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Original Article

Single one-off dose of Nigella Sativa does not attenuate indirect markers of exercise-induced muscle damage as a model of inflammation

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Abstract
Introduction Nigella sativa (NS) is widely known to have anti-inflammatory effects on various inflammation models; however, there are no known NS studies that include EIMD as a model of inflammation. This study aimed to investigate the effects of NS on EIMD as a model of inflammation. **Material and Methods** A total of 33 youth football athletes were assigned to three groups (i.e., NS3000, NS1500, and the control). All groups performed 10 sets of 10 repetitions of CMJ (with a 30-s intermittent rest between sets) by adopting a squat stance in starting and landing positions to induce muscle damage. Immediately after the exercise, they were administered a single dose of either 3000 mg or 1500 mg of NS or a placebo. A set of tests was conducted to measure muscular power, soreness, tenderness, thigh circumference, ROM, serum CK, and LDH at pre-exercise, 30 min, 1 d, 2 d, 3 d, and 4 d post-exercise. **Results** Changes in these variables over time were compared by a mixed factorial ANOVA. As expected, exercise decreased muscular power and ROM (30 min to 1 d post-exercise) ($P < 0.05$) and increased soreness, tenderness, thigh circumference, CK, and LDH (30 min to 1 d post-exercise) ($P < 0.05$). However, no significant interaction was observed between all measured parameters in three different groups for the measurement sessions ($P > 0.05$). **Discussions and Conclusion** The obtained results suggested that the CMJ protocol used in this study was sufficient to induce muscle damage. Thus,EIMD can be used as a model of inflammation; however, a single one-off intake of NS does not attenuate the indirect markers of EIMD. The effective dosage of NS to increase the recovery speed was not determined.
Key words: Eccentric exercise · Delayed-onset muscle soreness · Anti-inflammatory herbs · Thymoquinone

Abbreviations

NS	Nigella sativa
EIMD	Exercise-induced muscle damage
CMJ	Counter-movement jump
CK	Creatine kinase
LDH	Lactate dehydrogenase
DOMS	Delayed-onset muscle soreness
VAS	Visual analog scale
ROM	Range of motion

Introduction

By following unaccustomed exercises, one might experience pain sensation and stiffness in the muscles, which can arise from 24 to 72 hours after exercise. This sensation is known as 'Delayed onset muscle soreness (DOMS)" (Cheung et al., 2003), one of the symptoms of exercise-induced muscle damage (EIMD), which is a temporary, repairable damage of the muscles and connective tissues caused by physical exercises which occur depends on intensity and duration of the exercise (Owens et al., 2019).

Following EIMD, acute inflammatory response occurs as a defence mechanism in the body to repair the damage and to restore tissue function (Owens et al., 2019). It beginswhen the damaged muscle tissues attract monocytes that are then converted to macrophages and produce large amounts of pro-inflammatory prostaglandins. The production of prostaglandins can heighten vascular permeability (causing oedema), sensitized pain receptors and boost the protein degradation process(Fatouros, 2016). As a result, plenty of studies have examined the effective methods on preventing and treating EIMD, including an oral ingestion of anti-inflammatory substances.

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One of themis by taking non-steroidal anti-inflammatory drugs (NSAIDs)as to some extent NSAIDs can reduce the inflammation of the muscles. However, they can lead to the development of side effects if used for longer periodas reviewed by Puppala et al. (2020).Recently, there are many studies focusing on natural remedies that might have the same curing effects as NSAIDs. One of the potential herbs that are believed to have anti-inflammatory benefit is NS seeds, which is called *habbatussauda* in Malay language. It is known as black cumin, or 'the blessed seed' (Arabic), *kalonji* (India and Pakistan) or *siahdaneh* (Persian) (Hajhashemi et al., 2004). It is a member of the Ranunculaceace family and it has grown in western Asia, southern Europe, Middle East and northern Africa (Dietert and Dietert, 2007).

The seeds are believed to cure many ailments since more than three thousand years ago, as it was used by the family of pharaohs. Furthermore, the seeds are also used as traditional remedy for a number of illnesses including inflammatory disorders in the Indian subcontinent, Western Asia, the Arabian countries, and Europe (Majeed et al, 2020).

Continuous researches performed on NS suggested that it possess effects as an anti-tumor antibacterial, anti-oxidant, analgesic and it also carries the potential as an anti-inflammatory agent (Mazaheri et al., 2019; Bordoni et al., 2019)

Various studies have suggested that the anti-inflammatory activity of NS is due to its active component, which is thymoquinone (TQ). TQ is believed to act as a potent inhibitor of prostaglandins, histamine and leucotrienes production by inhibiting COX, thus inhibiting edema formation at the inflammation site (Parveen et al., 2011). Hence, pain will be lessened and the inflammation will be reduced.Previous researches of NS have been attempted on several inflammation models. However, according to researcher's knowledge, no study has been attempted to see its anti-inflammatory effects on inflammation following EIMD.Moreover, the dosages might not be applicable to treat inflammation problems related to skeletal muscle damage caused by exercises.

