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Effects of different doses of sports liniment with massage on changes of myoglobin among male Muay Thai fighters: A randomised cross-over pilot study

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Abstract

The pre-training application of sports liniment, whose pungent smell is a common feature of Muay Thai gyms, is routinely used by male Thai Muay Thai fighters, yet little research has examined its physiological effects on intramuscular oxygen stores. This study investigated the impact of different dosages (1, 3, and 5 mL) of Namman Muay (NM) liniment compared to a control (Baby Oil) on myoglobin oxygen saturation (Mb%) in the Rectus femoris muscle, crucial for kicking techniques. To enhance liniment permeation, two mechanical methods were utilized: Tape Stripping (TS) and Massage (MG). In a randomized, cross-over design, twelve male Muay Thai fighters participated in TS on Day 1, followed by MG on Day 2, receiving randomly allocated NM dosages or the control. A Moxy monitor measured Mb% at baseline, 20 min, and 45 min post-intervention. The results revealed all NM dosages combined with MG led to significant increases in Mb% at 20 minutes post-application. Notably, the 5 mL dosage with MG sustained significant increases at both 20 and 45 min post-application, showing a 26% increase at 20 min ($p < 0.001$) and a 17% increase at 45 min compared to baseline. The 5 mL TS group also exhibited a significant 9.1% increase post-application at 20 min ($p = 0.016$). These findings suggest that a 5 mL dosage of NM, particularly when applied with massage, significantly enhances myoglobin oxygenation. This implies that a higher amount of NM could potentially benefit the performance of male Muay Thai fighters.

Keywords: massage, Muay Thai, liniment oil, myoglobin

1. Introduction

Sports rubs have become synonymous with Muay Thai fighters, and one such product, Namman Muay (NM) also branded as the 'Boxing liniment', is gaining popularity both nationally and internationally [1,2]. Once applied topically to the skin, it is known for its pain-relieving benefits, which include its mechanism in the treatment of muscle pain [3]. This mechanism depends on compounds such as methyl salicylate and L-menthol, which are the two main components of NM. Methyl salicylate acts as a counterirritant, meaning it causes mild irritation to the skin, which diverts pain receptors away from the deeper source of pain [4]. The enzymes cyclooxygenase, which produce the inflammatory mediators prostaglandins and thromboxane that cause our pain, swelling and fever, are inhibited [5]. L-menthol is used as a cooling and analgesic agent. It reduces pain by activating the TRPM8 receptor, which is a cold-sensitive ion channel expressed in some nerve endings. By creating a sensation of cold, the pain signals from the affected area are masked [3]. There are other mechanisms that L-menthol uses to inhibit or activate systems that may interact with the modulation of pain in our body.

While the pain-relieving mechanisms of methyl salicylate and L-menthol are well-documented, there is a scarcity of research on their potential to increase muscle oxygen content (SmO2). Methyl salicylate, known for

its vasodilatory properties enhancing blood flow and oxygen delivery to muscles, while L-menthol is a cooling agent that can reduce temperature and oxygen consumption, however, is also a vasodilator [3]. This leads to a rather contradictory process where the two compounds may be inferred to cancel each other out. Nevertheless, previous studies have indicated a potential synergistic effect of these compounds in improving oxygenation in muscles [6], thus benefiting athletic performance and recovery.

Two mechanical enhancement techniques commonly employed in facilitating the permeation of topical substances are Tape Stripping and Massage. Tape stripping has been recognized as an effective method for enhancing drug penetration as a penetration enhancer, as demonstrated by [7]. This technique involves the application of adhesive tape on the rectus femoris muscle, followed by pressing and lifting it off, repeated 20 times to induce Stratum Corneum (SC) disturbance, thereby facilitating easier penetration of the active pharmaceutical ingredient (API), as elucidated by [8]. Research indicates that tape stripping yields superior outcomes compared to non-tape stripped SC, as evidenced by [9].

Studies emphasize the enhanced penetration of the SC and underlying structures when topical mediums are applied and massaged in, as highlighted by [10]. However, the effectiveness of medium penetration remains a topic of debate, with conflicting evidence, as noted by [11].

