Thermoregulatory effects of guava leaf extract-menthol toner application for post-exercise use

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ABSTRACT

Introduction

Thermoregulatory and cardiovascular processes, including skin blood flow and sweating, during sustained exercise are significantly associated with increased body heat content due to metabolic heat production (Kenny and McGinn 2017). Sweating mainly reduces core and skin temperature during exercise (Tansey and Johnson 2015); then at the cessation of exercise, sweat production rate decreases rapidly, and the principle route of heat dissipation is likely convection through the skin in the period following exercise (Gerrett et al. 2018). However, sweat alkalinity during excessive perspiration resulting from bicarbonate ions (HCO₃⁻) might alter the skin barrier and cause various skin diseases (Schmid-Wendtner and Korting 2006). Sweating from exercise was reported as the common factor which aggravated the symptoms of atopic dermatitis in school children (12–14 year-olds) (Williams et al. 2004). Moreover, secretion of sweat and leakage into tissues could promote itching for people with atopic dermatitis and aggravate dermatitis (Murota et al. 2013). Additionally, apocrine sweat glands secrete milk-like substances composed of electrolytes, steroids, proteins, vitamins, and lipid compounds, which might be transformed by skin flora bacteria and cause body odour (Fredrich et al. 2013). Therefore, post-exercise hygienic practices should quickly clean sweat stains from the skin, and if there is a way to decrease the remaining post-exercise perspiration, then the occurrence of the aforementioned problems will be prevented. Since the restoration of thermoregulation after exercise turns to be primarily convection through the skin instead, the body is still recovering after a workout.

Guava leaves [Psidium guajava L. (Myrtaceae)], which contain phytochemical phenolic compounds (Morais-Braga et al. 2017), are regionally used as an alternative medicine for disinfecting the human gastrointestinal tract (Daswani et al. 2011; de Souza et al. 2014). Moreover, guava leaf extracts have astringent properties resulting from tannin phenolics (Ashok and Upadhyaya 2012) and can be utilised as a skin toner for tightening skin pores and decreasing oily skin (Pongsakornpaisan et al. 2019). Further, menthol, at various concentrations, is generally used as a cosmetic cooling agent in healthcare products (Kamatou et al. 2013). Currently, it is known that applying menthol can excite the transient receptor potential melastatin 8 (TRPM8) that can cause cutaneous vasodilation (Craighead and Alexander 2016) and increase the process of convection heat dissipation through the skin (Wong and Hollowed 2017). To date, it remains unknown whether the proven astringent effects of guava leaf...
extract and cutaneous vasodilation, and heat-convection of menthol can be extrapolated during the post-exercise recovery period. Specifically, there is inadequate research on the utilisation of both substances to develop alternative skincare and healthcare products suitable for post-exercise application to resolve problems associated with post-exercise perspiration without affecting heat dissipation.

Therefore, this study aimed to investigate the influence of guava leaf extract-menthol toner (GMT) for post-exercise use on thermoregulatory effects including perspiration and skin temperature as well as recovery heart rate compared with that of the placebo (PCB) and baseline (BSL) values. It was hypothesised that the GMT toner could remarkably reduce perspiration after sweat stain cleaning due to the astringent properties of guava leaf extract. Nonetheless, exercise recovery, including the relative changes of heart rate (HR) and skin temperature reductions, should not be altered because the heat dissipation primarily goes on with the heat convection due to the TRPM8 activation of menthol which causing cutaneous vasodilation. These findings may potentially be helpful based on the potential future use of GMT to clean sweat stains, reduce post-exercise perspiration, and enhance post-exercise body heat dissipation, as well as allowing for better hygiene for individuals who exercise or those with hyperhidrosis before regular bathing.

Materials and methods

Materials

Commercial guava leaf extract was purchased from Specialty Natural Products Co., Ltd., Chonburi, Thailand (Lot No. HE-EL41-PSG) and quantified for the total phenolic and flavonoid contents at the Faculty of Pharmaceutical Sciences, Chulalongkorn University (Bangkok, Thailand). The GMT formulation contains 20% v/v of guava leaf extract, 1% w/v of menthol (1381709 USP, Sigma-Aldrich Pte. Ltd., Singapore), aluminium chlorohydrate (ICL performance products Ltd., China), ethanol (RCI Labscan Ltd., Thailand), Polysorbate 80 (Spectrum chemical Mfg., Corp., USA), perfumes, and purified water. The placebo formulation (PCB) composes the same toner base without the actives (i.e., guava leaf extract and menthol). All chemicals are either pharmaceutical or cosmetic grade. The studied preparations were prepared and evaluated for physical and chemical stabilities at the compounding laboratory, the Faculty of Pharmaceutical Sciences, Chulalongkorn University (Bangkok, Thailand).

