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# MULTIPLE INTAKES OF NIGELLA SATIVA BEFORE AND AFTER EXERCISE-INDUCED MUSCLE DAMAGE DOES NOT INFLUENCE MUSCLE DAMAGE AND INFLAMMATORY CYTOKINES

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## Abstract

**Introduction** Nigella sativa is known to have potent anti-inflammatory effects. Thus, the present study investigated the hypothesis that Nigella sativa ingestion would reduce muscle damage and inflammation after eccentric exercise. **Material and methods** Twenty sedentary men aged 19 to 27 years were assigned to 2 groups, either Nigella sativa (N) or control (C). N group was administered 2000mg/day of Nigella sativa from 2 weeks before exercise to 4 days post-exercise and C group was administered a placebo. They underwent 10 sets X 10 repetitions of counter-movement jump (CMJ) with 30-second rest between each set, employing squat in starting and landing position to facilitate muscle damage. A set of tests were conducted to measure indirect muscle damage and inflammation markers (muscular power, range of motion (ROM), thigh circumference, soreness, creatine kinase [CK]; interleukin-6 [IL-6] and interleukin-1β [IL-1β]) at pre-exercise, 30 minutes, 1-, 2-, 3- and 4-days post-exercise. Changes in these parameters over time were compared between N and C groups by two-way repeated-measures ANOVA. **Results** Significant changes were noted in muscular power, ROM, thigh circumference, soreness and CK from pre-exercise at most of the measurement sessions for N and C groups ($p<0.05$), but no significant changes were marked in IL-6 and IL-1β at all measurement sessions for N and C groups ($p>0.05$). There were also no significant differences between groups for all markers of muscle damage and inflammation at all measurement sessions ($p>0.05$). **Discussions and conclusion** Significant changes in muscular power, ROM, thigh circumference, soreness and CK from pre-exercise at most of the measurement sessions for both groups suggested that the eccentric exercise model used in this study was enough to induce muscle damage, but it might be too mild to activate the inflammatory cytokines. No significant differences between groups for all markers of muscle damage and inflammation at all measurement sessions suggested that Nigella sativa ingestion does not attenuate indirect markers of muscle damage and inflammation following mild eccentric exercise model.

**Keywords:** Eccentric exercise · Exercise-induced muscle damage · Nigella sativa · Anti-inflammation

## Abbreviations

EIMD Exercise-induced muscle damage

CMJ Counter-movement jump

CK Creatine kinase
VAS Visual analog scale
ROM Range of motion

IL-6 interleukin-6
IL-1β interleukin-1β

## Introduction

The sensations of pain, muscle tightness, swelling, and tenderness that may arise following novel exercise that involves a large number of eccentric contractions are widely recognised as delayed-onset muscle soreness (DOMS) (Close *et al.*, 2005). DOMS is one of the evidences of exercise-induced muscle damage (EIMD) besides losses of strength and power, local swelling, stiffness, structural disruption, and increase in proteins activities within the muscles and the circulation (Howatson and van Someren (2008). The mechanism following EIMD begins with the damage at the cellular level followed by an inevitable inflammatory response (MacIntyre *et al.*, 2001). The inflammatory process takes place by infiltration of inflammatory cells such as neutrophils and macrophages, and inflammatory proteins such as cytokines and cyclooxygenase (COX) within the exercised muscle (Tidball, 2005). These mediators are responsible for the impairment of muscle functions such as swelling, soreness, and tenderness (MacIntyre *et al.*, 2001). Hence, it is important to reduce muscle damage by minimising inflammatory responses following exercise. One of many initiatives for this aim could be an oral supplementation of anti-inflammatory substances.

Numerous anti-inflammatory substances were reported to have benefits in reducing the symptoms of EIMD, such as curcumin (Tanabe *et al.*, 2015), milk (Rankin *et al.*, 2015), ginger (Matsumura *et al.*, 2015), Panax ginseng (Jung *et al.*, 2011), beetroot juice (Clifford *et al.*, 2016), and black currant nectar (Hutchison *et al.*, 2016). In addition to the list, Nigella sativa (NS) seeds are also widely reported to carry anti-inflammatory benefits. The herb has been planted in many parts of the world such as the Middle East, western Asia, northern Africa, and Europe. The seeds are broadly used in daily cooking as spice additive, bread flavour, or aromatic enhancer (Ramadhan *et al.*, 2011). The anti-inflammatory effects of NS have been used in the treatment of several models of inflammation, such as rhinitis, encephalomyelitis, peritonitis, and arthritis (Salem, 2005; Cingi *et al.*, 2011; Balaha *et al.*, 2012).

