รายงานวิจัยฉบับสมบูรณ์

โครงการ ผลการใช้ไพลครีม(Zingiber cassumunar ROXB) ในการรักษาอาการปวดกล้ามเนื้อหลังการออกกำลังกาย

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บทคัดย่อ

ไพลเป็นสมุนไพรพื้นบ้านที่รู้จักการแพร่หลายในการรักษาอาการปวดและการอักเสบของโรคทางระบบกระดูกและกล้ามเนื้อ หลักฐานที่นำครีมไพลมาใช้ในการรักษาโรคยังไม่ชัดเจนนัก โดยเฉพาะอย่างยิ่งการนำครีมไพลมารักษาอาการปวดกล้ามเนื้อหลังการออกกำลังกาย นอกจากนี้ อาจจะนำครีมไพลมาใช้ร่วมกับ ultrasound เพื่อเสริมฤทธิ์ลดการอักเสบ อย่างไรก็ตามยังไม่มีงานวิจัยที่สนับสนุน

วัตถุประสงค์ 1) เพื่อศึกษาเปรียบเทียบไพลครีมใช้ความเข้มข้นของยาอยู่ที่ 14% กับ 7% ในการรักษาอาการปวดกล้ามเนื้อหลังการออกกำลังกาย 2) เพื่อศึกษาผลของ14%ไพลครีมร่วมกับเครื่อง ultrasound ในการรักษาอาการปวดกล้ามเนื้อหลังการออกกำลังกาย

วิธีการศึกษา เป็นการศึกษาแบบ randomized controlled trial ทำการศึกษาที่ ห้องปฏิบัติการภาควิชาเวชศาสตร์ฟื้นฟู คณะแพทยศาสตร์ มหาวิทยาลัยขอนแก่น การศึกษานี้แบ่งเป็น 2 โครงการ ศึกษาในอาสาสมัครสุขภาพแข็งแรง ไม่ใช่นักกีฬา ออกกำลังกายกล้ามเนื้อต้นขา quadriceps ด้วยเครื่อง isokinetic ชุดละ 25 ครั้ง ทั้งหมด 4 ชุด เพื่อให้เกิดอาการปวดกล้ามเนื้อหลังการออกกำลังกาย โครงการที่ 1 อาสาสมัครจำนวน 75 คน จะถูกสุ่มแบ่งเป็น 3 กลุ่ม กลุ่มที่ 1 ได้รับ 14% ไพลครีม กลุ่มที่ 2 ได้ 7% ไพลครีม และกลุ่มที่ 3 ได้ยาหลอก ทั้ง 3 กลุ่มจะทายาที่กล้ามเนื้อต้นขาทันทีหลังออกกำลังกายและ ต่อเนื่องทุก 8 ชั่วโมงเป็นเวลา 7 วัน โครงการที่ 2 ศึกษาในอาสาสมัคร จำนวน 75 คน ออกกำลังกายกล้ามเนื้อต้นขา quadriceps เช่นเดียวกับโครงการที่ 1 หลังจากนั้นอาสาสมัครจะถูกสุ่มแบ่งเป็น 3 กลุ่ม กลุ่มที่ 1 ได้รับ 14% ไพลครีม ทายาที่กล้ามเนื้อต้นขาทันทีหลังออกกำลังกายและ ต่อเนื่องทุก 8 ชั่วโมง กลุ่มที่ 2 รักษาโดยใช้ continuous ultrasound (1MHz, 1watt.cm-2) เป็นเวลา 5 นาที และกลุ่มที่ 3 รักษาโดยใช้ไพลครีม ร่วมกับ ultrasound ได้ กลุ่มที่ได้รับ ultrasound จะได้รับการรักษาวันละครั้ง เป็นเวลา 7 วัน

ผลการศึกษา แบ่งเป็นสองส่วนด้วยกัน

ส่วนที่1 ผลการศึกษา พบว่า 14%ไพลครีมสามารถลดอาการปวดได้อย่างชัดเจน เมื่อเทียบกับยาหลอก (p = 0.03) 7%ไพลครีมสามารถลดอาการปวดแต่ไม่แตกต่าง กับยา(p = 0.3) ส่วนการเปรียบเทียบผลของ14% ไพลครีมกับ 7% ไพลครีม พบว่าไม่มีความแตกต่างกัน (p = 0.2) ผลการศึกษาอื่นๆ ได้แก่ ความแข็งแรงของกล้ามเนื้อ ความสูงของการกระโดด เส้นรอบวงต้นขา ระดับของ creatine kinase (CK) ในเลือด พบว่าไม่แตกต่างกันทั้งสามกลุ่ม

ส่วนที่ 2 ผลการศึกษา พบว่า กลุ่มที่ 1 ใช้ 14%ไพลครีม กลุ่มที่ 2 ใช้ ultrasound และกลุ่มที่ 3 ใช้14%ไพลครีมร่วมกับ ultrasound ทั้งสามกลุ่มสามารถลดอาการปวดกล้ามเนื้อหลังการออกกำลังกายได้ แต่ไม่มีความแตกต่างกันอย่างมีนัยสำคัญทางสถิติ ผลการศึกษาอื่นๆ ได้แก่ ความไวต่อแรงกด ความเหนื่อย ความแข็งแรงของกล้ามเนื้อ เส้นรอบวงต้นขา การเคลื่อนไหวของข้อ และ ระดับของ creatine kinase (CK) ในเลือด พบว่าไม่แตกต่างกันทั้งสามกลุ่ม

สรุป จากผลการศึกษาสรุปว่า 14%ไพลครีม น่าจะเหมาะสมในการนำมาใช้รักษาอาการปวดกล้ามเนื้อหลังการออกกำลังกาย ส่วน 7%ไพลครีมน่าจะให้ผลในการรักษาบ้าง แต่น้อยกว่า 14% ไพลครีม และการใช้14%ไพลครีม หรือ ultrasound สามารถลดอาการปวดได้ แต่ ultrasoundไม่สามารถเสริมฤทธิ์ของ14%ไพลครีมในการลดอาการปวดกล้ามเนื้อหลังการออกกำลังกาย ซึ่งต้องการการศึกษาต่อไป

Abstract

Plai (Zingibe rcassumunar ROXB) is used as folk remedy for release pain and inflammation of musculoskeletal problems. The evidence of anti-inflammatory effect of Plai cream in clinical trial currently is rather limited, especially no evidence in treatment of delayed onset of muscle soreness (DOMS). In addition, anti-inflammatory effect of Plai cream that may be enhanced by phonoporesis or ultrasound therapy is questionable.

Objectives: To evaluate the effect of Plai cream in either 7 or 14% concentration on delayed onset muscle soreness (DOMS), and to evaluate effect of ultrasound combined with Plai cream in the treatment of delayed onset muscle soreness.

Study design: Randomized controlled trial study

Setting: exercise laboratory, Rehabilitation Department, KhonKaen University.

Methods: This study was conducted in 2 experiments. All participants were untrained healthy volunteers performed 4 sets of 25 eccentric repetitions of the dominant quadriceps muscle on an isokinetic dynamometry machine to induce delayed onset muscle soreness.

Experiment 1: Seventy-five participants were randomized into 3 groups; 14% Plai cream, 7% Plai cream and placebo cream. All participants immediately applied cream into the quadriceps muscles for 5 min immediately following the exercise and every 8 hours thereafter for 7 days. Experiment 2: Seventy-five volunteers were randomized into 3 groups, group 1 for 14%Plai cream, group 2 for ultrasound and group 3 for combined. Group 1 received Plai cream applied into the quadriceps muscles immediately following the exercise and every 8 hours. Group 2 received continuous ultrasound therapy (1MHz, 1watt.cm^{-2}) for 5 minutes. Group 3 received combined 14% Plai cream with ultrasound. All groups received continuous treatment every day until 7 days.

Results: Experimental 1, compared to the placebo cream the 14% Plai cream substantially reduced muscle soreness over the 7 days ($p = 0.03$), but had similar muscle soreness effects to 7% Plai cream ($p = 0.2$). Compared to the placebo cream the 7% Plai cream resulted in a small non-significant reduction in muscle soreness levels over the following 7 days ($p = 0.3$). Compared to placebo cream there was little effect of Plai cream (7% or 14%) on muscle strength, jump height, thigh circumference or creatine kinase concentration.

Experimental 2, there was no significant difference of visual analog pain score, pressure pain threshold, rate of perceived exertion, creatine kinase, muscle strength, range of motion and thigh circumference among three group ($p > 0.05$) among 14% Plai cream, ultrasound and combined.

Conclusion: Using 14% Plai cream over a 7 day period substantially reduced muscle soreness symptoms compared to 7% Plai cream or a placebo cream. We suggest the administration of 14% Plai cream is a useful alternative in the management of DOMS. However, application of 14% Plai cream may not be facilitated by phonophorosis process

Keywords : Plai cream; Muscle soreness; Muscle strength; Creatine kinase; Ultrasound

Executive Summary

Delayed onset muscle soreness (DOMS) indicated by muscle pain and tenderness typically occurs after a strenuous workout or undertaking unaccustomed exercise. The symptoms of DOMS include pain, loss of strength, swelling and stiffness that generally occur 24-48 hours after the exercise and resolves within 10 days. The underlying causes of DOMS are related to exercise induced muscle damage including sarcomere disruption and the ensuing secondary inflammatory response.[3-4] Numerous methods to prevent and reduce DOMS have been suggested including stretching exercises, massage, nutritional supplementation and non-steroidal anti-inflammatory drugs (NSAIDs)

Plai (Zingiber cassumunar ROXB) is a popular herb used for musculoskeletal disorders in Asia. Plai is in the ginger family that contains potent phytochemicals. In animal research Plai extracts reduced edema and inhibited the inflammation process.[9-11] In human use, 14% Plai cream has been used by clinicians to reduce inflammatory processes in musculoskeletal disorders. However, the effect of local application of Plai cream for the treatment of DOMS has not been investigated previously. There are currently two commercially available Plai creams on the market, a lower cost, but lower concentration cream (7%), and a more expensive but higher concentration brand (14%). Little is known about the effectiveness of either cream in the reduction of DOMS.

In addition, the anti-inflammatory effect of plai cream may be enhanced by ultrasound wave. Ultrasound is widely used in physiotherapy to relieve pain and inflammation. Ultrasound also is used for phonophoresis to enhance transdermal

penetration of pharmacological substances[15]. Ultrasound combined with Plai oil for anti-inflammatory effect has never been studied before.

The objectives of this study were 1) to evaluate whether application of Plai cream was effective at reducing DOMS and muscle performance loss after eccentric exercise and whether there is a dose-dependent response 2) to evaluate the effect of Plai oil that may be enhanced anti-inflammatory effect by phonoporesis process (application with ultrasound therapy).

Methods

This study was divided into two experiments. The protocols were approved by the Khon Kaen University Ethics Committee for Human Research. The untrained healthy volunteers were performed intensive eccentric exercise of the dominant quadriceps muscles by an isokinetic dynamometer to induce delayed onset muscles soreness. The participants performed 4 sets of 25 maximum eccentric knee extension contractions from flexion (90°) to full knee extension (0°) at a speed of $60°.s^{-1}$. A rest period of 10 seconds was given for recovery between repetitions and a rest period of 3 minutes was given between each set of contractions. All participants received the same verbal encouragement throughout the test.

