UNIVERSITI PUTRA MALAYSIA

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NURAIN IRDAYANI BINTI KAMISE

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EFFECTS OF PROPOLIS ON ALLERGIC DISEASES: A SYSTEMATIC REVIEW OF *IN VITRO*, *IN VIVO,* AND CLINICAL STUDIES

Nurain Irdayani Kamise[a], Hui Ming Ong[a], Fahmida Islam[b], Poi Yi Aw Yong[b], Kong Yen Liew[a], Chau Ling Tham[a]

[a]Department of Biomedical Sciences, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia

[b]School of Science, Monash University Malaysia

Introduction: The prevalence of allergic diseases, including atopic dermatitis, allergic rhinitis, and asthma has been increasing in the global population and the lack of safe and effective treatment for these diseases remains a major challenge. Propolis is a bee product collected from plant parts and exudates. Its phenolic constituents have demonstrated various biological activities, including anti-inflammatory and immunomodulatory effects. However, the potential application of propolis for allergic diseases has not been fully explored. **Objectives:** This study aimed to conduct a systematic review of studies investigating the effects of propolis on different allergic diseases to support its further development for therapeutic use. **Methodology:** Relevant articles were retrieved from three electronic databases, PubMed, Scopus and ScienceDirect, using the keywords "propolis" AND "allergy". All *in vitro, in vivo,* and clinical studies that met the eligibility criteria were included in the systematic review, followed by the extraction of related findings such as the types of propolis used and their effects in different experimental models. **Results**: A total of 12 eligible articles consisting of 7 *in vitro* studies, 7 *in vivo* studies, and 2 clinical studies were identified. *In vitro* studies demonstrated that propolis inhibited the release of allergic mediators and cytokines in different cellular models. Propolis ethanolic extract was also able to ameliorate the symptoms of atopic dermatitis and allergic rhinitis *in vivo*. Besides that, the anti-asthmatic effects of propolis were supported by both *in vivo* and clinical studies where propolis has been shown to suppress airway inflammation, airway hyperresponsiveness and improve pulmonary function. Notably, propolis treatment showed anti-allergic effects when administered orally for asthma and allergic rhinitis as well as topically for allergic rhinitis and atopic dermatitis in *in vivo* models, and these were observed regardless of the mode of treatment. Although a clinical study showed that propolis had no effect on allergic sensitization and eczema, it was demonstrated to be a safe supplement for lactating mothers. **Discussion:** The anti-allergic activities of propolis were likely to be contributed by the active constituents commonly found in propolis. Caffeic acid phenethyl ester (CAPE), tectochrysin and pinocembrin have been shown to have anti-asthmatic effects *in vivo*, whereas CAPE and chrysin have been shown to attenuate atopic dermatitis-like symptoms. Although

propolis in the form of extracts as well as active constituents demonstrated anti-allergic effects, the safety and effectiveness of the active constituents are yet to be evaluated in clinical trial. In contrast, propolis extract formulated as a nutritional food product has been shown to have therapeutic effects for asthma as an adjuvant therapy in the clinical setting and thus, such approach may be adopted for other allergic diseases. **Conclusion:** Overall, propolis demonstrated beneficial effects for various allergic diseases in pre-clinical studies; however, clinical evidence is lacking for allergic rhinitis and atopic dermatitis. With proven safety for human consumption, the effectiveness of propolis extract for allergic rhinitis and atopic dermatitis could be further evaluated in the clinical setting. It is also worthwhile to further investigate the clinical therapeutic potential of active constituents of propolis for different allergic diseases.

Keywords: propolis, allergy, asthma, allergic rhinitis, atopic dermatitis, systematic review

ABSTRAK

KESAN PROPOLIS KEATAS PENYAKIT ALAHAN: ULASAN SISTEMATIK DARIPADA *IN VITRO, IN VIVO*, DAN KAJIAN KLINIKAL

Nurain Irdayani Kamise[a], Hui Ming Ong[a], Fahmida Islam[b], Poi Yi Aw Yong[b], Kong Yen Liew[a], Chau Ling Tham[a]

[a]Jabatan Sains Bioperubatan, Fakulti Perubatan dan Sains Kesihatan, Universiti Putra Malaysia

[b]Sekolah Sains, Universiti Monash Malaysia

Pendahuluan: Kelaziman penyakit alahan, termasuk dermatitis atopik, rhinitis alergi, dan asma telah meningkat pada populasi global dan kekurangan rawatan yang selamat dan berkesan untuk penyakit ini tetap menjadi cabaran utama. Propolis adalah produk lebah yang dikumpulkan dari bahagian tanaman dan eksudat. Konstituen fenoliknya telah menunjukkan pelbagai aktiviti biologi, termasuk kesan anti-radang dan imunomodulator. Walau bagaimanapun, kemungkinan penggunaan propolis untuk penyakit alahan belum diterokai sepenuhnya. **Objektif:** Kajian ini bertujuan untuk melakukan tinjauan sistematik terhadap kajian yang menyiasat kesan propolis terhadap penyakit alahan yang berbeza untuk menyokong pengembangan selanjutnya untuk penggunaan terapi. **Metodologi:** Artikel yang relevan diambil dari tiga pangkalan data elektronik, PubMed, Scopus dan ScienceDirect, menggunakan kata kunci "propolis" DAN "alergi". Semua kajian *in vitro, in vivo,* dan klinikal yang memenuhi kriteria kelayakan dimasukkan dalam tinjauan sistematik, diikuti dengan pengekstrakan penemuan yang berkaitan seperti jenis propolis yang digunakan dan kesannya dalam model eksperimen yang berbeza. **Hasil**: Sebanyak 12 artikel yang layak terdiri daripada 7 kajian *in vitro*, 7 kajian *in vivo*, dan 2 kajian klinikal dikenal pasti. Kajian *in vitro* menunjukkan bahawa propolis menghalang pembebasan mediator alergi dan sitokin dalam model sel yang berbeza. Ekstrak etanolik propolis juga dapat memperbaiki gejala dermatitis atopik dan rhinitis alergi *in vivo*. Selain itu, kesan anti-asma propolis disokong oleh kajian *in vivo* dan klinikal di mana propolis terbukti dapat menekan keradangan saluran udara, hiperresponsif saluran udara dan meningkatkan fungsi paru-paru. Terutama, rawatan propolis menunjukkan kesan anti-alergi apabila diberikan secara oral untuk asma dan rhinitis alergi serta topikal untuk rhinitis alergi dan dermatitis atopik pada model *in vivo*, dan ini diperhatikan tanpa mengira cara rawatannya. Walaupun kajian klinikal menunjukkan bahawa propolis tidak berpengaruh pada kepekaan alergi dan ekzema, ia terbukti sebagai makanan tambahan yang selamat untuk ibu yang menyusui. **Perbincangan:** Kegiatan anti-alergi propolis kemungkinan dikaitkan oleh unsur-unsur aktif yang biasanya terdapat di propolis. Fenetil ester asid kafein (CAPE), tektochrysin dan pinocembrin telah terbukti

mempunyai kesan anti-asma secara *in vivo*, manakala CAPE dan chrysin terbukti dapat mengurangkan gejala seperti dermatitis atopik. Walaupun propolis dalam bentuk ekstrak dan juga unsur aktif menunjukkan kesan anti-alergi, keselamatan dan keberkesanan komponen aktif masih belum dinilai dalam percubaan klinikal. Sebaliknya, ekstrak propolis yang diformulasikan sebagai produk pemakanan telah terbukti mempunyai kesan terapeutik untuk asma sebagai terapi tambahan dalam tetapan klinikal dan dengan demikian, pendekatan tersebut dapat digunakan untuk penyakit alergi yang lain. **Kesimpulan:** Secara keseluruhan, propolis menunjukkan kesan yang baik untuk pelbagai penyakit alahan dalam kajian pra-klinikal; namun, bukti klinikal kurang untuk rhinitis alergi dan dermatitis atopik. Dengan keselamatan yang terbukti untuk penggunaan manusia, keberkesanan ekstrak propolis untuk rhinitis alergi dan dermatitis atopik dapat dinilai lebih lanjut dalam keadaan klinikal. Juga bermanfaat untuk menyelidik lebih lanjut potensi terapi klinikal konstituen aktif propolis untuk penyakit alahan yang berbeza.

Kata kunci: propolis, alergi, asma, rhinitis alergi, dermatitis atopik, ulasan sistematik

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LIST OF ABBREVIATIONS

AD	Atopic Dermatitis
AEP	Aqueous Extracts of Propolis
AIT	Allergen-specific Immunotherapy
AHR	Airway Hyperresponsiveness
AKT	Protein Kinase B
AR	Allergic Rhinitis
BALB	Bagg Albino
BALF	Bronchoalveolar Lavage Fluid
BGPP	Brazilian Green Propolis
BMBs	Bone Marrow Biopsies
CAI	Chronic Allergic Inflammation
CAPE	Caffeic Acid Phenethyl Esters
ConA	Concanavalin A
DNP	Dinitrophenyl
EEP	Ethanolic Extract of Propolis
E-NPP3	Ecto-Nucleotide Pyrophosphatase-3
ERK	Extracellular-Signal-Regulated Kinase
FA	Food Allergy
FC	Fragment Crystallizable
FcεRI	High-Affinity IgE Receptor
GATA	Zinc-Finger Transcription Factor
GF	Growth Factor

H1R	Histamine Receptor 1
HPLC	High Performance Liquid Chromatography
ICAM	Intercellular Adhesion Molecule
IFN	Interferon
IL	Interleukin
IgE	Immunoglobulin E
LECs	Lung Epithelial Cells
LTs	Leukotrienes
MAPKs	Mitogen-Activated Protein Kinases
mRNA	Messenger Ribonucleic Acid
MCP	Monocyte Chemoattractant Protein
Mcpt8	Mast Cell Protease 8
ND	Not Determined
NF- κB	Nuclear Factor-kappaB
NO	Nitric Oxide
OVA	Ovalbumin
PBS	Phosphate Buffer Saline
PBMCs	Peripheral Blood Mononuclear Cells
PKC	Protein Kinase C
PMA	Phorbol 12-Myristate 13-Acetate
PMN-MDSC	Polymorphonuclear-Myeloid-Derived Suppressor Cells
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analysis
PGs	Prostaglandins

RBL-2H3	Rat Basophilic Leukaemia
ROS	Reactive Oxygen Species
RPMCs	Rat Peritoneal Mast Cells
SCIT	Subcutaneously
SLIT	Sublingually
TH1	T-Helper Type 1 Cell
TH2	T-Helper Type 2 Cell
TNF	Tumour Necrosis Factor
TSLP	Thymic Stromal Lymphopoietin
WEP	Water Extract of Propolis
WHO	World Health Organization
WAO	World Allergy Organization