On the basis of evidences that NS could suppress the prostaglandins generation by inhibiting COX, the researcher believes that NS may also have the positive effects on inflammatory response following EIMD. If NS helps to reduce inflammation following EIMD, there is also a need to determine the specific dosage to attenuate the recovery of EIMD effectively.

In summary, the present study was initiated by two main problems; i) to researcher's knowledge, no studies have been carried out to examine effects of NS on EIMD, and ii) no anti-inflammatory studies of NS have used or included EIMD as one of the inflammation models even though inflammation response also occurred following EIMD. The purpose of this studywas to examine the effect of a single one-off intake of NS on changes in indirect markers of muscle damage and inflammation after counter-movement jump exercise. It was predicted that NS ingestion would attenuate indirect EIMD markers compared to placebo following exercise.

Methods

Subjects

Thirty three medically fit subjects were recruited randomly among all youth football players aged 15 to 17 years old. The subjects should be free from any history of musculoskeletal, liver and kidney problems. Ethical approval was obtained from the university's human ethics commitee prior to the study.Prior to data collection, subjects and their guardian were given informed consent and health assesment was done using a health history questionnaire to identify the risk for cardiovascular and orthopaedic problems during exercise or after intervention. Daily or weekly sports activity questionnairewas also given to be filled up as to see how intense he exercises to control the effects on inflammation response. Subjects were requested not to change their daily food intake pattern or medication during the study. In addition, they were also requested to be well-hydrated because the concentrations of various substances in blood could be affected by hydration status.

Experimental design

The present study was an experimental study which employed a randomized double-blind controlled trial. Subjects were allocated into three groups based on the therapeutic intervention that was given. Two groups were given NS with either 3000 mg or 1500 mg each, and another group was given placebo. Double-blind method was selected in allocating subjects into their groups of treatments, and it was done by an independent member of the research team.

On the first day, subjects underwent pre-exercise tests that measured muscular power, soreness and tenderness, thigh circumferences, range of motion (ROM) and knee joint angle, and blood serum parameters which were creatine kinase (CK) and lactate dehydrogenase (LDH). In order to induce symptoms of muscle damage to the muscles, all subjects were asked to perform a single bout of a counter-movement jump (CMJ) as a model of eccentric exercise on the same day.

CMJ model was selected because it involves eccentric contraction and it is very practical to be done in fields. The same parameters measured in the pre-exercise tests were repeated again at 30 min, 1 day, 2 days, 3 days and 4 days after the exercise bout. Supplementation of a single one-off dose of 3000 mg or 1500 mg of NS or placebo was done right after exercise protocol. Subjects were also given methyl salicylate as a standard analgesic treatment and they were allowed to rub the methyl salicylate on their legs if they feel pain.

Changes in muscular power, soreness and tenderness, thigh circumferences, ROM of the knee joint, CK and LDH activities following exercise were compared between each group.

NS and placebo

In this study, a single one-off dose of supplementation was administered orally in double-blind method by the subjects. It was either 3000 mg of NS, 1500 mg of NS or placebo. The allocation of supplementation groups was done by the assistant researcher and both of the researcher and the subjects have no knowledge of which subjects administered which supplements. The supplements were ingested by the subjects on the day they performed an eccentric exercise bout of counter-movement jump, and it was done right after the exercise bout.

Both NS and placebo were capsulated and the exact amount of NS in capsule is 500 mg. Starch was used as the placebo. All subjects in each group were given a pack of supplement which contained six capsules of supplements. The pack contained six capsules NS, or three capsules of NS and three capsules of placebo, or six capsules of placebo.

Exercise bout

CMJ was conducted for 100 repetitions with 10 repetition in a set. Subjects were given 30 seconds intermittent rests between every set. They performed maximal vertical jumps throughout the exercise and they were asked to adopt a squat stance on starting and landing position. Squat stance on starting and landing position were done to facilitate muscle damage to the muscles.

Muscle damage markers

Soreness and tenderness

Muscle soreness was measured subjectively using visual analog scale (VAS). Subjects had to perform a maximal vertical jump for three times and at the completion of the third jump, subjects indicated their pain level by marking at any point on a continuous line between 0 to 10, that was drawn on a piece of paper. 0 represented “not at all sore” and 10 represented “extremely sore”.

As for the muscle tenderness measurement, algometer was used in this study. It was applied at the midpoint of rectus femoris with the subjects lying supine. The midpoint of rectus femoris was marked with permanent marker so that the measurements could be made at the same point each time. The measurements of muscle soreness and tenderness were done six times; at pre-exercise, 30 min post-exercise, and day 1, 2, 3 and 4 after the exercise bout.