The kicking technique surpasses the punching method in Muay Thai, with skilled athletes utilizing diverse approaches based on their weight divisions. A detailed analysis of striking techniques by [12] across different weight classes highlighted variations in speed-power capabilities, with lighter fighters demonstrating greater velocity potential. In a significant study by [13] focusing on Elite Thai and UK Muay Thai Fighters, it was found that Thai Fighters favored the Rear Roundhouse Kick (RHK) as the predominant attacking strategy, employing it 50% more frequently than their UK counterparts, who leaned towards punching techniques by 25%. The RHK, particularly effective when targeting the mid-section, offers a substantial biomechanical advantage. This technique initiates from the ankle and knee joints to enhance velocity, with subsequent hip flexion during body rotation leading to leg extension, as highlighted by [14,15,16].

The primary muscle involved in hip flexion and knee extension, known as the "kicking muscle," is the rectus femoris, exhibiting heightened activity during the RHK compared to other muscles, as noted by [17]. Insights from studies in Taekwondo by [18,19] underscore the critical role of hip musculature strength, knee angular velocity, and hip torques in optimizing RHK performance.

Technological advancements, such as the Moxy monitor, have greatly enhanced the measurement of tissue oxygen saturation (SmO2). This non-invasive device utilizes near-infrared light to penetrate muscle tissue and accurately calculate SmO2 by detecting oxygenated and deoxygenated myoglobin (Mb) and hemoglobin (Hb) levels in the muscle [20,21]. The Moxy monitor provides athletes with continuous real-time data on muscle oxygen saturation during exercise, enabling them to adjust training intensity promptly for improved performance and recovery. Its versatility across various sports and activities makes it a valuable tool in optimizing athletic performance by offering immediate feedback on muscle oxygenation. This real-time feedback allows athletes to make timely adjustments to their training intensity based on physiological responses [22].

Despite the promising aspects of methyl salicylate and L-menthol in enhancing oxygen levels, the impact of sports liniments containing these compounds on athletic performance remains underexplored. One study [23] used a muscle rub cream (Tiger balm) containing 15% methyl salicylate and 5% L-menthol, using a dosage form of 3 grammes. The results of this study was that leg power increased by 1.7% $P = 0.028$) using horizontal jump distance as an outcome comparing to the control group. 3 grammes is the equivalent to 3 mL, therefore using 1 mL, 3 mL and 5 mL had been chosen for this study to fit in with the leaflets recommendation ‘apply liberally’ and noting this previous studies dosage form. What is an effective amount to increase oxygen levels remains ambiguous. Mechanical aids like massage and tape stripping have emerged as viable techniques for enhancing the penetration of active ingredients into muscle tissue, further underscoring the potential advantages of sports rubs [10,11, 24].

This study seeks to investigate the effects of different dosages of NM (1 mL, 3 mL, and 5 mL) compared to a control group on the dominant leg rectus femoris muscle of male Muay Thai fighters. By employing massage and tape stripping methods, the study aims to determine the optimal dosage and application technique for enhancing SmO2 levels. With a focus on the physiological increase in SmO_2 levels, this study can hypothesise that the physiological increase in SmO_2 (Mb) will be increased by the massage technique, and it will be more effective with 5 mL of NM. The study anticipates shedding light on the potential performance benefits of sports rubs containing methyl salicylate and L-menthol.

2. Materials and methods

2.1 Participants

This research is a randomised cross-over pilot study has recruited twelve male Muay Thai fighters from the Muay Thai Academy, Gum Pun, Nakhon Ratchasima city and Khon Kaen city, Northeast Thailand (Figure 1). Inclusion criteria; 19 to 36 years male Muay Thai fighters. All participants signed the informed consent form, and

the experimental procedures were verbally explained to each participant by the research assistant on the day of briefing before the start of the study.

The sample size for this randomized cross-over pilot study was determined based on existing literature, which emphasizes the inherent challenges in calculating appropriate sample sizes for feasibility and pilot studies. As noted by [25], many pilot or feasibility studies are intentionally not powered to detect statistically significant differences; rather, such considerations are typically addressed in the subsequent main study.

Following the recommendation to recruit a small number of participants, typically between 10 - 20, to assess study outcomes, data quality, and potential biases [26], 12 participants were deemed suitable. This decision was made considering the lack of prior data, the study's focus on outcome measurements, and the cautious approach taken to ensure the study's robustness and ability to provide valuable insights into the research question. Experimental randomised cross-over designs are advantageous for controlling inter-individual variability due to their within-subject comparison approach, increased statistical power, cost efficiency, analytical flexibility, and minimization of confounding variables.

