# STUDY PROTOCOL AND STATISTICAL ANALYSIS

## The Effect of Apple Fruit Extract (*Malus Sylvestris* Mill) as an Antioxidant and Anti-Inflammatory on Allergic Rhinitis

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# THE INFLUENCE OF APPLE FRUIT EXTRACT (MALUS SYLVESTRIS MILL) AS AN ANTIOXIDANT AND ANTI-INFLAMMATORY IN ALLERGIC RHINITIS

(Study on the levels of Malondialdehyde, Nuclear Factor Kappa Beta, Tumor Necrosis Factor Alpha, Interleukin 6, Interleukin 8, clinical symptoms of nasal congestion, sneezing, itchy nose, and rhinorrhea)

## CHAPTER I

## INTRODUCTION

### A. Background of the Problem

Allergic Rhinitis (AR) is a chronic disease that can affect the quality of life of its sufferers. As many as 57% of AR sufferers still experience symptoms despite receiving antihistamine therapy and intranasal corticosteroids (ICS). Allergic Rhinitis is an inflammatory process in the nasal mucosa that is initiated by hypersensitivity reactions due to exposure to allergens mediated by Immunoglobulin E (IgE) with several characteristic symptoms consisting of: nasal congestion, runny nose or watery nasal discharge (rhinorrhea), itching in the nose, and sneezing. The occurrence of AR can be triggered by contact with allergens. Several allergens that can cause AR symptoms are often found in both home and outdoor environments. Examples of allergens include: house dust mites, pollen, and fur or hair from pets (Akhouri & House, 2022).

Based on a survey by the World Health Organization (WHO), approximately 400 million people worldwide suffer from allergic rhinitis, and the incidence rate is expected to continue to rise. According to the ISA AC phase III study, the prevalence of children aged 13 to 14 years with allergic rhinitis ranges from 1.4% to 39.7% of the global population. According to the World Allergy Report, the incidence of allergic rhinitis in low- and middle-income countries in the Asia-Pacific region is 5% – 45% of the total population (Nurhutami et al., 2020). Allergen avoidance is often difficult to achieve due to the daily activities or jobs of sufferers that do not allow for avoiding allergens, thus necessitating long-term use of antihistamines.

Data from the AR patients in the ENT outpatient unit at the Sebelas Maret University Hospital, Surakarta, shows that the number of new AR cases in 2023 is 103 (37%) cases, and old cases are 170 (62%) cases. The number of new cases up to April 2024 is 159 (36%) cases, and old cases are 273 (63%) cases. This data indicates that the number of old cases each year is greater than the number of new cases, which shows.

2 that long-term patients still experience complaints and still come for treatment. This may be caused by the standard therapy used not being maximal in addressing

complaints comprehensively, thus complementary therapy is still needed to address RA symptoms. Complementary treatment is a group of additional medical care using modalities or products that are not included in conventional medicine, which are used on patients with allergic diseases including RA. Various complementary therapies for RA include acupuncture, herbal medicine, homeopathy, and physical therapy.

Pathogenetically, inflammation in RA begins with the sensitization phase, rapid allergic reaction phase (RAFC), and slow allergic reaction phase (RAFL). In RAFC, mast cell degranulation occurs, resulting in the production of several mediators such as histamine, prostaglandin, and leukotriene. In RAFL, infiltration of several inflammatory cells such as eosinophils occurs due to ICAM 1 (Intercellular adhesion molecule 1) molecules. Subsequently, several inflammatory cytokines such as interleukin (IL) IL 4, IL 6, Tumor Necrosis Factor Alpha, and others are produced. The pathway for cytokine and inflammatory molecule formation is through cytokine gene transcription by NFkB (Nuclear factor kappa B) in the cell nucleus. The activators of NFkB include oxidant free radicals, namely ROS (Reactive oxygen species) such as air pollution. In research that has been conducted, an increase in NFkB expression and mRNA ICAM 1 in the nasal mucosa of RA patients was observed (Zhao et al, 2008).