Range of motion (ROM)

To determine ROM, the flexed (FANG) and stretched (SANG) knee joint angle had first to be obtained. It is because ROM was calculated by subtracting SANG from FANG. To obtain FANG, subjects were asked to fully flex their knee by touching calf to their hamstring, and SANG was obtained when subjects straighten out knee as much as possible. To measure all the angles, three points were drawn on the lateral epicondyle, midline of the lateral femur and lateral fibula as the reference landmarks. Centre of the circular disk of the goniometer was hold on the drawn points on the lateral epicondyle, meanwhile the stationary arm of the goniometer was hold in line with the midline of lateral femur and the moving arm of the goniometer was hold in line with the lateral fibula. To obtain consistency during each measurements, all points were drawn using a permanent marker pen and were remained unchanged during the experimental period. The measurements of the angle were done for six times; pre-exercise, 30 minutes, 1 day, 2 days, 3 days and 4 days post-exercise.

Circumference

A constant tension tape measure was used to measure thigh circumference of four marked sites which were equally divided between 4 cm under gluteal fold until knee crease. The marks were maintained using a permanent ink marker during five days of the experimental period. The subjects were in standing position when the measurements were taken. The measurements of circumferences were done for six times; pre-exercise, 30 minutes, 1 day, 2 days, 3 days and 4 days post-exercise.

Blood sampling and analyses

Blood serum were analysed for the markers of muscle damage and inflammation. Muscle damage and inflammation markers were CK and LDH.Approximately 2 mℓ of venous blood was drawn for six times; pre-exercise, 30 minutes, 1 day, 2 days, 3 days and 4 days post exercise at the location of data collection. The blood samples were carefully brought to Sport Science lab in Universiti Sains Malaysia to be centrifuged to obtain blood serum, then kept frozen afterwards. The samples were sent to other labs to be analyzed.

Statistical analyses

Changes in these variables over time were compared between treatments and placebo conditions by a mixed factorial ANOVA.

Results

Table 1illustrated descriptive statistic for all measure parameters measured across measurement time, and table 2 displayed test of within-subjects effects on all parameters across the measurements.

From table 2, mixed factorial ANOVA revealed a non-significant interaction between groups across time for muscular power ($F= 0.753$, $p>0.05$), soreness ($F= 1.577$, $p>0.05$), tenderness ($F= 1.354$, $p>0.05$), circumference ($F= 1.135$, $p>0.05$), ROM ($F= 0.657$, $p>0.05$), CK ($F= 1.764$, $p>0.05$) and LDH ($F= 0.740$, $p>0.05$).

Muscular power

Table 3 showed muscular strength increases significantly from pre-exercise session to 30 min post-exercise session ($p<0.05$), and decreases significantly from 30 min post-exercise to 1 day post-exercise ($p<0.05$). From 1 day post-exercise to 2 days post-exercise, no significant changes is found ($p>0.05$) but shows significant incline from 2 days post-exercise to 3 days post-exercise ($p<0.05$). Then, from 3 days post-exercise to 4 days post-exercise, no significant changes occurred ($p>0.05$).

Soreness and tenderness

Soreness

Table 1 displayedsimilar trends of the mean changes of VAS of soreness of all groups; first, an incline from pre-exercise sessions to 1 day post-exercise sessions followed by a levelling out. Even though all groups showed similar trends, the scores for NS 3000mg group peaked lower than both NS 1500mg and placebo groups from pre-exercise to 1 day post-exercise sessions, then gradually decreased until the last day of the tests session.

Table 4 displayed pairwise comparison between measurements of soreness.

From the table it showed that there are significant incline at measurements from pre-exercise session to 30 min post-exercise session and from 30 min post-exercise session to 1 day post-exercise session ($p<0.05$). Meanwhile, from 1 day post-exercise to 2 days post-exercise, no significant changes is found ($p>0.05$) but shows significant decline from 2 days post-exercise to 3 days post-exercise and from 3 days post-exercise to 4 days post-exercise ($p<0.05$).

Tenderness (pain threshold)

Table 5 showed tenderness decreases significantly from pre-exercise session to 30 min post-exercise session, and from 30 min post-exercise to 1 day post-exercise ($p<0.05$). From 1 day post-exercise to 2 days post-exercise, no significant changes is found ($p>0.05$) but shows significant incline from 2 days post-exercise to 3 days post-exercise and from 3 days post-exercise to 4 days post-exercise ($p<0.05$).

Circumference (swelling)

Table 6 showed that there is significant incline at measurements from pre-exercise session to 30 min post-exercise session and from 30 min post-exercise session to 1 day post-exercise session ($p<0.05$). Meanwhile, from 1 day post-exercise to 2 days post-exercise, no significant changes is found ($p>0.05$) but shows significant decline from 2 days post-exercise to 3 days post-exercise and from 3 days postexercise to 4 days post-exercise ($p<0.05$).

Range of motion (ROM)

Table 7 showed that there are significant decline at measurements from pre-exercise session to 30 min post-exercise session and from 30 min post-exercise session to 1 day post-exercise session ($p<0.05$). Meanwhile, from 1 day post-exercise to 2 days post-exercise, no significant changes is found ($p>0.05$) but shows significant incline from 2 days post-exercise to 3 days post-exercise and from 3 days post-exercise to 4 days post-exercise ($p<0.05$).

