

Effect of Plai cream [*Zingiber montanum* (J.Koenig) Link ex A.Dietr. syn. *Zingiber cassumunar* Roxb.] combined with ultrasound on delayed onset muscle soreness

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Plai cream (*Zingiber cassumunar* Roxb.) has been used as a remedy for release pain and inflammation of musculoskeletal problems. The enhancement of the anti-inflammatory effect of Plai cream by phonophoresis or ultrasound therapy is questionable. The aim of this study was to evaluate the effect of Plai cream combined with ultrasound in the treatment of delayed onset muscle soreness (DOMS). Seventy-five volunteers were randomly allocated into 3 groups; 14 % Plai cream (5 cm long strip), ultrasound treatment (1MHz, 1watt.cm⁻²) for 5 min, and combined 14 % Plai cream and ultrasound for 5 min. The participants performed eccentric exercise (4 sets of 25 repetitions at a speed of 60°.s⁻¹) of dominant quadriceps using isokinetic dynamometry to induce DOMS. All participants received their allocated treatment once per day for the next 7 days. We found pain score, thigh circumference and serum creatine kinase were increased, while pressure pain threshold and muscle strength were decreased, but small changes of joint motion after intensive exercise (post-exercise day 1, 2, 3 & 7). However, there was no significant difference changes of all outcomes among three groups. In conclusion, combined 14 % Plai cream with ultrasound had no additional benefit at reducing DOMS compared to either 14 % Plai cream alone or ultrasound alone.

Keywords: Plai cream, Phonophoresis, Muscle soreness, Muscle strength, Creatine kinase

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Delayed onset muscle soreness (DOMS) illustrated as muscle pain and tenderness typically occurs after strenuous eccentric exercise, especially unfamiliar exercise¹. The symptoms associated with DOMS that normally occur 24-48 hrs after exercise² derive from exercise induced sarcomere disruption and the secondary inflammatory response^{3,4}. Non-steroidal anti-inflammatory drugs (NSAIDs) are one of numerous methods prescribed for DOMS management that may improve the muscle pain, stiffness, swelling and function loss accompanying DOMS. The NSAIDs inhibit cyclooxygenase activity, subsequently reducing prostaglandins and inflammatory processes^{5,6}.

Plai or *Zingiber cassumunar* Roxb. is widely used in Thailand as traditional medicine for anti-

inflammation and pain reduction. Plai cream is thought to inhibit cyclooxygenase, cyclooxygenase-2 and lipoxygenase pathways^{7,8}, similar to the anti-inflammatory effects of NSAID's. Previous clinical trials revealed application of Plai cream has beneficial effects in decreasing symptoms of ankle sprain⁹, knee osteoarthritis¹⁰ and muscle strain¹¹. In a recent study, Plai cream at a concentration of 14 % reduced DOMS pain significantly¹².

Ultrasound is commonly used in physiotherapy to relieve pain and inflammation. Ultrasound as phonophoresis has also been used to enhance transdermal penetration of pharmacological substances¹³. Ultrasound in combination with diclofenac gel for management to osteoarthritis patients was found to improve pain at rest and activity, the Western Ontario and McMaster

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Universities Arthritis Index (WOMAC) pain score and physical function after treatment compared to either diclogencic gel alone or ultrasound therapy alone¹⁴. Ultrasound and phonophoresis has also been showed with beneficial effects on other musculoskeletal disorders such as painful shoulder syndrome and lateral epicondylitis¹⁵⁻¹⁶. The evidence for the use of phonophoresis for the management of DOMS has some merit with a study showing that using ultrasound combined with trolamine salicylate cream on 3 consecutive days after eccentric exercise reduced muscle soreness by 24.4 % compared to trolamine salicylate cream alone and reduced muscle soreness by 51.7 % compared to ultrasound alone¹⁷. However, while phonophoresis has been successfully used with other products to reduce DOMS symptoms, the combination of ultrasound with Plai oil for the reduction of DOMS symptoms has not been investigated.

