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Edited by J.J.P. Baars & A.S.M. Sonnenberg

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Lignosus rhinocerotis on Airway Muscle Relaxation

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Background & Aim

- Lignosus rhinocerotis* (Tiger Milk Mushroom) has been used for 400 years by the communities in South East Asia for treating asthma and cough. It is from the family of Polyporaceae (Basidiomycota) (**Fig. 1**). The sclerotium is particularly of medicinal value¹.
- Patients with asthma and chronic cough are known to have airway smooth muscle contraction and hence airway narrowing, leading to breathing difficulty. Asthmatic attack consists of early phase (airway contraction) and late phase (airway inflammation). Current mainstay of treatment of asthma is bronchodilators for early phase and corticosteroid for late phase^{2,3}.
- No pharmacological studies published to date to support its most popular claims for treating cough and asthma. This study aims to investigate the direct relaxant effects of the *L. rhinocerotis* cold water extract on isolated rat airways.



Fig. 1. *L. rhinocerotis* comprises pileus (cap), stipe and sclerotium.

Methods

Cultivated sclerotia of *L. rhinocerotis* (TM02) were provided by Ligno Biotech Sdn. Bhd. (Selangor, Malaysia). Cold water extract (see ref. 4 for extraction method) was subjected to organ bath experiment to study the direct effect on airway smooth muscle contractility. Male Sprague-Dawley rats (260-360g; 2-3 months) were sacrificed and dissected. Trachea and bronchus were isolated and hooked in the bath⁵. Carbachol (muscarinic receptor agonist) $1 \times 10^{-6} \text{M}$ was added to induce tissue contraction.

Then, cold water extract was added cumulatively to the bath until maximal response reached. All the data were expressed as mean \pm standard deviation (SD) of n number of animals. E_{max} represents maximum relaxation response.

Results

Cold water extract of the mushroom exhibited marked relaxation on the rat tracheal (**Fig. 2a**) and bronchial rings (**Fig. 2b**) when compared to vehicle control. The extract fully relaxed the tracheal rings (E_{max} : $106.10 \pm 8.11\%$) at 3.75mg/ml and bronchus (E_{max} : $106.45 \pm 19.02\%$) at 2.5mg/ml .

In subsequent experiments (**Fig. 3**), tissues were exposed to the extract at varying duration between each concentration to investigate any impact of incubation time. The concentration required to fully relax trachea decreased with longer incubation time intervals, from 4.75mg/ml (5-minute; $49.30 \pm 13.57\%$) to 2.5mg/ml (30-minute; $118.19 \pm 17.13\%$) (**Fig. 3a**). Similarly, lower concentrations were required to fully relax bronchus with longer incubation time intervals, from 5.5mg/ml (5-minute; $96.68 \pm 8.56\%$) to 1.75mg/ml (30-minute; $116.25 \pm 19.56\%$) (**Fig. 3b**).