Creatine Kinase (CK)

Table 8 showed that there are significant changes at all measurements ($p<0.05$). Significant incline occur at measurements from pre-exercise session to 30 min post-exercise session and 30 min post-exercise session to 1 day post-exercise session ($p<0.05$). Meanwhile, significant decline occur at measurements from 1 day post-exercise afterwards ($p<0.05$).

Lactate Dehydrogenase (LDH)

Table 9 showed no significant changes at measurement from pre-exercise session to 30 min post-exercise session ($p>0.05$). However, there are significant incline at measurements from 30 min post exercise session to 1 day post-exercise session, 1 day post-exercise session to 2 days post-exercise session, and 2 days post-exercise session to 3 days post-exercise session ($p<0.05$). Meanwhile, from 3 days post-exercise session to 4 days post-exercise session, no significant changes is found ($p>0.05$).

Tables

Table 1 *Descriptive Statistics of All Parameters Mean Changes of Three Different Groups of Treatment across Measurements*

Independent variable	Mean±SD[Percentage of changes (%)]						
	Group	Pre	Post-30 min	Post-1 day	Post-2 day	Post-3 day	Post-4 day
Power (inch)	NS 3000mg	16.00 ±1.87 [0]	16.77 ±1.68 [+4.79]	15.71 ±1.90 [-6.32]	16.72 ±2.57 [+6.42]	16.61 ±2.16 [-0.65]	17.22 ±2.16 [+3.67]
	NS 1500mg	15.11 ±1.60 [0]	15.40 ±2.31 [+1.93]	15.08 ±2.96 [-2.06]	15.86 ±3.11 [+5.13]	17.26 ±3.82 [+8.89]	16.36 ±4.20 [-5.27]
	Placebo	15.51 ±2.80 [0]	17.30 ±2.85 [+11.54]	16.67 ±2.98 [-3.64]	16.92 ±2.13 [+1.50]	18.51 ±2.92 [+9.40]	18.25 ±2.50 [-1.40]
Soreness (cm)	NS 3000mg	1.21 ±1.55 [0]	4.83 ±2.53 [+299.17]	5.52 ±2.63 [+14.28]	4.85 ±2.72 [-12.14]	3.83 ±2.20 [-21.03]	2.33 ±1.10 [-39.16]
	NS 1500mg	0.86 ±1.67 [0]	2.96 ±1.91 [+244.19]	6.27 ±1.85 [+111.82]	6.14 ±1.97 [-2.07]	4.46 ±1.45 [-27.36]	3.07 ±1.44 [-31.17]
	Placebo	0.16 ±0.35 [0]	2.99 ±1.78 [+1768.75]	6.50 ±2.13 [+117.39]	5.67 ±3.38 [-12.77]	3.70 ±1.88 [-34.74]	1.88 ±1.71 [-49.19]
Tenderness (kg)	NS 3000mg	14.85 ±1.47 [0]	14.23 ±1.66 [-4.18]	10.81 ±2.12 [-24.03]	11.66 ±1.70 [+7.96]	12.80 ±1.55 [+9.78]	14.35 ±1.14 [+12.11]
	NS 1500mg	15.22 ±1.13 [0]	14.45 ±1.90 [-5.06]	10.71 ±2.54 [-25.88]	10.81 ±1.46 [+0.93]	11.79 ±1.59 [+9.07]	13.33 ±2.19 [+13.06]
	Placebo	14.21 ±1.60 [0]	13.51 ±1.36 [-4.93]	10.39 ±2.57 [-23.01]	9.99 ±2.15 [-3.85]	10.68 ±1.72 [+6.91]	12.14 ±1.86 [+13.67]
Circumference (cm)	NS 3000mg	47.45 ±4.27 [0]	48.16 ±4.43 [+1.50]	48.86 ±4.45 [+1.45]	48.42 ±4.45 [-0.90]	48.08 ±4.25 [-0.70]	47.62 ±4.27 [-0.96]
	NS 1500mg	46.82 ±3.83 [0]	47.73 ±4.20 [+1.94]	48.48 ±3.77 [+1.57]	47.95 ±3.67 [-1.09]	47.66 ±3.84 [-0.60]	47.55 ±3.74 [-0.23]
	Placebo	46.32 ±3.28 [0]	47.03 ±3.58 [+1.53]	47.84 ±3.47 [+1.72]	47.97 ±3.47 [+0.27]	47.48 ±3.54 [-1.02]	46.80 ±3.26 [-1.43]
ROM (°)	NS 3000mg	151.64 ±3.23 [0]	150.09 ±3.78 [-1.02]	146.00 ±2.24 [-2.73]	145.91 ±3.27 [-0.06]	150.00 ±3.00 [+2.80]	151.27 ±2.53 [+0.85]
	NS 1500mg	149.73 ±3.07 [0]	147.64 ±3.30 [-1.40]	145.82 ±3.55 [-1.23]	146.55 ±3.73 [+0.50]	148.36 ±4.93 [+1.24]	150.36 ±2.98 [+1.35]
	Placebo	151.20 ±4.29 [0]	149.50 ±3.60 [-1.12]	146.00 ±2.54 [-2.34]	147.00 ±3.62 [+0.68]	149.10 ±2.85 [+1.43]	150.60 ±3.69 [+1.01]
CK (U/L)	NS 3000mg	175.75 ±68.15 [0]	204.58 ±73.43 [+16.40]	440.75 ±224.25 [+115.44]	392.25 ±184.61 [-11.00]	319.75 ±118.10 [-18.48]	249.58 ±77.18 [-21.95]
	NS 1500mg	220.30 ±97.77 [0]	240.50 ±100.53 [+9.17]	422.70 ±209.46 [+75.76]	330.50 ±168.58 [-21.81]	248.70 ±122.18 [-24.75]	205.80 ±116.53 [-17.25]
	Placebo	158.10 ±74.83 [0]	177.10 ±85.51 [+12.02]	292.60 ±207.35 [+65.22]	279.00 ±231.56 [-4.65]	252.80 ±186.04 [-9.39]	232.80 ±169.45 [-7.91]
LDH (U/L)	NS 3000mg	116.33 ±29.55 [0]	118.83 ±21.70 [+2.15]	125.92 ±25.16 [+5.97]	144.17 ±40.87 [+14.49]	164.33 ±33.37 [+13.98]	160.33 ±37.01 [-2.43]
	NS 1500mg	130.70 ±24.45 [0]	131.70 ±24.24 [+0.77]	140.30 ±21.72 [+6.53]	157.10 ±35.64 [+11.97]	169.20 ±22.04 [+7.70]	172.20 ±26.27 [+1.77]
	Placebo	109.11 ±24.17 [0]	117.78 ±27.17 [+7.95]	131.56 ±31.36 [+11.70]	133.56 ±23.44 [+1.52]	140.78 ±29.66 [+5.41]	147.11 ±30.44 [+4.50]