Ethical approval

This study was approved by the Research Ethics Review Committee, Health Science Groups, Chulalongkorn University, Thailand (COA No. 176/2017), and was performed following the Declaration of Helsinki. Prior to the commencement of the experiments, the participants were informed about the procedures. Thereafter, a signed consent form for study participation was collected from each participant.

Participants

Healthy participants, consisting of 23 men and 41 women (age range: 18–24 years), were enrolled in this study. All participants underwent assessment for readiness to exercise using the Physical Activity Readiness Questionnaire (PAR-Q) (Shephard 2015; Whitfield et al. 2017), and did not have a history of severe skin allergy to any topical products requiring treatment.

Preliminary testing

One week prior to the main trials, the participants visited the laboratory where they were initially familiarised with the experimental protocol and equipment and were tested to identify individual treadmill speed and slope that properly allowed aerobic exercise. Briefly, the individual’s 75% HR reserve (HRR) was calculated by applying Karvonen’s formula (Camarda et al. 2008). Each participant exercised by running on the treadmill (Mercury® med, H/P/Cosmos Sports & Medical Gmbh, Nußdorf, Germany) with a warm-up speed of 2.7 km/h and a 10% slope for 3 min. Subsequently, each participant performed an incremental Modified Bruce protocol (Stefani et al. 2010) with increments of speed by 1.3 km/h and slope by 2% grade every 3 min to approximately 75% individual HRR (±5 beats/min). The speed and slope of the treadmill were maintained for 5 min followed by a cooling down period for 5 min. At this stage, participants who were unable to achieve a vigorous-intensity exercise at the assigned workload and time duration or to produce sufficient sweat were excluded. Furthermore, the mean speed and constant slope of the treadmill to preserve the 75% individual HRR were fixed for each participant during the main trials.

Main trial

All experiments were conducted during the summer (between March and April) and at the same time of the day. For 24 h before their first experimental trial, the participants were instructed not to exercise, consume high sodium (salt) content foods, or drink alcohol or coffee to ensure similar levels of nourishment and hydration. Further, a good night’s sleep was recommended before the experiments, and participants were advised to have food and drink at least 2 h and 1 h prior to the trials, respectively. These instructions were replicated before each experiment. All of the participants attended the laboratory at the same time of the day, to avoid circadian variation in skin temperature, on three separate occasions with a one-week interval. On the first experiment day, the baseline test without any treatment (BSL), all participants underwent acclimatisation to the test facility with the controlled conditions: 25 ± 2 °C and 60 ± 2% RH for 30 min. The test began with a 5 min warm-up session involving walking on a treadmill at a speed of 3.0 km/h and a 14% slope. Subsequently, the participants were instructed to exercise at the previously determined speed and slope to reach an exercise intensity of 75% HRR for 5 min. Then the participants cooled down for 5 min and rested for 5 min; then the skin temperature and HR measurements were taken. Before the post-exercise monitoring period, the participants cleaned their whole body with a clean towel to remove sweat stains. Perspiration was collected by attached sweat patches on different body parts of each participant’s skin while they were sitting in the study room for 20 min. Simultaneously, the recovery HR was measured at 2 min intervals till the end of 20 min, and the skin temperature was again measured at the end of the period (Figure 1).

A single-blind, randomised, placebo-controlled crossover trial was used in this study. On the other two separate experiment days, the same subjects were randomly assigned to groups that received alternative sequences of treatments (i.e., GMT followed by PCB (GMT-PCB) or PCB followed by GMT (PCB-GMT)) with a one-week washout period between them. On each
occasion, participants performed the same procedure as in the BSL test; however, 5 mL of GMT or PCB was sprayed on the chin, neck, chest, body, armpits, arms, and legs after obtaining the initial skin temperature and HR measurements and left for 30 s. Next, the whole body was cleaned with a towel until no moisture was left before proceeding to the next step.

Measurements

Post-exercise perspiration

The post-exercise perspiration was measured during the 20 min sitting period after thorough removal of sweat stains by wiping with a clean towel after the BSL test or by using either GMT or PCB toner and then wiping with a clean towel. This was achieved by fixing 200 cm² weighed sweat patches (SOS Plus, Wayson Medical, Thailand) on the back of the forearms, back, chest, and upper thighs. Further, a 42 cm² weighted sweat patch was placed on the forehead (Baker et al. 2009). At the end of the 20 min sitting, all the sweat patches were removed and weighed using the same balance (TE313S-DS, 310 g ± 0.001 g, Sartorius, Germany). The before-after weight difference was calculated for post-exercise perspiration.

Recovery heart rate

The first HR measurement was obtained in the post-exercise recovery stage (Polar A300 with heart rate Sensor, Polar Electro, Finland) at the end of the 5 min resting period after exercise. Subsequently, HR measurements were obtained at 2 min intervals for 20 min during the post-exercise monitoring period in each trial of with or without treatments, respectively. Relative changes in HR (AHR) during the 20 min resting period were calculated.