Reactive oxygen species originating from motor vehicle pollution can strengthen allergic inflammation by increasing IgE production through B cell stimulation, enhancing allergen-induced specific IgE production, stimulating the production of cytokines IL 4 and IL 6, stimulating Th2, inducing the influx of inflammatory cells by increasing ICAM 1 expression, enhancing chemokine production, and inducing NFkB in expressing IL 8, IL 6, and TNF α. Hwang et al (2006) concluded that exposure to pollutants NOx, CO, and SO2 would increase the incidence of RA. Then Sequeira et al (2012) stated that there is a role of oxidative stress in RA with moderate to severe degrees, so strategies to maintain the balance of ROS and total antioxidant capacity can be beneficial in RA.

**Apple Fruit Extract**

Apple fruit extract derived from raw apples has been reported to have anti-allergic activity. In human atopic dermatitis, itching, sleep disturbances, and peripheral eosinophil counts have been reported to significantly decrease in the group receiving oral apple polyphenols at 10 mg/kg for 8 weeks compared to the control group treated with placebo (Enomoto et al, 2006). The mechanism by which apple fruit extract inhibits allergic reactions is provided by the observation that apple polyphenols and procyanidin fractions prevent degranulation and histamine release by inhibiting the increase in calcium concentration in antigen-stimulated mouse basophils (RBL 2H3 cells). Apple fruit extract is suggested to prevent degranulation by enhancing granulocyte stability and inhibiting histamine release. Additionally, apple fruit extract and its procyanidin fractions may also suppress inflammation, as they have been reported to have a dose-dependent inhibitory effect on

hyaluronidase activity, an enzyme associated with inflammation and released by degranulation.

# CHAPTER II LITERATURE REVIEW

## A. Theoretical Basis

### Allergic Rhinitis

#### a. Definition

Rhinitis comes from the word rino which means nose or nasal and itis which means inflammation or inflammatory. Allergic rhinitis is an inflammatory disease of the nasal mucosa characterized by symptoms such as frequent sneezing, a blocked nose, nasal pruritus or itching in the nose, and clear rhinorrhea or discharge from the nose (Bousquet et al., 2020). The inflammatory reaction in allergic rhinitis is caused by an allergic reaction in patients with a history of atopy who have previously been sensitized to the same allergen (Kasim et al., 2020). In addition to symptomatic symptoms in the nose, patients with allergic rhinitis may also experience allergic conjunctivitis, nonproductive cough, dysfunction of the eustachian tube, and chronic sinusitis (Akhouri & House, 2022).

#### b. Classification

Initially, allergic rhinitis (AR) was divided into several classifications based on the time of exposure, becoming seasonal (occurring in certain seasons), perennial (occurring throughout the year), and occupational. This classification then changed to the latest clarification according to the guidelines of The Allergic Rhinitis and Its Impact on Asthma (ARIA). The ARIA guidelines classify allergic rhinitis based on the duration of symptoms and the severity of symptoms. The classification of AR based on symptoms according to ARIA is as follows:

1. Intermittent: classified as allergic rhinitis with symptoms ≤4 days per week or lasting ≤4 weeks.

2. Persistent: defined as allergic rhinitis with symptoms >4 days per week and duration >4 weeks.

Whereas based on the severity of allergic rhinitis symptoms, it can then be classified into:

1. Mild: Can sleep normally No disturbances during daily activities, sports, and relaxation Works and studies normally No bothersome complaints
2. Moderate to Severe: Sleep disturbed There are disturbances during daily activities, sports, and relaxation Disturbances while working and studying There are bothersome complaints

Figure 2.1. Classification of Allergic Rhinitis According to ARIA (Klimek et al., 2019)

Note: Classification of AR based on symptoms according to ARIA.

Intermittent: classified as allergic rhinitis with symptoms ≤4 days per week or duration ≤4 weeks, Persistent: defined as allergic rhinitis with symptoms >4 days per week and duration >4 weeks.