Table 2 *Test of Within-Subjects Effects on All Parameters across the Measurements*

Parameters	Type III Sum of Squares	df	Mean Square	F	P value
Strength	27.009	5.287	5.108	0.753	0.593
Soreness	52.456	10	5.246	1.577	0.119
Tenderness	21.322	5.370	3.971	1.354	0.248
Circumference	3.772	6.362	0.593	1.135	0.348
ROM	42.457	7.011	6.056	0.657	0.708
CK	151309.456	3.407	44407.552	1.764	0.160
LDH	2823.369	8.171	345.538	0.740	0.659

Table 3 *Pairwise Comparisons between Measurements of Muscular Power*

Measurement	Measurement	Mean Difference	P value
Pre-exercise	30 min post-exercise	- 0.95*	0.010
30 min post-exercise	1 day post-exercise	0.67*	0.034
1 day post-exercise	2 days post-exercise	- 0.68	0.106
2 days post-exercise	3 days post-exercise	- 0.96*	0.004
3 days post-exercise	4 days post-exercise	0.19	0.187

Table 4 *Pairwise Comparisons between Measurements of Soreness*

Measurement	Measurement	Mean Difference	P value
Pre-exercise	30 min post-exercise	- 2.85*	0.000
30 min post-exercise	1 day post-exercise	- 2.50*	0.000
1 day post-exercise	2 days post-exercise	0.54	0.258
2 days post-exercise	3 days post-exercise	1.56*	0.000
3 days post-exercise	4 days post-exercise	1.56*	0.000

Table 5 *Pairwise Comparisons between Measurements of Tenderness*

Measurement	Measurement	Mean Difference	P value
Pre-exercise	30 min post-exercise	0.70*	0.000
30 min post-exercise	1 day post-exercise	3.42*	0.000
1 day post-exercise	2 days post-exercise	- 0.18	0.526
2 days post-exercise	3 days post-exercise	- 0.94*	0.000
3 days post-exercise	4 days post-exercise	-1.52*	0.000

Table 6 *Pairwise Comparisons between Measurements of Circumference*

Measurement	Measurement	Mean Difference	P value
Pre-exercise	30 min post-exercise	- 0.78*	0.000
30 min post-exercise	1 day post-exercise	- 0.76*	0.000
1 day post-exercise	2 days post-exercise	0.28	0.136
2 days post-exercise	3 days post-exercise	0.37*	0.000
3 days post-exercise	4 days post-exercise	0.42*	0.001

Table 7 *Pairwise Comparisons between Measurements of Range of Motion (ROM)*

Measurement	Measurement	Mean Difference	P value
Pre-exercise	30 min post-exercise	0.78*	0.000
30 min post-exercise	1 day post-exercise	3.14*	0.000
1 day post-exercise	2 days post-exercise	- 0.55	0.407
2 days post-exercise	3 days post-exercise	- 2.67*	0.000
3 days post-exercise	4 days post-exercise	- 1.59*	0.013

