

## Effects of lycium and lycium-composed formula on the peripheral coldness induced by local cooling in mice

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### Abstract

**Background:** Recently people appealing for peripheral coldness increase. Lycium fruits have a long tradition of use in nutrition and traditional medicine in East Asia, while its effect on peripheral coldness is not yet investigated. We study the effects of lycium and lycium-rich formula (*Ligustrum lucidum* ait, LLA) on the peripheral coldness induced by local cooling in mice. **Methods:** 7-week old male ddY mice were administered either water (control) or lycium or LLA for 2 weeks. After an aesthesia, the mouse body except for head was immersed into 10 °C water for 10 min. Then the skin surface and rectal temperature of mouse were measured with a thermocamera and a thermocouple thermometer, respectively. And two laser Doppler flow probes were set to measure the skin blood flow of plantar and tail, respectively.

**Results:** In the case of 1-week administration on mice, 10% lycium- and 10% LLA-treatments showed good recovery of whole body surface temperature measured by a thermocamera. After 2-week administration on mice, there were not significant differences in the recovery rates of rectal temperature compared with those of the control group. And the 30 min- and 50 min-recovery rates of dorsal skin surface temperature by 1% and 10% lycium-treatment were significantly higher than those of the respective control. Further, the 30 min- and 50 min-plantar skin blood flow by 10% LLA-treatment were significantly higher than those of the respective control. The 50 min-tail skin blood flow by 10% LLA-treatment and 60 min-tail skin blood flow by 1% LLA-treatment were significantly higher than those of the control, respectively.

**Conclusion:** It can be concluded that the administration of lycium or LLA on mouse led to an increase of peripheral blood flow, and thus a recovery of skin surface temperature.

**Keywords :** peripheral coldness, thermoregulation, blood flow, lycium

### Introduction

Cold syndrome is a common complaint in Eastern women. Besides peripheral coldness in the hands and feet, other symptoms such as fatigue and depressive moods are often complicating [1]. The pathophysiology of peripheral coldness is not well established, however, it could be associated with reduction of peripheral blood flow to protect the body from heat loss [2]. The local mechanisms from in vitro and in vivo study suggest that the local cooling on cutaneous leads to the generation of reactive oxygen species (ROS), which stimulate intracellular signaling and the subsequent translocation of adrenoceptors to the plasma membrane for circulating catecholamines, thereby augmenting vasoconstriction [3, 4].

However, a uniform treatment for peripheral coldness has not been established. It has been suggested that the administration of traditional herbal medicine had vasodilating action on peripheral

vessels and increased the blood flow under cold stress [5, 6, 7]. The fruits of *Lycium barbarum*, have a long tradition of use in nutrition and traditional medicine in East Asia. The beneficial effect has been attributed to the presence of various functional components including polysaccharides, flavonoids, phenolic acids and carotenoids [8, 9]. The pharmacological investigations show that polysaccharides isolated from lycium fruits have antioxidative [10-13], immunomodulatory [14-17], hepatoprotective [17,18] and neuroprotective properties [19-21] in cell and/or animal model. Furthermore, it has been reported that polysaccharides prevented the increase of blood pressure in hypertension rats, and improved the arterial compliance by up-regulation of the eNOS expression and improvement of the endothelium-dependent vasodilatation in the rat aorta ring [22, 23]. In addition, hesperidin, a flavonoid composed in lycium fruits [9], resulted in increased peripheral circulation and skin temperature under cold intolerance in rabbit and rat models [24, 25]. On the other hand, *Ligustrum lucidum* ait

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(LLA), a lycium-rich formula, which is composed of the essences of *Lycii fructus*, *Crataegi fructus*, *Phyllanthi fructus*, *Chrysanthemi flos*, *Coicis semen*, *Ganoderma lucidum*, and *Zizyphi fructus*, has a significant folk history in Japan. The immunomodulation of LLA has been reported by Chien with a mice model, and we found that LLA can be utilized as an antifatigue agent by its protective effect against exercise-induced oxidative stress in aged mice [26, 27]. Concerning the vasoreactivity of the components composed in LLA, it has been also reported that *Crataegus* had vasodilatory activity in the isolated guinea pig hearts and rat aortic rings [28], and *Zizyphus* shown the upregulation of eNOS expression in human endothelial cells [29]. While the effects on cutaneous blood flow under cold stress by lycium fruit and LLA are not yet investigated. The aim of the current study is to evaluate the efficacy of lycium fruit and LLA on peripheral coldness by measuring mice skin blood flow, temperature, and their recovery rates after cold stress.

