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Original Article

Gelam honey attenuates ovalbumin-induced airway inflammation in a mice model of allergic asthma

Nur Salme Suhana Shamshuddin [a], Rozaini Mohd Zohdi [a, b, *]

[a] Faculty of Pharmacy, Universiti Teknologi Mara (UiTM), 40450 Shah Alam, Selangor, Malaysia
[b] Atta-ur-Rahman Institute for Natural Products Discovery (AuRIns), 40450 Shah Alam, Selangor, Malaysia

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ABSTRACT

Allergic asthma is a chronic inflammatory disorder of the pulmonary airways. Gelam honey has been proven to possess anti-inflammatory property with great potential to treat an inflammatory condition. However, the effect of ingestion of Gelam honey on allergic asthma has never been studied. This study aimed to investigate the efficacy of Gelam honey on the histopathological changes in the lungs of a mice model of allergic asthma. Forty-two Balb/c mice were divided into seven groups: control, I, II, III, IV, V and VI group. All groups except the control were sensitized and challenged with ovalbumin. Mice in groups I, II, III, IV, and V were given honey at a dose of 10% (v/v), 40% (v/v) and 80% (v/v), dexamethasone 3 mg/kg, and phosphate buffered saline (vehicle) respectively, orally once a day for 5 days of the challenged period. Mice were sacrificed 24 h after the last OVA challenged and the lungs were evaluated for histopathological changes by light microscopy. All histopathological parameters such as epithelium thickness, the number of mast cell and mucus expression in Group III significantly improved when compared to Group VI except for subepithelial smooth muscle thickness ($p < 0.05$). In comparing Group III and IV, all the improvements in histopathological parameters were similar. Also, Gelam honey showed a significant ($p < 0.05$) reduction in inflammatory cell infiltration and beta-hexosaminidase level in bronchoalveolar lavage fluid. In conclusion, we demonstrated that administration of high concentration of Gelam honey alleviates the histopathological changes of mice model of allergic asthma.

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1. Introduction

Asthma is a chronic inflammatory disorder of the pulmonary airways characterized by bronchial hyper-responsiveness and airway obstruction caused by inflammation, mucus hypersecretion and airway wall remodeling.[1] In susceptible individual, the inflammation causes the symptoms of wheezing, coughing, chest tightness and breathlessness.[2] The respiratory airways of an asthmatic patient show abnormalities such as epithelial denudation, goblet cell metaplasia, subepithelial thickening, increased airway smooth muscle mass, bronchial gland enlargement, angiogenesis and alterations in extracellular matrix components involving large and small airways.[3] The incidence of asthma diseases has increased dramatically in the last decade. Approximately 300 million people worldwide suffer from asthma and it can be fatal if left untreated. Currently, inhaled corticosteroids are the most effective available treatment for asthma and they are used as the first-line therapy for persistent asthma in adults and children in many countries. However, systemic absorption of inhaled corticosteroids may have deleterious effects over long term use.[4] Thus, pursuit on effective and safe alternative medication for this disease management has become part of the major interest in scientific field nowadays.

Honey as a complementary medicine is increasingly being used as an adjunct and also as a substitute for effective and proven therapies in asthmatics among Malaysians.[5] It has been shown that honey exhibits various biological properties including antibacterial, antioxidant, antifungal and as wound healing promoter. In addition to these biological effects, recent studies found honey to possess

Abbreviations: BALF, Bronchoalveolar lavage fluid; DXN, Dexamethasone; i.p., Intraperitoneal injection; PBS, Phosphate buffered saline; PAS, Periodic acid Schiff; OVA, Ovalbumin.

* Corresponding author. Pharmaceutical Life Sciences Department, FF1-Level 6 Faculty of Pharmacy, Uitm Puncak Alam Campus, 42300 Bandar Puncak Alam, Selangor, Malaysia. Fax: +60 3 32584602.
E-mail addresses: salme.suhana@yahoo.com.my (N.S.S. Shamshuddin), rozainizohdi@puncakalam.uitm.edu.my (R. Mohd Zohdi).

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anti-inflammatory and immunomodulatory properties.[6–9] Since the main pathological feature of allergic asthma is caused by complex interactions between immunological mediators that are produced by the inflammatory process, this study aims to evaluate the efficacy of honey as an immunotherapeutic agent based on the pathogenesis of asthma. Many reports have highlighted the efficacy of immunomodulatory and anti-inflammatory agents to treat patients with asthma, thus it is likely to hypothesize that honey, which is cheap and readily available natural product may also be a potential therapeutic agent for asthma.[10–13] To our knowledge, no comprehensive studies thus far have addressed whether ingestion of honey is effective to treat allergic asthma. Therefore, this study is aimed to investigate the efficacy of Malaysian Gelam honey on the mediator and lung histopathology in a murine model of allergic asthma. The present approach may be useful to evaluate novel therapeutic modalities for asthma treatment and provide an evidence-based recommendation for the efficacy of honey as a complementary treatment for asthma.

2. Materials and methods

2.1. Honey sample

Local *Apis mellifera* honey from the floral source of *Melaleuca* spp. (Gelam) tree was used in this study. The honey was supplied by the Department of Agriculture Malaysia and irradiated with 25 kGy gamma irradiation using radioactive source Cobalt 60 for sterilization purposes at Bizworth Gammarad, Malaysian Nuclear Agency.