In experimental 1, seventy five participants were allocated into 3 groups by block randomization (25 participants per group), group 1, 14% Plai cream, group 2, 7% Plai cream and group 3, placebo (control). All groups immediately applied 2 grams of cream (strips of 5 cm long) and gently rubbed into the quadriceps muscles for 5 min immediately following the exercise and every 8 hours thereafter for 7 days.

In experimental 2, seventy five participants were allocated into 3 groups by block randomization (25 participants per group), group 1, 14% Plai cream, group 2,

ultrasound and group 3, combined Plai cream and ultrasound. Group 1 received 5 cm long strip of Plai cream applied and gently rubbed into the quadriceps muscles for 5 min immediately following the exercise and every 8 hours. Group 2 received continuous ultrasound therapy (1MHz, 1watt.cm-2) for 5 minutes. Group 3 received combined 14% Plai cream with ultrasound. All groups received continuous treatment every day until 7 days.

Statistical analysis

Descriptive statistical analysis includes the mean and standard deviation. A generalized linear mixed model was used to analyze repeated measurements of the longitudinal study. Analysis of the statistic indicated the overall effects of treatment and the pattern of change difference of measured time points of all variables among three groups. The analyses were conducted by using STATA program version 13.0. Statistical significant difference was accepted at $p < 0.05$.

Results

Experimental 1, compared to the placebo cream the 14% Plai cream substantially reduced muscle soreness over the 7 days by -82% (95%CI = -155 to -6%, p = 0.03), but had similar muscle soreness effects to 7% Plai cream (-34%, -96 to 27%, p = 0.2). Compared to the placebo cream the 7% Plai cream resulted in a small non-significant reduction in muscle soreness levels over the following 7 days (-40%, -116 to 36%, p = 0.3). Compared to placebo cream there was little effect of Plai cream (7% or 14%) on muscle strength, jump height, thigh circumference or creatine kinase concentration.

Experimental 2, there was no significant difference of visual analog pain score, pressure pain threshold, rate of perceived exertion, creatine kinase, muscle strength, range of motion and thigh circumference among three group ($p > 0.05$) among 14% Plai cream, ultrasound and combined.

Conclusion

Using 14% Plai cream over a 7 day period substantially reduced muscle soreness symptoms compared to 7% Plai cream or a placebo cream. We suggest the administration of 14% Plai cream is a useful alternative in the management of DOMS. However, combined 14% Plai cream with ultrasound had no significant difference effect on DOMS compared to only 14% Plai cream or only ultrasound. Application of 14% Plai cream may not be facilitated by phonophorosis process

Content

Introduction

Delayed onset muscle soreness (DOMS) indicated by muscle pain and tenderness typically occurs after a strenuous workout or undertaking unaccustomed exercise. Eccentric exercise, especially of unfamiliar intensity, has a higher potential to develop muscle injury and DOMS than isometric and concentric exercise.[1] The symptoms of DOMS include pain, loss of strength, swelling and stiffness that generally occur 24-48 hours after the exercise and resolves within 10 days.[2] The underlying causes of DOMS are related to exercise induced muscle damage including sarcomere disruption and the ensuing secondary inflammatory response.[3-4] Inflammatory processes stimulate prostaglandin E2 release which sensitizes type III and IV pain afferents, and leukotrienes to attract neutrophils to produce free radicals that further exacerbate muscle cell damage.[2]

Delayed onset muscle soreness after eccentric exercise may result in reduction of muscle performance of athletes.[1] Numerous methods to prevent and reduce DOMS have been suggested including stretching exercises, massage and nutritional supplementation. Non-steroidal anti-inflammatory drugs (NSAIDs) have been used in an attempt to reduce DOMS by reducing inflammation, pain and improving function. The NSAIDs inhibit cyclooxygenase activity resulting in reduction of prostaglandins that are potent vasodilators and pain producing agents.[5] Balwin et al. (2001) revealed that three days after eccentric exercise of knee extensors, thigh soreness in participants who received naproxen sodium (220 mg 3 times a day) was 40% lower than participants who received a placebo treatment. In addition, strength decline associated with DOMS was halved in the naproxen group compared to the placebo group.[6] Rahnama et al. (2005) showed that muscle soreness and creatine kinase levels

48 hours after eccentric exercise in participants who received 2,800 mg Ibuprofen (400 mg on 7 occasions) was significantly lower than control subjects who did not receive any treatment.[7] Topical NSAIDs that have minimal systemic absorption is recommended to avoid enteral NSAID adverse effects.[8] Canavino et al. (2003) reported that local application of NSAID (transdermal ketoprofen, 1g of cream applied locally every 8 hours) reduced muscle soreness 24 hours post-exercise by 37% compared to placebo.

Plai (Zingiber cassumunar ROXB) is a popular herb used for musculoskeletal disorders in Asia. Plai is in the ginger family that contains potent phytochemicals. In animal research Plai extracts reduced edema and inhibited the inflammation process.[9-11] In human use, 14% Plai cream has been used by clinicians to reduce inflammatory processes in musculoskeletal disorders. Laupattarakasem et al. (1993) concluded that applying 2 grams of 14% Plai cream (2 times a day for 7 days) on an ankle sprain reduced pain by 90% on day 5 post-injury and reduced swelling by 55% on day 3 post-injury, compared to placebo.[12] Srirochana (2010) showed beneficial effects of applying 14% Plai cream (3 times a day for 4 weeks) over 1% diclofenac gel (reduced pain and improved physical and emotional function in patients with knee osteoarthritis).[13] To the best of the authors' knowledge, the effect of local application of Plai cream for the treatment of DOMS has not been investigated previously. There are currently two commercially available Plai creams on the market, a lower cost, but lower concentration cream (7%), and a more expensive but higher concentration brand (14%). Little is known about the effectiveness of either cream in the reduction of DOMS.

In addition, the anti-inflammatory effect of plai cream may be enhanced by ultrasound wave. Ultrasound is widely used in physiotherapy to relieve pain and

inflammation. Ultrasound also is used for phonophoresis to enhance transdermal penetration of pharmacological substances[14]. Deniz et al. (2009) revealed ultrasound with diclofenac gel in osteoarthritis patients more improved pain at rest and activity, WOMAC pain and physical function after treatment than only diclogenac gel and only ultrasound therapy[15]. Ultrasound and phonophoresis also showed beneficial effects on other musculoskeletal disorders such as painful shoulder syndrome and lateral epicondylitis[16-17]. The evidence of phonophoresis for DOMS management have been studied by Clccone et al. (1991) who showed using ultrasound with trolamine salicylate cream on 3 consecutive days after eccentric exercise reduced muscle soreness by 24.4% compared to only trolamine salicylate cream and 51.7% compared to only ultrasound[18]. However, ultrasound combined with Plai oil for anti-inflammatory effect has never been studied before.

The objective of this study was 1) to evaluate whether application of Plai cream was effective at reducing DOMS and muscle performance loss after eccentric exercise and whether there is a dose-dependent response 2) to evaluate the effect of Plai oil that may be enhanced anti-inflammatory effect by phonoporesis process (application with ultrasound therapy). If Plai cream has clinically anti–inflammation effect, it should be introduced to apply in exercise and sport performance.

Methods

This study was conducted at the Department of Rehabilitation Medicine, Faculty of Medicine, KhonKaen University, Thailand. Seventy-five healthy untrained volunteers, aged 18-60 years, who gave their written informed consent were included. The exclusion criteria were volunteers who had uncontrolled medical diseases such as diabetes, hypertension and heart disease or uncontrolled psychological problems.

Exercise induced DOMS

Delayed onset muscle soreness was induced by strenuous eccentric exercise of the dominant quadriceps muscles by an isokinetic dynamometer (Primus RS, BTE technologies, Hanover, MD, USA) (Figure 1). Prior to exercise, all subjects completed a warm-up consisting of stretching exercises of the major lower limb muscles for 15 minutes. The participants were then seated on an adjustable chair with their trunk reclined at 15°. The hip, thigh and non-dominant leg were secured to the chair (via webbing straps) while the dominant leg was strapped to the dynamometer's attachment with the knee flexion angle of 90°. The rotational axis of knee movement was aligned with the lateral femoral epicondyle. The participants performed 4 sets of 25 maximum eccentric knee extension contractions from flexion (90°) to full knee extension (0°) at a speed of $60°.s^{-1}$. A rest period of 10 seconds was given for recovery between repetitions and a rest period of 3 minutes was given between each set of contractions. All participants received the same verbal encouragement throughout the test.

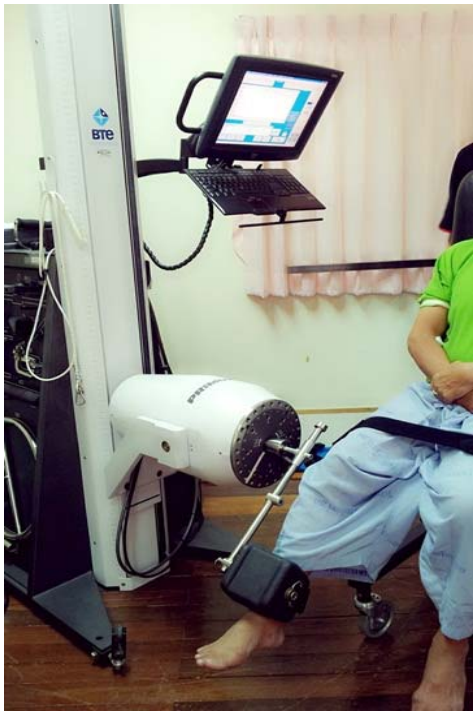

Figure 1. Exercise induced delayed onset muscle soreness

Study 1, the participants were allocated into 3 groups by block randomization (25 participants per group), group 1, 14% Plai cream, group 2, 7% Plai cream and group 3, placebo (control). The 3 creams were manufactured by the same company and consisted of the same color, odor and packaging (Figure 2). For all groups a dose of 2 g or approximately a 2.5 cm long strip of cream was gently rubbed into the quadriceps muscles (mid quadriceps area around 15 x 8 cm or 120 cm^2) until the cream was not visible around 5 min, immediately following the eccentric exercise. The participants were instructed to apply the cream every 8 hours after exercise for 1 week.

Figure 2. A = 14%Plai cream, B = 7% Plai cream, C = Placebo cream

Study 2, the participants were allocated into 3 groups by block randomization, group 1, 14% Plai cream, group 2, ultrasound and group 3, combined Plai cream and ultrasound. Group1, the14% Plai creams that a dose of 2 g or approximately a 2.5 cm long strip of cream was gently massaged into the quadriceps muscles until the cream was not visible, immediately following the eccentric exercise. The participants were instructed to apply the cream every 8 hours after exercise for 1 week. Group 2, the certified physiotherapist applied pulsed ultrasound wave therapy with 1 MHz frequency and 1 watt/cm^2 at the quadriceps muscle area of participants for 5 minutes, immediately following the eccentric exercise and then every day. Transducer head

was applied at right angle to ensure maximum energy absorption. The skin was coated with acoustic gel which was not contained pharmacological substances. Group 3, combined 14% Plai cream and ultrasound, the participants received ultrasound therapy with the same dose as group 2 and used 14% plai cream 2.5 cm long strip of cream as a replacement for acoustic gel (Figure 3).