## Methods

### Preparation of lycium- and LLA-contained food for mice

Seven herbs for the study were purchased from Ningxia Jibaoli Co. Ltd. (Ningxia, China), and were identified by a taxonomist. Their voucher specimens were deposited at the Institute of International Kampo Co. Ltd. (Fukushima, Japan) for future reference. Lycium were composed of the boiling water extract of *Lycii Fructus* from *Lycium chinense*, and were mixed with mice feed (Kyudo, Fukuoka, Japan) by the blending ratios of 1% and 10% (w/w), respectively. Lycium-rich LLA were prepared as previously described [27]. Briefly, LLA were composed of the boiling water extract of *Lycii Fructus* from *Lycium chinense*, *Crataegi Fructus* from *Crataegus cuneata*, *Phyllanthi Fructus* from *Phyllanthusemblica*, *Chrysanthemi Flos* from *Chrysanthemum morifolium*, *Coicis Semen* from *Zizyphus jujuba*, *Ganoderma lucidum* from *Ganoderma lucidum* (Leyessex Fr) Karst, and *Zizyphi Fructus* from *Coixlacryma-jobi*, then, were mixed with mice feed by the blending ratios of 1% and 10% (w/w, the concentration of LLA was calculated as equivalent of lycium), respectively.

### Animal administration

The study was submitted to, and approved by the Ethics Committee of Sojo University. All experiments were conducted in strict accordance with the Guidelines of the Japanese Pharmacological Society for the Care and Use of Laboratory Animals. Male ddY mice weighing 35–45 g were obtained from Kyudo Co., Ltd. (Fukuoka, Japan). The mice were housed in a 12 h light–dark cycle, with food and water available ad libitum. After there was a one-week adaptation period, the mice were administered either water or lycium-contained food (1% or 10%) or LLA-contained food (1% or 10%) for 2 weeks.

### Cold stress on mice

The mice were anaesthetized with the intraperitoneal (i.p.) administration of pentobarbital sodium (75 mg/kg) (Wako, Osaka, Japan), and placed on a heating pad in the dorsal position. After an aesthesia state of mouse had stabilized, the respective temperature and blood flow were measured. Then, the mouse body except for head was immersed into 10 °C water for 10 min. After the surface water was wiped, the rectal temperature, the skin surface temperature, the plantar and tail blood flows of the mouse were measured for an hour. The temperature and humidity of the laboratory were at 24±2°C and 55±10%, respectively.

### Measurement of skin surface temperature

After an aesthesia state had stabilized, the skin surface temperature of the 1-week treatment mouse was measured using a thermocamera (Thermo Shot F305/W, Nec/Avio, Tokyo, Japan). The dorsal skin surface temperature of the 2-week treatment mouse was measured using a probe (Ret-, Physitemp, NJ, USA) with a thermocouple thermometer (HD 2108.1, Delta Ohm, Caselle di Selvazano (PD), Italy).

### Measurement of rectal temperature

A rectal probe (Ret-3, Physitemp, NJ, USA) with a thermocouple thermometer (HD 2108.1, Delta Ohm, Caselle di Selvazano (PD), Italy) was inserted to measure rectal temperature of mouse.

### Measurement of skin blood flow of plantar and tail

A laser Doppler flow probe (NS type; Omega Wave, Tokyo, Japan) was set to the position about 5 mm apart from the centre of the plantar surface of the left foot to measure the plantar skin blood flow with a non-contact laser Doppler flow meter (ALF 21N; Advance, Tokyo, Japan) as described by Honda et al [4]. Another laser Doppler flow probe (NS type; Omega Wave, Tokyo, Japan) was set to the position about 5 mm apart from the bottom of the tail to measure the tail skin blood flow with a contact laser Doppler flow meter (ALF 21; Advance, Tokyo, Japan). The blood flow was expressed as arbitrary perfusion units (PU). The microvessels with the blood flow between 25 and 35 PU were selected for the measurement. Data were stored and analyzed on a Windows computer with an AD converter (Powerlab; ADInstruments, NSW, Australia).

