, 48, and 72 hours after exercise. Pain levels increased gradually during 4 hours after exercise until 72 hours after exercise, but the pain level did not change significantly during 24 hours after exercise until 72 hours after exercise. Research has shown that creatine kinase and lactate dehydrogenase enzymes are enclosed within the cell membrane, but their release in blood

steam may increase due to cell membrane rupture, induction of enzyme synthesis, increased cell proliferation and increased cellular degradation process. (4). Also, heavy and unusual muscle activity often causes pain, abnormal sensitivity to pressure or touch and stiffness (7) in skeletal muscles after several hours (12). This pain, which usually begins after six hours, is called delayed onset muscle soreness, (DOMS) which is associated with functional, biochemical and inflammatory symptoms (13, 14). Delayed onset muscle soreness (DOMS) is a grade one muscular strain, in which sensitivity to touch and muscle aches that can be felt by touch or motion can affect any skeletal muscle (15). It is worth noting that the DOMS range does not always reflect the extent of muscular damage and therefore it is best to be cautious when estimating DOMS levels using muscle injury indices. Symptoms of DOMS usually occur 12-6 hours after the activity, and the person goes to sleep with a slight discomfort and wakes up with severe and debilitating pain the

next morning. The peak in pain usually occurs 48-72 hours after the activity, which disappears within 7 to 5 days or reaches its minimum (16). The explanation of the changes in the fourth stage can be expressed in such a way that, when the active muscle is pulled, various components of the cell may be damaged. After severe eccentric contractions that cause delayed onset muscle soreness, muscular myofibriles, connective tissue around the fibers, adjacent layers of plasma membranes, plasma membranes of muscular myofibriles, sarcomers, and sarcoplasmic networks, and the like are damaged (17). The results of the study on the effects of weight training on serum and myoglobin levels showed that myoglobin significantly increased in all subjects after exercise, but this increase was lower in the training group, so it can be concluded that exercise with high intensity weights causes muscle damage, and this muscular injury occurs less in people with regular exercises due to making adjustments. Another study on the training-induced muscle injury concluded that myoglobin increased at the end of the training and reached a peak four hours after the end of the training (19-17). In very severe DOMSs, splitting of stripped muscle fibers may be along with excretion of myoglobin from the urine. High levels of myoglobin can lead to renal dysfunction. This complication (reported in animals and humans) occurs after severe and unusual exercise, especially in heat, and its characteristic symptoms include: increased serum enzymes, swelling, tenderness, muscle stiffness (17), nausea, abnormal tissue and the presence of myoglobin in the urine. Other symptoms of DOMS reported in the study include severe water loss, calcium loss, increased potassium, decreased blood albumin and increased plasma creatine kinase (17, 20); therefore, the present is consistent with most studies (4, 6, 21). However, the use of rosemary supplementation for 14 days reduced the neutrophil levels significantly compared to the pre-test and 4 hours after exercise, but the

rosemary supplementation at 0.25 ml 24 hours after exercise increased neutrophil in inactive men. However, there was no significant effect on creatine kinase, lactate dehydrogenase, lymphocyte and pain levels in inactive men. Many attempts have been made to investigate DOMS, but the underlying and precise cause remains uncertain, however the degree of damage, depending on severity, duration, and most importantly, type of activity performed are known (13), so that eccentric muscle contraction produces the most muscular damage after the activity compared to the intrinsic and static contractions (12, 21). Inflammatory responses include pain, swelling, increased white blood cell count, rapid increase in intra-muscle mediators and blood circulation, and accumulation of monocytes and lymphocytes in damaged muscle fibers, and eventually changes in the main inflammatory indices such as C-reactive protein (7). Rosemary, due to its abundance in anti-inflammatory and antioxidant combinations, such as camphor, rosmarinic acid, 1.8 cineole, etc. reduces neutrophil levels (22, 23). Studies have shown that rosemary, due to the presence of carnosol and phenolic compounds, prevents lipid oxidation, and hence enhances the ability of free radical inhibitors and inhibition of synthetic antioxidant BHT (butyl hydroxyl toluene) (25). However, in the present study, rosemary supplementation only maintained low levels of neutrophil in posttest and 4 hours after exercise, but did not significantly affect creatine kinase, lactate dehydrogenase, lymphocyte and pain levels in inactive men. In this regard, caffeine supplementation with doses of 6 and 9 mg / kg of body weight did not have a significant effect on serum lactate dehydrogenase and aspartate aminotransferase in elite male volleyball players (6); however, consumption of 2.52 g of powdered cinnamon per day for 10 days had a significant effect on decreasing creatine kinase and lactate dehydrogenase in inactive boys and had a positive effect on prevention of DOMS (4).

Differences in the results of previous studies with this study can be attributed to the type of exercise protocol, the intensity and duration of exercise, and the subjects participating in it. Since subjects in the present study comprised healthy men, it may be one of the reasons for keeping the levels of lactate dehydrogenase constant 24 to 72 hours after DOMS. Probably one more reason to keep the levels of lactate dehydrogenase constant after eccentric exercise is the intensity or duration of exercise, because the concentration of lactate dehydrogenase compared to creatine kinase is more affected by intensity of exercise and it may have not been so severe as to stimulate increase in the levels of lactate dehydrogenase, or to affect other factors that could have stimulated lactate dehydrogenase elevation, or these results were achieved because of the very short half-life of lactate dehydrogenase compared with creatine kinase. However, no similar results were obtained in other studies (25).

Conclusion

The results of this study indicate that the use of rosemary can reduce neutrophil and ultimately inflammation in skeletal muscle, however, rosemary with doses of 0.25 and 0.5 ml has no significant effect on delayed onset muscle soreness (DOMS) indices 4, 24, 48 and 72 hours after exercise in inactive men.

Ethical issues

Not applicable.

Authors' contributions

All authors equally contributed to the writing and revision of this paper.

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