CHAPTER 1

INTRODUCTION

1.1 Background

Allergy occurs when our immune system responds to a foreign substance called allergen. Allergen does not cause any reaction in most people, but atopic individuals have an increased risk of developing allergic disease. Allergic disease is a type of immune-mediated disorders due to IgE-dependent immunological reaction to an allergen (Ilaria *et al*., 2016). The prevalence of allergic diseases including allergic asthma, anaphylaxis and eczema have been drastically increased over the last 20 to 30 years, attacking children and adults worldwide in developed and developing countries (Pawankar *et al*., 2013). They sometimes cause life-threatening conditions such as anaphylaxis and fatal asthma as well as give negative impact on the quality of life. Generally, allergic reactions often are classified into the early-phase reactions and the late-phase reactions (Amin, 2012). Early-phase reactions occur rapidly within seconds after exposure to an allergen whereas late-phase reactions occur within several hours. Early-phase reactions mainly result in the secretion of granules and preformed mediators by mast cells. This process is known as mast cell degranulation where histamine and other proteases are being released into the environment (Amin, 2012; Schroeder, 2011). Late-phase reactions occur after activation of mast cells which involve the release of newly synthesized inflammatory mediators such as cytokines, leukotrienes, chemokines, prostaglandins, and growth factors. Production of these mediators thereby lead to bronchoconstriction, vasodilation, and potent immune

allergic and inflammatory responses (Sakai *et al*., 2010). Hence, activation of mast cells is well known as a crucial event in various allergic diseases including allergic asthma, allergic rhinitis, atopic dermatitis, and anaphylaxis (Galli & Tsai, 2012)

It is important to emphasize the current therapies to combat allergic disease because there are no established methods to prevent these diseases due to various underlying molecular mechanisms. Glucocorticosteroids and antihistamines are gold standard treatments for allergic diseases due to their symptom relief and anti-inflammatory properties respectively. At present, there are also other therapeutic strategies to combat allergic diseases such as allergen-specific immunotherapy and non-specific immunotherapy (anti-IgE). Although they remain the mainstay of therapy to fight against allergic diseases, limited efficacy, and adverse effects remain a major challenge. The common reported adverse effects for glucocorticosteroids and antihistamines are cardiac toxicity, chest congestion, drowsiness, and upset stomach whereas IgE-mediated side effects, injection site induration, itching and pain are the common reported adverse effects for allergen-specific immunotherapy and non-specific immunotherapy. Therefore, due to the side effects reported, researchers currently move their attention towards natural products to fight against allergic reactions with less side effects (Tang *et al*., 2015; Chung *et al*., 2013).

Propolis, or also known as bee glue, is a resinous natural product that bees collected from plant parts and exudates. Propolis consists of a mixture of phenolic constituents that has been demonstrated to exhibit many beneficial pharmacological activities such as anti-inflammatory, antioxidant, antiviral and immunomodulatory effects. Therefore, it is widely used in folk medicine for the management of various disorders and appears in various forms as food supplements, biocosmetics, and biopharmaceuticals. However, the potential application of propolis for allergic diseases has not been fully

explored yet. Hence, this systematic review was done to investigate the effects of propolis on allergic diseases such as allergic asthma, allergic rhinitis, atopic dermatitis, and anaphylaxis. Systematic review is an essential tool to summarize evidence of previous research accurately and reliably in order to provide clinicians a kickoff point to develop guideline for clinical practice and to support new research. Therefore, this study aimed to conduct a systematic review of studies investigating the effects of propolis on different allergic diseases to support its further development for therapeutic use.

1.2 Statement of Problem

The current therapies for allergic diseases have been reported to possess several undesirable adverse effects or with limited efficacy. Other than that, allergic disease is a lifetime condition where many people would prefer a complementary alternative compared to modern medicine. Also, as the effects of propolis on allergic diseases remain controversial and have not been fully explored, thus systematic review was conducted to determine the potential application of propolis. This systematic review may provide scientific basis for further development of propolis as prophylactic or therapeutic use in allergic diseases.

1.3 Objectives of Study

1.3.1 General Objective

To conduct a systematic review of studies investigating the effects of propolis on different allergic diseases (asthma, atopic dermatitis, allergic rhinitis, and

anaphylaxis) to support its further development for prophylactic or therapeutic use.

1.3.2 Specific Objectives

1. To determine the effects of propolis in different experimental models of allergic diseases such as *in vitro, in vivo,* and clinical studies.
2. To identify the types of propolis and their mode of treatment which contributed to their anti-allergic activities.

1.4 Significance of Study

Based on this study, we will be able to evaluate the effects of propolis on different cellular and animal models and their prophylactic or therapeutic effects in human trials along with the type of propolis that contributed to their anti-allergic effects by using various mode of treatments. This systematic review may provide scientific basis for further development of propolis as prophylactic or therapeutic use in allergic diseases. The findings from this systematic review would be useful to guide healthcare professionals in making decisions regarding the use, safety, and efficacy of propolis as an alternative or complementary treatment to the other modern therapies for various allergic diseases.

1.5 Hypothesis

Propolis in various forms of extracts may exhibit anti-allergic effects for asthma, atopic dermatitis, allergic rhinitis, and anaphylaxis in *in vitro, in vivo,* and clinical studies.

CHAPTER 2

LITERATURE REVIEW

2.1 Allergic Diseases

Allergic diseases become one of the most common types of disease worldwide. They may be not a major life-threatening disease but still give rise to substantial global health burden (Pawankar *et al*., 2013). Allergic asthma, atopic dermatitis, allergic rhinitis, gastrointestinal diseases, food, and drug allergy are the major chronic allergic diseases (Simon *et al*., 2010). Majority of the patients suffer from IgE-mediated reactions even though several mechanisms are involved. However, the relationship between the symptoms of allergy and positive IgE-sensitization remain unclear because neither all individuals with allergic diseases sensitized nor all sensitized individuals develop clinical symptoms (Bousquet *et al*., 2008). Usually, the symptom of allergic diseases starts on early stage of life, but the clinical phenotypes differ with age, hence making it more complex. Notably, the heterogeneity of asthma has been growing attention since in complex chronic diseases, heterogeneity is the important characteristics. Any other way, allergic diseases tend to accumulate in the same subject as multimorbid or otherwise follow an allergic march (Wenzel, 2012).

Generally, the risk factors for allergic diseases can be divided into two categories such as host and environmental variables (Grammatikos, 2008). Heredity, gender, ethnicity, and age are all host factors, but environmental risk factors account for the majority of allergic disease cases. Food (Boyce *et al*., 2014), medications, the hygiene theory, stress (Ninabahen, Xiang, Rehm, & Marshall Jr. 2012), and others are

examples of environmental risk factors. Increased exposure to environmental factors does, in fact, raise the risk of allergic diseases. Other than from the pain caused by the diseases, other adverse effects include lowered quality of life and an increase in the financial burden placed on the patients, both directly and indirectly (Pawankar, 2014).

2.1.1 Pathophysiology

During the acute response at the early-phase of allergic reactions, a type I hypersensitivity reaction to an allergen elicits a response in a kind of immune cell known as a T-helper type 2 cell (TH2) lymphocyte that produces the cytokine interleukin-4 (IL-4). These TH2 cells communicate with B cells, which are lymphocytes that produce antibodies. This interaction, when combined with IL-4 signals, induces the B cell to start producing a high amount of antibody known as IgE. In the blood, secreted IgE will circulate and binds to an IgE-specific receptor called FcεRI on the surface of immune cells that includes basophils and mast cells. At this early stage, the IgE-coated cells are sensitized to the allergen (Charles *et al.*, 2009). Following exposure to the same allergen occurs after allergen binds to the IgE molecules on their surface, activation of mast cells and basophils take place. When multiple IgE-receptor complexes connect with the same allergenic molecule and activate the sensitized cell, this is known as cross-linking of the IgE and Fc receptors.

Degranulation occurs when activated mast cells and basophils release histamine, preformed mediators including cytokines, leukotrienes, interleukins, and prostaglandins and other proteases into the neighbouring tissue from their granules thereby lead to various systemic effects such as vasodilation, nerve stimulation, contraction of the smooth muscle, and mucous secretion. This causes rhinorrhea,

itching, dyspnea, and anaphylaxis and it is determined by the individual, allergen, and mode of exposure, limited to certain physiological systems (Charles *et al.*, 2009). Later responses might arise when inflammatory mediators of the initial response are no longer present. To explain this, we must look at the migration of different leukocyte types to the site of origin. Following the initial reaction, the late-phase reaction normally occurs between 2 and 24 hours after the first. It is possible that mast cell cytokines play a role in long-term consequences. Even though they are still mediated by eosinophils and TH2 cells, late-phase asthmatic responses differ slightly from other allergy reactions (Holt & Sly, 2007).

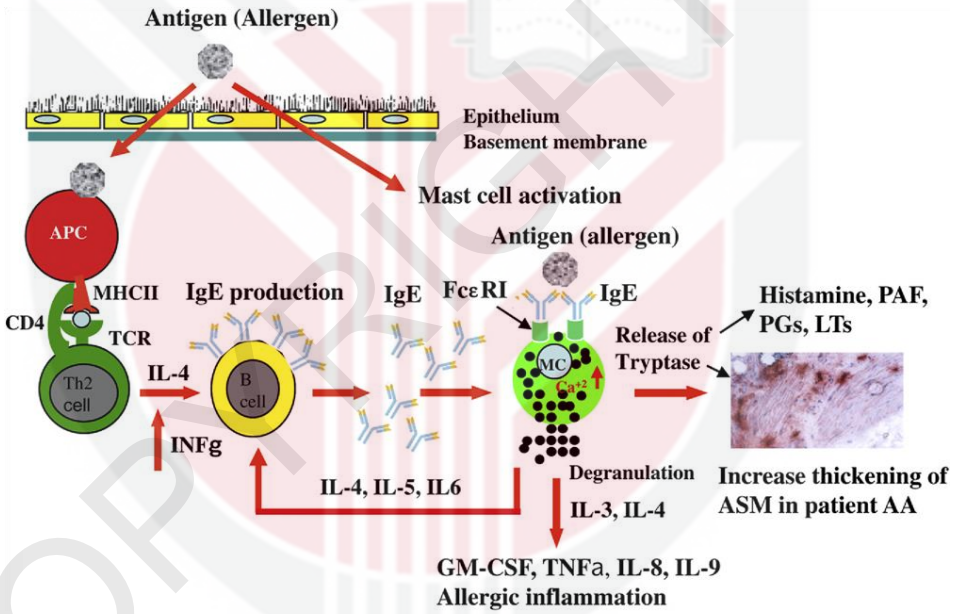

Figure 2.1.1: Induction and effector mechanisms in type I hypersensitivity.

In addition, the pathogenesis of allergic contact dermatitis involves a reaction that is more likely towards a type IV hypersensitivity reaction even though it is termed as an "allergic" reaction which commonly refers to type I hypersensitivity (Martin *et al.*, 2012). T cells (CD8+) are immune cells that damage target cells on contact in type IV hypersensitivity and activated macrophages which produce the hydrolytic enzymes.

2.2 Prevention, Treatment, and Therapy

Prevention methods for allergic diseases includes simply breastfeeding during the early pregnancy may reduce the risk of dermatitis and giving peanut goods early may reduce the chance of allergies (Greer *et al*., 2019), (Garcia-Larsen *et al*., 2018). However, there is no convincing evidence that a pregnant mother's diet or breastfeeding increases the risk (Greer *et al*., 2019). There is also no evidence that delaying the introduction of specific meals is beneficial. However, early exposure to possible allergies may be beneficial. Other than that, supplementing with fish oil during pregnancy has been linked to a decreased risk whereas probiotic supplements may help prevent atopic dermatitis during pregnancy or infancy (Garcia-Larsen *et al*., 2018). In terms of allergic disease management, it often entails avoiding what causes the allergy and taking medications to alleviate symptoms, with the treatment chosen based on the results of the diagnostic (National Institute of Allergy and Infectious Diseases (NIAID), 2015).