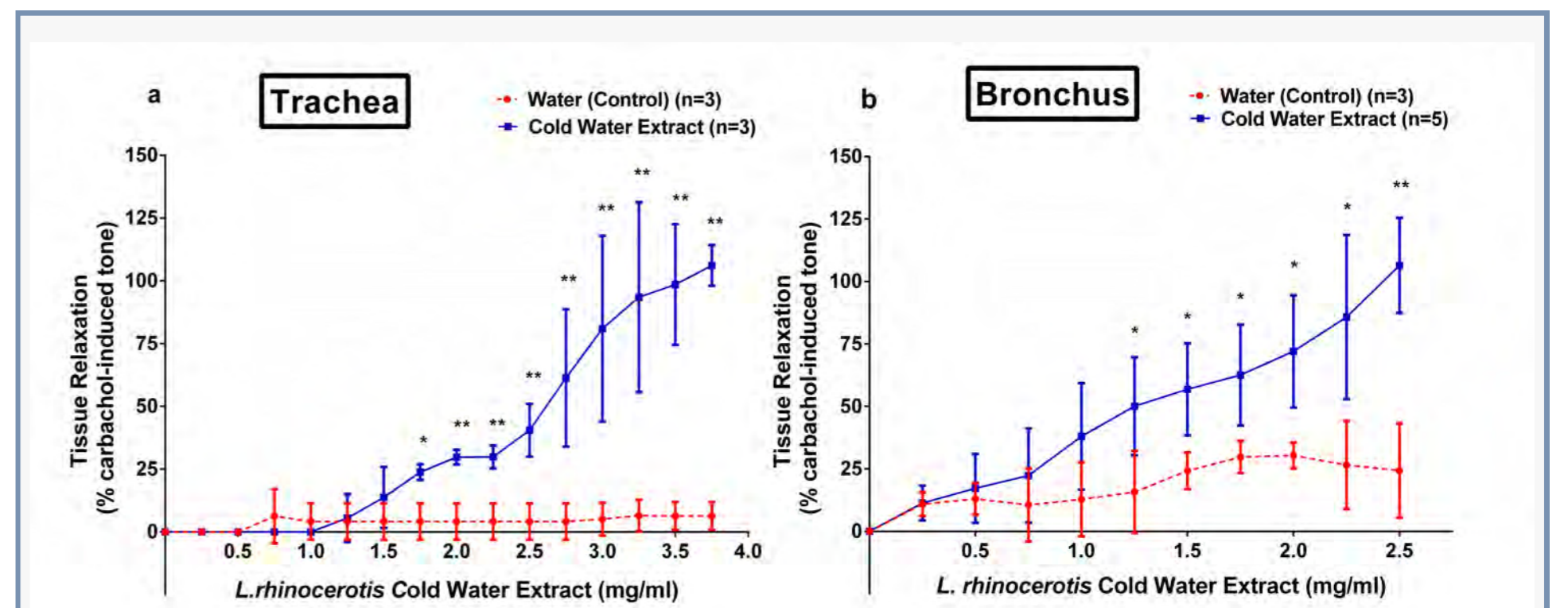


Fig. 2 The relaxation effect of *L. rhinocerotis* cold water extract on carbachol-induced contraction in isolated rat (a) trachea and (b) bronchus. Incubation time between the additions of each concentration was 15 minutes. The mushroom extract caused significant relaxation in tracheal and bronchial segments when compared to vehicle control (Unpaired T-test; * $p < 0.05$ and ** $p < 0.01$).

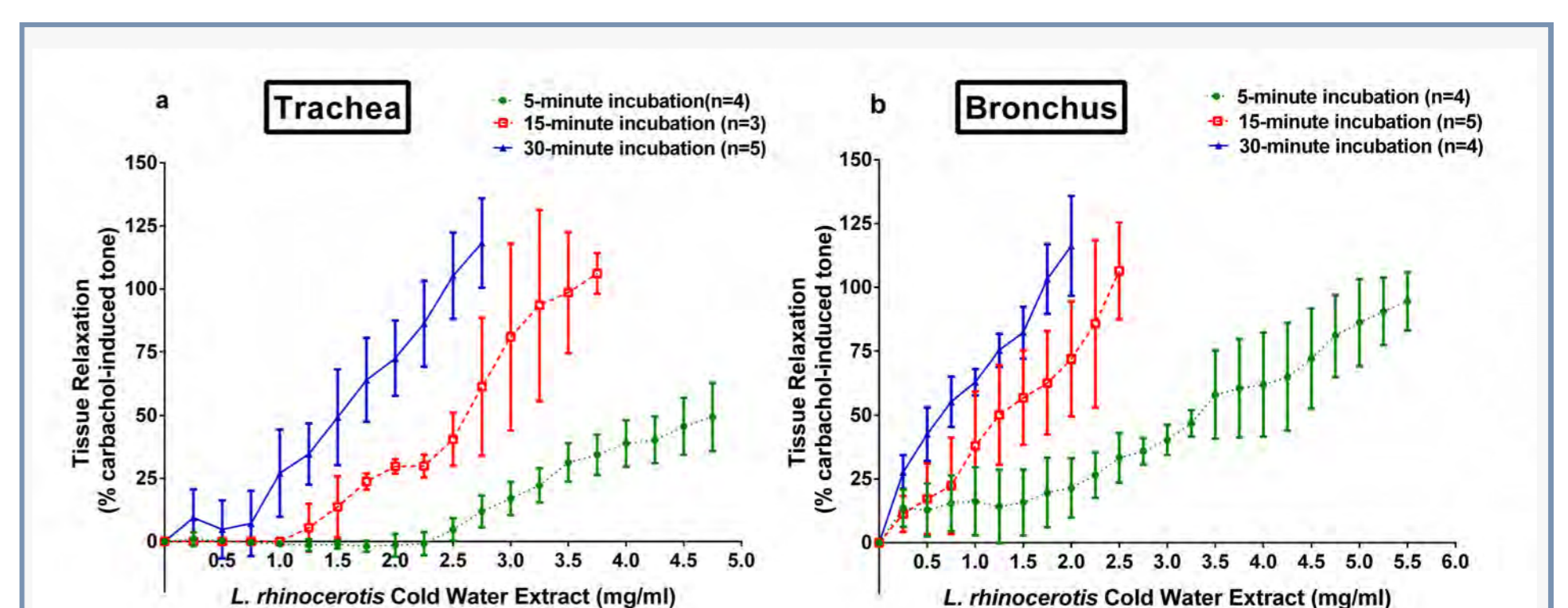


Fig. 3 Effect of different incubation time of *L. rhinocerotis* cold water extract in isolated rat (a) trachea and (b) bronchus. Overall, a longer incubation time resulted in a greater relaxation response for each concentration tested.

Overall, the findings suggest that the longer the tissues were exposed to the extract, a greater relaxation response was observed for each concentration tested.

L. rhinocerotis cold water extract was reported to contain mainly carbohydrates and proteins. β -glucan is the dominant form of glucan composition⁶. We postulate that the bioactive component in the cold water extract is large polysaccharide or polysaccharide-protein complex, which are less likely to penetrate cells directly. Therefore, a longer response time may be linked to the requirement of these large molecules to adsorb and bind to these surface proteins/receptors.

Conclusion

- Overall, our finding revealed a **direct relaxant effect of *L. rhinocerotis* cold water extract on pre-contracted rat airways**, in support for its most popular claims to treat cough and asthma.
- The airway relaxant effect is **time- and concentration-dependent**.

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