It has been demonstrated that thymoquinone (TQ), one of NS's active components, has an anti-inflammatory effect by suppressing both the cellular and fluid phases of inflammation. The possible mechanisms that might reduce the inflammation are by inhibiting eicosanoid generation, such as thromboxane $B_2$ and leukotrienes $B_4$ by inhibiting COX (Hajhashemi *et al.*, 2004), as well as by inhibiting inflammatory cytokines and growth mediators (Woo *et al.*, 2012). Thus, NS may be effective in attenuating EIMD in humans. However, we did not find any NS study investigating its anti-inflammatory effects on EIMD in humans except our previous work (Raimi *et al.*, 2020). In the study, we found that a single one-off intake of NS did not attenuate indirect markers of muscle damage and inflammation after a bout of counter-movement jump. Therefore, in this study, we aimed to examine the effect of supplementation of NS continuously from 2 weeks before exercise to 4 days post-exercise on indirect markers of muscle damage and inflammation following a bout of counter-movement jump (CMJ). It was hypothesized that multiple days of NS supplementation would attenuate markers of muscle damage and inflammation compared to placebo.

## Materials and methods

### Participants

Twenty sedentary young men aged 19 to 27 years old were randomly recruited among pre-university and university students. All participants were free from any history of musculoskeletal, liver, and kidney problems. Ethical approval was obtained from human ethics committee and complied with the Helsinki Declaration as revised in 2013. Each participant was given informed consent before the study. The risk for musculoskeletal and cardiovascular problems was assessed using a health history questionnaire; participation and intensity of exercise were assessed using a daily/weekly sports activity questionnaire.

During the study, participants were requested to be well-hydrated, maintain the pattern of their daily food intake, and be free from drugs. Any major changes in daily dietary intake had to be reported.

**Experimental design**

This study implemented a randomised double-blind controlled trial. Participants were allocated into two groups: either the NS group (N) or the placebo group (C). Before the group allocation, participants underwent physical assessment, health screening, and familiarisation. On the exercise day, they underwent a pre-exercise measurement to assess indirect markers of muscle damage and inflammation, which are muscular power, range of motion (ROM), thigh circumferences, soreness, creatine kinase (CK), and interleukin-6 (IL-6) and interleukin-1β (IL-1β). Approximately 5ml of blood was drawn to test their level of blood markers. To facilitate muscle damage, all subjects performed a bout of CMJ as a model of eccentric exercise. The measurement to assess indirect markers of muscle damage and inflammation were repeated at 30 minutes, 1 day, 2 days, 3 days, and 4 days post-exercise. Changes in the markers from pre-exercise were compared between groups using two-way repeated-measures ANOVA.

**Supplementation protocols**

The group allocations were fully performed by the lab staff, without both the participants and the researchers knowing the content of the supplements and which supplements belonged to which subject. N group was administered 2000mg of NS continuously from two weeks before to four days post-exercise. C group, the control group, was administered maltodextrin as a placebo. Both NS and maltodextrin were enclosed in a capsule with 500 mg each, and all participants were given a small bottle containing the supplements to be consumed for a total of 19 days.

**Eccentric exercise**

CMJ was conducted for 10 sets of ten repetitions in each set, and 32-second rests between sets were given. Participants performed maximal vertical jumps with a squat position at the beginning and landing phases to facilitate muscle damage.

**Measurements of markers**

*Muscular power*

To assess muscular power, a vertical jump test was implemented in the present study. Subjects were requested to reach up to the highest point they could ($T_1$) and mark the point three times on a board on the wall using the tip of their fingers. They were asked to jump as high as possible and make a mark ($T_2$) on the board. The mean height was then calculated. The difference between $T_2$ and $T_1$ was used to reflect the participants' muscular power.