2.2.1 Medications

The most common treatment in the form of drugs includes intranasal corticosteroids, H1-antihistamines (Scadding *et al*., 2008) or mast cell stabilizers (Finn & Walsh, 2013). Other than that, roxatidine acetate, a histamine H2-receptor antagonist which is a common treatment for gastric and duodenal ulcers, has been shown to have an anti-allergic inflammatory effect by suppressing the release of histamine and pro-inflammatory mediators (Lee *et al*., 2017). Cromolyn sodium has been used as a mast cell stabilizer since the 1960s as therapy for asthma and allergic complications, and it

has also been shown to be effective in downregulating the release of inflammatory mediators in both *in vitro* and *in vivo* studies. (Sinniah, Yazid, & Flower, 2017). Furthermore, ketotifen fumarate which also a mast cell stabilizer, perform by forbidding the release of mediators from activated mast cells (Finn & Walsh, 2013).

2.2.2 Immunotherapy

Immunotherapies are the modern interventions, as well as feasible innovative molecular and immunological approaches for allergic diseases which categorized into allergen-specific immunotherapy (AIT) to achieve immunological tolerance (Platts-mills *et al*., 2016) against allergen and non-specific therapies such as anti Ig-E. Notably, allergen-specific immunotherapy is useful for asthma and environmental allergies. However, its efficacy in food allergies is inconclusive, and it is not recommended. Immunotherapy works by exposing people to higher doses of allergen in order to alter the immune system's response. The basic principle of allergen tolerance training is to repeatedly administer higher doses of allergenic molecules to prevent new allergen sensitivities together with activate tolerance-inducing cells and mediators.

For non-specific therapies, anti-IgE therapy is one of the recommended and ideal as an anti-allergy treatment. Omalizumab is a humanized monoclonal anti-IgE antibody that specifically targets IgE's C3 domain. On mast cells or basophils, it can prevent IgE-FcεRI receptors binding and lower circulating free IgE levels, thereby inhibiting allergic responses. Omalizumab currently used in many countries to treat severe allergic asthma. Clinical studies have also demonstrated its efficacy in atopic dermatitis, allergic urticaria, allergic rhinitis, and anaphylaxis (Landolina & Levi-

Schaffer, 2015). On the surface of mast cells, IgE will upregulate FcεRI, hence lowering the amount of free IgE reduces the number of IgE receptors and the population of mast cell. Omalizumab was shown in studies to effectively lower both early and late-phase response in asthmatic (Price, 2008). Furthermore, Omalizumab has been shown to prevent systemic reactions to allergen immunotherapy (Incorvaia *et al*., 2014).

2.3 Prevalence of Allergic Diseases

Allergic disorders have risen to become a global public health concern in the last 20 years, owing to the disease's widespread prevalence. Despite the widespread impact of allergic diseases on the global population, the prevalence of allergic diseases is on the rise. For example, 300 million people worldwide suffer from asthma, and the World Health Organization (WHO) predicts that number will rise to 400 million by 2025 (Pawankar, 2014). The increase in incidence is not solely due to environmental factors such as climate change, dietary changes, and pollution (Nicolaou & Siddique, 2005), but also to a general improvement in public awareness in response to allergic cases, and people are more likely to seek medical advice or treatment for the diseases (Jarvis & Burney, 1998). According to Pawankar *et al*. (2011), the World Allergy Organization (WAO) summarized the global burden of allergic diseases with socioeconomic impact and recommended allergy education to healthcare professionals to improve patient service. By improving the effectiveness of allergy medication toward the patient, occurrence of allergic diseases such as allergic rhinitis, atopic dermatitis, allergic asthma, and anaphylaxis can be reduced.

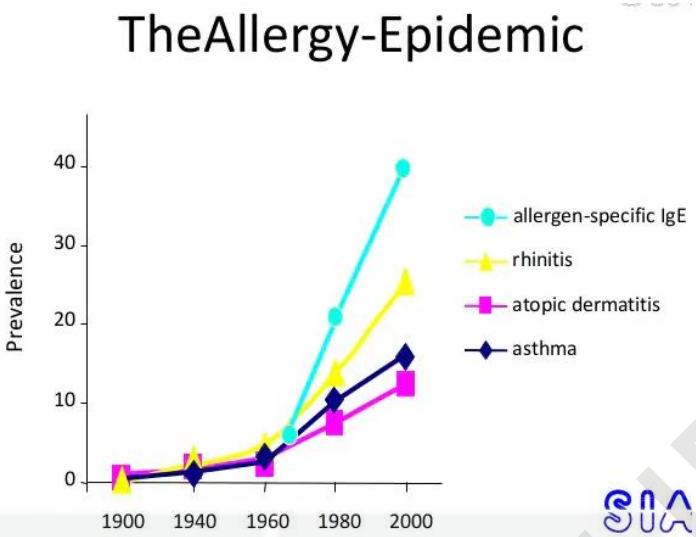

Figure 2.3: Prevalence of allergic diseases

2.4 Propolis is a Bee Product

Propolis is a resinous adhesive that contains resin, beeswax, essential oils, pollen grains, micronutrients, and trace amounts of vitamins (Chanchao, 2013). Propolis is used in beehives and act by repair damage and protect the hive from predation and microorganism invasion. Aside from that, bees that produce propolis and honey are regarded as high-value products (Muruke, 2014). The bioactive components of propolis are affected by the resin, nectar, and pollen sources available at the cultivation site. Studies have shown that propolis produced by bees has therapeutic effects due to their bioactive components. Bee propolis has been used in various countries such as Brazil, India, and China as a folk medicine to improve health and treat diseases. Because of the remarkable scientific data on the pharmacological activities of propolis, the propolis in various species of bees was investigated for many pharmacological properties, and its healing potentials in distinct aspects were recognized (Vijay, 2013).

2.4.1 Chemical Composition

Plant exudates, beeswax, and materials introduced during propolis elaboration are the three sources of compounds identified in propolis (Bankova & Castro, 2000). Generally, the composition of propolis is directly related towards the composition of bud exudates from poplars and other trees collected by honeybees. The chemical composition of propolis varies greatly, owing primarily to the diversity of plant species growing around the area where bees collect plant exudates. Propolis found in temperate zones differs remarkably from Brazilian propolis. Brazilian green propolis is found is high in artepillin C, diterpenic acids, and prenylated acetophenones. Flavonoids are only found in trace amounts. Isoflavonoids are important constituents of red propolis from Brazil and are acquired by bees from Dalbergia species which are also found in Cuba and Mexico. Furthermore, the variation of propolis components from different regions of Brazil categorized according to its geographical origin, chemical composition, and botanical source (Olivieri da Silva Frozza *et al*., 2013).

Table 2.4.1: Plant Origin and Major Constituents of the Most Common Propolis Types

Propolis Type	Geographic Origin	Botanical Sources	Major Constituents
Poplar	Europe, North America, China, New Zealand	Populus species of section *Aigeiros*, most often *P. nigra* L.	Flavones and flavanones (pinocembrin, pinobanksin, pinobanksin-3-O-acetate, chrysin, galangin), cinnamic acids (Notably caffeic acid) and their benzyl-, phenethyl-, and prenyl esters

Green (alecrim) Brazilian	Brazil	*Baccharis* species, predominantly B. *dracunculifolia* DC	Prenylated p-coumaric acids, diterpenic acids, prenylated acetophenones
Birch	Russia	*Betula verrucosa* Ehrh.	Flavones and flavonols (different from the poplar type): acacetin, apigenin, ermanin, rhamnocitrin, kaempferid, >-acetoxybetulenol
Red propolis	Cuba, Brazil, Mexico	*D. ecastophyllum* and other *Dalbergia* species	Isoflflavonoids (isoflavans, terocarpans)
Mediterranean	Sicily, Greece, Crete, Malta	*Cupressaceae* (Species unidentifified, possibly *C. sempervirens*) and Pinaceae	Diterpenes (mainly acids of labdane type), anthraquinones
‘‘Clusia’’	Cuba, Venezuela	*Clusia* species including *C. major, C. minor*	Polyprenylated benzophenones
‘‘Pacific’’	Pacifific region (Okinawa, Taiwan, Indonesia)	*Macaranga tanarius*	C-prenyl-flavanones

2.4.2 Properties and Application

Propolis have been used for a very long time and well reported as a medication in the 1600s. The medicinal preparations containing propolis has been used for mouth and throat infections treatment, as well as dental caries. Before bandages were available, it

was frequently used to shield raw skin and treat skin wounds (Walgrave, Warshaw, & Glesne, 2005). The balsam in propolis has antifungal, antibacterial, and antiviral properties, as well as anti-inflammatory, antioxidant, local anesthetic, antitumor (cytotoxic) (Chan, Cheung & Sze, 2013), immunomodulatory (Sforcin, 2007), spasmolytic, and other biological properties skin (Walgrave, Warshaw, & Glesne, 2005). The organic substances in propolis extracted with ethanol are responsible for these effects. The chemical composition of the propolis is determined by the composition of the botanical source, thus its pharmacological activity is strongly linked to the site of collection.

Flavonoids and other polyphenolics, primarily substituted cinnamic acids and their esters, are the chemical compositions of European type (poplar) propolis that are responsible for its beneficial biological activities (Popova *et al*., 2007), particularly its antibacterial and antifungal properties. Diterpenes and prenylated p-coumaric acids also known as substituted cinnamic acids are responsible for these activities in Brazilian propolis (Bankova, 2005). It has been used in dermatology to treat burns, leg ulcers, psoriasis, atopic dermatitis, warts, herpes labialis, and genitals, as well as wound healing and tissue regeneration (Gregory *et al*., 2002; Vynograd, Vynograd, & Sosnowski, 2000). Many propolis producers claim that their products are ‘‘all natural," which consumers frequently associate with safety.

CHAPTER 3

METHODOLOGY

3.1 Search Strategy

Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) guidelines was used to conduct this systematic review (Liberati *et al*, 2009). All articles related to the effects of propolis were obtained from three electronic databases: PubMed, Scopus and ScienceDirect. The keywords used for database searching in this study were "propolis" AND "allergy". The studies were screened according to the eligibility criteria by the first review authors (Nurain Irdayani Kamise, Hui Ming Ong, Fahmida Islam, Poi Yee Aw Yong). Eligible studies were then rechecked by the second review author (Kong Yen Liew). The last search for relevant articles in the databases was performed on 28 June 2021.

3.2 Eligibility Criteria

The studies were selected if they met the eligibility criteria (1) studies of propolis on allergic diseases, (2) the effects of propolis on allergic diseases in experimental studies such as *in vitro, in vivo* and clinical study on allergy, and (3) studies published in English language. The articles will be excluded if they are (1) studies not related to propolis and allergic diseases, (2) case report and reviews (3) observational studies (cross-sectional, case-control and cohort), and (4) studies published in other than English Language.