Table 8 *Pairwise Comparisons between Measurements of Serum Creatine Kinase (CK)*

Measurement	Measurement	Mean Difference	P value
Pre-exercise	30 min post-exercise	- 22.68*	0.000
30 min post-exercise	1 day post-exercise	- 177.96*	0.000
1 day post-exercise	2 days post-exercise	51.43*	0.001
2 days post-exercise	3 days post-exercise	60.17*	0.002
3 days post-exercise	4 days post-exercise	44.36*	0.001

Table 9 *Pairwise Comparisons between Measurements of Serum Lactate Dehydrogenase (LDH)*

Measurement	Measurement	Mean Difference	P value
Pre-exercise	30 min post-exercise	- 4.06	0.388
30 min post-exercise	1 day post-exercise	- 9.82*	0.032
1 day post-exercise	2 days post-exercise	- 12.35*	0.005
2 days post-exercise	3 days post-exercise	- 13.16*	0.019
3 days post-exercise	4 days post-exercise	- 1.78	0.759

Discussion

The main findings of the study included that; 1) markers of EIMD suggest that CMJ protocol used in this study was sufficient to induce muscle damage, 2) however,no significant interaction was found on all measured parameters (muscular power, soreness, tenderness, thigh circumferences, ROM, CK and LDH) of three different groups across the measurement sessions, 3) regardless the amount of NS given, a single one-off intake is of no significant benefit to increase the speed of recovery following EIMD.These results did not support the hypothesis that ingestion of NS would attenuate EIMD. Therefore, the effective dosage of NS to increase the recovery speed was unable to be determined and the intake of NS in a single one-off method might be the possible cause.

Previous studies indicated that soreness is developed between one and two days post-exercise, peaks between one and three days post-exercise (Clarkson et al., 1992) and lasts between three to seven days post-exercise. Similarly, in the present study, VAS score for soreness peaked at 1 day post-exercise and did not yet return to baseline at 4 days post-exercise. The pattern of incline and recovery in means of soreness scores also concurs with previous findings in study by Connolly et al. (2006). On the VAS score from 0 (“not at all sore”) to 10 (“extremely sore”), the eccentric exercise model used in this study produce soreness scores of 5.5 to 6.5 on 1 day post-exercise. Typically, mild eccentric exercise model such as DHR produce soreness scores of 4 to 5, meanwhile more intense model than DHR such as elbow flexors usually produce average peak soreness scores of 7 to 8 (Sayers and Hubal, 2008). It suggests that the eccentric exercise model used in this study has intermediate intensity, and the exercise protocol is enough to induce injury to the muscle as the soreness scores is in the middle between the soreness scores of DHR and elbow flexor model.

There are two mechanisms proposed for the sensation of soreness, which are; 1) swelling or edema and increased pressure within the damaged muscle trigger the sensory receptor that responds to mechanical pressure or distortion (mechanoreceptors), 2) increase of prostaglandins, bradykinins and histamines caused by infiltration of inflammatory components within the damaged muscle trigger the pain receptors that are sensitive to chemical signals (Sayers and Hubal, 2008).

Even though there were significant changes in soreness across time, there was no significant difference between all groups. Therefore, ingestion of NS regardless the dose was ineffective at reducing soreness following EIMD.

The tenderness score is the minimum pressure which induces pain in tender and trigger points of tissue. In the present study, the tenderness score in both NS group showed highest reduction at one day post-exercise while in placebo group showed highest reduction on two days post-exercise (Figure 4.3). This result is in concurs with the result of Connolly et al. (2006) which reported the group of treatment with tart cherry juice showed highest reduction at one day post-exercise while placebo group showed highest reduction at two days post-exercise.

However, in the present study, no significant interaction was found on tenderness between the treatment and placebo groups across the measurement sessions. The lack of effect of NS on muscle tenderness might reveal that the measurement were not sensitive to real differences between NS and placebo trials and sometimes, pain test is still considered as a very subjective measurement even though variety of measurement tools used.

Muscle group circumference is a common method used to measure the presence of edema (swelling). Edema is caused by fluid accumulation in the damaged muscle tissues. As reported earlier byChleboun et al. (1998) and Tanabe et al. (2015) in their study using elbow flexor as the muscle damaging exercise model, circumference increases following exercise, and it peaks at four to five days post-exercise.

However in the present study, the data yielded showed that circumference at every point measured peaks as early as one day post-exercise. The result is supported by Nunan et al. (2010), which had used DHR model, a milder damaging exercise protocol than elbow flexor model. Nunan et al. (2010) mentioned that the measurement for circumference peak at one day post-exercise, even though no significant difference of circumference between one day and two days post-exercise. This might be explained by the difference of the damaging exercise protocol used with the previous studies. As explained before, elbow flexor model of exercise is much more intense compared to the exercise model used in this study. Even though there was no direct explanation to the relationship between swelling time course and the intensity of the exercise, the researcher suggested that the higher intensity exercises can contribute to the longer time of fluid accumulation in the damage muscles and peak later than the mild intensity exercises.