*Range of motion (ROM)*

Determination of ROM, the flexed (FANG), and stretched (SANG) knee joint angle were obtained. ROM was calculated via subtracting SANG from FANG. ROM was determined as the difference between the FANG and the SANG. Three points were drawn on the lateral epicondyle, midline of the lateral femur and lateral fibula as the landmarks to measure the angles. The Centre of the circular disc of the goniometer was held on the drawn points on the lateral epicondyle, while the stationary arm of the goniometer was held in line with the midline of the lateral femur. The moving arm of the goniometer was held in line with the lateral fibula.

*Thigh circumference*

To assess swelling, thigh circumference was first measured. The measurement of thigh circumference was performed using a constant tension measure tape. The circumference at the middle point of the thigh was obtained at between 4 cm under the gluteal fold to knee crease.

*Soreness*

A visual analogue scale (VAS) was used to assess soreness. The measurement was performed after the vertical jump test. Subjects indicated their soreness level by putting a mark on a continuous straight 10 cm line. '0' indicates no soreness and '10' indicates extreme soreness.

*Blood sampling and analyses*

Blood samples were analysed to examine CK, IL-6, and IL-1β as the blood markers of muscle damage and inflammation. Approximately 5 mℓ of venous blood was drawn at every measurement. Some of the samples were carefully centrifuged at the USM lab to obtain serum and the other samples were directly sent to another lab to be analysed. Analyses of both interleukins were performed by staffs at USM lab using AssayMax™ Human Interleukin-6 and Interleukin-1β ELISA Kits. The kits were produced by Assaypro LLC, USA.

**Statistical analyses**

Two-way repeated measure ANOVA in SPSS was used to compare changes in all variables over time between treatments and placebo conditions.

## Results

**Muscular power**

The muscular power that was assessed using jumping height at pre-exercise was not significantly different between N (41.61±6.41cm) and C (40.99±8.08cm) groups. The percentage changes in muscular power from pre-exercise significantly decreased at most of the measurement sessions for both groups (Fig. 1[A]), but no significant interaction effect was found in the changes between the groups.

**ROM**

At pre-exercise, ROM was similar between N (151.00°±2.98°) and C (151.44°±2.30°) groups. After exercise, ROM significantly decreased at all measurement time points from the pre-exercise, but no significant interaction effect was found for the changes between groups (Fig 1[B]).

**Thigh circumference**

No significant difference in the pre-exercise thigh circumference between N (46.13±2.03 cm) and C (52.51±3.93cm) groups was evident. Thigh circumference significantly increased after exercise at 1-day post-exercise for N group and at 2 days post-exercise for C group, but no significant differences were noted between groups (Fig. 2[A]).

**Muscle soreness**

Both groups showed significant increases in VAS score between 30 min and 4 days post-exercise. The peak of VAS for C (6.60±1.55cm) group was higher than N (5.40±1.63cm) group, but no significant differences between groups were evident (Fig**.** 2[B]).

**Serum CK**

Before the exercise, serum CK was within the normal range for the N (156±54.24U/L) and C (150± 44.10U/L) groups. It increased significantly from pre-exercise at most of the measurement sessions for both groups (Fig. 3).

**IL-6 and IL-1β**

No significant differences were noted between groups before exercise (IL-6; 0.010± 0.007pg/ml vs 0.022± 0.037pg/ml, IL-1β; 5.93±2.61pg/ml vs 20.43±20.09pg/ml) for N and C groups, respectively. There were also no significant changes at all measurement sessions for both groups (Fig. 4).