Epidemiology of Allergic Rhinitis

Allergic Rhinitis affects about 25 to 40% of children and adults globally. About 80% of AR symptoms appear before the age of 20 years and peak at ages 20 to 40 years before gradually declining. (Nur Husna, 2022) The incidence rate of AR in children during the first 5 years of life is reported to be 17.2%, with the peak age at diagnosis between 24 and 29 months (2.5%). Meta-analysis studies have shown specific gender differences in the prevalence of AR, with a male predominance in childhood and a female predominance in adolescence. (Pinart, 2017). The incidence of AR is also influenced by the living environment. This is mainly due to increased pollutant levels (e.g., traffic-related pollutants) that can exacerbate clinical AR. (Nur Husna, 2022)

Pathophysiology of Allergic Rhinitis

Type I hypersensitivity is an allergic reaction mediated by IgE antibodies in response to allergens. Type I hypersensitivity reactions occur rapidly (+/- 20 minutes after exposure to allergens), activating mast cells and inflammation as well as their infiltration into tissues. (Gangwar, 2015) The allergic response in AR can be divided into two phases: the early phase and the late phase. The early phase begins within 20 minutes after exposure to harmful allergens. Antigen-presenting cells such as dendritic cells in the mucosal surface uptake, process, and present peptides from allergens on major histocompatibility complex (MHC) class II molecules. The antigen-MHC class II complex serves as a ligand for the T cell receptor on naive CD4+ T cells, resulting in the differentiation of naive CD4+ T cells into allergen-specific Th2 cells. Cytokines such as IL-4 and IL-13 released from activated Th2 cells interact with B cells to produce allergen-specific IgE. This allergen-specific IgE binds to high-affinity Fc receptors for IgE (FcεR) present on mast cells, leading to mast cell activation. (Nur Husna,2022). The cross-linking of FcεR on mast cells causes the release of allergy mediators consisting of histamine, protease, and lipid mediators such as leukotriene (LT) C4, and prostaglandin D2 (PGD2) which cause blood vessel leakage, bronchoconstriction, inflammation, and intestinal hypermotility (Justiz, 2018). These mediators induce mucosal edema and characteristic watery rhinorrhea of allergic rhinitis (AR) by causing blood vessels to leak. Histamine is the main mediator in AR where it activates H1 receptors on sensory nerve endings and causes sneezing, pruritus, and reflex secretion responses, and also interacts with H1 and H2 receptors on mucosal blood vessels, causing

swelling of blood vessels (nasal obstruction) and plasma leakage (Nur Husna, 2022).

After 4 to 6 hours of allergen exposure, the final phase of the allergic response begins. In this phase, inflammation of the nasal mucosa occurs with the entry and activation of various inflammatory cells such as T cells, eosinophils, basophils, neutrophils, and monocytes into the nasal mucosa (Nur Husna, 2022). The recruitment of these inflammatory cells is triggered by cytokines such as IL-4 and IL-5. These cytokines regulate the expression of adhesion molecules such as vascular cell adhesion molecule 1 (VCAM-1) on endothelial cells that facilitate the entry of inflammatory cells. Activation of structural cells in the nasal mucosa, such as epithelial cells and fibroblasts, can promote the release of additional chemokines (e.g., eotaxin, RANTES, and TARC) that facilitate cellular entry from peripheral blood (Nur Husna, 2022).

Helper T cells 2 (Th2) activate a type 2 response by stimulating B cells to proliferate and differentiate into plasma cells through the production of Th2 cytokines including IL-4, IL-5, IL-6, and IL-13. Th2 cells are the main contributors to IgE-producing B cells, and Th2 cells play a key role in the pathogenesis of allergic rhinitis. Together with eosinophils and basophils, Th2 cells infiltrate the nasal mucosal tissue, producing the final phase allergic response. IL-4 is a key cytokine.

17 degrees allergy and the initial sensitization dose can be determined, known to be slightly more sensitive compared to serological tests and more cost-effective.

Contraindications of SET include patients with uncontrolled/severe asthma, unstable cardiovascular disease, pregnancy, and/or concurrent beta blocker therapy. H2 receptor antagonists, tricyclic antidepressants, and anti-IgE monoclonal antibodies omalizumab can interfere with allergic reactions in skin tests. Therefore, it is recommended to stop use before testing (Schoeman et al., 2021).

Another in vitro test that can be performed is the eosinophil count. Eosinophil levels may be normal or increased. Total IgE tests (Pristopaper radioimmunosorbent test) often show normal values unless the patient shows signs of allergy to more than one type of disease such as bronchial asthma or urticaria (Schoeman et al., 2021).