These features make them particularly suitable for studies in fields such as medicine and psychology, where individual differences can significantly impact treatment outcomes [27]. A total of 12 participants participated in this experimental randomised cross-over design pilot study, resulting in 24 samples, with the preferred kicking leg designated as the dominant leg. Participants with cardiac disease, musculoskeletal injury in the last 6 months, allergy to aspirin, contraindications to sports rub, massage or tape, and allergies were excluded from this study. Ambient conditions inside the Muay Thai gyms, the temperature was set at 21^0 C, same time of day (11.00 am to 6 .00 pm). Participants declared that they had not consumed any sports energy drinks or alcohol in the previous 24 hours. They were asked to maintain their normal physical/exercise-related activities throughout the experiment. The NM used contained 31% methyl salicylate and 1% L-menthol came from batch No: 56107, with 3 years before the expired date.

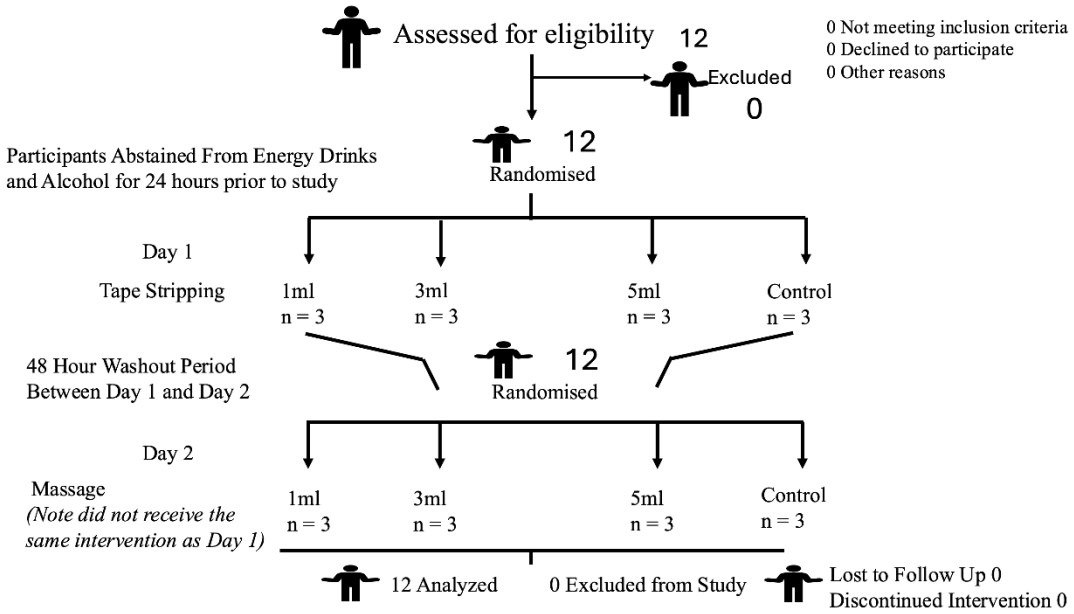

Figure 1 Methodology diagram.

2.2 Experimental Procedures

Current health status was assessed using the 2023 Physical Activity Readiness Questionnaire. Weight, height, and body temperature were also recorded. Participants were randomized using the block method, with three individuals allocated to one of the following dosage forms of NM (1 mL, 3 mL, 5 mL) or to the control, Baby Oil (BO). The control (BO) was treated as one of the conditions within the experiment's crossover sequence. After a dosage form was assigned on Day 1, it was excluded from the selection for Day 3 to ensure that no participant received the same dosage form on both days.

Participants performed the experiment in their usual gym environment. While lying supine on a massage table, a 9 cm × 6 cm rectangle (see Figure 2) was placed on the identified dominant leg to expose the rectus femoris muscle. The Moxy monitor was positioned within the rectangle over the rectus femoris, and an initial measurement was taken. The muscle oxygen saturation (Mb%) was recorded for two min.

A second two-minute recording was obtained after the assigned dose had been applied within the rectangular

area and allowed to permeate for 20 min. Subsequently, participants performed a Muay Thai warm-up exercise known as the RAMP protocol (Raise temperature, Activate muscle groups, Mobilise joints, and Potentiate contractile properties in the muscle) for 15 min. They then returned to the massage table in a supine position, where the final Mb% measurement was recorded for two min, 45 min after the dosage form had been applied. A ten-minute cool-down was then performed.