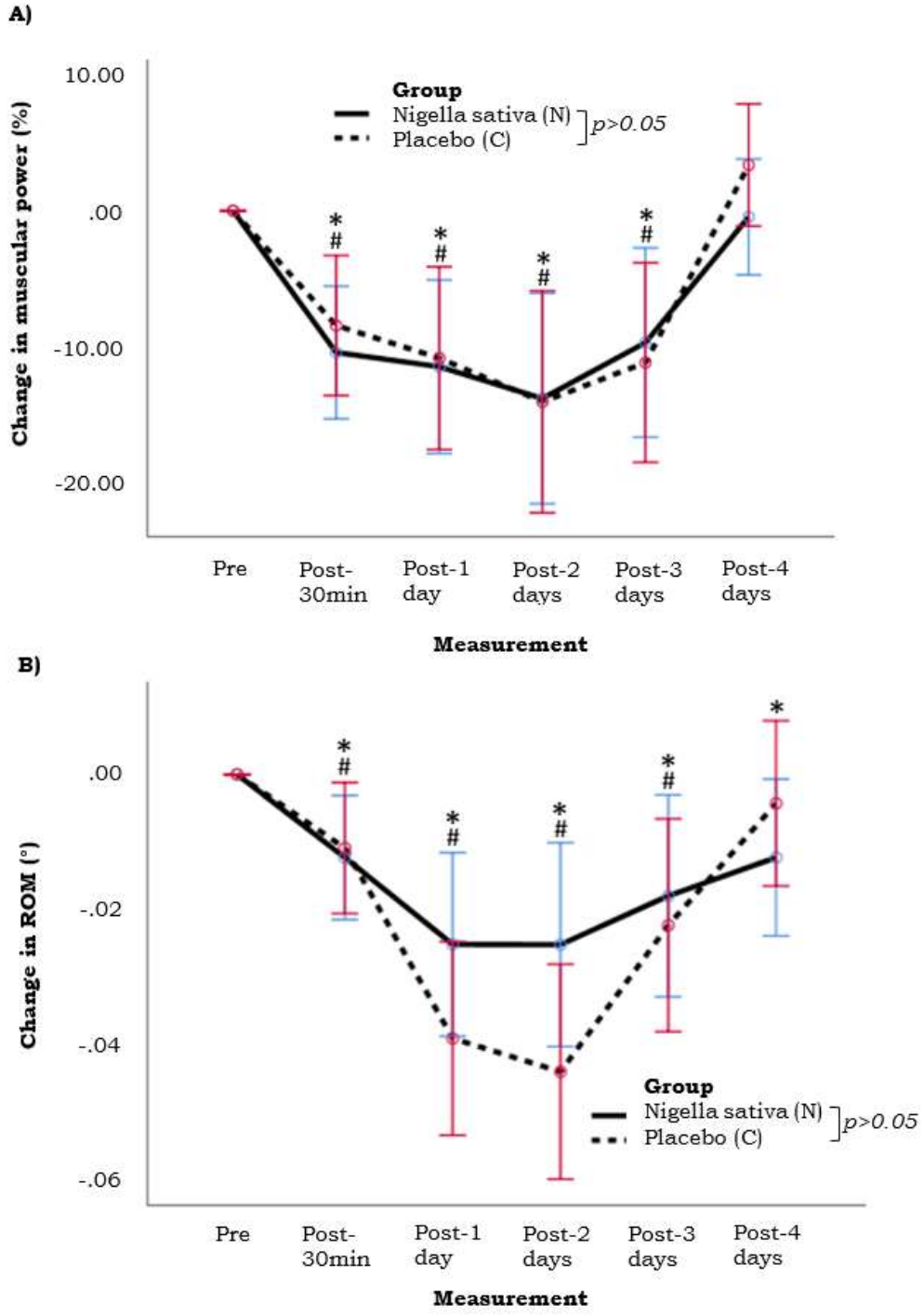


**Fig. 1** Percentage changes in muscular power (**A**) and changes in ROM (**B**) from the baseline (Pre), at 30 minutes-4 days after CMJ for the Nigella sativa (N) and placebo (C) groups.

*$p < 0.05$ vs. Pre in N group; #$p < 0.05$ vs. Pre in C group; $p > 0.05$: no significant interaction effect between groups.