2.2. Reagents

Aluminum hydroxide was purchased from Fisher Scientific (Waltham, MA, USA). OVA (Grade V), Dexamethasone (DXN), Phosphate buffered saline (PBS), Toluidine Blue O and Periodic Acid Schiff stain were purchased from Sigma Chemical Co. (St. Louis, MO, USA).

2.3. Animals

All animal studies were conducted in accordance with the criteria of the investigations and Universiti Teknologi MARA Committee of Animal Research and Ethics (UiTM CARE) guidelines concerning the use of experimental animals. A total of forty-two female Balb/c mice, 8–12 weeks old, were obtained from Laboratory Animal Facility and Management (LAFAM), Faculty of Pharmacy, UiTM Puncak Alam Campus. The animals underwent acclimatization period for one week and were given normal mouse diet along with filtered tap water *ad libitum*.

2.4. Experimental design

Mice were sensitized and challenged with OVA, as previously described with slight modification.[14] Mice were randomly divided into seven groups ($n = 6$). The mice in study groups I, II, III, IV, V and VI were immunized on days 0, 7, and 14 by intraperitoneal (i.p.) injection of 50.0 μg chicken ovalbumin (OVA) with 1 mg aluminum hydroxide as an adjuvant in a total volume of 100 μl of PBS. The mice were then anesthetized and challenged by intranasal instillations of 100 μg OVA in 50 μl PBS on days 14, 25, 26 and 27. The mice in the control group were given PBS without OVA.

Treatment for groups I, II, III and IV started on days 23–27 where mice were fed via oral gavage (10 μl/g body weight) with 10%, 40%, 80% (v/v) honey and 3 mg/kg DXN diluted in PBS respectively, 1 h prior to OVA administration (Fig. 1). Groups V and control received PBS only while group VI received no treatment. Animals recovered quickly from the procedure with only mild discomfort. At the end of the experiment (day 28), the mice were sacrificed by dissecting the thoracic region under anesthesia using an intraperitoneal injection of pentobarbital (75 mg/kg).

2.5. Histopathological analysis of lung

2.5.1. Inflammatory cell infiltration

At 24 h after the last challenge, mice were sacrificed and the lungs were removed and fixed in 10% buffered formalin prior to embedding in paraffin. The lung tissues were sectioned into 5 μm thick and stained with hematoxylin and eosin (H&E) to assess inflammatory cell infiltration. Inflammation was evaluated using a semi-quantitative scoring system with a grading scale ranging from 0 to 3 as described previously by Jang et al.[15] A value of 0 was assigned when no inflammation was detected; [1] was assigned when occasional cuffing with inflammatory cells was observed; [2] was assigned when most bronchiole or vessels were surrounded by a thin layer (one to five cells) of inflammatory cells; and a value of [3] was assigned when most bronchiole or vessel were surrounded by a thick layer (>5 cells) of inflammatory cells.

2.5.2. Quantitation of airway mucus expression

Mucus secretion was visualized using Periodic-acid Schiff staining. Paraffin embedded lung tissue sections were de-waxed and hydrated. Following rehydration, tissue sections were treated with Periodic-acid Solution (Aqueous solution, 1 g/l, Sigma–Aldrich) for 10 min. After washing repeatedly in water, slides were covered with Schiff's reagent solution (Sigma–Aldrich) for 15 min. Slides were then washed in water and immersed in Harris Hematoxylin, followed by a differentiation in acid alcohol (HCL concentration 4 ml; 95% EtOH—396 ml) followed by water again. The tissue sections were dehydrated through different concentrations of alcohol, and sections were mounted in mounting medium and the coverslips were applied. Mucus expression levels in the airway were quantified by counting PAS-positive and PAS-negative epithelial cells in each bronchiole. Results are expressed as the percentage of PAS-positive cells per bronchiole, which was calculated as the number of PAS-positive epithelial cells per bronchiole divided by the total number of epithelial cells in each bronchiole.[15]

2.5.3. Toluidine blue staining for mast cell

Number of mast cells was evaluated via toluidine blue staining in the connective tissue and smooth muscle layer underneath the epithelial of the bronchiole.[16] Photomicrographs were taken by Leica MC170 HD camera which was adapted on Leica DM2500 model microscope. The histopathological analysis was carried out with Leica Application Suite version 4.3.0 software.

2.6. Total cell counting

Bronchoalveolar lavage fluid (BALF) was obtained by intratracheal instillation. The lungs were lavaged three times with 0.8 ml of sterile PBS. The BALF from each sample was centrifuged (4 °C, 420 g, 15 min), and supernatants were stored at −80 °C for beta hexosaminidase analysis. Cell pellets were resuspended in 200 μl sterile PBS for total cell counts using hemacytometer and 100 μl of cell suspension were applied to a slide by cytospinning followed by staining with Giemsa. At least 200 cells were counted per slide.[14]

2.7. Beta hexosaminidase assay

Beta hexosaminidase activity was determined as a measure of mast-cell degranulation. BALF (50 μl) was incubated with 50 μl 5 mM p-nitrophenyl N-acetyl-b-D-glucosaminide in 50 mM sodium citrate buffer (pH 4.5) at 37 °C for 2 h. Reactions were terminated

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