Figure 3. Ultrasound therapy on quadriceps muscle

The participants were requested not to use other treatments such as an oral NSAID, muscle relaxants, physiotherapy or massage. If the participants received other therapies, all details were recorded. Simple analgesic drugs or paracetamol were allowed for management of severe and intolerant pain, and the number of paracetamol tablets or drugs used per day were recorded .The compliance of cream application was recorded on the daily record checklist. All participants completed the study. The participants were evaluated at pre-test, day 1, day 2, day 3 and day 7 post-exercise.

Plai cream preparation

Plai cream is an ordinary oil in water emulsion technique. Plai oil was extracted by stream distillation from Zingiber cassumunar ROXB rhizome. Plai and placebo creams were produced by Bangkok lab & Cosmetic Co., Ltd., Bangkok, Thailand that have GMP standard and manufacture with high quality standard of PIC/S GMP.

Muscle soreness and swelling

Subjective muscle soreness of the quadriceps was determined by using a visual analogue scale, ranged from 0 to 10 (0 indicating no pain, 10 indicating the worst pain). The visual analog scale has been widely used as a reliable tool for pain intensity assessment.[19]

Swelling of the thigh was evaluated by measuring thigh circumference at mid-level (midpoint between inguinal fold and anterior aspect of the patella) in the supine position. The thigh circumferences were measured to the nearest 0.1 cm by using a standard tape (Rollfix, Hoechst. Mass, Germany). The mean of the two closest thigh circumference measurements was recorded. The research assistant taking the tape measurement was blinded to the participant's group.

Muscle strength

Muscle strength was evaluated by 3-second maximal voluntary concentric (MVC) of the dominant quadriceps muscle using isokinetic dynamometry. The dominant leg was secured to the isokinetic attachment with the knee in flexion at 45^{o}. Each subject performed three MVCs, separated by a 2-min rest. Verbal

encouragement was given in order to motivate the subject for the maximal force exertion.

Explosive muscle power was assessed via the countermovement jump test using standard testing equipment (Yardstick, Swift Performance Equipment, New South Wales, Australia). The participants jumped from a standing position and their fingertips reached to the highest level. Participants completed a total of 3 jumps (with a 60 second rest between jumps).The highest jump height of 3 performances was recorded for analysis. 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)

A 5-ml blood sample for CK was obtained from the median cubital vein by a certified medical technologist at Srinagarind Hospital. The blood CK levels were analyzed by an automated analyzer (Cobas® 6000 analyzer; Roche Diagnostics Corp., Indianapolis, IN, USA). Plasma CK was measured at pre-test, day 1, day 2 and day 7 post-exercise.

Rate of perceived exertion (RPE)

The Perceived exertion was rated by using the Borg scale (ranged from 6 to 20, 6 for no exertion at all and 20 for maximal exertion) after 5 min rest and as soon as the participants arrived at the laboratory each day[15].

Range of motion (ROM)

The motion of tested knee joint was measured by a blinded researcher assistance to evaluate the degree of joint stiffness from muscle soreness. The goniometer (BASELINE® dynamometric, USA) was used to measure degree of movement from knee extension in prone position to full knee flexion.

Pressure pain threshold

The threshold of pain pressure of quadriceps was measured by a manual algometer (BASELINE®, New York, USA). A blinded research assistance pressed an algometer perpendicularly to quadriceps and observed minimal pressure which produces painful sensation, while the participants sat down and extended their knee joint. The average of three points of measured on quadriceps (5, 10, and 15 cm above the apex of patella) was recorded.

This study has been approved by the KhonKaen University Ethics Committee for Human Research reference number HE 561436, and has been registered at Thai Clinical Trial Registry identification number TCTR20140215001.

Statistical analysis

Descriptive statistical analysis includes the mean and standard deviation. A generalized linear mixed model was used to analyze repeated measurements of the longitudinal study. Analysis of the statistic indicated the overall effects of treatment and the pattern of change difference of measured time points of muscle pain score, pain pressure threshold, rate of perceived exertion, creatine kinase, muscle strength, range of motion and thigh circumference among three groups. The analyses were

conducted by using STATA program version 13.0. Statistical significant difference was accepted at $p < 0.05$.

Results

Study 1

There were 75 healthy volunteers enrolled in this study, aged 28.7 ± 13.7 years, 28 males, 47 females, body weight 67.5 ± 10.5 kg, BMI 23.3 ± 4.0 $kg.m^{-2}$. The characteristics of each group are presented in Table 1.

Table 1. Demographic data of participants

	Placebo n= 25	7% Plai n = 25	14% Plai n = 25
Male/Female	9/16	9/16	10/15
Age (years)	26.2 ± 12.0	31.3 ± 16.7	28.7 ± 13.7
Body weight (kg)	57.6 ± 11.6	57.5 ± 7.2	61.5 ± 10.5
BMI ($kg.m^{-2}$)	21.4 ± 3.7	21.9 ± 2.3	23.1 ± 3.9

Data are means ± SD.

Pain score

Subjective pain of the quadriceps muscles were similar in all 3 groups at baseline and increased as a result of the eccentric exercise (Figure 4). Overall, perceived pain levels over the 4 recovery periods were substantially reduced in the 14% Plai group by -82% (95%CI = -155 to -6%, p = 0.03) compared to the placebo group. In addition, when analyzing the individual day pain scores, pain was substantially reduced in the 14% Plai group compared to the placebo group on post-exercise day 1 (-26.8%, -52.4 to -1.2%, p = 0.04). The pain levels in the 7% Plai group were not significantly different to the placebo group (-40%, -116 to 36%, p =

0.3) and 14% Plai group (-34%, -96 to 27%, p = 0.2) throughout the recovery period. No oral analgesic drug was required during the study.

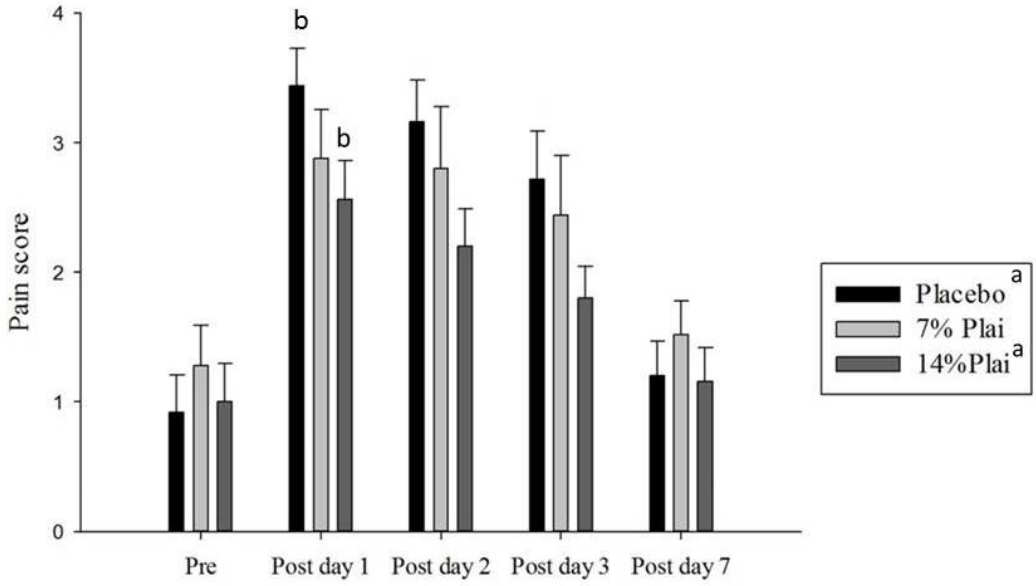

Figure 4. Pain score of Plai (7 and 14%) and placebo groups at baseline, post exercise day 1, day 2, day 3 and day 7 (mean ± SE).

[a]14% Plai was statistically lower over all days combined compared to Placebo. [b]14% Plai was statistically lower on individual days compared to Placebo.

Muscle strength

The 3-s maximal voluntary contraction force of the quadriceps can be seen in Table 2. All groups showed a substantial decrease in maximal force 24 hours after the eccentric exercise bout and tended to follow a similar recovery pattern over the next 2 days. At 7 days post eccentric exercise, maximal isometric force was substantially higher in the 14% Plai group compared to the placebo group (22%, 0.01 to 43.8%, p = 0.04).

Creatine kinase

Serum creatine kinase levels at post exercise day 1 increased significantly from baseline as a response to the eccentric exercise in all groups ($p < 0.01$), and creatine kinase level had recovered almost back to baseline levels by recovery day 7. There was no overall difference between groups (area under the curve for the 4 measurements) as a result of the separate interventions.

Jump height and thigh circumference

Finally there was no substantial difference in the countermovement jump height or thigh circumference measures over the period of the study between groups.

Table 2. Pain score, muscle strength and creatine kinase concentration of three groups (mean ± SD).

	Placebo[a]	7% Plai	14% Plai [a]
Pain score			
Baseline	0.9 ± 1.4	1.1 ± 1.2	1.0 ± 1.5
Post exercise day 1	3.4 ± 1.5[b]	2.9 ± 1.9	2.6 ± 1.5[b]
Post exercise day 2	3.1 ± 1.7	2.8 ± 2.4	2.2 ± 1.4
Post exercise day 3	2.6 ± 1.9	2.4 ± 2.3	1.8 ± 1.2
Post exercise day 7	1.5 ± 1.5	1.8 ± 1.7	1.2 ± 1.3
Muscle strength (kg)			
Baseline	24.5 ± 6.3	25.2 ± 6.7	25.8 ± 5.6
Post exercise day 1	23.6 ± 6.9	23.4 ± 5.9	23.9 ± 5.9
Post exercise day 2	24.8 ± 6.5	25.1 ± 5.5	27.4 ± 6.8
Post exercise day 3	25.6 ± 7.5	25.5 ± 5.4	28.0 ± 7.9
Post exercise day 7	25.9 ± 8.4[b]	25.8 ± 6.3	29.5 ± 6.7[b]
Creatine kinase ($mmol.L^{-1}$)			
Baseline	123.0 ± 49.4	110.4 ± 42.4	113.8 ± 51.0
Post exercise day 1	175.0 ± 96.2	167.0 ± 127.7	170.6 ± 110.8
Post exercise day 2	180.9 ± 113.7	151.6 ± 80.8	154.2 ± 88.4
Post exercise day 7	134.1 ± 53.5	132.2 ± 63.9	136.4 ± 64.1

[a] = Overall significant difference of pain score between 14% Plai and placebo cream.
[b]= Significant difference on individual day of measurement.

Study 2

There was 75 participants, average age 41.5 ± 9.3 year, average BMI 24.2 ± 3.9kg.m^{-2}, 6 male and 44 female. The participants were divided into 3 groups, 14% Plai cream, ultrasound and combined 14% Plai cream with ultrasound. The characteristics of each groups were presented in the Table 3.