3.3 Study Selection and Data Extraction

Articles obtained from the three electronic databases were sorted out according to their publication dates using EndNote software. Duplicates were removed by using EndNote software and the remaining articles were screened independently according to the eligibility criteria. The articles were first accessed by reviewing the abstracts, which was performed independently by four review authors in an unblinded but standardised manner. Any disagreements between the review authors were sorted out through discussions. The eligible studies were then subjected to full-text screening to further evaluate the suitability of the articles. Articles that did not fulfil the eligibility criteria were excluded. The full text of selected articles was reviewed for relevant data studying the effects of propolis on allergic diseases involving the type of allergic models, type of propolis used, cell or animal model, treatment method and the experimental outcome. All first reviewers performed the data extraction using the same extraction form. The second review author checked for accuracy and contact the authors if the data was incomplete, or any mistakes present.

CHAPTER 4

RESULT

4.1 Literature Search

The search by using keywords “propolis” AND “allergy” from three electronic databases produced 1149 records whereby out of which 131 were duplicates. By screening through title and abstracts of these 1018 records, we examined their potential relevance to be reviewed. All the potentially relevant articles matching these criteria were selected regardless of publication year. In total, there are 918 records to be excluded and the remaining 100 records were retrieved. From the 100 records, the full text screening was performed to assess on their potential to be included in the review. We finally excluded another 88 records due to not meeting the eligibility criteria. The reasons for exclusion include allergic effects of various substances but not specifically propolis (12), other than *in vitro, in vivo,* and clinical studies (cross sectional and cohort studies) (21), case report or reviews (22), full text cannot be retrieved (7), bioactive compounds that is not specifically isolated from propolis (16) and other than English language (Poland, Russia, France) (10 studies). All the 12 eligible articles were retrieved throughout the study selection process and all 12 articles consisting of three types of studies including *in vitro* (6 studies), *in vivo* (7 studies) and clinical (2 studies) and data such as type of allergic diseases, study design, treatment concentration/dosage and experimental outcome were then extracted from the 12 articles as shown in figure 4.1 and table 4.1.

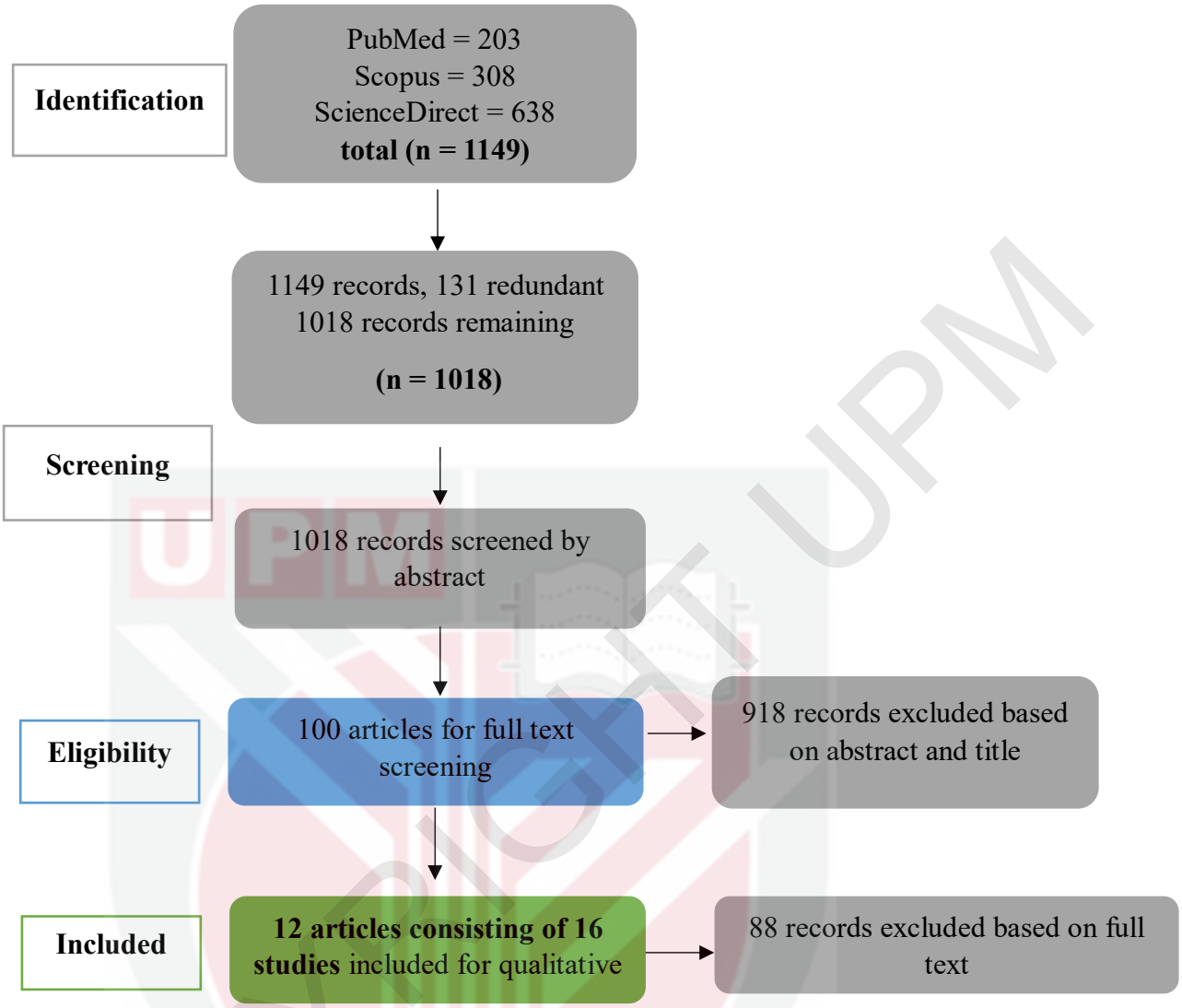

Figure 4.1: Study selection process in flowchart

Table 4.1: 12 articles consisting of 16 studies (*in vitro, in vivo,* clinical trial)

Category	Number of studies
Type of studies	
In vitro	7
In vivo	7
Clinical Trial	2

4.2 Data Extraction and Organisation

The 100 papers were further screened on their full text articles and only 12 papers were found relevant for this systematic review. The studies were as categorized as *in vitro* studies, *in vivo* studies, and clinical studies in which data such as type of allergic diseases, study design, treatment concentration or dosage and experimental outcome were then extracted from the 12 articles as shown in Table 4.2.

Table 4.2: *In vitro, in vivo,* and clinical studies were categorized based on type of allergic diseases, study design, treatment concentration or dosage and experimental outcome.

***In Vitro* Studies**							
Type of allergic disease	Type of Propolis origin	Cell model; Inducer	Treatment method	Propolis Treatment Grouping; Effective concentration	Experimental outcome	Study conclusion (anti-allergic effects of Propolis & their components)	References

Allergen-induced inflammation	Ethanolic extract of Brazilian Green Propolis	Peripheral leukocytes; Cry j1	Pre-treatment	3, 10, 30 and 100 µg ml; 10 µg/ml (cys-LTs release) & 100 µg/ml (histamine release)	↓ cys-LTs ↓ histamine	Yes	Tani et al. (2009)
		Peripheral blood mononuclear cells (PBMCs); Cry j1	Pre-treatment	3, 10, 30 µg ml; 30 µg/ml	↓ IL-5 and IL-13 release		

Aspergillus-induced asthma	Propolis ethanolic extract (Iran)	Mouse lung epithelial cells (TC-1 JHU-1), A. fumigatus conidia	Simultaneous treatment with propolis and A. candida	25mg/mL; 25mg/mL	↓ IL-12, IFNγ, and IL-13 ↓ IL-17 level	Yes	Khosravi et al., (2018)
Basophil-dependent allergic inflammation	Standardized Japanese propolis	Bone marrow cells (BMBs), anti DNP-IgE & DNP_{23}-HSA	Pre-treatment	0-100 mg/ml; 100 mg/ml	↓ levels of IL-4, IL-6, and IL-13 ⇔ IL-9 production ↓ FcεRI signaling molecules (Lyn, Akt, ERK) phosphorylation levels	Yes	Kashiwakura et al., (2020)

Mast cell degranulation	Brazillian propolis water & ethanolic extract (A, E, G, G2), Chinese propolis water & ethanolic extract (C), and Chrysin	Rat basophilic leukemia (RBL-2H3) cells; DNP-IgE & DNP-BSA	Pre-treatment	- 0%, 0.01%, 0.1%, 1%; 0.1% (C) & 10 µg ml (Chrysin)	(C) ↓ degranulation > (A, E, G, G2) ↓ IL-4 and MCP-1 production by Chrysin	Yes	Nakamura et al., (2010)
	Propolis ethanolic extract (Brazil)	Rat peritoneal mast cells; Compound	Pre-treatment	3, 10 & 30 µg/ml; 10 & 30 µg/ml	↓ histamine release	Yes	Shinmei et al., (2009)

		48/80 & antigen					
	Propolis ethanolic extract (Brazil)	Rat peritoneal mast cells; Compound 48/80	Pre-treatment	3, 10 & 30 µg/ml; 10 & 30 µg/ml	↓ histamine release	Yes	Shinmei et al., (2010)
	Ethanolic extract of Brazilian Green Propolis	RBL-2H3. Ionomycin	Pre-treatment	25, 75 and 100 µg/mL; 25, 75 and 100 µg/mL	↓ ionomycin-induced upregulation of IL-9 expression	Yes	Shaha et al., (2018)
In Vivo Studies							

Type of allergic disease	Type of Propolis origin	Animal model; Inducer	Mode of Treatment; Route of administration	Propolis Treatment Grouping: Effective dose	Experimental outcome	Study conclusion (anti-allergic effects of Propolis & their components)	References
Allergic asthma	Standardized green propolis extract EPP-AF® (Brazil)	Female C57BL/6 mice (6–8 weeks old); Ovalbumin (OVA)	Post-treatment; Oral	150 mg/kg (17/22 days, daily); 150 mg/kg	In BALF: ↓ eosinophils ↓ IL-5 & IL-13 ↓ eosinophils ↓ il13 gene expression in the lungs ↓ mucus production & cellular infiltration	Yes	Piñeros et al., (2020)

	Propolis water extract (Taiwan)	Female BALB/c mice (6–8 weeks old); OVA	Post-treatment; Oral	65mg/kg (low dose) & 325mg/kg (high dose); 65mg/kg	↓significantly of the OVA-specific IgE titer ↓ level of OVA specific IgG_1 titer (Th2 response) ↑ level of OVA specific IgG_2 (Th1 response) ↓ methacholine-induced airway hyperresponsiveness (AHR)	Yes	Sy et al., (2006)

					↓airway resistance Cytokine release in ConA-stimulated splenocytes: ↑ level of IFN-γ (Th1 response ↓ IL-10 (Th2 response) Cytokine release in OVA-stimulated cultured splenocytes:		

					↓ level of IFN-γ, IL-6, and IL-10 ↓IL-5 in BALF		
Allergic rhinitis	Propolis ethanolic extract (Brazil)	1.Male BALB/c mice (5 weeks old); OVA, antigen & histamine	Co-treatment; Oral	200, 500 & 1000 mg/kg, (Daily for 4 weeks from day 25 – day 53); 1000 mg/kg (repeated administration)	Sensitized: ↓ sneezing & nasal rubbing ⇔ total serum IgE level Non-sensitized: no sig. inhibition	Yes	Shinmei et al., (2009)