Day 2: No intervention was conducted, allowing for a 48-hour washout period following the initial dosage form.

Day 3: Forty-eight hours after the first intervention, the procedure from Day 1 was repeated. However, the dosage form used previously was excluded from randomization to prevent participants from receiving the same dosage form twice.

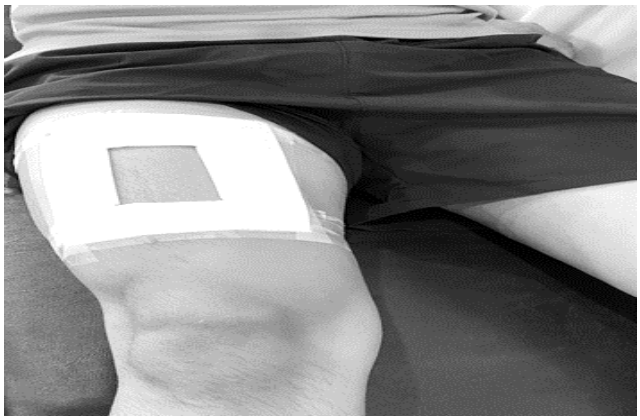

Figure 2 9 cm x 6 cm patch used to contain the selected dosage form within the dominant leg rectus femoris.

2.3 Interventions

The TS group received 20 x 2 cm x 6 cm tape strips, before placing the 9 cm x 6 cm rectangle on the rectus femoris muscle. Applied pressure to the tape from medial to lateral and then removed lateral to medial. The selected dosage form was then applied to the rectangle using a pharmaceutical-grad pipette of 100 1000µL, (SCILogex LLC, Connecticut, USA) (See Figure 3) In the MG group, the 9 cm x 6 cm rectangle was placed over the thigh and secured so that the rectus femoris muscle was exposed. The randomly selected dosage form was applied using the pharmaceutical-grade pipette, ensuring the correct millilitres was filled into the rectangle.

Figure 3 Pharmaceutical-grade pipette 100-1000 µL.

2.4 Outcome measurements: Muscle Oxygenation

The oxygen supply to the muscle was measured with the Moxy monitor (Moxy5, Fortiori Design, Minnesota, USA) and the antenna using near-infrared spectroscopy (NIRS). The Moxy monitor was placed in the centre of the 9 x 6 cm patch directly above the rectus femoris muscle and recorded the wavelength spectrum between 670 and 810 nm [28]. The measurements were performed three times and lasted two min, the baseline measurement

without the dosage form, and 20 and 45 min after the dosage form whilst laying in the supine position by the principal investigator.

The validity and reliability of the Moxy monitor have been reported as good in various studies where the device has been used to collect data in the field and the laboratory, particularly when used in the field of sport science [29,30]. One study reported on 0% to 100% scale having applied an arterial occlusion method which assessed repeatability, reproducibility and face validity using four sensors x3 on the quadriceps and x1 on the lateral gastrocnemius. The passive and active conditions measured revealed good robust 0%-100% scale on all muscle sites during the passive conditions M_{range} 67% ±9.9 (M_{min} = 10.1% ± 5.7; M_{max} = 78.1% ± 6.0). SmO_2 90% CI for minimum and 95% CI for Maximum [19]. Another study looking at an incremental cycle protocol revealed SmO_2 had a strong correlation between trials (ICC: r = 0.773-0.992, $p < 0.01$). It is also worth noting however, that the high correlations were seen during low intensity and decreased as the relative exercise increased which may have been attributed to tissue ischaemia or increases due to more muscle contractions [31]. A recent study by researchers at the Institute of Sport and Preventive Medicine explored the test-retest reliability of the Moxy Monitor in male cyclists.

The research revealed that the SmO_2 test-retest reliability was around 6%, indicating that day-to-day variations could account for differences in measurements. Interestingly, there were no consistent differences observed between dominant and non-dominant legs, although muscle oxygenation levels could vary by up to 20%. The analysis showed no significant systematic differences between trials and sides. The Intraclass Correlation Coefficient (ICC) ranged from 0.79 to 0.92 for both legs and all intensities, with the Standard Error of Measurement (SEM) ranging from 5 to 9% SmO2 and the Minimal Detectable Change (MDC) from 14 to 18% SmO2. The bias and Limits of Agreement (LoA) between legs were -2.0% ± 19.9% SmO2. Overall, the relative reliability of SmO2 was considered good to excellent based on current standards, although the adequacy of test-retest reliability may vary depending on the specific research objectives. The wide LoA suggest potential differences in muscle oxygenation between sides during exercise that are not solely explained by leg dominance [32]. The Moxy monitor offered the study valuable insights into muscle oxygenation and performance through key metrics such as SmO2 (Muscle Oxygenation Saturation) and Total Hemoglobin (THb). SmO2 indicates the level of oxygen saturation in the muscle, ranging from 0% to 100%, shedding light on oxygen utilization during activity.