Table 3. Demographic data of participants

	Plai cream n= 25	Ultrasound n = 25	Combined n = 25
Male/Female	3/22	3/22	3/22
Age (years)	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

Data are means ± SD.

The study revealed similar pattern of changes in all outcomes after eccentric exercise among three groups. One day after eccentric exercise, pain score, RPE, thigh circumference and CK increased, and then slowly decreased. While PPT, muscle strength, range of motion were decreased after exercise one day and then gradually improved. However, there was no significant difference of visual analog pain score, PPT, RPE,CK, muscle strength, range of motion and thigh circumference among three group ($p > 0.05$). The results were shown in the Table 4 and Table 5.

Table 4. Pain score, muscle strength and creatine kinase concentration of three groups (mean ± SD).

	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	33.2 ± 7.5	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	34.9 ± 6.5
Post exercise day 7	32.9 ± 7.9	34.7 ± 7.9	34.8 ± 7.9
Creatine kinase ($mmol.L^{-1}$)			
Baseline	116.1 ± 41.6	128.9 ± 44.9	128.3 ± 42.4
Post exercise day 1	168.6 ± 101.9	167.3 ± 83.6	169.8 ± 114.5
Post exercise day 2	148.9 ± 90.0	148.6 ± 67.0	181.4 ± 116.3
Post exercise day 7	131.1 ± 56.2	140.8 ± 78.0	139.6 ± 55.5

Table 5. Pain pressure threshold, rate of perceived exertion (RPE), range of motion, thigh circumference of three groups (mean ± SD).

	Plai cream	Ultrasound	Combined
Pain pressure threshold			
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			
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			
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	48.0 ± 4.2
Post exercise day 2	48.0 ± 5.7	47.8 ± 3.4	47.4 ± 3.9
Post exercise day 3	48.1 ± 5.7	47.7 ± 3.2	48.2 ± 4.5
Post exercise day 7	47.9 ± 5.8	47.7 ± 3.3	47.9 ± 4.4

Discussion

Study 1

Applying 14% Plai cream over eccentrically worked muscles every 8 hours for at least 3 days is likely to have a beneficial effect on DOMS and subsequent recovery of muscle strength, compared to no treatment (placebo). Using 7% Plai cream produced little beneficial effect.

It has been suggested that Plai extracts have anti-inflammatory and analgesic effects. Previous research indicates various phytochemicals that were isolated from Plai including curcumin, cassumunar, and phenylbutenoids (i.e. (E)-4(3',4'-dimethylphenyl) but-3-en-I-ol, (E)-1-(3,4-dimethoxyphenyl) but-3-en-2-ol and (E)-1-(3,4-dimethoxyphenyl) butadiene) may have beneficial effects on the inflammatory process.[10-11, 20] Curcumin (diferuloylmethane) has an anti-inflammatory effect on reducing activities of cyclooxygenase-2 (COX-II), lipoxygenase, inducible nitric oxide synthase and inflammatory cytokines.[20] Cassumunar extracts have been found to have an anti-inflammatory effect on chemically induced edema of the mouse ear stronger than curcumin.[10] In another previous experiment in rats it was revealed that (E)-1-(3,4-dimethoxyphenyl) but-3-en-2-ol, reduced edema in induced rat paw edema and inhibited exudate formation, leukocyte accumulation and prostaglandin-like activity of the exudates.[11] The anti-inflammation effect of (E)-1-(3,4-dimethoxyphenyl) butadiene (DMPBD) has been found through the inhibition of cyclooxygenase, and lipoxygenase pathways.[9]

The topical Plai preparation can be manufactured in several forms; however, the cream form is the best preparation because a high volume of oil is present as the

active ingredient. The properties of the cream are easily spread and do not feel greasy and sticky after application. In the balm form, although it has a similar level of active ingredient to the cream, it produces a sticky or tacky feeling especially in a tropical climate. In the gel form, the hydrophilic ingredient is not compatible with a high percentage of Plai oil and therefore the gel has a lower percentage of active ingredient. Topical Plai cream is readily absorbed through the skin and helps reduce inflammation [12-13]; however, little research exists on other topical forms.

Previous clinical trials showed Plai cream reduced musculoskeletal pain in injured individuals. Laupattarakasem et al. (1993) showed Plai cream significantly reduced pain from ankle sprains at days 5 and 6 post treatment and required less analgesic drug at days 1 and 2 than placebo.[12] Srirochana (2010) revealed positive effects of Plai cream in treatment of osteoarthritis compared to diclofenac gel after two weeks.[13] The duration of effectiveness may depend on the type and lesion of inflammation. This study showed an acute analgesic effect of 14% Plai cream on reducing muscle soreness 24 hours post treatment.

It has been found that topical NSAIDs can help with reducing pain and DOMS[8]; however, such drugs also carry the adverse effects of application including dry skin, rash and pruritis.[21] Additionally, topical NSAIDs has been associated with systemic adverse effects such as dyspepsia or gastritis.[21] The current study found a significant analgesic effect of topical 14% Plai cream and significant improvement of muscle strength (3-s MVC) at post-exercise day 7 compared to the placebo group. We suggest the topical 14% Plai cream would be an effective alternative to NSAID in DOMS management.

The mechanism of NSAIDs is anti-inflammation resulting in reduced muscle damage. This study, however, did not find any significant difference in creatine kinase concentration between 14% Plai cream and the placebo. The results from the current study indicate that creatine kinase concentration was dampened in the Plai groups compared to the control group. It is possible that Plai cream may help reduce the early release of creatine kinase, however this will remain speculation until further investigation is conducted with a larger sample size which will help to decrease the effect of the high variability in this parameter.

A secondary objective of this study was to gauge the effect of Plai cream dosage on DOMS and force recovery. Pain scores after days 1, 2 and 3 in participants given the 7% Plai cream were lower than participants in the placebo group but higher than participants given the 14% Plai cream. The effect of Plai cream on DOMS may be related to its concentration. It seems that the lower concentration Plai cream had little effect at either reducing DOMS or helping in the recovery of force production. The dose related effect of Plai cream may be similar to NSAIDs such as diclofenac or ibuprofen.[22] Taking our results into consideration we would recommend clinicians use the 14% Plai cream for analgesic effects.

Study 2

The study found no substantial difference effects of 14% Plai cream, ultrasound and combined on DOMS in terms of pain score, pain pressure threshold, rate of perceived exertion, creatine kinase, muscle strength, range of motion and thigh circumference. The combination of 14%Plai cream and ultrasound had not been detected greater effect than 14% Plai cream or ultrasound Application of 14% Plai cream may have similar effect on DOMS to ultrasound therapy.

The mechanisms of DOMS have been found that are from exercise induced muscle damage including sarcomere disruption and secondary inflammatory response [3-4]. Inflammation process stimulates Prostaglandin E2 to sensitize type III and IV pain afferents, and leukotrienes to attract neutrophil for producing free radicals that exacerbate muscle cell damage[2]. The previous studies revealed the various phytochemical extracts from Plai have effect to reduce pain and inflammation[10-11, 23]. The 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) Plai reduced edema and inflammation through inhibition of cyclooxygenase, lipoxygenase pathways[9], exudate formation and leukocyte accumulation[11]. Previous clinical trials studies found Plai cream significantly reduced pain from ankle sprain [12] and pain from osteoarthritis [13]. Recent clinical trial found the dose related of Plai cream and suggested concentration of 14% Plai cream is proper to reduce pain on DOMS. This study used 14% Plai cream with the same treatment protocol of the previous study was expected to effectively reduced pain on DOMS.

It has been suggested ultrasound therapy generates oscillatory movement in the tissue caused decrease pain and inflammation[24]. Hasson S et al. (1990) suggested the pulsed ultrasound had significant effect to reduce pain form DOMS[25]. Craig JA et al (1999) found there was no substantial effect of ultrasound on DOMS[24]. 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 but did not find different effect between ultrasound and sham[26]. However, our study found the effect of ultrasound was not different to Plai cream. Ultrasound may have similar pain reduction effect to Plai cream on DOMS

The anti-inflammatory effect of plai cream enhanced by ultrasound wave may be from both thermal and mechanical effect. Ultrasound was successfully used for

phonophoresis to enhance transdermal penetration to improve inflammation in musculoskeletal disorders such as osteoarthritis, painful shoulder syndrome and lateral epicondylitis to improve pain and physical function [14-17]. The evidence of phonophoresis for DOMS management have been studied by Clccone et al. (1991) who showed using ultrasound with trolamine salicylate cream on 3 consecutive days after eccentric exercise reduced muscle soreness by 24.4% compared to only trolamine salicylate cream and 51.7% compared to only ultrasound [18]. Phonophoresis with Plai cream in this study used compound phytochemical substances that may be different from tropical NSAID.

Previous studies regarding phonophoresis with phytochemical substances showed various results. Phonophoresis with Aloe vara gel reduced edema and inflammation of rat paw with collagenase-induced tendinitis [27], whereas Phonophoresis with Arnica montana had no anti-inflammatory effect of acute muscle inflammation of rat [28]. Our study found ultrasound combined with Plai cream did not have substantial greater effects than Plai cream or ultrasound. We speculated that various phytochemical substances in Plai extract may have little effect on phonophoresis enhancement. In addition, muscle soreness from DOMS in this study did not produce serious pain. Therefore, little improvement from phonophoresis may not be obviously observed. Phonophoresis with Plai cream study in other conditions such as tendinitis or arthritis would be recommended.

Conclusions

Compared to placebo, 14% Plai cream reduced DOMS perception at 24, 48 and 72 hours after eccentric exercise, but no significant pain reduction was observed with 7% Plai cream. In addition, those participants that used the 14% Plai cream showed increased force production recovery compared to the placebo group by the

end of the 7-day intervention period which was not found in the participants using the7% Plai cream. Given these results it is recommended that when using Plai cream as an analgesic the higher concentration (14%) is likely to be more effective (7%). Additionally, combined 14% Plai cream with ultrasound had no additional effect on DOMS compared to only 14% Plai cream or only ultrasound. Ultrasound may not enhance Plai extract to penetrate skin by phonophorosis process.

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เอกสารแนบหมายเลข 3

Output จากโครงการวิจัยที่ได้รับทุนจาก สกว.