	Propolis ethanolic extract (Turkey)	Male Sprague-Dawley rats (≥6 weeks); OVA	Co-treatment; Intranasal & oral	200 mg/kg (21 days after 1st); 200 mg/kg	Intranasal (topical route): ↓ chondrocytes, vascular congestion,eosinophils and symptom scores 1, 3, and 4 ⇔ ciliary loss, inflammation, increase in goblet cells, vascular proliferation and symptom scores 2 and 5	Yes	Yasar et al., (2016)

					Oral: ↓ Ciliary loss, inflammation, increase in goblet cells, vascular proliferation, eosinophils and symptom scores 1, 2, 3,4 ⇔ chondrocytes, vascular congestion, and symptom score 5 Mometasone furoate and systemic		

					Propolis: ↓ inflammation, goblet cells and symptom scores 2, 3, and 4 Ketotifen and systemic propolis: ↓ inflammation, goblet cells, chondrocytes, and symptom scores 2 and 3		

					Intranasal (topical route) or systemic (oral route) propolis: ↓ inflammation, increase in goblet cells, chondrocytes, vascular proliferation, and symptom scores 1, 2 and 3 in the systemic propolis group		
	Ethanolic extract Brazilian Green	Male brown Norway rats (6 weeks old);	Co-treatment; Oral	40 mg/kg (low dose) & 80 mg/kg (high dose) Daily (day 1-22); 40	↓ sneezing ↓ nasal score (watery rhinnorhea, swelling and redness)	Yes	Shaha et al., (2018)

	Propolis (BGPP)	solution toluene 2,4-diissocyanate (TDI)		mg/kg (nasal symptoms) & 80 mg/kg (H1R and Th2 genes suppression)	↓ H1R mRNA expression levels ↓ IL-4, IL-5 and IL-9 mRNA expression levels		
Chronic allergic inflammation (CAI) & Food Allergy (FA)	Standardized Japanese propolis	Balb/c mice; anti-DNP IgE, DNP11-OVA	Pre-treatment; Intragastric	**CAI** 0.3 mg propolis (day 1); 0.3 mg **FA** 0.3 mg propolis (twice a week from day 27); 0.3 mg	**CAI** ↓ IgE-CAI through ear thickness ↓ leukocytes infiltration in ears ↓*Mcpt8* expression in the ear skin	Yes	Kashiwakura et al., (2020)

					↓ FcεRI-mediated basophil activation **FA** ↓ occurrence of diarrhea ↓ mMCP-1 serum down-regulated IL-4 expression in jejuna		
Allergic atopic dermatitis	Propolis ethanolic extract (Brazil)	Female ICR mice (6–10 weeks old); Compound	Co-treatment; Topical	3mg/site (15, 30 & 60 min); compound 48/80-induced scratching	↓ compound 48/80-induced and histamine-induced scratching behaviour	Yes	Shinmei et al., (2010)

		48/80, histamine		(0 min = 3mg), (15 min = 3mg), (30 min = 1 & 3mg, (60 min = 1 mg) ; Histamine-induced scratching (o min = 3mg); Vascular permeability (60 min = 3mg)	No sig. inhibition histamine-induced scratching behaviour at 15, 30 and 60 min. ↓ vascular permeability		
Clinical Studies							

Type of allergic disease	Type of Propolis origin	Number of patients	Age and gender	Grouping and treatment method	Propolis Treatment frequency	Parameters; Experimental Outcome	Study outcome (improvement of disease symptoms)
Asthma	Aqueous extract of propolis (Denmark, China, Uruguay, Brazil)	46	Age: 19 to 52 years old Males: 36 Females: 10	1) Placebo + Theophylline: n=24 2) Propolis + Theophylline: n=22 Age, sex, and asthma severity	Once a day for 2 months	↓ Nocturnal attacks: coughing fits, tightness in the chest, wheezing and shortness of breath Inflammatory mediators:	Yes

				were nearly evenly distributed.		↑ > 3-fold of IL-10 ↓ TNF-a, ICAM-1, IL-6 and IL-8 by 52, 65, 44 and 30%, respectively ↓ Serum levels of PGE2, PGF2a and LTs by 36, 39 and 28% of	

						their initial values.	
Allergic sensitization and eczema	Brazillian green propolis extract	80	Mother's age: >20 years Infant's age: 2-8 months Gender (ND)	1) Allocated to propolis group: n= 40 (1 did not received intervention) 2) Allocated to placebo group: n= 40	4 years and 3 months (June 2015-September 2018)	⇔ Atopic sensitization: levels of total IgE and antigen-specific IgE ⇔ Improvement of non-specific symptoms: eczema, in mothers and their offspring	Neither yes nor no

4.2 Description of the Studies

For *in vivo* studies, the sample size of these experiments ranged from three to thirty mice or rats involving both gender and regardless of their ages. A systematic review was carried out to summarize a total of seven *in vivo* studies regarding the effects of propolis on allergic diseases, in which the studies were aimed to assess the effects of propolis on allergic rhinitis (3 studies), asthma (2 studies), atopic dermatitis (1 study), chronic allergic inflammation and food allergy (1 study). In *in vitro* studies, there were in total seven studies reviewed to investigate the effects of propolis on allergic diseases with different cellular models. All studies consist of various allergic models including allergen-induced inflammation (1 study), aspergillus-induced asthma (1 study), basophil-dependent allergic inflammation (1 study) and mast cell degranulation (4 study). For clinical studies, there are two studies investigate the anti-allergic effects of propolis on asthma and allergic sensitization with eczema via placebo-controlled trial.

4.4 Allergic Response Assessed and Outcome Measured (*In Vitro*)

4.4.1 *Aspergillus*-induced Asthma

A study conducted by Khosravi *et al.* (2018) investigated the effect of propolis collected from the hives of *Apis mellifera L.* bees of southern Iran on cytokine modulation. The study reported that *Aspergillus fumigatus* candida stimulated mouse lung epithelial cells (TC-1 JHU-1) to produce IL-12, IL-13, IFNγ, and IL-17 cytokines, with the latter the cytokines being significantly upregulated after 6 hours of exposure. Simultaneously treated with propolis (25µg/mL) and exposed to *A. fumigatus* candida showed a significant downregulation of Th2 cytokines, IL-13, and IL-17 and an increase in Th1 cytokines, IL-2 and IFNγ. Results obtained from this study Suggest

the potential use of propolis to attenuatae Th2 responses in allergic disorders caused by *Aspergillus spp.* including asthma, allergic bronchopulmonary aspergillosis, and allergic *Aspergillus* sinusitis.

4.4.2 Allergen-induced Inflammation

A preclinical study by Tani *et al.* (2008) has shown that Brazilian Green Propolis (BGPP) in effective against allergen-induced inflammation by inhibiting the pro-inflammatory cytokines and histamine release. In this study, thirty mililiters of blood were collected from ten allergic patients with positive specific IgE to Cry j1/2 antigen that have clinical history of pollinosis. Subsequently, peripheral leukocytes and peripheral blood mononuclear cells (PBMCs) were prepared from the allergic patients. The authors found that a pre-treatment of BGPP ethanol extract was able to inhibit the release of cys-LTs and histamine in Cry j1-induced peripheral leukocytes at 5.8 µg/mL and 100 µg/mL of BGPP respectively. Besides that, the release of IL-5 and IL-13 was also slightly inhibited by BGPP ethanol extract in Cry j1-induced PBMCs. This study shows the potential of BGPP in attenuating allergic inflammation in different cellular models.

4.4.3 Basophil-dependent Allergic Inflammation

Kashiwakura *et al.* (2020) conducted a study to investigate the inhibitory effect of propolis on basophil activation, cytokine production and its signal transduction. The study revealed that bone marrow cells from Balb/c mice (BMBs) treated with 100µg/mL propolis (obtained from Yamada Bee, Okayama, Japan) for 6 hours in the presence of 0.5µg/ml anti-DNP IgE mAb did not inhibit FcεRI expression.

Furthermore, it was reported that anti-DNP IgE mAb sensitized BMBs, stimulated with DNP_{23}-HAS (0-100ng/mL) in the presence of propolis (1-100µg/mL) inhibited the release of inflammatory cytokines, IL-4, IL-6 and IL-13, with 100µg/mL propolis treatment showing significant inhibition. However, it had no effect on the spontaneous production of IL-9 by BMBs. The inhibitory effect of propolis was brought about by its ability to inhibit the phosphorylation of FcεRI signaling molecules Lyn, Akt, and ERK. Pretreatment with 100µg/mL of propolis for 6 hours significantly reduced the phosphorylation levels of all three signaling molecules in sensitized BMBs, stimulated with 100ng/mL Ag.

4.4.4 Mast Cell Degranulation

Nakamura *et al.* (2010) carried out a study to compare the inhibitory potential of water (WEP) and ethanol (EEP) extracts of propolis obtained from different origins (Brazil and China) on mast cell degranulation and cytokine production. Pre-treating rat basophilic leukemia (RBL-2H3) cells with propolis extracts 30 minutes before Ag stimulation followed by evaluation of β-hexosaminidase release revealed that both WEP and EEP obtained from China were stronger inhibitors of mast cell degranulation compared to those of Brazilian propolis. Results further showed that regardless of the origin, EEP exhibited greater inhibitory potential than WEP. Ethanol extracts of Chinese propolis underwent further analysis where chrysin, kaempferol and its derivative were discovered as the active compounds contributing to the anti-allergic property of propolis. In antigen-stimulated cells, chrysin had shown to inhibit IL-4 and MCP-1 in a dose-dependent manner, with 10µg/mL exhibiting the greatest inhibition. HPLC quantification later revealed that Brazilian propolis contained smaller quantities

of chrysin and kaempferol compared to Chinese propolis, thereby explaining their differences in anti-allergic potential.

Similarly, another study by Shaha *et al*. (2018) has also demonstrated that BGPP are able to attenuate the allergic diseases in two *in vitro* models, namely HeLa and RBL-2H3 cells. Histamine and PMA were added into HeLa cells as the inducer after the cells were pre-treated with BGPP (25 – 75 µg/mL) or RJ (25 – 200 µg/mL). Based on their findings, the expression of H1 Receptor (H1R) gene and PMA-induced phosphorylation of PKCδ was decreased in dose dependent manner with the presence of BGPP. The compounds were found to be effective against allergic rhinitis in ionomycin-induced RBL-2H3 cells as well. Inhibition of IL-9 expression was reported in BGPP-pretreated group (25 – 100 µg/mL) as compared to the induced group in the study. Taken together, these two studies have shown the potential of BGPP in attenuating release of granules and chemical mediators during allergic response.

In an allergic rhinitis model by Shinmei *et al*. (2009), Brazilian propolis produced significant and dose-related inhibition against histamine release from antigen- and compound 48/80-induced rat mast cells (at concentration > 10 µg/ml). Therefore, the authors concluded that the inhibitory action of Brazilian propolis ethanolic extract on sneezing and nasal rubbing allergic rhinitis model were perhaps caused by its ability to interrupt the release of inflammatory mediators such as histamine from mast cells. Another study by Shinmei *et al*. (2010) demonstrated that Brazilian propolis (3, 10, 30 µg/ml) showed dose-dependent inhibition on compound 48/80-induced histamine release from rat peritoneal mast cells. The inhibitory effects of Brazilian propolis were greater especially at the higher concentrations of 10 and 30 µg/ml. Combining the *in vivo* and *in vitro* results in this study, the authors speculated

that Brazilian propolis inhibits topical-induced scratching behaviours through local anaesthetic and systemic routes by suppressing mast cell-related mechanism.