### Statistical Analysis

All data are expressed as mean±S.E.M, where n equals the number of animals. The comparisons between the two groups were carried out using a Student's t-test. Multiple comparisons were

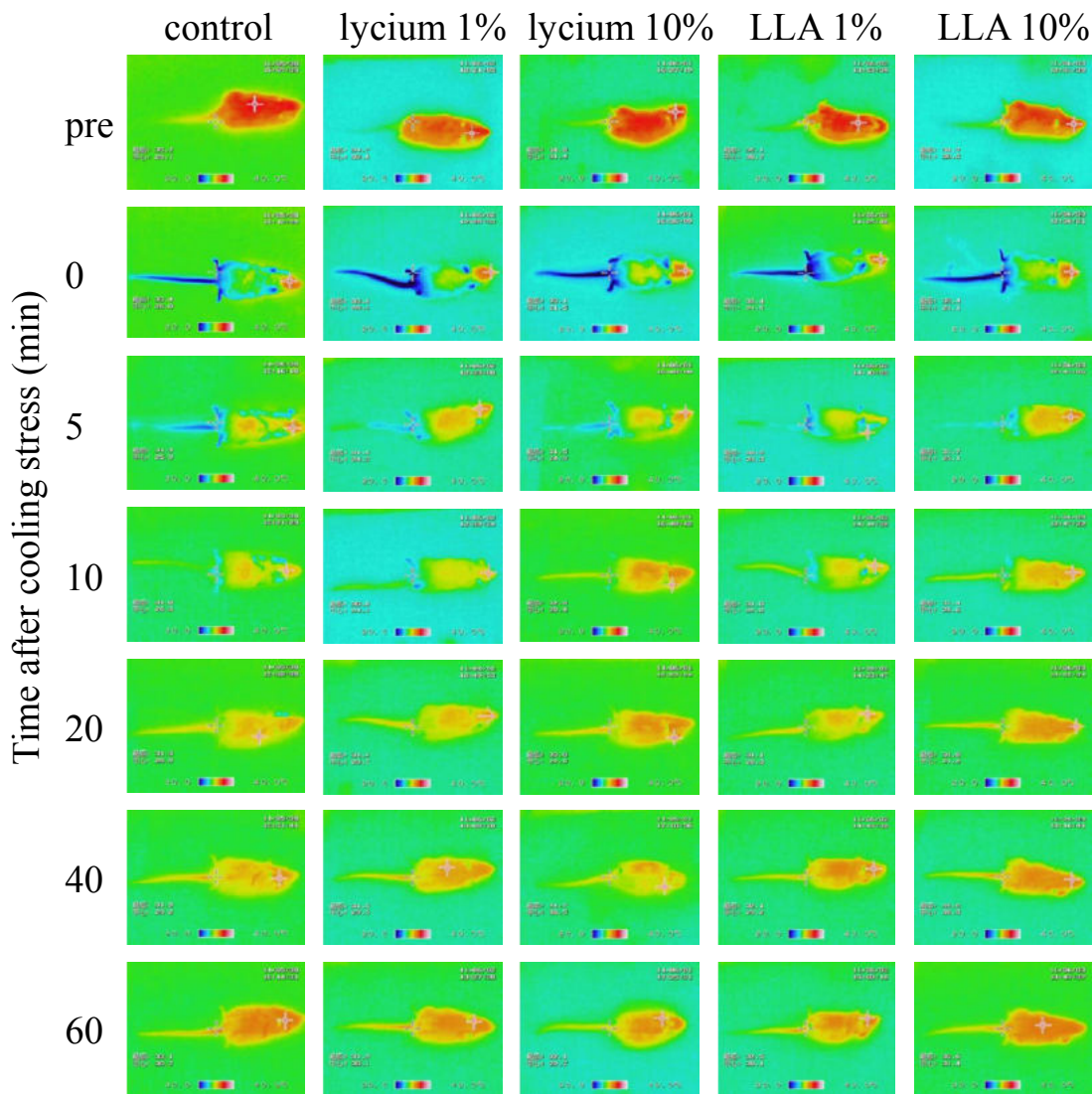
performed using Dunnet-test. Probability (p) values less than 0.05 were considered to be statistically significant.

## Results

### Effects on skin surface temperature by 1-week administration

After 1-week administration with water (control) or lycium or LLA, data from the thermocamera showed that the recovery of skin

surface temperature by lycium or LLA is concentration-dependently faster than those of the control group, especially the recovery for 10 min after cold stress as shown in Figure. 1. On the other hand, there were not significant differences in the recovery rates of rectal temperature by lycium or LLA compared with those of the control group (data not shown).