Skin temperature

The skin temperatures (Skin-thermometer ST500, Courage + Khazaka electronic GmbH, Germany) were measured at the end of the 5 min post-exercise resting period and after the 20 min sitting period. Skin temperatures were measured at the forehead, chest, dorsal aspect of forearms, thighs, calves, lower abdomen, and back of the participants (Powers and Howley 2012). The mean skin temperature of all measured positions was reported.

Table 1. Descriptive characteristics of the participants.

| Characteristics | Male (n = 23) | Female (n = 41) | Total (n = 64) |
|-----------------|--------------|----------------|---------------|
| Age (years)     | 20.0 ± 1.3   | 19.4 ± 1.0     | 19.6 ± 1.1    |
| Weight (kg)     | 70.0 ± 12.6  | 54.8 ± 9.4     | 60.3 ± 12.9   |
| Height (cm)     | 175.1 ± 6.3  | 162.3 ± 5.5    | 166.9 ± 8.4   |
| RSBP (mm Hg)    | 122.1 ± 10.4 | 106.0 ± 10.0   | 111.8 ± 12.7  |
| RDBP (mm Hg)    | 76.3 ± 5.6   | 69.8 ± 5.9     | 72.1 ± 6.6    |
| RHR (beats/min) | 72 ± 12      | 81 ± 11        | 78 ± 12       |

Values are presented as mean ± SD. RSBP, resting systolic blood pressure; RDBP, resting diastolic blood pressure; RHR, resting heart rate.

Statistical analysis

All data analyses were conducted using the SPSS version 23 program (IBM Corporation, USA). Initially, the data of each parameter were examined for variance homogeneity to assume the variances were either homogeneous or heterogeneous, using a particular analysis of variance (ANOVA) measurements to analyse the mean differences. Later, repeated two-way ANOVA (3 × 9: group × time pair) was used for between-group comparisons of the relative changes in HR (AHR) during the 20 min sitting period. To evaluate differences between groups in the recovery HRs after the 5 min post-exercise resting period and the changes between skin temperatures after the 5 min post-exercise rest and the 20 min post-cleaning period, one-way ANOVA for between-group comparisons was used. After a significant F value, the Bonferroni post hoc tests were conducted to identify pairwise differences. Due to heterogeneous variance data, one-way ANOVA utilising the Brown-Forsythe method to examine the mean equality followed by the Games-Howell post hoc tests were used to identify significant post-exercise perspiration pairwise differences. Significant differences were determined at \( p < 0.05 \). Data are presented as the mean ± standard deviation.

Results

Preliminary stability data revealed that the light brown clear GMT toner was chemically and physically stable when stored in a light-protected container at room temperature for at least 6 months (data not shown). Moreover, GMT was reported to pass an allergic skin test upon examination in 40 male and female volunteers.

There were no unusual occurrences regarding the examined products and experimental designs for all the enrolled 64 participants (Table 1). The average age of the participants was 19.6 ± 1.1 years with a weight and height range of 47.4–73.2 kg.
and 158.5–175.3 cm, respectively, and an average resting HR range of 66–90 beats/min. All participants had no record of skin product allergy and passed the pre-exercise readiness assessment using the PAR-Q questionnaires.

**Perspiration or sweating amounts in the post-exercise stage**

Post-exercise perspiration in the GMT test was reduced by about half when compared with that in PCB and BSL experiments, whereas the PCB test showed insignificantly different results from the BSL values. Table 2 presents the post-exercise perspiration in all the tests.

**Skin temperature characteristics**

After the 5 min post-exercise resting period, skin temperatures in all trials were approximately one degree Celsius lower than those before exercise (data not shown). The skin temperatures tended to rise after the 20 min post-cleaning sitting period; the PCB test showed the highest skin temperature change, while the GMT and BSL tests showed comparable changes (as shown in Table 2). Nevertheless, the changes in the skin temperature following the 20 min post-cleaning (Tsk) were statistically insignificant (p = 0.38) for all the tests.

**Recovery heart rate responses**

As shown in Table 2, the recovery HRs obtained at the end of the 5 min post-exercise resting period were comparable among all the three tests (p = 0.10). Figure 2 also shows comparative declining curves of the recovery HR in each experiment. Furthermore, as shown in Table 3, between-group comparisons of the relative changes in HR (ΔHR) during the 20 min post-
exercise monitoring period after complete cleaning showed no significant difference among all the three tests ($p = 0.39$).