4.5 Allergic Response Assessed and Outcome Measured (*In Vivo*)

4.5.1 Allergic Rhinitis (AR)

The beneficial effect of propolis against allergic rhinitis was reflected in *in vivo* models. According to Shaha *et al*. (2018), BGPP were effectively inhibiting the allergic symptoms and expression of H1R and cytokines in toluene 2,4-diisocyanate (TDI)-induced Brown Norway rats. Co-treatment of 40 and 80 mg/kg of BGPP was orally administered to the sensitized rats in order to evaluate the allergic symptoms based on sneezing and nasal score (watery rhinorrhoea, swelling and redness). All the allergic symptoms were significantly relieved with the propolis treatment. Besides, the mRNA expression levels of H1R and cytokines include IL4, IL-5 and IL-9 were inhibited in the nasal mucosa of TDI-induced rats. Hence, the authors highlighted the anti-allergic effect of BGPP via the decrease in the amount of H1R protein and cytokines in the nasal mucosa.

Besides, another reported study by Shinmei *et al*. (2009) demonstrated the activity of Brazilian propolis ethanolic extract (55 wt.%/vol.%) in murine allergic rhinitis model by observing its possible inhibitory effect on sneezing and nasal rubbing behaviors, along with elucidating its mechanisms on total IgE level and histamine release. Brazilian propolis (200, 500 & 1000 mg/kg, p.o.) was administered daily to OVA-sensitized male BALB/c mice for 4 weeks (from day 25 to day 53), together with daily intranasal sensitization by antigen (from day 18 to day 53). The sneezing and nasal rubbing activities were observed on day 32, 39, 46 and 53. Interestingly,

Brazilian propolis was found to require repeated administration for a gradual inhibitory effect on sneezing and nasal rubbing as compared to single administration, in which significant inhibition was observed at 1000 mg/kg after repetitive treatment for 2 weeks in antigen-induced animals. However, repeated administration of Brazilian propolis at 1000 mg/kg did not cause significant difference in total IgE in mouse serum as compared to control animals. For histamine-induced nasal rubbing and sneezing model, Brazilian propolis failed to exhibit significant inhibition at a dose of 1000 mg/kg in non-sensitized mice. In addition, Brazilian propolis produced significant and dose-related inhibition against histamine release from antigen- and compound 48/80-induced rat mast cells (at concentration > 10 µg/ml). Therefore, the authors concluded that the inhibitory action of Brazilian propolis ethanolic extract on sneezing and nasal rubbing allergic rhinitis model were perhaps caused by its ability to interrupt the release of inflammatory mediators such as histamine from mast cells.

Next, similar study by Yasar *et al.* (2016) carried out a study to investigate the potential of propolis as an anti-allergic agent in allergic rhinitis by using OVA-induced rat model. This study evaluates any changes in mucosal histology and rated symptoms such as nasal irritation, sneezing, and nasal secretion by scoring. In this study, prior to propolis treatment the Sprague-Dawley rats were sensitized with 0.3 mg of OVA and 30 mg aluminium hydroxide daily via intraperitoneal administration at the first phase of study and then 10 µL of OVA were applied at each nostril for the second phase. Both propolis treatment group via intranasal and oral route shows significant improvement in histological and symptoms (nasal irritation, sneezing and nasal secretion) score assessment as compared to the control group. Rats that received intranasal propolis shows decreased level of eosinophils, chondrocytes, vascular congestion, and symptom scores 1,3 and 4 whereas rats that received oral propolis

shows decreased level of, inflammation, ciliary loss, increase in goblet cells, eosinophils, vascular proliferation, and symptom scores 1,2,3 and 4 compared to control group. Although route of administration of propolis may cause different effect on allergic rhinitis, this study demonstrated that propolis has anti-allergic activity on allergic symptoms and mucosal histology.

4.5.2 Atopic Dermatitis (AD)

An *in vivo* study carried out by Shinmei *et al*. (2010) with the aim to investigate the anti-itching effect of Brazilian propolis ethanolic extract (55 wt.%/vol.%, topical application) in compound 48/80- and histamine-induced scratching models. Brazilian propolis (0.3, 1 & 3 mg/site) was applied topically on female ICR mice for compound 48/80 (10 μg/site) and histamine (100 nmol/site)-induced scratching behavior assessments. The highest dose of Brazilian propolis (3 mg/site) significantly inhibited compound 48/80- and histamine-induced scratching behaviour at 0 min. The remaining doses of Brazilian propolis also significantly suppressed the scratching behaviors at 15, 30 and 60 min after drug application. However, similar inhibitory pattern was not observed for Brazilian propolis at any doses in histamine-induced scratching model from 15 – 60 min after drug application. The investigation also showed that topical application of propolis (3 mg/site, after 60 min) demonstrated significant suppression on compound 48/80 (0.5 μg/site)-generated increased vascular permeability of the animal skin. Furthermore, Brazilian propolis (3, 10, 30 μg/ml) showed dose-dependent inhibition on compound 48/80-induced histamine release from rat peritoneal mast cells, especially at the higher concentrations of 10 and 30 μg/ml. Based on these results, it is speculated by the authors that Brazilian propolis

inhibits topical-induced scratching behaviors through local anesthetic and systemic routes by suppressing mast cell-related mechanism.

4.5.3 Asthma

A study by Sy *et al.* (2006) conducted an *in vivo* investigation of propolis activities via the serum levels of ovalbumin (OVA)-specific IgE titer and cytokines profiles by using an ovalbumin-induced asthma model. The serum levels and cytokines were analyzed specifically from the bronchoalveolar lavage fluids (BALF) and cultured splenocytes. In this study, six BALB/c mice were used in each treatment group of low-dose propolis and high-dose propolis water extracts via tube feeding. All mice undergo immunization procedure prior to treatment via intraperitoneal injection at 10 and 12 weeks old with 20 and 50µg/ml OVA in phosphate-buffered saline (PBS) with aluminium hydroxide respectively. It was reported that low-dose propolis group reduce the OVA-specific IgE titer significantly compared to positive control group. Besides, the study shows low level of OVA-specific IgG_1 (Th2 response) titer in low-dose propolis group whereas high level of IgG_2 (Th1 response) titer in high-dose propolis group. These results suggest that the propolis may have complex active components that may not only act as Th1 response stimulators but also Th2 response inhibitors. The Th2 response inhibitory effect also seen via decreased level of IL-10 in cultured splenocytes and IL-5 secretion in BALF. Other than that, propolis also appeared to inhibit airway hyperresponsiveness in response to 25mg/ml methacholine in the OVA-induced asthma mice with low dose of propolis. Therefore, the results of this study suggested that propolis extracts may have the potential to be therapeutic agent for asthma.

A study reported by Piñeros *et al.* (2020) also investigated the anti-inflammatory effect of standardized Brazilian green propolis extract via an ovalbumin-induced allergic asthma model in female C57BL/6 mice. The mice were orally treated with 150 mg/kg of propolis daily after last ovalbumin (OVA) sensitization for 17 days. BALF was obtained from mice three days after second challenge. The anti-inflammatory potential of propolis was characterized by the reduced total cell number, eosinophils and IL-5 level in the BALF of propolis-treated allergic mice. The lower il13 gene expression of lungs, reduced IL-13 and mucus production and decreased cellular infiltration by pre-treatment of propolis also showed the attenuation of Th2 inflammation in allergic mice. Apart from this, propolis treatment also promoted differentiation of lung PMN-MDSC and CD4+Foxp3+ regulatory T cells in *in vitro* setting, as well as for *in vivo* frequency. Thus, the authors suggested that Brazilian green propolis-mediated immunomodulatory role in asthmatic mice is associated with regulatory T cells and myeloid-derived suppressor cells.

4.5.4 Chronic Allergic Inflammation (CAI)

A study led by Kashiwakura *et al.* (2020) on BALB/c mice found that intragastric administration of 0.3mg Japanese propolis followed by intravenous injection of 50µg anti-DNP IgE mAb and intradermal challenge with Ag 24 hours after IgE injection significantly suppressed the IgE-CAI response as measured by the reduction in ear thickness when compared to untreated mice. It was further revealed that propolis treatment reduced the infiltration of leukocytes in mice ear and significantly suppressed *Mcpt8* expression levels. Results from this study showed that attenuation

of the IgE-CAI was brought about by propolis inhibiting basophil migration without affecting its population.

4.5.5 Food allergy

A study also conducted by Kashiwakura *et al.* (2020) examining Ag-induced intestinal anaphylaxis showed that treatment with Japanese propolis significantly reduced diarrheal symptoms in OVA-sensitized Balb/c mice when compared to untreated and PBS-treated mice. It was also found that propolis treatment greatly reduced serum mMCP-1 levels, an indicator for the severity of intestinal inflammation and suppressed IL-4 expression levels. The total levels of IgE, IgG1 and OVA-specific IgE and IgG1 were comparable to the untreated and PBS-treated mice. The study also investigated E-NPP3 expression levels in jejuna since its upregulation in basophils is linked to Ag-induced intestinal anaphylaxis. The E-NPP3 levels in propolis treated food allergy mice was found to be comparable to the untreated and PBS-treated mice.

4.6 Allergic Response Assessed and Outcome Measured (Clinical Studies)

4.6.1 Asthma

Khayyal *et al*. (2002) had demonstrated a clinical trial on asthmatic patients by given the aqueous extract of propolis (AEP) as an adjuvant theraphy in the form of milk-based product as a nutritional supplement daily for 2 months. This study assesses the improvement on clinical status of forty-six human volunteer patients (36 males and 10 females), age ranging from 19 to 52 years who had experienced mild to moderate asthma for the last 2 to 5 years. Propolis group (silver sachets) with theophylline were

compared with the placebo group (white sachets) with theophylline in terms of pulmonary ventilator functions and inflammatory mediators release. The results show a significant reduced in frequency of nocturnal attacks such as coughing fits, wheezing and shortness of breath and improvement in pulmonary ventilatory functions of the propolis group compared to placebo. Propolis group also reported significant decrease in inflammatory mediators level such as TNF-a, ICAM-1, IL-6 and IL-8 and serum levels of prostaglandin E_2 (PGE2) and F_2 (PGF2) and leukotrienes (LTs). Thus, the comparison in this study shows that AEP may be adjuvant therapy in asthmatic patients.

4.6.2 Allergic Sensitization and Eczema

A study reported by Igarashi *et al*. (2019) investigated placebo-controlled trial to assess the efficacy of propolis supplementation on the risk of atopic sensitization in lactating women and their offspring. Eighty pairs of mothers and their offspring were participated and divided into propolis group and placebo group. They were randomly assigned with propolis supplement in the form of 3 capsules given daily while placebo capsule contains safflower oil. The primary outcome which is the atopic sensitization shows that no significant different in serum levels of total IgE and antigen specific IgE of the offspring between the groups. This study also evaluates the improvement of non-specific symptoms such as eczema in both mother and offspring. Therefore, propolis supplementation neither improved nor worsened the subjective symptoms. This study shows that propolis has no effects on the adverse events in lactating mother and their offspring and both may take propolis safely.