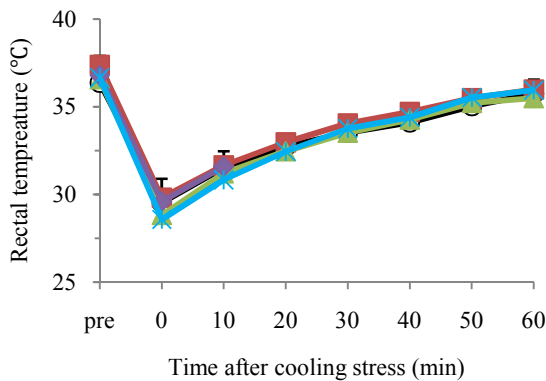
**Figure 1.** Effects on skin surface temperature by lycium or LLA administration for 1-week in mice. Time-course of changes in the skin surface temperature are measured with a thermocamera before (pre) and after cold stress (n=3). Mice are fed for 1-week with water, 1% lycium, 10 % lycium, 1% LLA and 10% LLA, respectively.



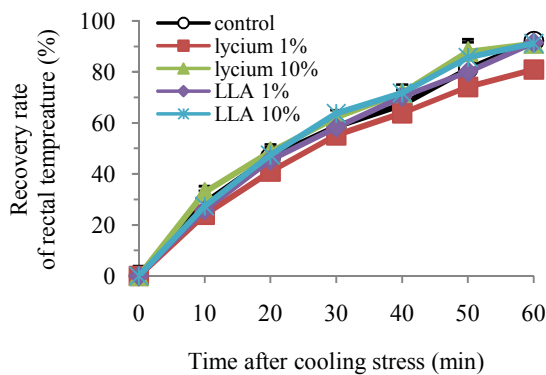
### Effects on rectal temperature by 2-week administration

In the case of 2-week treatment on mice, after cooling stress, there were not significant differences in the rectal temperature and the respective recovery rates by lycium or LLA compared with those of the control group (Figure. 2).

A.



B.



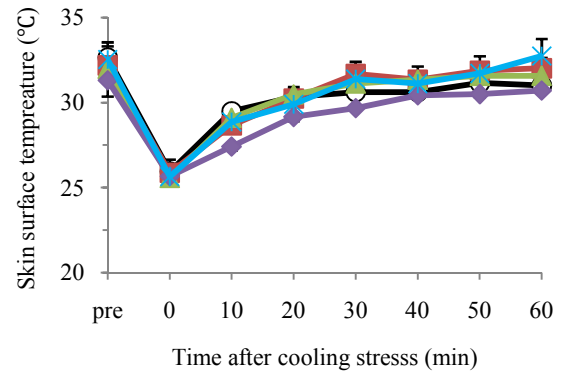
**Figure 2.** Effects on rectal temperature by lycium or LLA administration for 2-week in mice. (A). Time-course of changes in the rectal temperature are measured with a thermocouple thermometer before (pre) and after cold stress (n=3-6). Mice are fed for 2-week with water, 1% lycium, 10 % lycium, 1% LLA and 10% LLA, respectively. (B). The recovery rate is expressed as a percentage of the rectal temperature before (pre) and after cooling stress. Values were means±SEM.

### Effects on skin surface temperature by 2-week administration

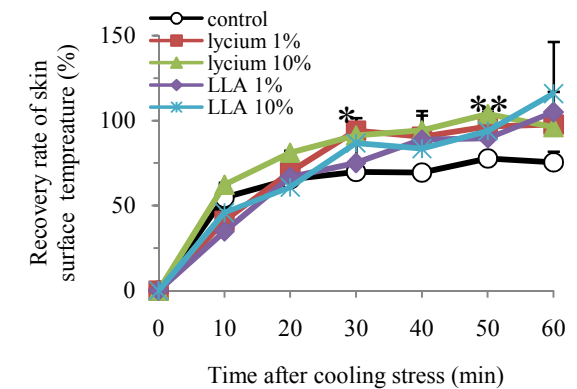
Figure. 3A showed the effects of lycium and LLA on the cooling stress-induced change of dorsal skin surface temperature measured with the thermocouple thermometer. Figure. 3B showed that from 30 min after the cooling stress, the recovery rates of skin

surface temperature by lycium and LLA groups were well over the control group. Furtherly, the 30 min-recovery rates of skin surface temperature by 1% lycium and the 50 min-recovery rate by 10% lycium were significantly higher than those of the respective control.

A.



B.