The findings also showed there were significant changes in circumference across time, however no significant difference between all groups. Therefore, ingestion of NS regardless the dose was ineffective at reducing swelling following EIMD.

Following EIMD, decreasing ROM at the affected joint is said to be caused by the increase in passive muscle stiffness resulted by muscle swelling as well as disruption of myofibrils (Chleboun et al., 1998).In the previous study by Chapman et al. (2007), which had used higher intensity exercise protocol (elbow flexor model) to induce muscle damage, they reported the highest reduction in ROM was seen at three days post-exercise. Meanwhile, Tartibian et al. (2009) who had used moderate intensity exercise protocol (knee extensor model) documented that the highest reduction in ROM is occurred at two days post-exercise. In another study which had also used moderate intensity exercise protocol (DHR model) by Nunan et al. (2010), there was no changes in ROM in a drug treatment group. However, in a control group a greatest reduction of ROM occurred at one day post-exercise, earlier compared to other studies, and it returned to pre-exercise level at three days post-exercise.

In the present study, we found that the greatest reduction in ROM at one day post-exercise (Figure 4.8), was similar to what has been found by Nunan et al. (2010). This might indicate that the exercise model used in this study has moderate intensity as the one used by Nunan et al. (2010). On the basis of the previous studies and this study, it can be concluded that ROM in the higher intensity exercise shows highest reduction later compared to ROM in the moderate intensity exercise. This conclusion is supported by the study by Saka et al. (2009), which examined the differences of ROM between higher (elbow flexor) and moderate intensity (knee extensor) of eccentric exercise. They found that the highest reduction of ROM for elbow flexor is occurred at three days post-exercise andfor knee extensor is occurred at one day post-exercise. They added that DOMS and reduction in ROM were higher for elbow flexor than for knee extensor.

The findings also showed there were significant changes in ROM across time, however no significant difference between all groups. Therefore, ingestion of NS regardless the dose was ineffective at reducing the magnitude of decrease in ROM following EIMD.

Elevation of CK in blood has been widely considered to be one of the indirect indices of muscle damage. When muscle is damaged, muscle cells and plasma membrane of muscle fibres disrupt and release CK into the blood serum (Clarkson et al. 1992). To analyse CK, researchers used either blood plasma or serum. In the present study, blood serum was used rather than plasma. Researchers believed both do not have much difference because the composition of serum and plasma is similar but only exclude blood clotting factors.

The timeline for the release and clearance of the CK is dependent on the exercise protocol used to induce damage to muscles. In the earlier report by Byrnes et al. (1985), mild eccentric exercise models such as DHR produce peak plasma CK from 300 to 600 IU and it peaks from between 12 hours to 1 day post-exercise. Later studies support the findings by reporting peak plasma CK produced by DHR model are in the range of 300 to 390 IU (Sacheck et al., 2002). Compared to milder eccentric exercise model, CK in higher force or intensity eccentric exercise model such as elbow flexor and knee extensor model begin to elevate (one to two days post-exercise), and peak later (four to seven days post-exercise) (Byrne et al., 2001). These eccentric exercise models produce CK ranging from 800 to 16000 IU (Nosaka et al., 2002).

In this study, the findings showed the CK activity was peaking at 1 day post-exercise. It was in line with the study by Nosaka et al. (2002) andNunan et al. (2010) but in contrast to the temporal pattern associated with EIMD following intense or high force eccentric exercise, which indicated more of a later response peaking at four to seven days following exercise (Byrne et al., 2001). The peak CK yielded ranges from 207 to 441 IU and the graph mirrors the classic temporal pattern associated with EIMD following mild eccentric exercise. It indicated that the eccentric exercise model used in this study was mild but sufficient to induce damage to muscles.

Even though there were significant changes in CK across time, there was no significant difference between all groups. Therefore, ingestion of NS regardless the dose was ineffective at reducing CK following EIMD.

The elevation of LDH enzyme in blood indicates the breakdown of tissues. The breakdown of tissue following EIMD explained the elevation of LDH in blood serum.In the present study, the finding showed LDH peaks at three to four days post-exercise. In NS1500 mg and placebo group, LDH increases continuously from post-exercise and remained elevated until end of measurement session at four days post-exercise. Meanwhile in NS3000 mg group, it peaks at three days post-exercise and decreases afterwards. The findings can be possibly supported by recent study by Shariatifar et al. (2014), which proved that NS could reduce LDH. This is the only one study focused on the effects of NS on LDH as a muscle damage parameter, however the study was for rat, not for human.

Regardless treatment, the elevations of LDH on the days following the eccentric exercise were consistent with the literatures. Mastaloudis et al. (2006) also reported similar pattern of LDH following race. LDH increased at midrace, peaked at post-race and remained elevated through four days post-race.

The early start but delayed peaks of LDH activity in the circulation after exercise might be explained by the early destruction of damaged muscle tissues after exercise, and the completion of the destruction process might take longer time. In the previous study, it was said that if there was no further increase in LDH, it indicates that the damaged muscle already resistant to the destruction process.