2.5 Statistical analysis

The statistical analysis section of the study was conducted with meticulous attention to detail and transparency. All participants with available baseline data were included in the analysis, with results presented in Tables 1 and 2 showing means ± standard deviation (SD) and means ± standard error (SE) respectively. To ensure the robustness of the analysis, the Shapiro-Wilk test was used to assess data normality, while the Levene test was employed to check for homogeneity. Table 1 showcased a comprehensive "two-way ANOVA with repeated measures", with a "multivariate F-Test" and "Bonferroni corrected post hoc tests", complemented by "Pillai's trace" test. These statistical tests were instrumental in evaluating significant changes across time and conditions for both primary (the Mb%) and secondary outcomes (the tape stripping or massage technique), particularly focusing on muscle oxygen saturation at three different time points.

In maintaining methodological rigor, Mulchy analysis was utilized to examine the sphericity assumption, with the application of Huynh-Feldt correction when necessary to address any violations of this assumption. Furthermore, Table 2 presented a pairwise comparison to ascertain mean differences, with a Bonferroni post hoc test applied to adjust for multiple comparisons. The percentile difference was visually represented in Figure 4 to enhance data interpretation.

The analysis presented in Table 1 and Table 2 delves deep into uncovering potential disparities in group effects and interactions between groups and time. It also thoroughly examines the differences across various timelines within each group, providing a holistic and detailed overview of the study's findings.

Statistical analyses were conducted using IBM SPSS software version 28 for Windows, with a significance level set at $p < .05$ to determine statistical significance. This meticulous approach not only ensures the reliability of the findings but also enhances the clarity and transparency of the research methodology.

3. Results

Twelve Muay Thai fighters participated in the study and were included in the final analysis. Their anthropometric characteristics were as follows: 19 – 37 years, with a mean age of 24.7 ± 5.1 years, height of 171.5 ± 6.1 cm, weight of 63.3 ± 6.3 kg, and BMI of 21.4 ± 1.6 kg/m². Table 1 shows the comparison of Mb% between each mechanical enhancer and dosages at baseline, 20 min, and 45 min post within both MG and TS groups. Table 2 shows the pairwise comparison examining Mb% at baseline, 20 min, and 45 min post within MG and TS groups. There were also no significant differences seen within both BO dosage forms in the MG and TS groups. There

were also no significant differences seen between the groups. Figure 4. The mean differences in myoglobin percentages (%) among the MG group's participants, measuring before dosage, 20 min after dosage and 45 min after dosage.

Table 1 Comparison of Myoglobin percentage within each mechanical enhancer and dosages at baseline, 20 min and 45 min post.

Group (Condition)	Baseline	20 min post	45 min post	*p* Value *	Effect Size *
Tape Stripping					
1 mL	72.58 ± 15.29	73.91± 2.14	77.32 ± 3.87	.643	.057
3 mL	73.75 ± 2.04	81.29 ± 5.75	81.89 ± 1.58	.122	.245
5 mL	82.50 ± 3.69	90.40 ± 0.76	82.69 ± 1.60	.053	.324
BO	78.15 ± 11.48	76.97 ± 12.35	75.24 ± 9.99	.772	.034
Massage					
1 mL	81.84 ± 2.37	90.24 ± 3.65	84.69 ± 4.83	.044*	.341
3 mL	77.38 ± 6.32	90.53 ± 1.64	76.82 ± 11.23	< .001*	.643*
5 mL	64.53 ± 9.51	83.55 ± 5.87	76.31 ± 13.63	< .001*	.729*
BO	88.05 ± 0.36	91.48 ± 0.76	90.15 ± 1.24	. 599	.066

NOTE. Data are expressed as mean difference ± standard deviation (*SD). Obtained with Repeated-measures ANOVA. Effect size=partial eta square*
There are no significant difference to report across the myoglobin percentage baselines
* Multivariate tests including the F-Test, Bonferroni corrected post hoc test, and Pillai's trace test, the results revealed findings in terms of both p-values and effect sizes.
5ml of NM with massage exhibits a significant difference in myoglobin percentage at $p < 0.001$and the largest effect size of .729
3 ml NM with massage exhibits a significant difference in myoglobin percentage at $p < 0.001$ and an effect size of .643
Pillai's trace reveals significant difference in time effect $p < 0.001$ and significant difference in Group by Time effect (Interaction) $p = .049$

Table 2 Pairwise comparison examining Myoglobin at baseline, 20 min, and 45 min post.