This study aims to evaluate the therapeutic effect of Plai cream combined with ultrasound on subsequent DOMS symptoms compared to only Plai cream or ultrasound alone. If the anti-inflammatory effect of Plai cream can be enhanced by phonophoresis, it could be an alternative management for individuals with DOMS.

Methods

This clinical trial was conducted at the exercise laboratory, Rehabilitation Department, KhonKaen University, Thailand. Seventy-five healthy volunteers aged 18–60 yrs were included in this study. Participants who suffered serious medical diseases such as diabetes, hypertension and heart disease were excluded. The included participants were asked to sign consent forms before commencing the experiment. The study was approved by the KhonKaen University Ethics Committee for Human Research (Ref. No. HE 561436), and was registered at Thai Clinical Trial Registry (ID. No. TCTR20150628001).

Exercise induced DOMS

Strenuous eccentric exercise of the quadriceps muscle was performed to generate DOMS using an isokinetic dynamometer (Primus RS, BTE technologies, Hanover, MD, USA). Fifteen minutes of low intensity and active stretching exercises were used to warm-up the lower limbs prior to start the eccentric exercise. Four sets of 25 maximum eccentric contractions of the dominant knee was performed at a speed of $60^{\circ} \cdot s^{-1}$ with 10 s rest between repetitions and

3 min rest between each set. The knee movement started from flexion (90°) to full extension (0°) with the rotational axis of knee movement of the dynamometer positioned at the lateral femoral epicondyle. The participants were verbally encouraged to give maximal efforts during the exercise. The exercise protocol has been used previously to successfully induce DOMS¹². The participants were allocated by block randomization into 3 groups; group 1, (Plai cream), group 2 (ultrasound), and group 3 (combined Plai cream and ultrasound). For group 1, a dose of 2 gm or approximately a 2.5 cm long strip of 14 % Plai cream was gently massaged with hand on to the quadriceps muscles around 5 min or until the cream was not visible, immediately following the eccentric exercise. The participants were then instructed to apply a similar amount of Plai cream every 8 hrs after exercise. The Plai cream was manufactured by Bangkok Lab & Cosmetic Co., Bangkok, Thailand that has a GMP (Good Manufacturing Practice) and PIC/S GMP standard. For group 2, a certified physiotherapist applied continuous ultrasound wave therapy¹⁷ with 1 MHz frequency and $1.5 \text{ watt} \cdot \text{cm}^{-2}$ on to the quadriceps muscle area of participants for 5 min immediately following the eccentric exercise and then every day. The transducer head was applied at a right angle to ensure maximum energy absorption. The skin was coated with acoustic gel which did not contain any pharmacological substances. Finally for group 3, the participants received the same ultrasound therapy as group 2 and used a 2.5 cm long strip of Plai cream (14 % Plai cream) as a replacement for acoustic gel. The compliance of participants using the treatments was observed on daily basis along with any other treatments they required (including analgesic relief, NSAID, physiotherapy or massage).

Measurement

The muscle strength, perceived muscle soreness, pressure pain threshold, rate of perceived exertion, range of motion and thigh circumference were measured at baseline, day 1, day 2, day 3, and day 7 post-exercise. Plasma creatine kinase activity was measured at baseline, day 1, day 2 and day 3 post-exercise.

Pain score

The perceived muscle soreness was evaluated by a visual analogue scale ranged from 0 for no pain, to 10 for the worst pain. The visual analog scale has been widely accepted as a reliable method to assess the level of pain intensity¹⁸

Muscle strength

Participant's muscle strength was evaluated during a 3-second maximal isometric voluntary concentric (MVC) of their dominant quadriceps by using dynamometry. All participants were seated while their dominant leg was secured to the isokinetic attachment. Each participant performed three MVCs (2min rest between each) with the knee flexed at 90° with verbal encouragement. The average of two closest trials was recorded. Muscle strength and muscle soreness were measured immediately prior to eccentric exercise, and then again at the same time of day on days 1, 2, 3, 4 and 7 post-exercise.