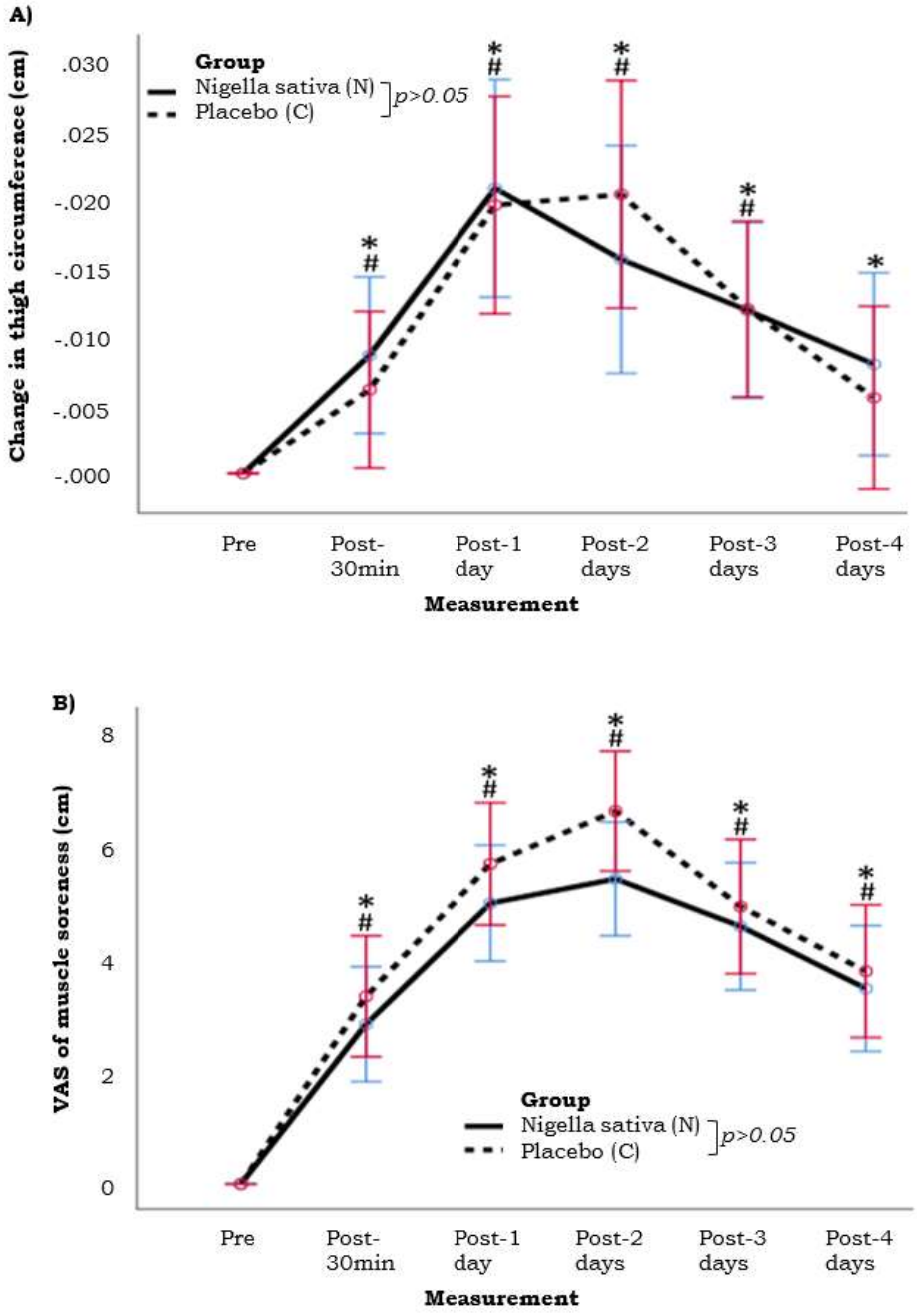


**Fig. 2** Changes in thigh circumference from the baseline (Pre) at 30 minutes-4 days after CMJ (**A**) and VAS of muscle soreness (**B**) at Pre, 30 minutes-4 days after CMJ for the Nigella sativa (N) and placebo (C) groups.

*$p < 0.05$ vs. Pre in N group; $^{\#}p < 0.05$ vs. Pre in C group; $p > 0.05$: no significant interaction effect between groups.