CHAPTER 5

DISCUSSION

5.1 Interpretation of Findings

5.1.1 *In Vitro* and *In Vivo* Studies

Mast cells are one of the most important effector cells in allergic reactions. Mast cell activation occurs when the mast cell's IgE-FcRI receptor surface is exposed to the same allergen again. The signalling pathway of mast cell degranulation is well understood, with activation of mast cells generally initiating the stimulation of protein kinase C, mitogen-activated protein kinases (MAPKs), and nuclear factor-B (NF-B) (Bradding *et al*., 1993). Then, the degranulation of mast cells is followed by subsequent release of pro-inflammatory mediators such as histamine and cytokines (Metcalfe, Kaliner, & Donlon, 1981; Miyajima *et al*., 1997). In the acute stage of hypersensitivity, histamine is widely acknowledged as the most potent vasoactive mediator (Petersen, Mosbech, & Skov, 1996). Along with a variety of mast cell-derived inflammatory mediators, histamine has a strong broncho-constricting action, which plays an important role in the pathogenesis of allergic responses (Nader, 2013). A study by Tani *et al*. (2009) and Nakamura *et al.* (2010) shows that Brazilian propolis able to inhibit the release of allergic mediators such as histamine and cytokines. In *in vivo* and *in vitro* anti-allergic models, caffeic acid phenethyl esters (CAPE) significantly reduced histamine-induced permeability and histamine release from rat peritoneal mast cells (RPMCs) (Nader, 2013). These findings are in line with the anti-allergic effects of ethanolic extract of Brazilian propolis, which may contain small amount of CAPE, on mast cells (Berreta *et al.*, 2016).

Although propolis extracts have shown anti-allergic effects, they may be relying on their chemical composition, which varies greatly due to bee species, seasonality, geographic and climatic factors, plant species, collector type, extraction method, extraction solvent type, and solvent ratio (Uran *et al*., 2021; Toreti *et al*., 2013). Results obtained from the study conducted by Nakumara *et al*. (2010) established that the components of propolis varies based on its geographic location which in turn affects their anti-allergic potential. WEP and EEP obtained from China showed a greater inhibitory effect on antigen-induced degranulation compared to those from Brazil. Similar results were obtained from a previous study investigating the effects of Brazilian/Chinese propolis on β-hexosaminidase release from rat peritoneal mast cells induced by Concanavalin A (ConA) or Compound 48/80 (Miyataka *et al*. 1998).

In this study by Nakamura *et al*. (2010), chrysin, kaempferol and its derivatives were identified as the key anti-allergic components in ethanol extracts of Chinese propolis. Several studies have shown that chrysin reduces eosinophil counts in the blood, total IgE serum levels, inflammatory cell infiltration, and airway hyperresponsiveness in murine asthma models. Chrysin inhibits the expression of pro-inflammatory cytokines (IL-1, TNF, IL-6, and IL-4) in mast cells by altering caspase 1 and NF-B. (Bae *et al*., 2011). Likewise, results obtained from this study also showed that chrysin inhibited cytokine IL-4 release from mast cells following antigen stimulation. Similar to the mechanism of action of chrysin, kaempferol too inhibits degranulation (Shim *et al*., 2009) and cytokine production (Kempuraj *et al*., 2005). Therefore, chrysin and kaempferol may be the major anti-allergic components in EEP of Chinese propolis which inhibited degranulation of antigen-stimulated RBL-2H3 cells. Besides that, the finding that EEP of Chinese propolis inhibited mast cell

degranulation more significant than EEP Brazilian propolis may be attributed to the larger quantities of chrysin and kaempferol in Chinese propolis compared to Brazilian propolis as revealed by the quantification of flavonoids by HPLC. Since the bioactivity of propolis may vary significantly according to its geographic and climatic zone, it is essential to characterize each type of propolis e.g., by determining the quantities of active constituents commonly present in propolis extracts.

Another observation made from this study was, regardless of the origin, EEP exhibited greater inhibitory potential than WEP. This may be due to active-substances in propolis that are ethanol-soluble; the number of phenolic compounds in water extracts is 10-fold lower than in ethanolic extracts (de Moura *et al*., 2011; Mello *et al*., 2010). To increase the solubility of biologically active substances in water, propolis should be extracted at a higher temperature of 70°C in the presence of an additional solvent, polyethylene glycol (Kubbiliene *et al*., 2015).

Furthermore, in another study by Brazilian green propolis (BGPP) also significantly inhibited mast cell degranulation, a key event in allergic reaction that led to the release of mediators to further contribute to the inflammatory response. Results reported by Shaha *et al*. (2018) had shown the suppressive effect of BGPP ethanol extract in the upregulation of H1R mRNA expression in both PMA and histamine-induced HeLa cells. In the case of allergy and inflammation, involvement of H1R has been widely documented in many previous studies, for instance, Th2-type immune responses can be enhanced via H1R activation (Thangam *et al*., 2018). Gene expression of H1R is also correlated with the allergic symptoms severity (Kitamura *et al*., 2015). Similar inhibitory effect on H1R expression in HeLa cells was reported for quercetin (Hattori *et al*., 2013). Although the quercetin was not directly extracted from propolis sources, it was reported that quercetin and its derivatives are commonly

present in BGPP (Andrade *et al*., 2017; Xu *et al*., 2020). Hence, the inhibitory activity of BGPP might be attributed to quercetin that is possibly presented in the BGPP ethanol extract.

Both PMA and histamine increased H1R mRNA expression, which is a common finding in allergic rhinitis (AR). PMA is a protein kinase C (PKC) activator, whereas histamine production involves PKC activation and calcium influx, ultimately leading to degranulation (Branco *et al*., 2018; Shin *et al*., 2019). As a result, PKC translocates from the cytosol to the Golgi, allowing phosphorylation of MAPK/ERK kinase (MEK) and extracellular signal-regulated kinase (ERK) to occur (Kitamura *et al*., 2015). Besides, the suppression of IL-9 expression in RBL-2H3 cells by BGPP was also reported by Shaha *et al*. (2018). Overexpression of IL-9 in AR patients is known to promote Th-2 allergic responses, airway inflammation and asthmatic symptoms in allergic rhinitis and asthma model. It was suggested that neutralization of IL-9 expression level was effective to ameliorate the symptoms of AR (Cheng *et al*., 2002; Gu, Wang, & Cao, 2017). The study had demonstrated the ability of BGPP to inhibit the allergic inflammatory responses in several ways collectively.

Other than mast cells, basophils are another type of primary effector cells which play an important role in allergic diseases. Both mast cells and basophils directly respond to allergen challenge through immunoglobulin dependent or independent mechanisms (He *et al*., 2013). In the study by Kashiwakura *et al*. (2020) propolis treatment able to inhibit the release of proinflammatory cytokines IL-4, IL-6 and IL-13 from activated basophils without affecting FcεRI expression. Also, using IgE-CAI which depends on basophils it was found that propolis inhibited basophil activation *in vivo*. Lyn is a member of the Src kinase family and is important in the IgE/FcεRI signaling pathway, which regulates allergy in both mast cells and basophils. In

contrast, Charles *et al*. (2009) found that in Lyn deficient mice, there was marked basophilia, increased basophil GATA-3 expression, and IgE-dependent TH2 differentiation. These results indicated Lyn to also have a negative regulatory function on basophil homeostasis. However, propolis treatment only affected the positive regulatory function of Lyn since propolis treatment significantly suppressed Lyn phosphorylation and therefore the cytokine release from basophils.

Despite the study by Kashiwakura *et al*. (2020) not identifying the bioactive components of this Japanese propolis, based on previous studies polyphenols such as fisetin, apigenin, and luteolin may contribute to the inhibitory effect of Japanese propolis observed in basophil activation of IgE-CAI and food allergy. These polyphenols have shown to be the strongest inhibitors of both IL-4 and IL-13 production by basophils (Hirano *et al*., 2004; Kawai *et al*., 2007). Other flavonoids including kaempferol and quercetin also substantially inhibited cytokine release from basophils (Higa *et al*., 2003). CAPE, another abundant flavonoid, is a potent anti-inflammatory agent that can modulate NF-B signaling (Armutcu *et al*., 2015). It has been shown to modulate ERK MAPK signaling in mastocytes as well as the PI3K/Akt pathway in human cell lines (Cho *et al*., 2014; Li *et al*., 2017). In the same study by Kasiwakura *et al*. (2020), it has been shown that IL-4 expression levels jejuna of propolis-treated food allergy mice was significantly down-regulated compared to untreated food allergy mice. Thymic stromal lymphopoietin (TSLP) is a master regulator of Th2 immunity, and TSLP-primed basophils play an important role in the pathogenesis of food allergy. Epicutaneous sensitization induces TSLP, which primes basophil migration and causes rapid and sustained basophil activation (Siracusa *et al*., 2011), causing them to release IL-4 and eliciting the Th2 response (Salter *et al*., 2015). Results obtained from this study showed propolis greatly suppressed the basophil-IL-

4 axis thereby, indicating it as a potential agent for attenuating Th2 mediated food allergy. However, further research must be done to establish the exact inhibitory mechanism of propolis on basophil activation.

Next, propolis also shows inhibitory effects on allergen-induced inflammation cellular models. The onset of allergic rhinitis started when the allergen that the person is sensitized with were crosslinked with IgE presented on the mucosal mast cells. Subsequently, it resulted in the release of mediators and vasoactive substances such as histamine and cysteinyl leukotrienes (cys-LTs) which contributed to the nasal symptoms (Wheatley & Togias, 2015). In this study by Tani *et al*. (2010), BGPP ethanol extract was effective in inhibiting the release of cys-LTs and histamine in PBMCs, suggesting that the reduction in cys-LTs and histamine may lead to improvements in allergic nasal obstruction as leukotrienes are important mediators in allergic nasal obstruction, and cys-LTs receptor antagonist and synthesis inhibitors also been demonstrated to be effective in the treatment of nasal obstruction (Scow *et al*., 2007). A decrease in IL-5 and IL-13 levels was also observed in this study when BGPP treatment was given. Significant reduction in IL-13 levels was similar to a previous study of BGPP ethanol extract testing on J774A.1 macrophages but the inhibitory effect of IL-5 was not significant (Szliszka, Kucharska *et al*., 2013). The difference might possibly be due to the different cellular models used for these two studies. However, the finding was accordant with a study by Piñeros *et al*. (2020) which showed the inhibitory effect of a Brazilian propolis extract on the IL-5 and IL-13 secretion on OVA-stimulated cells to alleviate Th2 immune response by increasing differentiation of polymorphonuclear myeloid-derived suppressor cells (PMN-MDSC) and CD4+Foxp3+ regulatory (Treg) cells. In general, BGPP is very rich in various

phenolic and flavonoid compounds that may be responsible for their anti-allergic effects (Andrade, Denadai, de Oliveira, Nunes, & Narain, 2017).