Plasma creatine kinase (CK)

The level of plasma CK was analyzed from a 5-ml blood sample which was taken from the median cubital vein after 5 min seated rest. The plasma CK was collected at the same time in the morning. The analysis of plasma CK was performed by using an automated analyzer (Cobas® 6000 analyzer; Roche Diagnostics Corp., Indianapolis, IN, USA) at Srinagarind Hospital Laboratory at baseline, day 1, day 2 and day 3 post-exercise.

Pressure pain threshold

Pressure pain threshold of the dominant quadriceps was measured by a manual algometer (Baseline® algometer, Fabrication Enterprises Inc., White Plains, NY, USA). An allocation-blinded research assistance pressed the algometer perpendicularly to quadriceps muscle and recorded the minimal pressure which produced a painful sensation. The pressure test was conducted while participants were on an examination bench with their knee joint extended. The average of three points measured on the quadriceps (5, 10, and 15 cm above the apex of patella) was recorded.

Rate of perceived exertion (RPE)

The participant rated perceived exertion by using the Borg scale (ranged from 6 to 20)¹⁵ recorded at the laboratory after 5 min rest each testing day.

Range of motion (ROM)

The range of motion of the tested knee joint was measured to evaluate the degree of joint stiffness from muscle soreness. The research assistance used a goniometer (Baseline® goniometer, Fabrication Enterprises Inc., White Plains, NY, USA) to measure the degree of knee motion in a prone position from full extension to full knee flexion.

Thigh circumference

Thigh circumference of participants was evaluated to indicate muscle swelling in supine position by a blinded research assistant. The measurement was performed at the midpoint between inguinal fold and anterior aspect of the patella by using a standard tape (Rollfix, Hoechst. Mass, Germany). The circumferences were measured to the nearest 0.1 cm and the mean of the two closest measurements (of a total of 3 measures) was recorded.

Statistical analysis

The study results are presented by descriptive statistics including the mean and standard deviation. Overall effects and differences in pain score, pressure pain threshold, rate of perceived exertion, creatine kinase, muscle strength, range of motion and thigh circumference among three groups during the baseline and recovery time periods were analyzed using a repeated measures analysis of variance (ANOVA) and post hoc analysis. The statistical analysis of statistic was conducted using STATA (version 13.0) with a $p < 0.05$.

Results

The characteristics of the participant groups are presented in the Table 1 ($n = 75$, age 41.5 ± 9.3 yrs, BMI 24.2 ± 3.9 kg.m⁻², mean \pm SD, 6 male and 44 female). There was no drop out and additional treatments such as analgesic, NSAID or massage were not observed. The visual analog pain score significantly increased post-exercise day 1 ($p < 0.01$) in all 3 groups and slowly decreased until post-exercise day 7. The pain score of the combined Plai cream with ultrasound group was lower than Plai cream group alone (mean difference = -2.0, 95 % CI - 8.8 to 4.8, $p = 0.6$) or ultrasound group alone (-2.1, -8.9 to 4.6, $p = 0.5$), but this difference did not reach statistical significance (Table 2). The muscle strength (MVC) decreased within the first 2 days' post-exercise ($p < 0.01$) similar to pressure pain sensation ($p < 0.01$) and gradually improved over the week. The serum CK and thigh circumference was significantly increased at post-exercise day1 ($p < 0.01$) and slowly returned to baseline. Little changes of range of motion and rate of perceived exertion were observed throughout the experiment. Statistical testing showed there was no significant difference of MVC, pressure pain threshold, serum CK, range of motion, rate of perceived exertion and thigh circumference among the three groups (Tables 2&3).

Table 1 — Demographic data of participants

	Plai cream n= 25	Ultrasound n = 25	Combined n = 25
Male/Female	3/22	3/22	3/22
Age (yrs)	41.7 ± 9.9	41.3 ± 9.0	41.6 ± 9.4
Body weight (kg)	60.4 ± 13.3	58.9 ± 8.8	59.9 ± 6.7
BMI (kg.m ⁻²)	24.6 ± 4.9	24.1 ± 3.5	23.5 ± 3.2

Table 2 — Pain score, muscle strength and creatine kinase concentration of the three groups (mean ± SD) at baseline, and post-exercise days.