Mallus sylvestris Mill (Apple Fruit)

The apple fruit has the Latin name Mallus sylvestris mill and is a perennial fruit plant originating from the western Asia region with a subtropical climate. Apple plants can grow and bear fruit well at altitudes of 700 to 1200 m above sea level, with an optimal altitude of 1000 to 1200 m above sea level. Apple plants have a taproot, which is a root that is perpendicular to the ground that supports the plant and absorbs nutrients from the soil. The bark is usually brown and scaly. The simple leaves are oval-shaped and usually have fine teeth along their edges. Apple flowers are striking with five white petals, often tinged with pink, and many stamens. The

flowers are pollinated by bees and other insects, and most varieties require cross-pollination for fertilization (Botannica, 2023).

Apple plants have very varied fruits, which are green, red, and also reddish with oval or round shapes. The skin of the apple fruit is thin and rough and has large pores. However, after.perfect ripe will become shiny and also smooth the surface of the fruit.

Apple is one type of fruit that is high in fiber and phytochemical content, especially phenolic and flavonoid (Lobo, 2019).

Flavonoids are one group of phenolics that have antioxidants obtained from food sources derived from plants believed to be protection against oxidative stress. One of the most important flavonoid contents is quercetin. The quercetin content in apples is quite high, in 100 grams of apple contains about 4.42 mg of quercetin aglycone and 13.2 quercetin glycosides (Mahardinar, 2015).

The compounds most commonly found in apple skin consist of procyanidins, catechin, epicatechin, chlorogenic acid, phloridzin, and quercetin conjugates. Inside the apple flesh, there are several catechins, procyanidins, epicatechins, and phloridzin, but these compounds are found in much lower concentrations than in the skin (Patricia et al., 2016).

Apples are produced globally. Apples are consumed raw and in processed products, such as dried fruit, juice, apple cider, brandy, jam, and vinegar. Apple consumption reduces the risk of coronary heart disease, which is related to the presence of high concentrations of polyphenols in apples (Wojdyło & Oszmiański, 2020).

Apples contain five different structural subclasses, including phenolic acids and flavonoids, which consist of phenolic carboxylic acids (e.g., chlorogenic acid), anthocyanins (e.g., cyanidin glycosides), flavonols (e.g., quercetin glycosides), dihydrochalcones (e.g., phloridzin), flavan 3-ols (e.g., epicatechin) as well as their oligomers and polymers, and procyanidins. Chlorogenic acid is the most abundant polyphenol in apples as a single polyphenol. Among these polyphenols, 70% of it consists of flavan 3-ols and procyanidins. The concentration of procyanidins in apples is significantly correlated with antioxidant activity, and various clinical studies in animals and humans show that flavan 3-ols and procyanidins are involved in various health benefits, such as the prevention of dementia, cardiovascular diseases, type II diabetes, and cancer (Shoji et al., 2021).

The Taxonomic Position of Flavonoids

The taxonomic position of flavonoids is viewed from the level of oxidation of the structure and the substitution pattern of the C bonds. In one class, different from one another, are flavonoids with substituents on bonds A and B. Flavonoids have the following taxonomic position: flavon, flavanon, isoflavon, flavonol, flavanonol,

flavan 3 ol, and anthocyanidin (Simamora, 2009). According to Simamora (2010), the basic structure of flavonoids can be seen in Figure 2.5 as follows:

4. Antioxidant Polyphenols in Apples

Quercetin (3,31,41,5,7 pentahydroxyflavone) is a natural polyphenol flavonoid found in several fruits and vegetables, such as: apples, tomatoes, grapes, red onions, onions, legumes, and grains. Quercetin is an antioxidant flavonol from the flavonoid group and becomes the glycoside Que. This glycoside can conjugate with glucose, xylose, or rutinose and then attach to one of the Que hydroxyl groups, which ultimately creates various forms of glycoside Que. Nutritional flavonoids like Que are a type of antioxidant that is better than vitamins C and E. Que and its derivatives have major biological effects in the development of cycles and cellular signal transduction pathways. (Jafarinia et al., 2020).

he chemical structure of quercetin is an anti-flavan with carbon atoms, namely 15 atoms in three rings (C6 C3 C6), which are marked with labels A, B, and C. Quercetin is one of the flavonoids needed by the body, around 5 to 40 mg per day. Quercetin can be metabolized in the intestines and liver. Quercetin has 3 rings and a 5-hydroxyl group.