1. ผลงานตีพิมพ์ในวารสารวิชาการนานาชาติ (ระบุชื่อผู้แต่ง ชื่อเรื่อง ชื่อวารสาร ปี เล่มที่ เลขที่ และหน้า) หรือผลงานตามที่คาดไว้ในสัญญาโครงการ
   ส่งการศึกษาแรก ไปตีพิมพ์ ที่วารสาร Journal of Integrative Medicine รับตีพิมพ์แล้วอยู่ระหว่างรอตีพิมพ์ในวารสาร
   การศึกษาที่ 2 อยู่ระหว่างเตรียม manuscript ให้สมบูรณ์ และ ส่งให้ผู้เชี่ยวชาญภาษาอังกฤษตรวจสอบแก้ไข
2. การนำผลงานวิจัยไปใช้ประโยชน์
   ประโยชน์เชิงพาณิชย์ เป็นการศึกษาเกี่ยวกับ สมุนไพร ที่ปลูกได้เองในประเทศ และดำเนินการผลิตยาโดยบริษัทของคนไทย บริษัท Bangkok Lab & Cosmetic, Co, Ltd. เป็นการสนับสนุนการใช้ยาสมุนไพรในเชิงพาณิชย์
   ประโยชน์ เชิงสาธารณะ ใช้เป็นหลักฐานในการบอกประสิทธิภาพของครีมไพลซึ่งนำมาใช้รักษาผู้ป่วยที่มีอาการปวดกล้ามเนื้อ ข้อต่อ เส้นเอ็น เพื่อลดการอักเสบ ซึ่งขณะนี้ ยามีใช้ที่ รพ.ศรีนครินทร์ และ รพ.อื่นทั่วประเทศ
   ประโยชน์ เชิงวิชาการ การศึกษานี้มีนักศึกษาปริญญาเอก ช่วยวิจัยด้วย ซึ่งเป็นการพัฒนาการเรียนการสอน และ สร้างนักวิจัยใหม่
3. อื่นๆ (เช่น ผลงานตีพิมพ์ในวารสารวิชาการในประเทศ การเสนอผลงานในที่ประชุมวิชาการ หนังสือ การจดสิทธิบัตร)
   ได้นำเสนอผลงานในที่ประชุมวิชาการระดับนานาชาติ MedSci Congress 2015 Queentown New Zealand 31 August – 2 September 2015

ภาคผนวก

Experimental 1: Effect of Zingiber cassumunar ROXB (Plai cream) in the treatment of delayed onset muscle soreness

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Abstract

OBJECTIVE: To evaluate the effect of Zingiber cassumunar (Plai cream) in either 7% or 14% concentration on delayed onset muscle soreness (DOMS).

METHODS: Seventy-five untrained healthy volunteers, (28 males and 47 females), performed 4 sets of 25 eccentric repetitions of the dominant quadriceps muscle on an isokinetic dynamometry machine. Participants were then randomized into 3 groups; 14% Plai cream, 7% Plai cream and placebo cream. All groups immediately applied 2 g of cream (strips of 5 cm long) and gently rubbed into the quadriceps muscles for 5 min immediately following the exercise and every 8 h thereafter for 7 d. Muscle soreness, muscle strength, jump height, thigh circumference and creatine kinase were measured pre and post eccentric exercise.

RESULTS: Compared to the placebo cream the 14% Plai cream substantially reduced muscle soreness over the 7 days by -82% (95%CI = -155 to -6%, $P = 0.03$), but had similar muscle soreness effects to 7% Plai cream (-34%, -96 to 27%, $P = 0.2$). Compared to the placebo cream the 7% Plai cream resulted in a small non-significant reduction in muscle soreness levels over the following 7 d (-40%, -116 to 36%, $P = 0.3$). Compared to placebo cream there was little effect of Plai cream (7% or 14%) on muscle strength, jump height, thigh circumference or creatine kinase concentration.

CONCLUSION: Using 14% Plai cream over a 7-day period substantially reduced muscle soreness symptoms compared to 7% Plai cream or a placebo cream. We suggest the administration of 14% Plai cream is a useful alternative in the management of DOMS.

KEYWORDS: Plai cream; Muscle soreness; Muscle strength; Creatine kinase

1 Introduction

Delayed onset muscle soreness (DOMS) indicated by muscle pain and tenderness typically occurs after a strenuous workout or undertaking unaccustomed exercise. Eccentric exercise, especially of unfamiliar intensity, has a higher potential to develop muscle injury and DOMS than isometric and concentric exercise[1]. The symptoms of DOMS include pain, loss of strength, swelling and stiffness that generally occur 24–48 h after the exercise and resolves within 10 d[2]. The underlying causes of DOMS are related to exercise induced muscle damage including sarcomere disruption and the ensuing secondary inflammatory response[3,4]. Inflammatory processes stimulate prostaglandin E2 release which sensitizes type III and IV pain afferents, and leukotrienes to attract neutrophils to produce free radicals that further exacerbate muscle cell damage[2].

DOMS after eccentric exercise may result in reduction of muscle performance of athletes[1]. Numerous methods to prevent and reduce DOMS have been suggested including stretching exercises, massage and nutritional supplementation. Non-steroidal anti-inflammatory drugs (NSAIDs) have been used in an attempt to reduce DOMS by reducing inflammation, pain and improving function. The NSAIDs inhibit cyclooxygenase activity resulting in reduction of prostaglandins that are potent vasodilators and pain producing agents[5]. Balwin *et al*[6] revealed that three days after eccentric exercise of knee extensors, thigh soreness in participants who received naproxen sodium (220 mg 3 times a day) was 40% lower than participants who received a placebo treatment. In addition, strength decline associated with DOMS was halved in the naproxen group compared to the placebo group. Rahnama *et al*[7] showed that muscle soreness and creatine kinase levels 48 h after eccentric exercise in participants who received 2 800 mg Ibuprofen (400 mg on 7 occasions) was significantly lower than control subjects who did not receive any treatment. Topical

NSAIDs that have minimal systemic absorption is recommended to avoid enteral NSAID adverse effects[8]. Canavino *et al*[8] reported that local application of NSAID (transdermal ketoprofen, 1g of cream applied locally every 8 h) reduced muscle soreness 24 h post-exercise by 37% compared to placebo.

Plai (*Zingiber cassumunar* ROXB) is a popular herb used for musculoskeletal disorders in Asia. Plai is in the ginger family that contains potent phytochemicals. In animal research Plai extracts reduced edema and inhibited the inflammation process[9–11]. In human use, 14% Plai cream has been used by clinicians to reduce inflammatory processes in musculoskeletal disorders. Laupattarakasem *et al*[12] concluded that applying 2 g of 14% Plai cream (2 times a day for 7 d) on an ankle sprain reduced pain by 90% on day 5 post-injury and reduced swelling by 55% on day 3 post-injury, compared to placebo. Srirochana[13] showed beneficial effects of applying 14% Plai cream (3 times a day for 4 weeks) over 1% diclofenac gel (reduced pain and improved physical and emotional function in patients with knee osteoarthritis). To the best of the authors' knowledge, the effect of local application of Plai cream for the treatment of DOMS has not been investigated previously. There are currently two commercially available Plai creams on the market, a lower cost, but lower concentration cream (7%), and a more expensive but higher concentration brand (14%). Little is known about the effectiveness of either cream in the reduction of DOMS. The objective of this study was to evaluate whether application of Plai cream was effective at reducing DOMS and muscle performance loss after eccentric exercise and whether there is a dose-dependent response.

2 Methods

This study was conducted at the Department of Rehabilitation Medicine, Faculty of Medicine, Khon Kaen University, Thailand. Seventy-five healthy untrained volunteers, aged 18–60 years, who gave their written informed consent were included. The exclusion criteria were volunteers who had uncontrolled medical diseases such as diabetes, hypertension and heart disease or uncontrolled psychological problems.

2.1 Exercise induced DOMS

Delayed onset muscle soreness was induced by strenuous eccentric exercise of the dominant quadriceps muscles by an isokinetic dynamometer (Primus RS, BTE technologies, Hanover, MD, USA). Prior to exercise, all subjects completed a warm-up consisting of stretching exercises of the major lower limb muscles for 15 min. The participants were then seated on an adjustable chair with their trunk reclined at 15°. The hip, thigh and non-dominant leg were secured to the chair (via webbing straps) while the dominant leg was strapped to the dynamometer's attachment with the knee flexion angle of 90°. The rotational axis of knee movement was aligned with the lateral femoral epicondyle. The participants performed 4 sets of 25 maximum eccentric knee extension contractions from flexion (90°) to full knee extension (0°) at a speed of $60^{\circ} \cdot s^{-1}$. A rest period of 10 s was given for recovery between repetitions and a rest period of 3 min was given between each set of contractions. All participants received the same verbal encouragement throughout the test. The participants were allocated into 3 groups by block randomization (25 participants per group), group 1, 14% Plai cream, group 2, 7% Plai cream and group 3, placebo (control) (Figure 1). The 3 creams were manufactured by the same company and consisted of the same color, odor and packaging. For all groups a dose of 2 g or approximately a 2.5 cm long strip of cream was gently rubbed into the quadriceps muscles (mid quadriceps

area around 15×8 cm or 120 cm^2) until the cream was not visible (approximately 5 min), immediately following the eccentric exercise. The participants were instructed to apply the cream every 8 h after exercise for 1 week.

The participants were requested not to use other treatments such as an oral NSAID, muscle relaxants, physiotherapy or massage. If the participants received other therapies, all details were recorded. Simple analgesic drugs or paracetamol were allowed for management of severe and intolerant pain, and the number of paracetamol tablets or drugs used per day were recorded .The compliance of cream application was recorded on the daily record checklist. All participants completed the study (Figure 1). The participants were evaluated at pre-test, day 1, day 2, day 3 and day 7 post-exercise (Figure 2).