Discussion

During post-workout recovery, the routine should generally include a cool-down period before you take a shower, but if time does not allow for it then the use of a natural extract toner would offer an extra benefit including a reduction in perspiration, antibacterial, etc. The main findings indicate that GMT efficiently decreased the post-exercise perspiration during resting without significantly altering the body recovery after physical exercise. The efficient decrease in post-exercise perspiration could be attributed to the properties of guava leaf extract. Previous studies have shown that guava leaf extract contains tannins (Biswa et al. 2013; Mailoa et al. 2014), which have astringent properties capable of perspiration inhibition. The astringent effect of tannins might involve allowing denaturation of superficial layer proteins in the stratum corneum and obstruction of the sweat pores, which reduces the rate of perspiration (Piéard et al. 2003). Moreover, another reason for GMT to decrease perspiration greater than PCB and BSL was likely due to some constituents in the formulation. Guava leaf extract, the main ingredient in the GMT, contains glycerine as a solvent. Glycerine is well known as an efficient humectant and has an excellent hygroscopic property by which it can hydrate the stratum corneum and make it swell (Leelapornpisid et al. 2014). The mechanism of skin moisturisation by glycerine is probably by drawing water from other epidermis layers to the stratum corneum; additionally, the glycerine film could prevent evaporation of sweat from the skin (Flynn et al. 2001; Rawlings et al. 2004). Therefore, the occlusive mechanism of glycerine in the guava leaf extract on the skin may have caused a significant decrease in perspiration observed with GMT application compared to that of BSL and PCB tests.

Moreover, the GMT formulation contains another active substance, menthol, which stimulates the TRPM8 channel expressed in a subpopulation of sensory neurons and is also activated by cold sensation. Besides, this receptor also regulates conscious human skin blood flow (Johnson et al. 2009). Previous study has shown that applying menthol could also excite this receptor and could cause cutaneous vasodilation (Craighead and Alexander 2016). Further, menthol could stimulate TRPM8 on the aorta smooth muscle, causing the decreases of extracellular and intracellular calcium ions, resulting in smooth muscle relaxation from depolarisation and causing vasodilatation (Yang et al. 2006). The cutaneous vasodilation may have a beneficial effect on the heat dissipation of the body because it may increase the heat dissipation from the core body to the environment by convection via the skin (Wong and Hollowed 2017).

The Skin-Thermometer ST5300 equipment is equipped with a special sensor in which the measurement is based on contact-free infra-red recognition of heat emission from the skin. Skin temperature is defined by its microcirculation; the more the circulation at a certain area on the skin, the higher the skin temperature. During the recovery period after exercise, evaporation of sweat and increased skin blood flow are effective mechanisms for heat dissipation from the body. The slightly elevated skin temperatures after the 20 min post-cleaning period may relate to the convection heat loss (Tori et al. 1992). The current findings revealed that the changes in the skin temperature following the 20 min post-cleaning ($T_{sk}$) showed no significant differences.

The novel formulated GMT toner contains guava leaf extract and menthol, which reduced perspiration by astringent effects and affected cutaneous vasodilation by TRPM8 receptor activation, respectively. Even though sweating is mainly a primary means of cooling during exercise; on cessation of exercise, it is likely that the main route of heat loss is convection from the skin (Gerrett et al. 2018). Therefore, GMT toner could alleviate skin problems causing by sweat strain and promote heat dissipation through convection. Moreover, the changes in HR during the 20 min rest showed comparable reductions in all tests, and the relative changes ($\Delta HR$) were not significantly different among the tests, which could imply that the reduction in perspiration or cutaneous vasodilation of GMT did not significantly alter the body recovery process.

There is a global acceptance that physical activity is an important component of a healthy lifestyle in children and adolescents. Physical education programs have been modified to increase more class period and frequency engaging in moderate-to-vigorous physical activity. As a result, there is no time to shower before moving on to the next class. Our findings implied that the topical application of guava leaf extract-menthol toner could reduce post-exercise perspiration and facilitate cleaning of sweat strain. In this study, considering that most of our participants are adolescents involved in a vigorous-intensity exercise, which is usually in regular physical education program, applying GMT toner for the primary cleaning before taking a shower could be suggested for better personal hygiene. Additional investigation on antibacterial properties would fulfil the benefits of this natural toner. However, an assessment of antibacterial capability should be properly designed in future study to conform to briefly apply of the product and wipe off.

Conclusions

Sweat strain after a workout could adversely affect the skin. Further, bacteria might transform sweat stain accumulated on the skin into body odour. GMT application decreased post-exercise perspiration; therefore, it may resolve problems associated with excessive sweat on the skin, especially when there is no time for regular body cleaning. The current findings showed that lowered post-exercise perspiration did not influence heat dissipation and did not affect cardiovascular mechanism compared to baseline and placebo. In summary, natural GMT could offer an alternative means for improving post-exercise self-hygienic practices. In addition, it may alleviate skin problems associated with sweat strain commonly seen nowadays in our busy lifestyle.

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Disclosure statement

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