An important benefit of quercetin is as an anti-inflammatory, antioxidant, and its ability to inhibit lipid peroxidation, platelet aggregation, and capillary permeability. Quercetin is known to be able to inhibit lipopolysaccharide (LPS) induced production of tumor necrosis factor alpha (TNF α) in macrophages. LPS can induce the mRNA levels of TNF α and IL 1α, producing inflammatory enzymes such as cyclooxygenase (COX) and lipoxygenase (LOX). Based on this, quercetin as an antioxidant plays an important role in addressing inflammation (Jafarinia et al., 2020).

The antioxidant mechanism possessed by quercetin is reflected in its effects on glutathione (GSH), signal transduction pathways, ROS, and enzyme activity (Deepika and Maurya 2022). Quercetin can enhance the antioxidant capacity in the body by regulating GSH levels. This is because free radicals are produced by the body during metabolic processes, leading to genetic mutations, membrane cell damage, and causing various diseases such as heart disease, liver disease, diabetes mellitus, and accelerating body aging.

When the body produces free radicals, Superoxide Dismutase (SOD) will immediately convert O2 into H2O2, which will then be catalyzed into non-toxic H2O and GSH. Quercetin can convert H2O2 into H2O, and GSH will be oxidized and then oxidize glutathione (Michala and Pritsa, 2022).

Research by Granado Serrano et al. shows that Que increases the antioxidant capacity of cells by enhancing the intracellular p28 MAPK pathway, increasing GSH levels, and providing hydrogen donors to scavenge free radicals present in the

body. It was also found that Que inhibits atherosclerosis by blocking Nf kB signaling, thereby increasing NADPH expression. By affecting signal transduction, Que can modulate enzymes or antioxidant substances and enhance antioxidant properties, thus preventing disease progression. Que can prevent oxidative damage by inhibiting oxidative stress. Oxidative stress is caused by an imbalance between antioxidants and oxidants in the body and reactions that tend toward oxidation, where once oxidized, it will lead to increased secretion of protein enzymes and neutrophil infiltration. Que can regulate the balance of antioxidants and oxidants to suppress oxidative stress. Two enzymes, namely acetylcholinesterase and butyrylcholinesterase, are related to oxidative properties, while the OH group on the lateral side of the benzene ring in Que binds to amino acid residues on both active sides of the enzyme, thus preventing oxidative effects (Michala and Pritsa 2022) (Qi et al., 2022).

Anti-Inflammatory Apple Polyphenols

Inflammation is a normal biological process in response to tissue injury, microbial pathogen infection, and chemical irritants. This process begins with the migration of immune cells and the release of mediators, followed by the recruitment of inflammatory cells, the release of pro-inflammatory cytokines, and tissue repair. Flavonoids, such as hesperidin, apigenin, luteolin, and quercetin, have anti-inflammatory and analgesic effects and can influence the enzyme systems involved in the inflammatory process. Flavonoids can inhibit the expression of inducible nitric oxide synthase isoforms, cyclooxygenase, and lipoxygenase, which produce cytokines, prostanoids, leukotrienes, and other mediators of the inflammatory process. They also inhibit phosphodiesterase involved in cell activation. Flavonoids can reverse the inflammatory effects induced by carrageenan and inhibit adhesion, aggregation, and secretion of platelets (Wang et al., 2018).

Cytokines pro-allergic, IL 4 and IL 13, produced by Th2 and ILC2 at the early stage of the sensitization phase, promote the occurrence of isotype class switching of IgE in B cells and their transformation into plasma cells that secrete allergen-specific IgE in large quantities. This IgE then binds to high-affinity FcεRI receptors on the surface of mast cells and basophils, causing an allergic sensitization state.

Polyphenols are suspected to affect the recruitment, maturation, and function of B cells; however, these effects have not been thoroughly investigated and explained. On the other hand, the capacity to inhibit antigen-specific IgE production in a dose- and time-dependent manner has been well documented in in vitro and in vivo studies for several polyphenols such as curcumin, rosmarinic acid, quercetin, ferulic acid, tea catechins (EGCG, ellagitannins, and gallic acid), and red wine polyphenols.