The findings also showed there were significant changes in LDH across time, however no significant difference between all groups. Therefore, ingestion of NS regardless the dose was ineffective at reducing LDH following EIMD.

Effective dose of NS to benefit recovery

There were three experimental groups in this study, representing two different doses of NS and a placebo. The findings of the present study revealed that there were no significant differences in the measured parameters between all experimental groups. It means, the NS dose 3000mg nor 1500mg did not have significant effect on enhancing recovery following EIMD even though in some parameters the intake of NS had slight effects on increasing speed of recovery following EIMD compared to placebo. However, the effect was statistically not significant. So, effective dose dosage of NS to increase the recovery speed was unable to be determined in this study and the researcher believed the intake of NS in a single one-off method might be the possible cause.

Single one-off intake of NS

The present study found that NS ingestion had no positive effects on recovery of strength, soreness, tenderness, circumference, ROM, serum CK and LDH. Even so, the pattern of changes in all the muscle damage parameters were similar to those reported in the literatures, meaning that the EIMD is occurred and exercise protocol is sufficient to induce damage to muscle. It was expected that a single one-off dose of NS ingestion would benefit recovery, but no such effects found. The amount 1500mg and 3000mg of NS have been shown to be safe for a single one-off dose (Zaoui et al., 2002). However, it had not been known before this study how much doses of NS would be effective to prevent muscle damage in humans, so the researcher assumed that if any positive effect of NS ingestion on muscle damage existed, a significant difference between the NS and placebo conditions will be shown by the dose (1500mg and 3000mg).

It has been documented by Davis et al. (2007) that post-exercise inflammation starts to present around four to twelve hours after eccentric exercise, and further develops in one to two days after exercise. Considering the time taken for the NS to get into the blood circulation, it was assumed that the ingestion of NS at right after exercise could reduce the development of initial inflammation, resulted to the positive effects on recovery.

However, the expectation was to not take the absorption and clearance rate of NS into main consideration. It has been reported by Alkharfy et al. (2015) that TQ, the anti-inflammatory compound in NS possess rapid clearance and slower absorption rate following oral administration in animals. They had used the oral doses around 10 to 20mg/ kg body weight and the calculated time of clearance half of the compound from blood was found to be 4.49 to 4.58 hours, meanwhile the time of absorption half of the compound in blood was found to be 3.62 hours. The time needed to reach maximum concentration in blood was found to be 3.96 hours. Even though it is not known how equivalent the half-time clearance and absorption rate for animal was to the human, their findings clearly proved that the compound possess rapid clearance and slower absorption rate. Since there was no difference in the rate to human, the administration of a NS only once at 30 min after exercise as done in this study is not sufficient to make sure the compound still remain in the blood at least for 24 hours post-exercise. It is because the compound might be already cleared from blood as early as in 12 hours post-exercise.

It is also reported by Nosaka et al. (2006) that administration of essential amino acid 30 min before and right after eccentric exercise appeared to have no effect to muscle damage markers. However, when the supplementation was done continuously for the next four days after exercise, the increase in muscle soreness and ROM were successfully attenuated. Thus, it is possible that the effects of NS ingestion on muscle damage would have been found if the NS had been given not only once but continuously on recovery days, and/ or prior to the exercise bout.

Conclusions

In conclusion, the present study was unable to demonstrate the efficiency of Nigella sativa (NS) in attenuating EIMD as expected seeing that the findings showed no significant interaction was found on all measured parameters (muscle strength, soreness, tenderness, thigh circumferences, ROM, CK and LDH) of three different groups across the measurement sessions. In addition, the effective dose of NS to increase the speed of recovery following EIMD was also unable to be determined as there were no significant effects of both NS doses (3000mg and 1500mg of NS) and placebo on recovery following EIMD.This unexpected finding is believed to be explained by the single one-off method of supplementation that have been used in this study. It is because thymoquinone (TQ), the anti-inflammatory compound in NS possess rapid clearance from blood and slower absorption rate in blood. The results also led us to speculate that it might have some positive effects of NS supplementation if the NS had been given multiple doses continuously from prior to the exercise bout to the recovery days.

The pattern of changes in all the muscle damage parameters used in this study were similar to those reported in the literatures indicated the exercise model used is mild. Even so, it is still sufficient to induce damage to muscle. However, researcher believed that the results might be influenced by the age of the subjects chosen and their daily activities. It was said that boys are less prone to muscle damage due to their muscles flexibility and active daily routine. If severe damage to muscles are to be induced, the subjects should not be active boys but sedentary adult men, or/ and the milder damaging exercise model should be replaced by the higher intensity exercise model such as the elbow flexor.

To obtain positive effects of NS in attenuating inflammation or muscle damage following exercise, the limitations of the present study should be overcome.

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Conflict of interest
The authors declare that they have no conflict of interest.
Ethical approval
All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.
Informed consent
Informed consent was obtained from all individual participants included in the study.

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