Group (Condition)	20 min vs Baseline	45 min vs Baseline	45 min vs 20 min
Tape Stripping			
1 mL	+1.33 ± 2.93	+4.74 ± 4.95	+3.41 ± 5.13
3 mL	+7.54 ± 3.58	+8.14 ± 6.06	+0.60 ± 6.28
5 mL	+7.90 ± 2.93	+0.19 ± 4.95	-7.71 ± 5.13
BO	-1.19 ± 2.53	-2.91 ± 4.29	-1.72 ± 4.44
Massage			
1 mL	+8.41 ± 2.93*	+2.86 ± 5.00	-5.60 ± 5.13
3 mL	+13.15 ± 2.53**	-0.55 ± 4.29	-13.70 ± 4.44†
5 mL	+19.02 ± 2.92**	+11.78 ± 4.95*	-7.24 ± 5.13
BO	+3.80 ± 3.58	+3.80 ± 3.58	-1.69 ± 6.28

Note: Data are expressed as mean difference ± standard error. The level of statistical significance for the pairwise comparisons obtained with Bonferroni post hoc test, adjusted for multiple comparisons, is $p < 0.05$.
* Represents a significant difference in Percentage at less than $p < 0.05$
** Represents a significant difference in Percentage at less than < 0 .001
† Represents the < p 0.001 is significant however not at the higher level of significance
The tape stripping and 5ml NM reports the increase at 20 min vs baseline and almost significant at $p = 0.053$
The massage with 1, 3 and 5ml observed significant increases 20 mins vs baseline reporting $p = 0.044$, $p < 0.001$ and $p < 0.001$ respectively.
At 45 min vs 20 mins the 3ml observes a significant decrease
At 45 mins vs baseline the 5ml NM remains significant increase

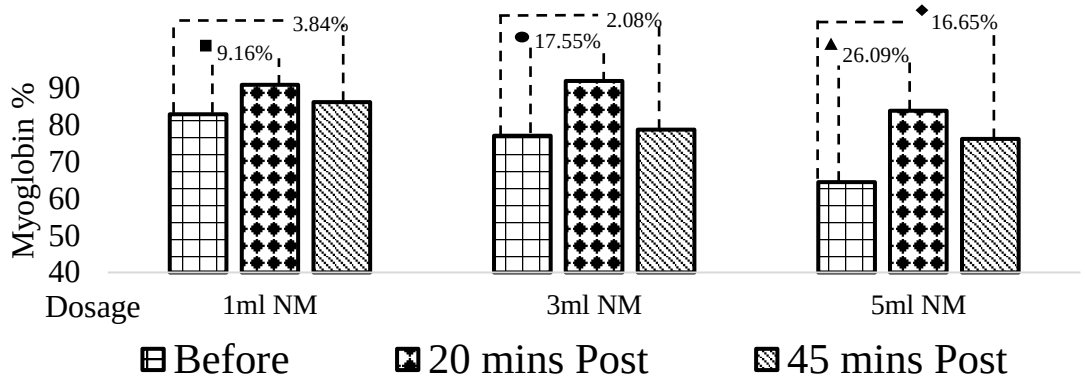

Figure 4 The mean differences in Myoglobin percentages (%) among the MG group's participants, measuring before dosage, 20 min after dosage, 45 min after dosage. MG group mean values of participants who received NM and their Mb percentages using measurements with percentage differences between pairs.
Revealing that through the pairwise comparison 5 mL of NM is significantly increased at both time points of before and 20 min ▲($p < 0.001$) and before and 45 min ◆($p = 0.016$). Whilst also revealing that the 1 mL ■($p = 0.011$) and 3 mL ●($p < 0.001$) are only significantly increased in the time points before and 20 min.