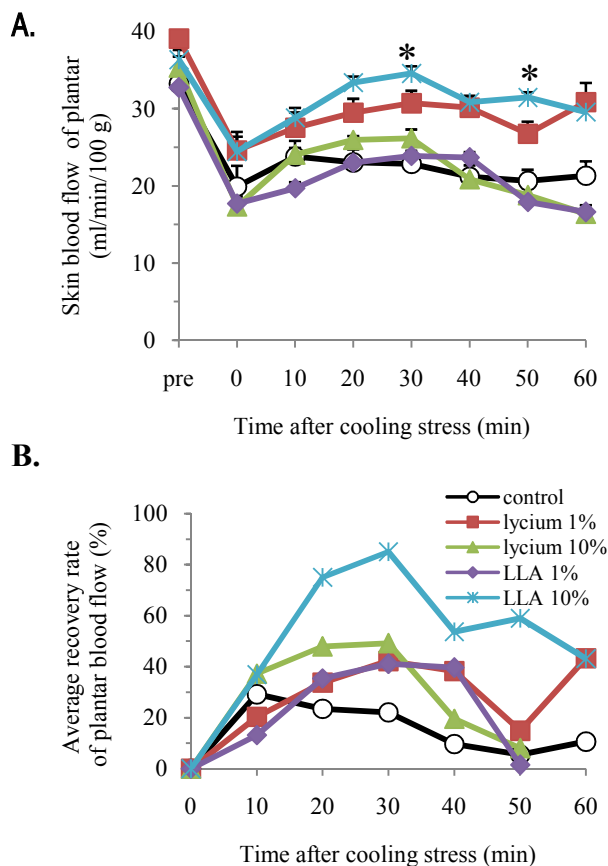


**Figure 3.** Effects on dorsal skin surface temperature by lycium or LLA administration for 2-week in mice. (A). Time-course of changes in the dorsal skin surface temperature are measured with a thermocouple thermometer before and after cold stress (n=3-6). Mice are fed for 2-week with water, 1% lycium, 10 % lycium, 1% LLA and 10% LLA, respectively. (B). Therecovery rate is expressed as a percentage of the dorsal skin surface temperature before (pre) and after cooling stress. Values were means±SEM. \**P*<0.05, \*\**P*<0.01 vs corresponding control.

### Effects on plantar skin blood flow by 2-week administration

Figure. 4A showed the effects of lycium and LLA on the cooling stress-induced change of plantar skin blood flow measured with non-contact flow meter. The 30 min- and 50 min-plantar blood flow by 10% LLA are significantly higher than those of the control. Fig. 4B showed that there were well over the control group at the 20 min-, 30 min- and 40 min-average recovery rates of the plantar skin blood flow by lycium- and LLA-treatment.

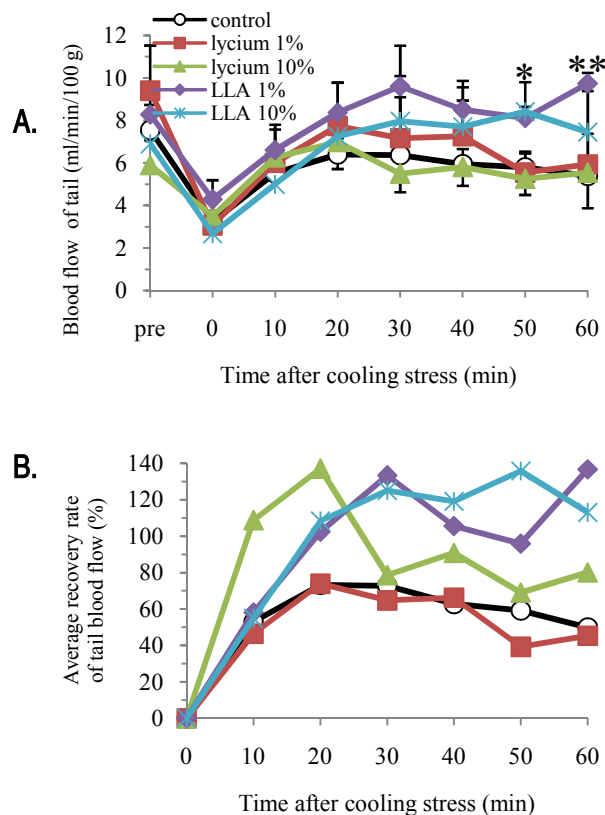




**Figure 4.** Effects on plantar skin blood flow by lycium or LLA administration for 2-week in mice. **(A).** Time-course of changes in the plantar skin blood flow are measured with a non-contact laser Doppler flow meter before and after cold stress (n=3-6). Mice are fed for 2-week with water, 1% lycium, 10 % lycium, 1% LLA and 10% LLA, respectively. Values were means±SEM. \**P*<0.05 vs corresponding control. **(B).** The average recovery rate is expressed as a percentage of the average of plantar skin blood flow before (pre) and after cooling stress.