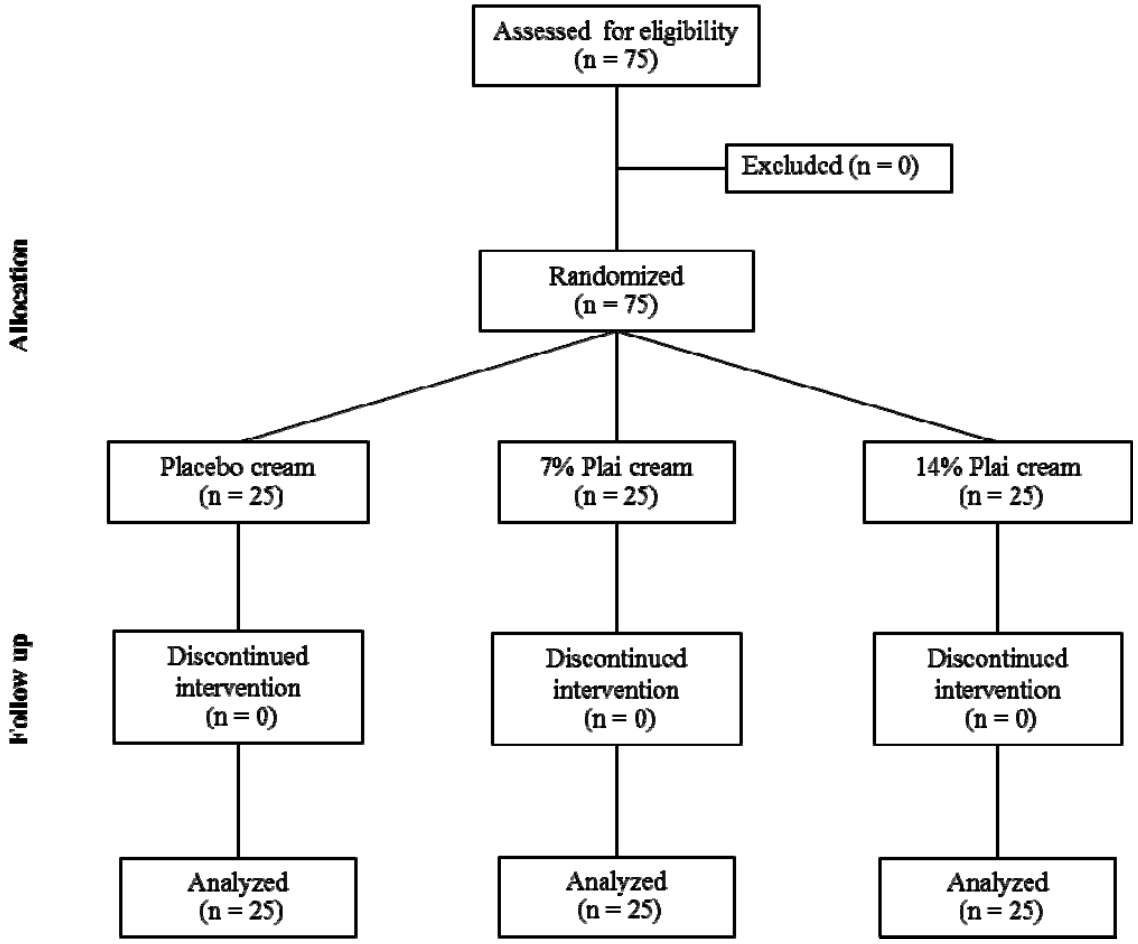

Figure 1 Flow diagram

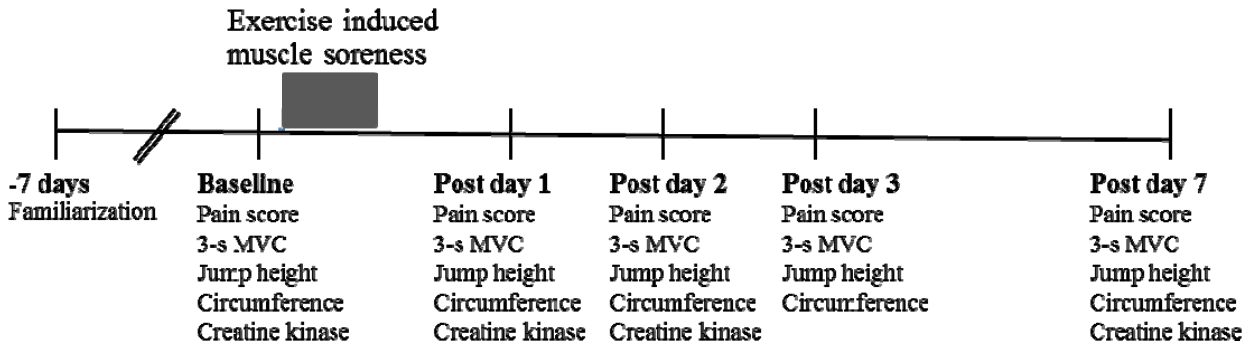

Figure 2 Summary of outcomes measurement in this study (3-s MVC, 3-second maximal voluntary contraction)

2.2 Plai cream preparation

Plai cream is an ordinary oil in water emulsion technique. Plai oil was extracted by stream distillation from *Zingiber cassumunar* ROXB rhizome. Plai and placebo creams were produced by Bangkok lab & Cosmetic Co., Ltd., Bangkok, Thailand that has a GMP (Good Manufacturing Practice) standard and manufactures with high quality standards of PIC/S GMP.

2.3 Muscle soreness and swelling

Subjective muscle soreness of the quadriceps was determined by using a visual analogue scale, ranged from 0 to 10 (0 indicating no pain, 10 indicating the worst pain). The visual analog scale has been widely used as a reliable tool for pain intensity assessment[14].

Swelling of the thigh was evaluated by measuring thigh circumference at mid-level (midpoint between inguinal fold and anterior aspect of the patella) in the supine position. The thigh circumferences were measured to the nearest 0.1 cm by using a standard tape (Rollfix, Hoechst. Mass, Germany). The mean of the two closest thigh

circumference measurements was recorded. The research assistant taking the tape measurement was blinded to the participant's group.

2.4 Muscle strength

Muscle strength was evaluated by 3-second maximal voluntary concentric (MVC) of the dominant quadriceps muscle using isokinetic dynamometry. The dominant leg was secured to the isokinetic attachment with the knee in flexion at 45°. Each subject performed three MVCs, separated by a 2-minute rest. Verbal encouragement was given in order to motivate the subject for the maximal force exertion.

Explosive muscle power was assessed via the countermovement jump test using standard testing equipment (Yardstick, Swift Performance Equipment, New South Wales, Australia). The participants jumped from a standing position and their fingertips reached to the highest level. Participants completed a total of 3 jumps (with a 60-second rest between jumps).The highest jump height of 3 performances was recorded for analysis. 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.

2.5 Plasma creatine kinase

A 5-ml blood sample for creatine kinase (CK) was obtained from the median cubital vein by a certified medical technologist at Srinagarind Hospital. The blood CK levels were analyzed by an automated analyzer (Cobas® 6000 analyzer; Roche Diagnostics Corp., Indianapolis, IN, USA). Plasma CK was measured at pre-test, day 1, day 2 and day 7 post-exercise.

This study has been approved by the KhonKaen University Ethics Committee for Human Research reference number HE 561436, and has been registered at Thai Clinical Trial Registry identification number TCTR20140215001.

2.6 Statistical analysis

The sample size was calculated based on repeated measures control study. The values were set at significant level for 0.05, power of study for 0.8, difference of mean pain score between two groups for 2, and standard deviation of pain score for 2.8[8]. Therefore, the estimated sample size was 25 participants per group.

Descriptive statistical analysis includes the mean and standard deviation. A generalized linear mixed model was used to analyze repeated measurements of the longitudinal study. Analysis of the statistic indicated the overall effects of treatment and the pattern of change difference of measured time points of muscle soreness score, muscle strength, jump height, thigh circumference and plasma creatine kinase among three groups. The analyses were conducted by using STATA program version 13.0. Statistical significant difference was accepted at $P < 0.05$.

3 Results

3.1 Demographic data of participants

There were 75 healthy volunteers enrolled in this study. The characteristics of each group are presented in Table 1.

Table 1 Demographic data of participants

Index	Placebo (*n*= 25)	7% Plai (*n* = 25)	14% Plai (*n* = 25)
Male/Female	9/16	9/16	10/15
Age (years)	26.2 ± 12.0	31.3 ± 16.7	28.7 ± 13.7
Body weight (kg)	57.6 ± 11.6	57.5 ± 7.2	61.5 ± 10.5
BMI ($kg.m^{-2}$)	21.4 ± 3.7	21.9 ± 2.3	23.1 ± 3.9

Data are presented as means ± standard deviation.

3.2 Pain score

Subjective pain of the quadriceps muscles were similar in all 3 groups at baseline and increased as a result of the eccentric exercise (Figure 3). Overall, perceived pain levels over the 4 recovery periods were substantially reduced in the 14% Plai group by -82% (95%CI = -155 to -6, $P = 0.03$) compared to the placebo group. In addition, when analyzing the individual day pain scores, pain was substantially reduced in the 14% Plai group compared to the placebo group on post-exercise day 1 (-26.8%, -52.4 to -1.2%, $P = 0.04$). The pain levels in the 7% Plai group were not significantly different to the placebo group (-40%, -116 to 36%, $P = 0.3$) and 14% Plai group (-34%, -96 to 27%, $P = 0.2$) throughout the recovery period. No oral analgesic drug was required during the study.

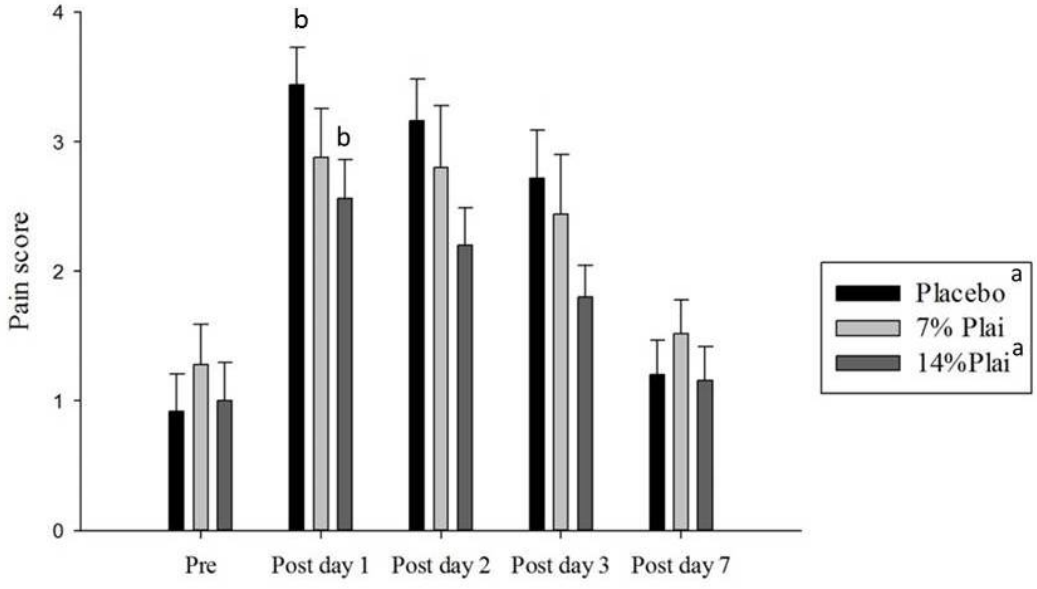

Figure 3 Pain score of Plai (7 and 14%) and placebo groups at baseline, post exercise day 1, day 2, day 3 and day 7 (mean ± SE).

[a] =4% Plai was statistically lower over all days combined compared to Placebo.

[b]=14% Plai was statistically lower on individual days compared to Placebo.

3.3 Muscle strength

The 3-s maximal voluntary contraction force of the quadriceps can be seen in Table 2. There was no significant difference in muscle strength at baseline between groups, but all groups showed a substantial decrease in maximal force 24 hours after the eccentric exercise bout, and tended to follow a similar recovery pattern over the next 2 days. At 7 days post eccentric exercise, maximal isometric force was substantially higher in the 14% Plai group compared to the placebo group (22%, 0.01 to 43.8%, $P = 0.04$).

Table 2. Muscle strength (kg) of three groups (mean ± SD).

Group	*n*	Baseline	Post exercise day 1	Post exercise day 2	Post exercise day 3	Post exercise day 7
Placebo	25	24.5 ± 6.3	23.6 ± 6.9	24.8 ± 6.5	25.6 ± 7.5	25.9 ± 8.4[b]
7% Plai cream	25	25.2 ± 6.7	23.4 ± 5.9	25.1 ± 5.5	25.5 ± 5.4	25.8 ± 6.3
14% Plaicream	25	25.8 ± 5.6	23.9 ± 5.9	27.4 ± 6.8	28.0 ± 7.9	29.5 ± 6.7[b]

[b]= Significant difference on individual day of measurement.

3.4 Creatine kinase

Serum creatine kinase levels increased significantly in all groups ($P < 0.01$) from baseline (where there no significant differences between groups) to post exercise day 1, as a response to the eccentric exercise, and had recovered almost back to baseline levels by recovery day 7 (Table 3). There was no overall difference between groups (area under the curve for the 4 measurements) as a result of the separate interventions.