Zhang et al. illustrated the modulatory effects of polyphenols with the example of dihydromyricetin, a natural flavonoid, which effectively suppresses the sensitization phase by reducing B cell populations and antigen-specific IgE

production as well as blocking the interaction of IgE with FcεRI. Similarly, phlorotannins (i.e., eckol, dieckol) and tea catechins can interact with FcεRI by directly binding to the α chain, thereby blocking the possibility of specific antigen IgE binding to FcεRI and thus suppressing the mast cell sensitization phase.

Furthermore, evidence has been provided that phlorotannins, saponins, catechins, as well as quercetin, kaempferol, and resveratrol may contribute to the weakening of allergic reactions by reducing the expression of FcεRI receptors, which are crucial for persistent sensitization and subsequent degranulation during the effector phase (Satitsuksanoa, 2021).

Considering the fundamental role of allergic inflammation in the development and progression of respiratory allergic diseases, it can be assumed that polyphenols, due to their anti-inflammatory and immunomodulatory properties, may be beneficial in the prevention and treatment of asthma and allergic rhinitis. In addition to anti-allergic actions, in vitro studies indicate that polyphenols may also function as anti-mucus secretion agents and possess antioxidant and antifibrotic activities.

so that it not only targets allergic inflammation, but also oxidative stress caused by inflammation and structural changes in the oral mucosa. The respiratory tract causes airway hyperreactivity and airway remodeling (Debinska, 2023).

The Role of Apple Polyphenol Antioxidants in Reducing Signs and Symptoms of Allergic Rhinitis

Quercetin has multiple biological activities. Quercetin plays a role in slowing the progression of diseases, such as: allergic diseases, malignancies, metabolic syndrome, and autoimmune diseases. Quercetin is capable of inhibiting inflammation by suppressing apoptosis and epithelial cell cytokines. Quercetin alleviates the symptoms of allergic rhinitis through the role of inhibiting neuropeptide production (Th1/Th2 cytokine imbalance) and eosinophil activation. Significant symptoms of allergic rhinitis include sneezing and frequent nose rubbing. Quercetin is known to reduce the frequency of sneezing and nose rubbing, thus improving RA symptoms (Xia et al., 2023).

Allergic rhinitis is an inflammatory nasal disease due to allergens mediated by IgE, driven by Th2 cells. IgG1 antibodies, IL-4, and IL-5 serve as markers for Th2 cells, while IgG2 antibodies and Interferon γ serve as markers for Th1 cells. Histamine promotes cytokine release in Th2 cells and decreases Th1 cell production. Quercetin can inhibit the increase of IgE, IgG1, histamine, IL-4, and IL-5 and the decrease of IgG2a and IFN γ, indicating that quercetin prevents Th1/Th2 imbalance. Quercetin is also capable of inhibiting the increase of inflammatory cells, such as: eosinophils, neutrophils, lymphocytes, and macrophages (Xia et al., 2023).

The imbalance of Treg/Th17 cells has been shown to be closely related to inflammation. Foxp3, IL-10, and TGF β are important regulators for Treg cells,

while IL-17 is a regulator for Th17 cells. IL-6 and TNF α are pro-inflammatory factors that are also regulators of Treg/Th17 balance. These findings indicate that quercetin enhances the balance of Treg/Th17 cells (Xia et al., 2023).

NF κB Pathway

The NF κB pathway is a classic pro-inflammatory signaling pathway that increases the expression of pro-inflammatory cytokines and chemokines. The entry and exit of nuclear NF κB regulate the inflammatory response. When exposed to drugs, the nuclear translocation of p65 undergoes significant changes. Cyclooxygenase 2 is a pro-inflammatory chemokine that can be transcriptionally regulated by the NF κB pathway. Previous research has reported that the NF κB pathway significantly participates in the development of RA, particularly the effects of drugs on the Treg/Th17 or Th1/Th2 balance. In this regard, it was found that quercetin reduces the regulation of COX 2, p IKBα, nuclear p65, and increases the levels of IKBα and cytoplasmic 65, indicating that quercetin inactivates the NF κB pathway (Xia et al., 2023).