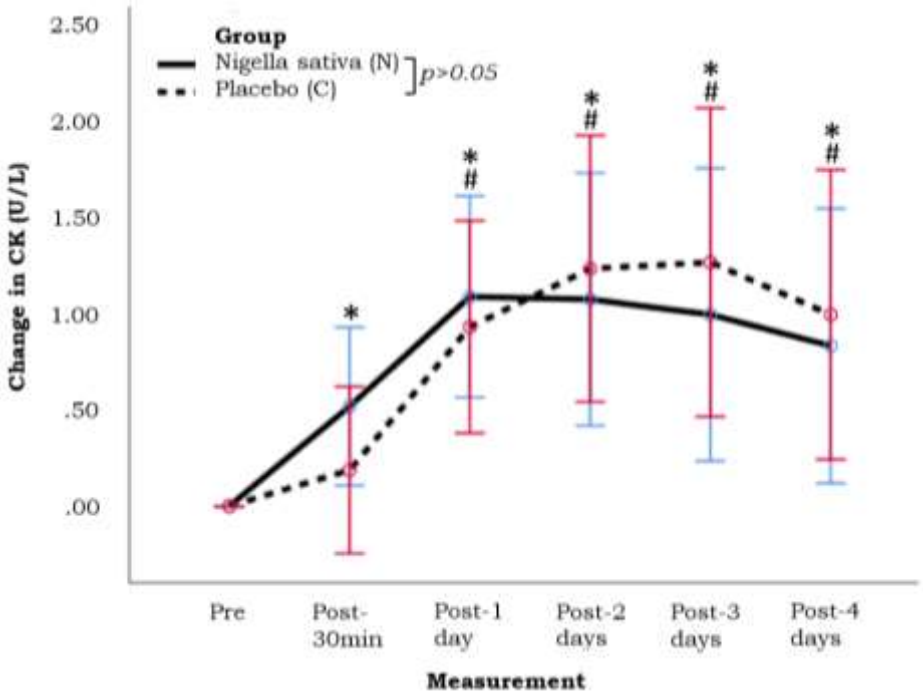


**Fig. 3** Changes in CK activity at Pre, 30 minutes-4 days after CMJ for the Nigella sativa (N) and placebo (C) groups.

*$p < 0.05$ vs. Pre in N group; #$p < 0.05$ vs. Pre in C group; $p > 0.05$: no significant interaction effect between groups.