Table 3. Creatine kinase concentration (mmol.L^{-1}) of three groups (mean ± SD).

Group	*n*	Baseline	Post exercise day 1	Post exercise day 2	Post exercise day 7
Placebo	25	123.0 ± 49.4	175.0 ± 96.2	180.9 ± 113.7	134.1 ± 53.5
7% Plai cream	25	110.4 ± 42.4	167.0 ± 127.7	151.6 ± 80.8	132.2 ± 63.9
14% Plai cream	25	113.8 ± 51.0	170.6 ± 110.8	154.2 ± 88.4	136.4 ± 64.1

3.5 Jump height and thigh circumference

Finally there was no substantial difference in the countermovement jump height or thigh circumference measures over the period of the study between groups.

4 Discussion

Applying 14% Plai cream over eccentrically worked muscles every 8 h for at least 3 d is likely to have a beneficial effect on DOMS and subsequent recovery of muscle strength, compared to no treatment (placebo). Using 7% Plai cream produced little beneficial effect.

It has been suggested that Plai extracts have anti-inflammatory and analgesic effects. Previous research indicates various phytochemicals that were isolated from Plai including curcumin, cassumunar, and phenylbutenoids (*i.e.*, (E)-4(3',4'-dimethylphenyl) but-3-en-I-ol, (E)-1-(3,4-dimethoxyphenyl) but-3-en-2-ol and (E)-1-(3,4-dimethoxyphenyl) butadiene) may have beneficial effects on the inflammatory process[10,11,15]. Curcumin (diferuloylmethane) has an anti-inflammatory effect on reducing activities of cyclooxygenase-2 (COX-II), lipoxygenase, inducible nitric oxide synthase and inflammatory cytokines[15]. Cassumunar extracts have been found to have an anti-inflammatory effect on chemically induced edema of the mouse ear stronger than curcumin[10]. In another previous experiment in rats it was revealed that (E)-1-(3,4-dimethoxyphenyl) but-3-en-2-ol, reduced edema in induced rat paw edema and inhibited exudate formation, leukocyte accumulation and prostaglandin-like activity of the exudates[11]. The anti-inflammation effect of (E)-1-(3,4-dimethoxyphenyl) butadiene (DMPBD) has been found through the inhibition of cyclooxygenase, and lipoxygenase pathways[9].

The topical Plai preparation can be manufactured in several forms; however, the cream form is the best preparation because a high volume of oil is present as the active ingredient. The properties of the cream are easily spread and do not feel greasy and sticky after application. In the balm form, although it has a similar level of active ingredient to the cream, it produces a sticky or tacky feeling especially in a tropical

climate. In the gel form, the hydrophilic ingredient is not compatible with a high percentage of Plai oil and therefore the gel has a lower percentage of active ingredient. Topical Plai cream is readily absorbed through the skin and helps reduce inflammation[12,13]; however, little research exists on other topical forms.

Previous clinical trials showed Plai cream reduced musculoskeletal pain in injured individuals. Laupattarakasem *et al*[12] showed Plai cream significantly reduced pain from ankle sprains at days 5 and 6 post treatment and required less analgesic drug at days 1 and 2 than placebo. Srirochana[13] revealed positive effects of Plai cream in treatment of osteoarthritis compared to diclofenac gel after two weeks. The duration of effectiveness may depend on the type and lesion of inflammation. This study showed an acute analgesic effect of 14% Plai cream on reducing muscle soreness 24 h post treatment.

It has been found that topical NSAIDs can help with reducing pain and DOMS[8]; however, such drugs also carry the adverse effects of application including dry skin, rash and pruritis[16]. Additionally, topical NSAIDs has been associated with systemic adverse effects such as dyspepsia or gastritis[16]. The current study found a significant analgesic effect of topical 14% Plai cream and significant improvement of muscle strength (3-s MVC) at post-exercise day 7 compared to the placebo group. We suggest the topical 14% Plai cream would be an effective alternative to NSAID in DOMS management.

The mechanism of NSAIDs is anti-inflammation resulting in reduced muscle damage. This study, however, did not find any significant difference in creatine kinase concentration between 14% Plai cream and the placebo. The results from the current study indicate that creatine kinase concentration was dampened in the Plai groups compared to the control group. It is possible that Plai cream may help reduce the early release of creatine kinase, however this will remain speculation until further

investigation is conducted with a larger sample size which will help to decrease the effect of the high variability in this parameter.

A secondary objective of this study was to gauge the effect of Plai cream dosage on DOMS and force recovery. Pain scores after days 1, 2 and 3 in participants given the 7% Plai cream were lower than participants in the placebo group but higher than participants given the 14% Plai cream. The effect of Plai cream on DOMS may be related to its concentration. It seems that the lower concentration Plai cream had little effect at either reducing DOMS or helping in the recovery of force production. The dose related effect of Plai cream may be similar to NSAIDs such as diclofenac or ibuprofen[17]. Taking our results into consideration we would recommend clinicians use the 14% Plai cream for analgesic effects.

5 Conclusions

Compared to placebo, 14% Plai cream reduced DOMS perception at 24, 48 and 72 h after eccentric exercise, but no significant pain reduction was observed with 7% Plai cream. In addition, those participants that used the 14% Plai cream showed increased force production recovery compared to the placebo group by the end of the 7-day intervention period which was not found in the participants using the7% Plai cream. Given these results it is recommended that when using Plai cream as an analgesic the higher concentration (14%) is likely to be more effective (7%).

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7 Conflict of interest statement

None declared.

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Experiment 2: The effect of Plai cream (Zingiber cassumunar ROXB) combined with ultrasound on delayed onset muscle soreness

Abstract

Plai (Zingiber cassumunar ROXB) is used as folk remedy for release pain and inflammation of musculoskeletal problems. The evidence of anti-inflammatory effect of Plai cream in clinical trial currently is rather limited, especially no evidence in treatment of delayed onset of muscle soreness (DOMS) of athletes. In addition, anti-inflammatory effect of Plai cream that may be enhanced by phonoporesis or ultrasound therapy is questionable. This study will evaluate the effect of Plai cream and Plai cream combined with ultrasound in DOMS treatment.

Objective: To evaluate effect of ultrasound combined with Plai cream in the treatment of delayed onset muscle soreness.

Study design: Randomized controlled trial study

Methods: This study was conducted in laboratory, Rehabilitation Department, KhonKaen University. Seventy-five volunteers were included in this study and were randomized into 3 groups, group 1 for 14%Plai cream, group 2 for ultrasound and group 3 for combined. The participants performed eccentric exerciser of dominant quadriceps by using isokinetic dynamometry to induce DOMS for 4 sets of 25 repetitions at a speed of $60°.s^{-1}$. Group 1 received 5 cm long strip of Plai cream applied. Group 2 received continuous ultrasound therapy (1MHz, $1watt.cm^{-2}$) for 5 minutes. Group 3 received combined 14% Plai cream with ultrasound. All groups received continuous treatment every day. Pain score, Pain pressure threshold (PPT), rate of perceived exertion (RPE), 3-sec maximal voluntary contraction, range of motion and thigh circumference were evaluated at pre-test, day 1, 2, 3 and 7 post-exercise. Plasma creatine kinase (CK) was measured at pre-test, day 1, day 2 and day 7

post exercise. A generalized linear mixed model was conducted for overall and each time point among three groups.

Results: Seventy five participants, average age 41.5 ± 9.3 year,6 male and 44 female were included. This study found there was no significant difference of visual analog pain score, PPT, RPE,CK, muscle strength, range of motion and thigh circumference among three group ($p > 0.05$) among 14% Plai cream, ultrasound and combined.

Conclusion: Combined 14% Plai cream with ultrasound had no significant difference effect on DOMS compared to only 14% Plai cream or only ultrasound. Application of 14% Plai cream may not be facilitated by phonophorosis process

1. Introduction

Delayed onset muscle soreness (DOMS) illustrated as muscle pain and tender typically occurred after strenuous exercise. Eccentric exercise especially unfamiliar exercise has a potential to develop muscle injury or DOMS rather than isometric and concentric exercise[1]. The symptoms of DOMS include pain, loss of strength, swelling and stiffness that generally occurs 24-48 hours after exercise and resolves within 10 days[2]. The mechanisms of DOMS have been found that are from exercise induced muscle damage including sarcomere disruption and secondary inflammatory response[3-4]. Inflammation process stimulates Prostaglandin E2 to sensitize type III and IV pain afferents, and leukotrienes to attract neutrophil for producing free radicals that exacerbate muscle cell damage[2].

The prevention and reduction of DOMS will improve subsequent sport performance of athletes. The numerous methods to prevent and reduce DOMS have been suggested including stretching exercise, massage and nutritional supplement. The non-steroidal anti-inflammatory drugs (NSAIDs) have been suggested for DOMS treatment that may reduce pain and improve function. The NSAIDs inhibit cyclooxygenase activity leading to reduce prostaglandins that are a potent vasodilators and pain producing agent [5-6]. Balwin et al. (2001) revealed three days after eccentric exercise of knee extensor, thigh soreness in participants who received naproxen sodium (220 mg three times a day) was lower than placebo by 40%, and isometric strength declined for NSAID less than placebo by 100%[7]. Rahnama et al. (2005) showed muscle soreness and creatine kinase after eccentric exercise 48 hours in participants who received Ibuprofen 2,800 mg (400 mg on seven occasions) significant lower than control [8]. Canavino et al. (2003) showed the local application of NSAID local application (transdermal ketoprofen, 1g of cream local apply every 8 hours)

reduced muscle soreness 24 hours post exercise by 37% compared to placebo, and the systemic absorption of NSAID was minimal that may avoid adverse effects of NSAID [9].

Plai or Zingiber cassumunar ROXB is widely used in Thailand as traditional medicine for anti-inflammation and pain reduction. The experiments in rats revealed that the extract from Zingiber cassumunar ROXB reduced edema in induced paw rat edema, and inhibited exudates formation, accumulation of leukocytes and prostaglandin-like activity of exudates[10]. The anti-inflammation effect have been found through the inhibition of cyclooxgenase, cyclooxygenase-2 and lipoxygenase pathway[11-12].

Plai cream has been introduced for clinicians to reduce inflammatory process in musculoskeletal disorders. Laupattarakasem et al. (1993) revealed applying of 2 grams of Plai cream (2 times a day for 7 days) reduced pain and swelling in the treatment of ankle sprain compared to placebo [13]. Srirochana (2010) showed the beneficial effect of applying Plai cream (3 times a day for 4 weeks) to reduce pain and improve physical and emotional function of knee osteoarthritis patients compared to diclofenac gel [14]. The positive effect of Plai cream for local application in DOMS treatment has been studied in the previous study (waiting for reference).