	Plai cream	Ultrasound	Combined
Pain score			
Baseline	0.5 ± 0.6	0.5 ± 0.5	0.4 ± 0.4
Post-exercise day 1	3.0 ± 2.2*	3.1 ± 2.1*	2.7 ± 1.9*
Post-exercise day 2	2.1 ± 1.7*	2.3 ± 2.1*	1.9 ± 1.7*
Post-exercise day 3	1.4 ± 1.2*	1.5 ± 1.5*	1.2 ± 1.5*
Post-exercise day 7	0.6 ± 1.0	0.5 ± 0.7	0.4 ± 0.4
Muscle strength (kg)			
Baseline	35.6 ± 7.6	34.8 ± 7.9	36.7 ± 7.0
Post-exercise day 1	34.4 ± 7.8*	32.2 ± 6.7*	34.9 ± 5.5*
Post-exercise day 2	32.8 ± 7.4*	32.8 ± 6.0*	34.0 ± 7.2*
Post-exercise day 3	34.3 ± 8.4	34.0 ± 6.9	35.5 ± 6.2
Post-exercise day 7	33.3 ± 8.7	34.7 ± 7.9	35.7 ± 7.0
Creatine kinase (mmol.L⁻¹)			
Baseline	116.1 ± 41.6	128.9 ± 44.9	127.8 ± 43.3
Post-exercise day 1	168.6 ± 101.9*	167.3 ± 83.6*	170.7 ± 116.9*
Post-exercise day 2	148.9 ± 90.0	148.6 ± 67.0	153.4 ± 91.2
Post-exercise day 7	131.1 ± 56.2	140.8 ± 78.0	134.1 ± 49.4

*Significant difference compared to baseline (p < 0.05)

Discussion

This study showed the eccentric exercise program is effective at inducing DOMS by significantly increasing the pain score, thigh circumference and creatine kinase release, and decreasing pressure pain threshold and muscle strength compared to baseline in each group; 14 % Plai cream, ultrasound and 14 % Plai cream combined with ultrasound. However, this study found no substantial difference in pain score, pain pressure threshold, rate of perceived exertion, creatine kinase, muscle strength, range of motion and thigh circumference during a recovery from eccentric exercise among the 3 intervention groups. The combination of 14 % Plai cream and ultrasound did not show any greater effect than 14 % Plai cream or ultrasound alone. Application of 14 % Plai cream may have similar effect on DOMS to ultrasound therapy.

The mechanisms that were thought to be responsible for DOMS include sarcomere disruption and secondary inflammation from muscle damage^{3,4}. The inflammation process stimulates Prostaglandin E2 to sensitize type III and IV pain afferents, and leukotrienes to attract neutrophils for producing free radicals that exacerbate

Table 3 — Pressure pain threshold, rate of perceived exertion (RPE), range of motion, thigh circumference of the three groups (mean ± SD) at baseline, and post-exercise days.

	Plai cream	Ultrasound	Combined
Pressure pain threshold (kg.cm⁻²)			
Baseline	2.9 ± 1.1	3.0 ± 1.0	3.5 ± 1.8
Post-exercise day 1	2.3 ± 1.4*	2.5 ± 1.3*	2.6 ± 1.4*
Post-exercise day 2	2.4 ± 1.4*	2.7 ± 1.3	2.8 ± 1.4*
Post-exercise day 3	2.4 ± 1.4*	2.7 ± 1.4	2.8 ± 1.4*
Post-exercise day 7	2.5 ± 1.3	3.0 ± 1.3	3.1 ± 1.4
RPE			
Baseline	8.1 ± 1.6	8.2 ± 2.0	8.4 ± 1.9
Post-exercise day 1	8.8 ± 1.9	8.4 ± 1.6	9.3 ± 2.4
Post-exercise day 2	7.9 ± 1.2	8.2 ± 1.6	8.5 ± 1.9
Post-exercise day 3	7.9 ± 2.3	7.9 ± 1.9	8.3 ± 1.8
Post-exercise day 7	7.9 ± 1.7	7.4 ± 1.2	7.2 ± 0.9
Range of motion (degree)			
Baseline	128.2 ± 9.9	125.8 ± 8.5	128.2 ± 7.8
Post-exercise day 1	125.8 ± 8.4	126.6 ± 7.0	127.0 ± 6.3
Post-exercise day 2	125.8 ± 8.0	126.6 ± 6.7	126.0 ± 6.1
Post-exercise day 3	126.2 ± 8.1	127.0 ± 6.6	126.2 ± 6.2
Post-exercise day 7	126.8 ± 8.4	126.6 ± 6.3	127.2 ± 6.8
Thigh circumference (cm)			
Baseline	47.5 ± 6.0	47.3 ± 3.1	47.1 ± 4.1
Post-exercise day 1	48.0 ± 5.5*	47.8 ± 3.4*	47.9 ± 4.2*
Post-exercise day 2	48.0 ± 5.7*	47.8 ± 3.4*	47.8 ± 4.3*
Post-exercise day 3	48.1 ± 5.7*	47.7 ± 3.2*	47.9 ± 4.3*
Post-exercise day 7	47.9 ± 5.8*	47.7 ± 3.3*	47.9 ± 4.4*