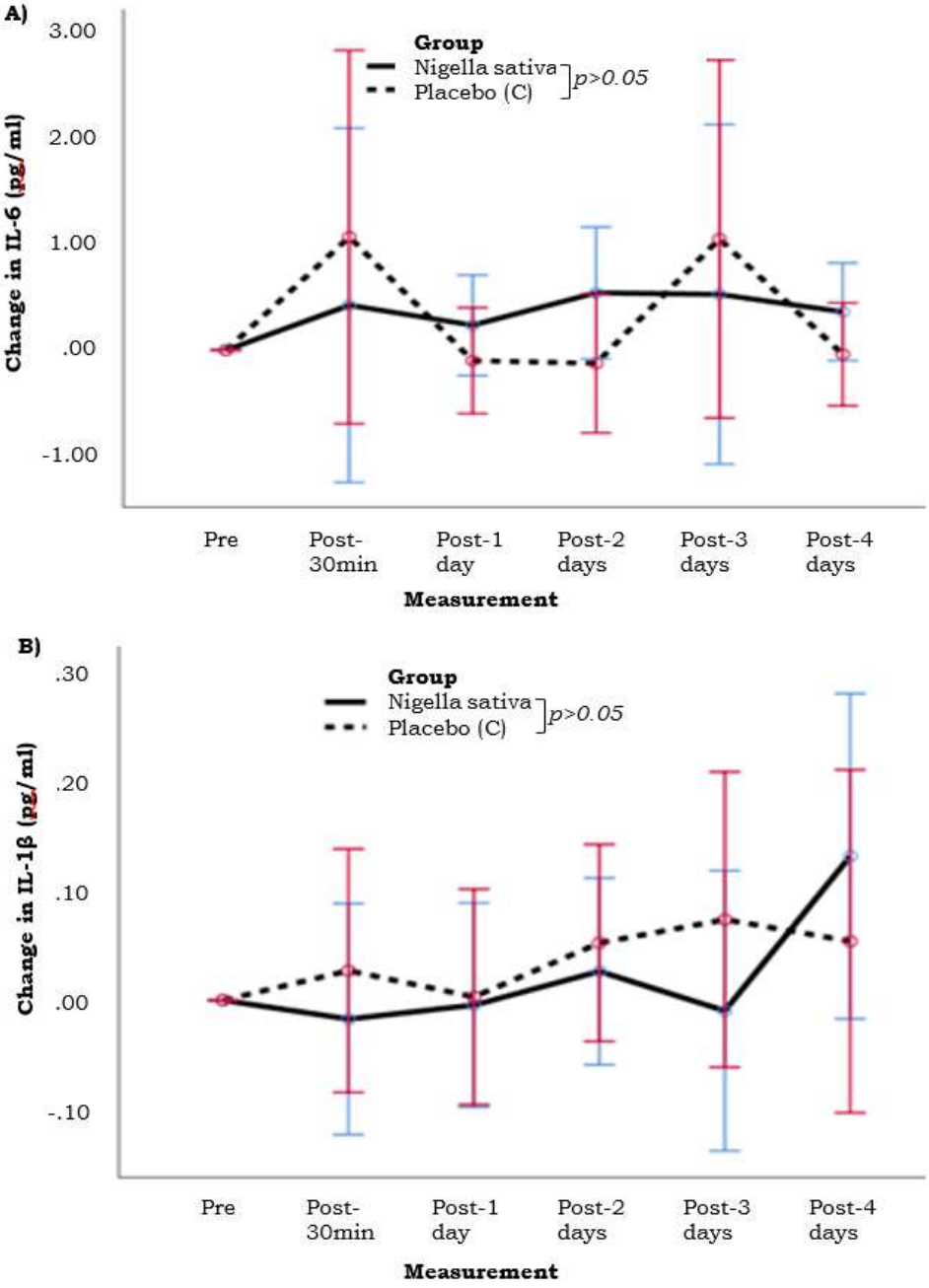


**Fig. 4** Changes in IL-6 (**A**) and IL-1β (**B**) at Pre, 30 minutes-4 days after CMJ for the Nigella sativa (N) and placebo (C) groups.

*$p > 0.05$: no significant interaction effect between groups.

## Discussions

This study investigated whether the supplementation of Nigella sativa continuously from 2 weeks before exercise to 4 days post-exercise would attenuate muscle damage and inflammation in sedentary young men. Themain findings of the study showed that no significant differences between the groups were evident for all markers of muscle damage and inflammation. These results did not support the hypothesis that Nigella sativa supplementation would attenuate muscle damage and inflammation markers after eccentric exercise.

Nigella sativa did not offer any significant effect to limit muscular power loss following EIMD. Despite the lack of significant differences

between groups, significant changes were noted in muscular power across measurement time. These changes were reasonable to expect because EIMD has been shown in many studies to attenuate force production. Such ability might be attributed to the cycle of muscle stretch-shortening, strength reduction, and increase in soreness during jumping following EIMD (Black and Dobson, 2012).

Nigella sativa ingestion was found ineffective at limiting ROM reduction following EIMD. However, significant decreases were noted in ROM from pre-exercise at all measurement sessions for both groups. It is reasonable to expect the decrease in ROM following EIMD based on the earlier notion that myofibrils disruption and local oedema resulting from the eccentric exercise may increase passive muscle stiffness (Chleboun *et al*., 1998).

Nigella sativa ingestion was also found not beneficial in reducing swelling following EIMD because no significant differences were found on thigh circumference between the groups across the measurement sessions. The increases in thigh circumference from pre-exercise at most of the measurement sessions for both groups seem to concur with the findings of a previous study that employed a mild intensity eccentric exercise model (Nunan, *et al.*, 2010).

The findings in this study showed that soreness began many hours after exercise and reached the highest score from one to two days after exercise, corresponding to the finding of a previous study (Clarkson and Hubal, 2002). However, no significant differences in VAS peak scores were found between groups at all measurement sessions. This finding indicates that ingestion of Nigella sativa was ineffective at attenuating soreness following EIMD. A later study proposed that a potential mechanism for the feeling of soreness resulting from swelling within the damaged muscle is due to the accumulation of substances that are responsible for the repair process that may trigger the mechanoreceptors, thus resulting in the feeling of soreness (Sayers and Hubal, 2008). This notion seems to concur with the present study due to the similar time frame of the onset and recovery of soreness and circumference. However, previous literature indicates that higher muscle soreness may not indicate higher muscle damage because soreness has a poor correlation with other muscle damage markers (ROM, circumference and CK) (Nosaka *et al.*, 2002).