The results of this pilot study indicate that there were notable increases within the time points of the MG group, in all participants after the NM dosage had taken effect after 20 min of application, which can be visualised in Figure 4 and statistically proven as a significant difference. The largest increase was seen with the application of 5 mL at 20 min vs baseline with an increase of 26.09%, and the largest effect size of .729 is also seen. In addition, this dosage form showed a significant increase at 45 min vs baseline. For the other dosage forms, 1 mL and 3 mL, no significant difference were observed between 45 min vs baseline. Looking at Table 2, which shows the analysis of the TS group and their dosing and time points, with the largest increase between 20 min vs baseline, with the highest dosing of 5 mL. The control was the use of a baby oil (BO), which did not contain any of the Namman Muay 'Boxing liniment' compounds or any active pharmaceutical ingredients that may have contributed to the same effects. The use and comparison of the BO control, therefore revealed no significant differences in the Mg and Ts groups and no significant differences between the three NM dosage forms and subsequent time points. This shows that NM dosing of 1 mL 3 mL and 5 mL affects myoglobin levels and supports the research question.

4. Discussions

The main objective of this study was to evaluate the effects of different dosages (1 mL, 3 mL, and 5 mL) of NM sports liniment—containing methyl salicylate and L-menthol—on myoglobin percentage (Mb%) in the rectus femoris muscle of male Muay Thai fighters, and to assess how application techniques such as tape stripping and effleurage massage influence these effects over three time points. Key findings demonstrated that a 5 mL dosage of NM, particularly when combined with effleurage massage, led to the most pronounced increase in Mb% at 20 min post-application. This effect was less notable with lower dosages and in the control, group using baby oil. Additionally, tape stripping and massage techniques appeared to enhance the permeation and efficacy of NM, resulting in greater physiological responses. These results suggest that both the dosage of NM and the method of application are important determinants of its effectiveness in increasing myoglobin levels, which may have implications for athletic performance and recovery in combat sports.

In the MG group, the 5 mL dosage of NM demonstrated an increase in Mb% at 20 min post-application, similar to the 1 mL and 3 mL dosages. Figure 4 indicates that the 1 mL dosage exhibited the smallest increase at 20 min, followed by the 3 mL dosage, with the highest percentage seen at 5 mL. While the dosage form appears to be a contributing factor, further research in this area is needed for confirmation. Wang [3] conducted a study using an analgesic cream containing 20% methyl salicylate and 6% L-menthol, which resulted in increased muscle oxygenation saturation and blood flow. The study showed a significant elevation in skin blood flow upon cream application compared to the control group. However, the study maintained a constant dosage of 0.4 mL throughout, making it challenging to draw comparisons with the effects of different dosage forms as seen in this study. This supports the notion that skin blood flow increases rapidly upon topical application, potentially affecting Mb% levels.

The 5 mL dosage of NM showed a significant increase within the TS group before and 20 min after application, unlike the 1 mL and 3 mL dosages in the TS group. This difference in results may be attributed to the dosage amount applied. Absorption of topically applied substances relies on their permeability through the skin. Research

is focused on overcoming the skin barrier to enhance delivery of compounds through the stratum corneum (SC) into underlying tissues. Tape stripping (TS) involves removing SC layers using adhesive products to facilitate topical application penetration [32]. A study on dermal penetration mechanisms indicated that tape stripping can remove approximately 34% of the SC [32]. Although Scotch tape was used in this study for consistency, the adhesive substance was not evaluated. A stronger adhesive tape may yield better results in removing the SC.

Effleurage massage demonstrated significant increases in all dosage forms when applied, suggesting its potential to aid in compound permeation through the SC [11]. One study proposed that massage exerts a penetration-reducing effect due to mechanical stress, leading to a reduction in SC thickness and intercellular space density, affecting compound flux [11]. However, these findings contradict another study advocating for massage therapy combined with topical analgesics as more effective than massage alone [33]. Massage, known for its vasodilatory effects, was shown to increase microcirculatory flow motion when effleurage was applied to the lower leg, resulting in enhanced perfusion in the massaged limb ($P < 0.001$) [34]. It is plausible that the combination of massage and the application of methyl salicylate and L-menthol contributes to a significant vasodilatory response, thereby increasing blood and Mb oxygen values.