Based on results by Tani *et al*. (2010) from HPLC analysis, artepillin C, baccharin and kaempferide were likely to be the main active components causing the suppression of cys-LTs release. Previous research has shown that these polyphenols can suppress the production of), nitric oxide (NO), reactive oxygen species (ROS), and various cytokines such as IL-5 and IL-13, as well as NF-B activation (Szliszka, Mertas, Czuba, & Król, 2013). Significant reduction on recruitment of total leukocytes, production of NO, secretion of cytokines and level of eicosanoid lipids by baccharin from green propolis was also reported in a recent study (Ferreira *et al*., 2021). Besides, Tang *et al*. (2021) showed that the expression of antioxidant enzymes and non-enzymatic antioxidants, level of inflammation factors (TNF-α and MCP-1), and mRNA expression of inflammatory genes was effectively regulated using kaempferide. Nonetheless, other bioactive components present in BGPP may be responsible for its anti-inflammatory effect in allergic reactions in addition to the components described above. All of the polyphenols may also have a synergistic effect, resulting in a greater alleviation effect in allergic inflammation.

Other than showing anti-allergic effects during mast cell and basophils activation, propolis was also capable of reducing airway inflammation and allergic asthma in both *in vitro* and *in vivo*. *Aspergillus fumigatus conidia*, the most prevalent fungal allergen, plays a critical role in asthma pathogenesis. The study by Khosravi *et al*. (2018) showed stimulating lung epithelial cells (LECs) with *A. fumigatus* significantly increased the release of cytokines IFNγ, IL-13, and IL-17 after 6 hours of exposure. IL-13 is a key Th2 cytokine responsible in airway inflammation and remodeling in asthma patients and is involved in allergic reactions to *Aspergillus*

(Becker *et al*., 2015; Niranjan *et al*., 2013). IL-17 is a prominent pro-inflammatory cytokine, contributing to the production of inflammatory cytokines including IL-6, IL-1β and TNF-α, prostaglandins- involved in airway remodeling. Treating cells simultaneously with Iranian propolis and the allergen showed a significant decrease in Th2 cytokine release-IL-13 and IL-17. These results are in accordance with previous studies on Brazilian green propolis that has also been shown to suppress IL-13, IL-5 and IL-17 release (Hirota *et al*., 2012; Szliszka *et al*., 2013; Tanaka et al. 2011). An increase in Th1 cytokines, IL-2 and IFNγ was also observed upon propolis treatment. IFNγ have been reported to play a protective role against aspergillosis (Shao *et al*., 2005). In asthma models, IFNγ reduces eosinophils and lymphocyte recruitment and inhibits airway hyperresponsiveness and mucus overproduction (Mitchell *et al*., 2011). Results indicate that propolis has the potential to inhibit Th2 mediated allergic response by shifting the Th1/Th2 balance to Th1 by promoting Th1 cytokine secretion. Even though the study did not identify the bioactive compounds responsible for the anti-allergic property of propolis, it can be attributed to the polyphenols and flavonoids present in it. The propolis extract is primarily composed of phenolic acids such as ferulic acid and caffeic acid, according to gas chromatography mass spectroscopy. Previous research has shown that they can significantly suppress Th2 dominated responses with the production of cytokines IL-4, IL-5, and IL-13, as well as reduce allergic airway inflammation and hyperresponsiveness in asthma murine models (Brasil Carneiro *et al*., 2014; Jun *et al*., 2008; Sin Singer Brugiolo *et al*., 2017).

Besides, Shinmei *et al*. (2009) and Yasar *et al*. (2016) reported that the symptoms of allergic rhinitis (AR) sere suppressed by ethanolic extracts of Brazilian propolis in *in vivo* model of AR. Nasal symptoms such as sneezing, airflow obstruction and nasal discharge can be stimulated within minutes after exposure to allergens and

this condition was mimicked in the tolerable daily intake (TDI) -stimulated rats. In the study conducted by Shaha *et al*. (2018), BGPP ethanol extract reportedly ameliorated the allergic symptoms with BGPP treatment. Interestingly, BGPP was only showing significant impact at 40 mg/kg concentration but not significantly suppressing at the higher dose of 80 mg/kg in terms of sneezing frequency and nasal score. The authors suggested the BGPP extract may contain activators of histamine signaling which then neutralizes the possible inhibitory components in the extract, and the impacts can be observed more significantly at higher dose of propolis extracts. However, this finding is contradicting with a study demonstrated by Shinmei *et al*. (2009) as they reported a single dose-administration of Brazilian propolis showed no significant effect in inhibiting the sneezing and nasal rubbing even a concentration of 1000 mg/kg was given to the murine model. The significant inhibitory effect was only observed after repeated administration for 14 days, suggesting the effect of propolis might only be significantly observable after long term consumption. Remarkable suppression of H1R and Th2 cytokines (IL-4, IL-5 and IL-9) gene expression was observed in TDI-sensitized rats at higher concentration. As the H1R expression was upregulated upon TDI sensitization, the BGPP possibly suppressed the transcription of the gene to eventually decrease the amount of H1R protein (Shaha *et al*., 2018). Previous studies had evidenced the gene expression of H1R and Th2 cytokines including IL-4 and IL-5 are correlated whereby the expression of H1R affected negatively on the production of Th2 cytokines (Lee, Kim, & Choi, 2018). Furthermore, the nuclear factor of activated T-cells (NFAT) signaling mediated IL-9 gene was identified as an AR-sensitive gene. When histamine and NFAT signaling were inhibited in *in vitro* models, nasal symptoms improved significantly. This is due to IL-9 promotes the upregulation of Th2 cytokines, the expression of IL-4, IL-5, and IL-13 causes allergic inflammation

and asthmatic symptoms, while suppressing IL-9 expression also reduces Th2 cytokine expression (Mizuguchi et al., 2016). However, the inhibitory effect of BGPP on Th2 cytokines expression was only observed at higher doses of treatment which is slightly contradicting with the results from sneezing and nasal scores. Therefore, the inhibitory activity of BGPP may be due to the suppressive effect of histamine signalling pathway involving the suppression of PKCδ activation. Besides, the suppression of NFAT-mediated IL-9 possibly play an important role in the pathogenesis of AR.

Based on the studies of Shinmei and associates in different in vivo allergic models, oral treatment of Brazilian propolis typically required repeated administrations (more than 2 weeks) or extremely high dosage in single administration for significant inhibition on mast cell degranulation (Shinmei *et al*., 2004; Shinmei et al., 2009). Hence, the authors also justified the safe oral usage of propolis since natural products generally need consecutive consumptions for desired effects (Shinmei *et al*., 2004). A previous study stated that oral administration of propolis (4000 mg/kg/day) for 2 weeks showed no sign of toxicity in mice (Kaneeda & Nishina, 1994). Despite the justification of repeated oral administration of propolis, we would suggest the future investigations on propolis could also consider finding out the bioavailability of propolis and its bioactive components via oral means. This is because the propolis significantly inhibited histamine release from in vitro rat mast cells directly whereas repeated treatment or relatively high dosage of propolis *in vivo* are necessary for equivalent inhibitory activities. Moreover, the same group of researchers also recommended topical application of propolis to suppress compound 48/80-induced scratching behaviors, instead of oral administration in atopic dermatitis (AD) in *in vivo* model (Shinmei, Kagawa, Yano, Hossen, & Kamei, 2010). Altogether, these results strongly affirm that the routes of administration of propolis to mice would lead to

varying experimental outcomes, and therefore more detailed studies on propolis's oral bioavailability and barrier absorption should be taken into considerations.

Thus, when looking on the route of administration of propolis extracts in *in vivo* studies, we found that propolis showed anti-allergic effects when administered orally for asthma and allergic rhinitis as well as topically for allergic rhinitis and atopic dermatitis in *in vivo* models and these were observed regardless of the mode of treatment. Thus, most study shows significant by oral administration and the effective doses range from 65 to 1000 mg/kg administered daily. Although propolis seems to be a potential anti-allergic agent, there were cases of occupational contact allergy caused by propolis in beekeepers since 1970s. This also occurred less frequently in musicians and people who make stringed musical instruments. There are currently concerns about the topical use of propolis for medicinal and cosmetic purposes, as there have been reports of immediate allergic reactions on skin when in contact with propolis (de Groot, 2010). Nevertheless, this relates to what we have found in our systematic review, where propolis was commonly administered via oral route. A particular study by Yasar *et al*. (2016) which compared both oral and intranasal (topical route) has also shown that oral route propolis treatment was more effective. Thus, it is likely that oral propolis is relatively harmless and safe and might even more effective as an anti-allergic agent compared to topical use of propolis.

5.1.2 Clinical Studies

Other than showing anti-asthmatic effects in *in vivo* models, there is also one clinical study which has shown that aqueous extract of propolis improved pulmonary function of asthmatic patients. In this study, the propolis extract was given in the form

of nutritional food product for the asthmatic patients. Placebo-controlled study by Khayyal *et al*. (2002) shows significant decrease in TNF-α level of the asthmatic patients in propolis and theophylline group after two-months of treatment. Theophylline is an oral medication for asthma. TNF-α is a powerful immunomodulator cytokine that is elevated in many inflammatory conditions, including bronchial asthma. TNF-α levels in bronchoalveolar lavage fluid from patients with symptomatic asthma have been found to be significantly elevated in numerous studies. The release of PGs may occur because of the cytokines IL-1 and/or IL-8. TNF-α levels were either unchanged or increased in patients given white-coded (placebo) sachets. When the effect of aqueous propolis extract (silver sachets) was compared to that of a placebo (white sachets), propolis group shows significant advantages in reducing the frequency and severity of attacks and improve the pulmonary functions pertaining to both the large and small airway passages, possibly by improving the patients' immunological reactivity. Thus, this finding indicates that propolis could act effectively as an adjuvant therapy for asthma.

Although propolis has no effects on allergic sensitization and eczema in the placebo-controlled, multicenter, and double-blind study by Igarashi et al., it was shown to be a safe supplement for both lactating mothers and their infants. This is because propolis supplementation did not improve or worsen subjective symptoms, such as eczema, in lactating women or their offspring when compared to placebo in pairs of mothers and their offspring at risk for atopic sensitization. Hence, it is considered as safe to be taken by lactating mothers and their offspring since this study showed that propolis did not appear to be associated with frequent or severe adverse events in lactating mothers and their offspring.

5.2 Limitations

Therefore, there are some limitations of this study. Firstly, cross-sectional, case-control and cohort study reporting immediate allergic reactions of allergic individuals to bee product were excluded. However, it should be noted that these studies did not investigate the mechanism of action and the causal association was not verified. Besides that, studies investigating the effects of active constituents that are not specifically isolated from propolis extracts were also excluded.

CHAPTER 6

CONCLUSION

To conclude, propolis in various forms of extracts had shown beneficial effects in various allergic disease models (asthma, allergic rhinitis, atopic dermatitis, and anaphylaxis) in *in vitro* and *in vivo* studies. Clinically, it has also been shown to have therapeutic effects in asthmatic individuals. Besides, the anti-allergic activities of propolis were likely to be attributed to active constituents such as CAPE, pinocembrin, chrysin, tectochrysin and galangin. Finally, future studies are required to further evaluate the therapeutic potential of propolis extract for allergic rhinitis and atopic dermatitis in the clinical trials as well as to investigate the safety and effectiveness of the active constituents of propolis on various allergic diseases in the clinical setting. This is important to support the development of propolis for the clinical management of allergic diseases.

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