Increases in circulatory CK following EIMD have been seen as the result of the disruption of muscle cells and plasma membrane of muscle fibres, and it is widely used as an indirect marker of muscle damage (Chleboun *et al*., 1998). The time frame of the onset release and clearance of the circulatory CK is dependent on the intensity of the eccentric exercise model used to induce muscle damage. It is reported that when the intensity of exercise is categorised as mild to moderate, the muscle tissue is stretched without noticeable changes in the permeability of the membrane but when the intensity of exercise is high, the permeability of the membrane changes and CK are released (Brancaccio *et al*., 2007). It seemed the changes in CK that occurred in this study were similar to the study by Nosaka *et al.* (2002) and Nunan *et al.* (2010) which showed the early onset of increase in CK when using mild intensity eccentric exercise. It was reported that in mild intensity exercises, circulatory CK was found below 1000 U/L with limited inflammation and faster recovery (Chatzinikolaou *et al.*, 2014). Even though there were significant changes in circulatory serum CK across time, no significant differences between all groups were evident. It reflected that NS was ineffective at reducing circulatory serum CK following EIMD.

IL-6 and IL-1β are classified as pro-inflammatory cytokines, which possess the role to promote inflammation process. The rise in IL-6 and IL-1β following exercise was mentioned due to; i) the declined in the storage of muscle glycogen, ii) the production of reactive oxygen species (ROS), and iii) the contraction of the skeletal muscle cell (Montero-Junior *et al., 2018;* Peterson and Pederson, 2006). In the present study, IL-6 and IL-1β were expected to be increased remarkably in blood serum but no significant changes found from pre-exercise to four days after exercise in all groups, regardless of which interventions had been given. This concurred with what has been reported in the study which implemented a −4° to −8° downhill running (Malm, 2001).

Because of no significant changes found in IL-6 and IL-1β after exercise, it seemed reasonable to suggest that there might be no remarkable inflammatory process activated following the exercise model that was chosen. This suggestion

is agreeable based on the notion stated that the magnitude increases of cytokines might be dependent on the intensity and duration of the exercise (Suzuki, 2018).

## Limitations

There were some limitations in this study. The exercise protocol chosen, CMJ, is enough to induce alteration in most of the functional parameters of muscle damage, but not enough to cause marked changes in biochemical parameters of muscle damage and inflammation. In any study, abundant literature reviews are needed to identify the work scopes in the study area that have been done so far. The findings are to be used as the groundwork for the researcher to create new study and objectives. However, we were unable to find the literature about the effects of Nigella sativa on EIMD that have used humans as their subjects. Even in a few studies focusing on the effectiveness of Nigella sativa on muscle damage, the subjects used were animals. It was not known to what extent the results from animal studies are generalisable to human studies.

The lack of effects of Nigella sativa on markers might reveal several possible factors. One of the factors was that the NS ingested was in the form of oil and the important anti-inflammatory substance in Nigella sativa, such as thymoquinone (TQ), which might not be readily absorbed by the target tissues. Although TQ has been widely studied and its inflammatory properties have been proven, evidence from previous studies revealed that TQ has poor absorption and rapid clearance (Alkharfy *et al.*, 2013; Abdelwahab *et al.*, 2013). It has been reported that TQ is hydrophobic (poor solubility to water) and has poor bioavailability (Elmowafy *et al.*, 2016). Thus, to increase bioavailability and longer circulation of TQ, a few new formulations should be implemented in the future, such as by loading TQ into nanoparticles carriers so that it can be easily absorbed into the bloodstream. In the present study, it was not known whether the amounts of NS given to the subjects contained enough TQ to reach into circulation, and it was not known how long it remains in the circulation.

## Conclusion

It has been concluded that the eccentric exercise model used in this study was enough to induce muscle damage, but it might be too mild to activate the inflammatory cytokines. Moreover, ingesting Nigella sativa two weeks prior and continuously until recovery following mild eccentric exercise was found to be ineffective in attenuating muscle damage and inflammation cytokines. These unexpected findings are believed to be explained by several factors as stated in the limitations. In future studies, the limitations should be studied before conducting similar research on Nigella sativa's effectiveness in attenuating muscle damage and inflammation following exercise.

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## Conflict of interest

The authors declare that they have no conflict of interest.

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