The control group using baby oil (BO) did not exhibit any significant increases compared to the intervention groups or different NM dosage forms, this lack of effect validates its role as a control. The commercial product, Babi oil, likely has a higher molecular weight exceeding > 500 Daltons, making it challenging to permeate the SC and lacking active pharmaceutical ingredients. In contrast, methyl salicylate and L-menthol are produced as nanoparticles ranging from 1-100 nanometres, enhancing skin penetration and safety, with a quantified molecular weight below < 500 Daltons [35].

A consideration is also the skin thickness, which is made up of, epidermis, dermis and the hypodermis (adipose). The adipose thickness may affect the NIRS Moxy monitor by weakening the signals, thereby giving invalid data [36]. The BMI of this athletic population of Muay Thai fighters were a mean of 21.4kg/m^2, which is well within the healthy weight range ergo, should not have impacted the NIRS signals.

The significance of oxygen in athletic recovery is widely acknowledged. Increased oxygen consumption post-exercise aids in restoring adenosine triphosphate (ATP) levels and facilitates the breakdown of lactic acid by the liver. Elevated levels of reactive oxygen species (ROS) in skeletal muscles during training and performance can lead to fatigue and reduced muscle power production [37,38]. In intense sports like Muay Thai fighting, managing ROS levels can be a decisive factor in determining victory or defeat. Prior to competition, applying NM 'Boxing-Liniment' with a 5 mL dosage, combined with mechanical enhancer massage (effleurage technique), can enhance myoglobin (Mb) levels throughout the competition, thereby supporting the fighters' recovery process.

The guidance in the package leaflet of NM recommends liberal application, which may result in misinterpretations. Extensive research literature highlights the adverse effects, potential side effects, and risks of overdose associated with methyl salicylate [39]. Salicylate toxicity is a valid concern, emphasizing the importance of precautions when using products containing methyl salicylate. Establishing a specified dosage ensures that Muay Thai fighters can confidently achieve the optimal effect for their upcoming performance.

This study is, to our knowledge, the first study to investigate different dosage forms of NM boxing oil on the rectus femoris muscle and to observe the physiological myoglobin data over three different time points. A positive aspect of this study is the correspondence to a typical Muay Thai fight or training session, which can then be related to a real-life situation. A professional Muay Thai fight consists of five rounds, each lasting three min, with a two minute break in between. This results in a total duration of 25 min if you go the distance. If you include the warmup and the performance of the Wai Khru ritual, these time spans are consistent with the application and post-measurements for data collection. The results could therefore be transferred to similar combat sports and also to sports with a short duration with rest periods such as ice hockey or basketball. For other sports it may not be appropriate to see the same benefits, future studies into other sports with consideration to how long they last is recommended. Randomised cross-over pilot studies allows for a smaller sample size and with Muay Thai fighters being a unique population, therefore difficult for large recruitment, this study was able to get 24 samples as they participated in both Day 1 and Day 2. Limitations to this study were also encountered, firstly the post application two time points set may benefit from seeing recordings at 15 min intervals post application and record four times as seen in a previous study [40]. This would allow for a possible understanding as to, when possible, increases in myoglobin % and subsequent decreases in the % occur. There were no physical outcomes seen and with Muay Thai being a very physical sport, it would be recommended to look at these and see whether a 5 mL NM with massage (effleurage technique) would have an effect. The literature on the mechanism of Methyl salicylate and L-menthol on the Mb% after being applied topically is sparse, therefore, a recommendation would be for this area of research to be increased.

5. Conclusions

Five mL of NM boxing liniment using the mechanical enhancer massage (effleurage technique) can increase Mb percentage within the rectus femoris muscle at 20 min post intervention and continues 45 min post

intervention. The clinical implications are that an identified dosage form of NM to increase Mb percentage to help the Muay Thai fighter as a pre-cursor to their warmup may help with preventing an overuse of the product.

6. Ethical approval

The study protocols were reviewed and approved by the Centre for Ethics in Human Research, Khon Kaen University, Thailand (Ref No: HE662203).

7. Conflicts of interest

The authors declare no conflict of interest.

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9. Author Contributions

Lee David Johnson conceived the study, collected the data, analyzed and interpreted the results, created the tables and figures, and drafted the manuscript. Chawin Sarinukul collected data and contributed to data analysis and interpretation. Kwanchayanawish Machana prepared the manuscript, contributed to the study, and managed submission. Kurusart Konharn contributed to study conception and provided critical input on data interpretation. Theera Rittirod conceived the study design, analyzed and interpreted the data, and critically revised the manuscript. All authors read and approved the final manuscript.

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