In summary, oral administration of quercetin can alleviate RA symptoms by inhibiting inflammation, Th1/Th2 imbalance, Treg/Th17 imbalance, and the NF κB pathway. Therefore, quercetin can weaken the inflammatory response of RA by enhancing the Th1/Th2 and Treg/Th17 imbalances and inactivating the NF κB pathway (Xia et al., 2023).

a. Side Effects

High doses of quercetin consumed together with certain types of drugs can affect the pharmacokinetics of those drugs. Quercetin has a weak inhibitory effect on CYP enzymes. Quercetin, when consumed in large amounts, will affect the transport of drugs mediated by organic anion transporting polypeptide (OATP), for example, statin drugs (Mohos et al., 2020).

b. Weaknesses

Quercetin has not been widely developed in the treatment of RA because quercetin has poor bioavailability, poor water solubility, and is unstable when passing through the gastrointestinal tract and when facing first-pass drug metabolism effects (Rajesh & Dhanaraj, 2023). If obtained from natural sources, a specific dose must be achieved for quercetin to provide benefits.

Formulation of Quercetin

The formulation of quercetin in apple polyphenols can be found in powder form, and functions as an antioxidant, anti-inflammatory, prevents platelet aggregation, and enhances relaxation of cardiovascular smooth muscle.

## Complementary Therapy

Complementary therapy is an additional therapy using certain modalities that are not included in conventional medicine (Ibnu et al., 2019). The goal of this complementary therapy is to improve the degree of health. Complementary therapy includes promotive, preventive, curative, and rehabilitative programs while considering quality, safety, and effectiveness, and is based on biomedical science. Complementary therapy serves as an additional therapy outside of the main therapy and functions to improve quality of life and enhance the immune system (Ismail et al., 2018).

Apple polyphenols are extracted and purified from raw apples and mainly consist of procyanidins, and the concentrated procyanidin fraction that has been purified has been confirmed to have higher activity in tests using rat cells. Mast cell and basophil stabilization, immune tolerance control has been suggested as a possible mechanism underlying the action of polyphenols (Sonji et al., 2006).

Apple polyphenols consist of 50% to 60% procyanidins, which are oligomers and polymers of catechins, along with low molecular weight polyphenols such as chlorogenic acid and catechins. However, macromolecular procyanidins are not absorbed and are largely considered to be excreted. The percentage of T cells in intestinal epithelial cells of food allergy index has been shown to decrease in rat models induced by continuous oral consumption of ovalbumin, but this decrease is prevented by the administration of procyanidin fractions derived from apples. In contrast, the decrease is not inhibited when catechins alone are administered. These results indicate that the procyanidin fraction derived from apples can induce oral immunotolerance (Akiyama et al., 2005).

## Malondialdehyde

Malondialdehyde is a small and reactive organic molecule widely distributed within human cells, formed by three carbon molecules with two aldehyde groups at carbon positions 1 and 3.

Malondialdehyde exists in various forms in aqueous solution due to its tautomeric chemical properties that depend on pH. At pH higher than pKa 4.46, the dominant form is the enolate anion, which shows low chemical reactivity, whereas at lower pH occurring under oxidative stress conditions, MDA appears in equilibrium between protonated enol (α β unsaturated carbonyl) aldehyde and dialdehyde form (Morales & Munné Boschb, 2019).

Malondialdehyde is one of several products of lipid peroxide decomposition formed in fats and oils, food, and tissues. This substance is the most widely investigated product due to its reactivity with various biological macromolecules and its relationship with the pathophysiology of several disease conditions.

Malondialdehyde is enzymatically formed as a product of the cyclooxygenase reaction in the synthesis of prostaglandins and thromboxanes. Undoubtedly, there are a number of free MDA in food, as indicated by its accumulation as a volatile product during cooking, but the amount present is difficult to determine due to its artifact formation (Wang et al., 2022).

MDA derivatives excreted in urine are interesting to know as potential indicators of the form of MDA in food and its reaction properties in vivo. The cross-linked glycine adenin related to MDA is obtained by reacting siximal 9 ethyladenine with methyl ester glycine, which indicates that cross-linking of nucleic acid amino acids mediated by MDA can occur in vivo (Draper et al., 2019).