*Significant difference compared to baseline (p < 0.05)

muscle cell damage². Previous studies revealed various phytochemical extracts from Plai can reduce pain and inflammation^{19,21}. Extracts from Plai such as curcumin (diferuloylmethan), casumunar, (E)-1-(3,4-dimethoxyphenyl) but-3-en-2-ol and (E)-1-(3,4-dimethoxyphenyl) butadiene (DMPBD) are thought to reduce edema and inflammation through inhibition of cyclooxygenase, lipoxigenase pathways⁸, resulting in less exudate formation and leukocyte accumulation²⁰.

Previous clinical trials found Plai cream significantly reduced pain from ankle sprain⁹, osteoarthritis¹⁰ and muscle strain¹¹. A recent clinical trial found a dose-response effect of Plai cream and suggested a concentration of 14 % Plai cream is required to reduce pain associated with DOMS¹². This study using the same treatment protocol and the same concentration of Plai cream (14 %) corroborated this earlier study by showing a reduction in pain associated with DOMS.

It has been suggested ultrasound therapy generates oscillatory movement in the tissue which may cause decreased pain and inflammation²². Hasson *et al.* (1990) suggested pulsed ultrasound therapy could

reduce pain associated with DOMS²³. Craig *et al.* (1999) found no substantial effect of ultrasound on DOMS²². However, the participants in the group who received high pulsed ultrasound had the lowest level of muscle soreness. Parker *et al.* (2014) suggested placebo effect to relieve pain of ultrasound therapy for DOMS since they did not find different effect between ultrasound and sham²⁴. Accordingly, our study found the effect of ultrasound was not different to 14 % Plai cream. Phonophoresis in this study was unsuccessful for enhancing any beneficial effects of the Plai cream. While phonophoresis with other phytochemicals (e.g. *Aloe vera*, *Arnica montana*) has shown mixed results²⁵⁻²⁶, evidence exists for a beneficial effect of phonophoresis using continuous ultrasound (1MHz, 1.5 Watt.cm⁻², 5 min) and trolamine salicylate for DOMS management¹⁷. This study, we used the ultrasound with the same parameters as Ciccone's study; however, our study found ultrasound combined with Plai cream did not have any substantially greater effect than Plai cream or ultrasound treatments alone. We hypothesize that the phonophoresis with Plai cream in this study which used compound phytochemical substances (e.g. curcumin or casumunar) may act differently under ultrasound than tropical trolamine salicylate.

The limitation of this study is there was no placebo cream group to reveal the profound beneficial effect of 14 % Plai cream and ultrasound. In addition, the higher than expected variation in the measures taken resulted in the study being inadequately powered. We would recommend any future research in this area to incorporate larger sample sizes.

Conclusion

Combining 14 % Plai cream with ultrasound had no additional benefit on symptoms associated with DOMS compared to 14 % Plai cream or ultrasound intervention alone. Ultrasound may not be useful at enhancing Plai extract skin penetration by phonophoresis.

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