### Effects on blood flow of tail by 2-week administration

Figure 5A showed the effects of lycium and LLA on the cooling stress-induced change of tail skin blood flow measured with contact flow meter. The 50 min-tail blood flow by 10% LLA and the 60 min-tail blood flow by 1% LLA are significantly higher than those of the control. Figure 5B showed that from 20 min after cooling stress, the average recovery rates of the tail skin blood flow by 10% lycium- and 1%, 10% LLA-treatment were well over the control group.



**Figure 5.** Effects on skin blood flow of tail by lycium or LLA administration for 2-week. **(A).** Time-course of changes in the skin blood flow of tail are measured with a contact laser Doppler flow meter before and after cold stress (n=3-6). Mice are fed for 2-week with water, 1% lycium, 10 % lycium, 1% LLA and 10% LLA, respectively. Values were means±SEM. \**P*<0.05, \*\**P*<0.01 vs corresponding control. **(B).** The average recovery rate is expressed as a percentage of the average of tail skin blood flow before (pre) and after cooling stress.

### Discussion

Here, it is reported that the oral administration of lycium and lycium-rich LLA on mice led to the increase of peripheral blood flow, and thus the recovery of skin surface temperature. Concerning the recovery of skin surface temperature after cold stress, in the case of 1-week treatment on mice, their recovery by lycium or LLA were well over the control (Figure. 1), and for 2-week treatment, the recovery rates of dorsal skin surface temperature by lycium or LLA were higher than those of the control from 30 min (Figure. 3B). Thus, the administration of lycium or lycium-rich LLA for 1 or 2-week facilitated the recovery of the skin surface temperature after cooling stress, and is expected to be useful for the treatment of cold syndrome in women. On the other hand, the



recovery rates of rectal temperature were not affected by lycium- or LLA-treatment even for 2 weeks (Figure. 2). Lycium and LLA induced the different effects on the recovery of skin surface temperature and body temperature after cold stress in mice, there were similar with royal jelly, a honey bee secretion, which was beneficial to the recovery of skin surface temperature in women [7]. The etiology of cold syndrome could be associated with decreased peripheral blood flow [5], indeed, the average recovery of the plantar or tail skin blood flow by lycium- or LLA-treatment were well over the control group (Figure. 4 and Figure. 5), and lycium or LLA led to the faster recovery of the skin surface temperature, thus it is expected to protect the body from heat loss. Hesperidin composed in lycium fruits and LLA, which can increase peripheral circulation and skin temperature under coldness [24, 25], thus supports the present study. Furthermore, the polysaccharides of lycium fruits shown antihypertensive effect by a significant decrease in the concentration of phenylephrine in isolated aortic rings [22], and improved the thoracic aorta vasoreactivity in rats under exhaustive exercise [27]. In addition, it has been reported the vasoreactivity by *Crataegus* and *Zizyphus* [28, 29], two components in LLA. Therefore, it seems that a synergistic effect by these components could contribute to the faster recovery of the skin blood flow by lycium or LLA.

Since cold stress on cutaneous leads to the generation of ROS [3], the mechanism of the faster recovery of skin blood flow by lycium or LLA may contribute to its antioxidant. Many pharmacological investigations on lycium fruits have focused on antioxidative [12, 13]. And we have reported that lycium-rich LLA most likely functions as an antioxidant agent to alleviate fatigue in aged mice [27], thus, it is also expected to improve the complications such as fatigue in cold syndrome. Further study may be needed to elucidate the details of the mechanisms involved in the increase of peripheral

blood flow and the recovery of skin surface temperature by lycium and lycium-rich LLA.

## Conclusions

Thus, the present fundamental study suggests that lycium and lycium-rich LLA may constitute a little contribution to the treatment of cold syndrome.

## Competing interests

All authors have confirmed that no competing financial interests exist.

## Authors' contributions

J-R Zhou conceived of the study, participated in its design and drafted the manuscript. B Ishikawa carried out the *in vivo* studies. M Nakashima performed the statistical analysis. K Yokomozo helped to draft the manuscript. All authors read and approved the final manuscript.

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