In addition, the anti-inflammatory effect of plai cream may be enhanced by ultrasound wave. Ultrasound is widely used in physiotherapy to relieve pain and inflammation. Ultrasound also is used for phonophoresis to enhance transdermal penetration of pharmacological substances[15]. Deniz et al. (2009) revealed ultrasound with diclofenac gel in osteoarthritis patients more improved pain at rest and activity, WOMAC pain and physical function after treatment than only diclogenac gel and only ultrasound therapy[16]. Ultrasound and phonophoresis also showed beneficial effects on other musculoskeletal disorders such as painful shoulder syndrome and lateral

epicondylitis[17-18]. The evidence of phonophoresis for DOMS management have been studied by Clccone et al. (1991) who showed using ultrasound with trolamine salicylate cream on 3 consecutive days after eccentric exercise reduced muscle soreness by 24.4% compared to only trolamine salicylate cream and 51.7% compared to only ultrasound[19].However, ultrasound combined with Plai oil for anti-inflammatory effect has never been studied before.

This study aims to evaluate the effect of Plai oil that may be enhanced anti-inflammatory effect by phonoporesis process (application with ultrasound therapy). If Plai cream has clinically anti–inflammation effect, it should be introduced to apply in exercise and sport performance.

2. Methods

A randomized controlled trial study was conducted at Rehabilitation Department KhonKaen University. This study included 75 healthy participants who have age between 18 – 60 years. The volunteers who had uncontrolled psychological disorders or uncontrolled medical diseases such as diabetes, hypertension and heart disease were excluded.

Delayed onset muscle soreness was induced by strenuous eccentric exercise of the dominant quadriceps muscles by an isokinetic dynamometer (Primus RS, BTE technologies, Hanover, MD, USA). All participants performed 15 minutes stretching lower limbs muscles for warm-up before strenuous eccentric exercise. The participants sit on the comfortable chair in secured position of lower limbs with 15^{o}trunk reclined. The dominant leg was fastened to the dynamometer's attachment with the rotational axis of knee movement on the lateral femoral epicondyle. The 4 sets of 25 maximum eccentric knee exercise began from flexion (90°) to full extension

(0°) at a speed of $60°.s^{-1}$ with 10 seconds rest between repetitions and 3 minutes rest between each set. The verbal reinforcement was performed throughout the exercise. The participants were allocated into 3 groups by block randomization, group 1, 14% Plai cream, group 2, ultrasound and group 3, combined Plai cream and ultrasound. Group1, the14% Plai creams were manufactured by Bangkok Lab & Cosmetic Co., Bangkok, Thailand. A dose of 2 g or approximately a 2.5 cm long strip of cream was gently massaged into the quadriceps muscles until the cream was not visible, immediately following the eccentric exercise. The participants were instructed to apply the cream every 8 hours after exercise for 1 week. Group 2, the certified physiotherapist applied pulsed ultrasound wave therapy with 1 MHz frequency and 1 watt/cm^2 at the quadriceps muscle area of participants for 5 minutes, immediately following the eccentric exercise and then every day. Transducer head was applied at right angle to ensure maximum energy absorption. The skin was coated with acoustic gel which was not contained pharmacological substances. Group 3, combined 14% Plai cream and ultrasound, the participants received ultrasound therapy with the same dose as group 2 and used 14% plai cream 2.5 cm long strip of cream as a replacement for acoustic gel.

The compliance of participants was observed on daily checklist and other treatments including analgesic, NSAID, physiotherapy or massage was recorded if the participants required.

Measurement

The muscle strength and muscle soreness will be measured at pre-test, 1 hour after exercise and on day 1, 2, 3, 4, 7 and 10 post-exercise. Plasma creatine kinase will be measured at pre-test, day 1, day 2 and day 3 post-exercise.

Pain Score

The perceived muscle soreness was evaluated by pain 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 level of pain intensity[20].

Thigh circumference of participants was evaluated 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 was recorded.

Muscle strength

The participants were tested 3-second maximal voluntary concentric (MVC) of their dominant quadriceps by using isokinetic dynamometry. All participants seated on comfortable chair while their dominant legs were secured to the isokinetic attachment. Each one performed three MVCs (2min rest) in position knee flexion $90^{o}$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 indicated the severity of muscle damage was analyzed from5-ml blood sample which was taken from the median cubital vein. The plasma CK was measured by using an automated analyzer (Cobas® 6000 analyzer; Roche

Diagnostics Corp., Indianapolis, IN, USA) at Srinagarind Hospital Laboratory. The plasma analysis was performed at pre-test, day 1, day 2 and day 7 post-exercise.

Rate of perceived exertion (RPE)

The Perceived exertion was rated by using the Borg scale (ranged from 6 to 20, 6 for no exertion at all and 20 for maximal exertion) after 5 min rest and as soon as the participants arrived at the laboratory each day[15].

Range of motion (ROM)

The motion of tested knee joint was measured by a blinded researcher assistance to evaluate the degree of joint stiffness from muscle soreness. The goniometer (BASELINE® dynamometric, USA) was used to measure degree of movement from knee extension in prone position to full knee flexion.

Pressure pain threshold

The threshold of pain pressure of quadriceps was measured by a manual algometer (BASELINE®, New York, USA). A blinded research assistance pressed an algometer perpendicularly to quadriceps and observed minimal pressure which produces painful sensation, while the participants sat down and extended their knee joint. The average of three points of measured on quadriceps (5, 10, and 15 cm above the apex of patella) was recorded.

This study has been approved by the KhonKaen University Ethics Committee for Human Research reference number HE 561436, and has been registered at Thai Clinical Trial Registry identification number TCTR20140215001

3. Statistical analysis

The study results were presented by descriptive statistic with mean and standard deviation. The overall effects and difference of time point changes of pain score, pain pressure threshold, rate of perceived exertion, creatine kinase, muscle strength, range of motion and thigh circumference among three group of study were analyzed by using a generalized linear mixed model. Analysis of statistic was conducted by using STATA program version 13.0 with accepted significant difference at $p < 0.05$.

4. Results

There was 75 participants, average age 41.5 ± 9.3 year, average BMI 24.2 ± 3.9kg.m^{-2}, 6 male and 44 female. The participants were divided into 3 groups, 14% Plai cream, ultrasound and combined 14% Plai cream with ultrasound. The characteristics of each groups were presented in the Table 1.

The study revealed similar pattern of changes in all outcomes after eccentric exercise among three groups. One day after eccentric exercise, pain score, RPE, thigh circumference and CK increased, and then slowly decreased. While PPT, muscle strength, range of motion were decreased after exercise one day and then gradually improved. However, there was no significant difference of visual analog pain score, PPT, RPE,CK, muscle strength, range of motion and thigh circumference among three group ($p > 0.05$). The results were shown in the Table 2 and Table 3.

“Table 1 around here”

“Table 2 around here”

“Table 3 around here”

5. Discussion

The study found no substantial difference effects of 14% Plai cream, ultrasound and combined on DOMS in terms of pain score, pain pressure threshold, rate of perceived exertion, creatine kinase, muscle strength, range of motion and thigh circumference. The combination of 14%Plai cream and ultrasound had not been detected greater effect than 14% Plai cream or ultrasound Application of 14% Plai cream may have similar effect on DOMS to ultrasound therapy.

The mechanisms of DOMS have been found that are from exercise induced muscle damage including sarcomere disruption and secondary inflammatory response [3-4]. Inflammation process stimulates Prostaglandin E2 to sensitize type III and IV pain afferents, and leukotrienes to attract neutrophil for producing free radicals that exacerbate muscle cell damage[2]. The previous studies revealed the various

phytochemical extracts from Plai have effect to reduce pain and inflammation[10, 21-22]. The 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) Plai reduced edema and inflammation through inhibition of cyclooxygenase, lipoxygenase pathways[12], exudate formation and leukocyte accumulation[10]. Previous clinical trials studies found Plai cream significantly reduced pain from ankle sprain [13] and pain from osteoarthritis [14]. Recent clinical trial found the dose related of Plai cream and suggested concentration of 14% Plai cream is proper to reduce pain on DOMS. This study used 14% Plai cream with the same treatment protocol of the previous study was expected to effectively reduced pain on DOMS.

It has been suggested ultrasound therapy generates oscillatory movement in the tissue caused decrease pain and inflammation[23]. Hasson S et al. (1990) suggested the pulsed ultrasound had significant effect to reduce pain form DOMS[24]. Craig JA et al (1999) found there was no substantial effect of ultrasound on DOMS[23]. 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 but did not find different effect between ultrasound and sham[25]. However, our study found the effect of ultrasound was not different to Plai cream. Ultrasound may have similar pain reduction effect to Plai cream on DOMS

The anti-inflammatory effect of plai cream enhanced by ultrasound wave may be from both thermal and mechanical effect. Ultrasound was successfully used for phonophoresis to enhance transdermal penetration to improve inflammation in musculoskeletal disorders such as osteoarthritis, painful shoulder syndrome and lateral epicondylitis to improve pain and physical function [15-18]. The evidence of phonophoresis for DOMS management have been studied by Clccone et al. (1991) who showed using ultrasound with trolamine salicylate cream on 3 consecutive days

after eccentric exercise reduced muscle soreness by 24.4% compared to only trolamine salicylate cream and 51.7% compared to only ultrasound [19]. Phonophoresis with Plai cream in this study used compound phytochemical substances that may be different from tropical NSAID.

Previous studies regarding phonophoresis with phytochemical substances showed various results. Phonophoresis with Aloe vara gel reduced edema and inflammation of rat paw with collagenase-induced tendinitis [26], whereas Phonophoresis with Arnica montana had no anti-inflammatory effect of acute muscle inflammation of rat [27]. Our study found ultrasound combined with Plai cream did not have substantial greater effects than Plai cream or ultrasound. We speculated that various phytochemical substances in Plai extract may have little effect on phonophoresis enhancement. In addition, muscle soreness from DOMS in this study did not produce serious pain. Therefore, little improvement from phonophoresis may not be obviously observed. Phonophoresis with Plai cream study in other conditions such as tendinitis or arthritis would be recommended.

6. Conclusion

Combined 14% Plai cream with ultrasound had no additional effect on DOMS compared to only 14% Plai cream or only ultrasound. Ultrasound may not enhance Plai extract to penetrate skin by phonophorosis process.

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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 (years)	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

Data are means ± SD.

Table 2. Pain score, muscle strength and creatine kinase concentration of three groups (mean ± SD).

	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	33.2 ± 7.5	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	34.9 ± 6.5
Post exercise day 7	32.9 ± 7.9	34.7 ± 7.9	34.8 ± 7.9
Creatine kinase ($mmol.L^{-1}$)			
Baseline	116.1 ± 41.6	128.9 ± 44.9	128.3 ± 42.4
Post exercise day 1	168.6 ± 101.9	167.3 ± 83.6	169.8 ± 114.5
Post exercise day 2	148.9 ± 90.0	148.6 ± 67.0	181.4 ± 116.3
Post exercise day 7	131.1 ± 56.2	140.8 ± 78.0	139.6 ± 55.5

Table 3. Pain pressure threshold, rate of perceived exertion (RPE), range of motion, thigh circumference of three groups (mean ± SD).

	Plai cream	Ultrasound	Combined
Pain pressure threshold			
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			
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			
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	48.0 ± 4.2
Post exercise day 2	48.0 ± 5.7	47.8 ± 3.4	47.4 ± 3.9
Post exercise day 3	48.1 ± 5.7	47.7 ± 3.2	48.2 ± 4.5
Post exercise day 7	47.9 ± 5.8	47.7 ± 3.3	47.9 ± 4.4