Stimulation by bacterial and viral antigens can cause PMN cells to produce and release ROS in large amounts, leading to oxidative stress. Proteins can become targets of ROS attack, such as those arising from lipid peroxidation and/or degradation products. Malondialdehyde or 4-hydroxynonenal derived from lipid peroxidation can form stable cross-linking products with certain amino acids. Malondialdehyde can be formed during the peroxidation of polyunsaturated fatty acids through the action of thromboxane synthase in human platelets on prostaglandin PGH2, PGH3, and PGG2, as well as through the action of polyamine oxidase and amine oxidase on spermine (Jové et al., 2020).

Malondialdehyde is metabolized in the liver into semialdehyde malonic acid, which is an unstable molecule and spontaneously decomposes into acetaldehyde, which is then converted into acetate by aldehyde dehydrogenase and finally into carbon dioxide and water. After this, the next steps include conformational changes, enzymatic activity, or binding and inactivation of receptors, increased susceptibility to proteases, and changes in immunogenicity (Cherian et al., 2019). Reactive oxygen species have a very short lifespan, making them difficult to detect; however, tissue damage related to ROS can be observed by the end products of lipid peroxidation such as MDA. The level of tissue damage can be assessed by measuring the concentration of lipid peroxidation products and the levels of endogenous antioxidants in the body. Increased lipid peroxidation can burden the antioxidant defense system and trigger cell apoptosis and pathological processes that lead to increased serum MDA levels, reflecting increased free radical production. Increased MDA and other biomarkers of oxidative stress contribute to increased morbidity and mortality.

Cytokines Involved in Allergic Rhinitis

Cytokines are peptides produced in response to microbial stimuli and other antigens and act as mediators in immune and inflammatory reactions. Cytokine secretion occurs quickly and only briefly. Their action is often pleiotropic, meaning one cytokine acts on various types of cells and produces various effects. Cytokines often influence the synthesis and effects of other cytokines (Subowo, 2009).

Cytokine products related to inflammation include TNF α, IL 1, IL 6, and IL 8. These pro-inflammatory cytokines are released by activated tissue macrophages (Baratawijaya, 2006).

## Cytokine Products

In RAFL, there is infiltration of a number of inflammatory cells such as eosinophils due to ICAM 1 (Intercellular adhesion molecule 1) molecules. Subsequently, a number of inflammatory cytokines such as interleukin (IL) IL 4, IL 6, TNF α, and others are produced. The pathway for cytokine and inflammatory molecule formation is through cytokine gene transcription by NFkB (Nuclear factor kappa B) in the cell nucleus. Activators of NFkB include oxidant free radicals such as ROS (Reactive oxygen species) like air pollution. Zhao et al (2008) found an increase in NFkB expression and mRNA ICAM 1 in the nasal mucosa of patients with allergic rhinitis.

## Tumor Necrosis Factor Alpha

Tumor Necrosis Factor Alpha is the first pro-inflammatory cytokine identified in allergic rhinitis. The concentration of TNF α is related to age; older children have higher levels and concentrations of TNF α. Children who experience recurrent effusion otitis media caused by allergic rhinitis also have higher levels of TNF α than children who are undergoing tympanostomy for the first time.

## Interleukin 6

Interleukin 6 is one of the most important pro-inflammatory cytokines in the body's immune response. The IL 6 cytokine is a four-helix protein that binds to the membrane-bound IL 6 receptor or IL 6 receptor (IL 6R), and the IL 6 and IL 6R complex is the second receptor protein that joins. The 130 kDa glycoprotein (gp130) transduces intracellular signals through Janus kinase (JAK)/signal transducer and activator of transcription (STAT). Interleukin 6 shows measurable affinity only for IL 6R, but not for gp130, and IL 6R itself does not bind to gp130. Only the IL 6 and IL 6R complex binds gp130 and induces its dimerization. All cells in the body express gp130, but only a few cells, such as liver cells and some white blood cells, express IL 6R. Therefore, cells that only express gp130 and not IL 6R are not stimulated by IL 6 (Rose John, 2020).

## Interleukin 8

Interleukin 8 (IL 8) is a member of the chemokine family that is related to the CXC motif. It is associated with two chemokine receptors, CXC 1 (CXCR1) and 2 (CXCR2), which are included in the superfamily of G protein-coupled receptors with high affinity. CXCR1 is also associated with granulocyte chemotactic protein 2 (GCP 2), neutrophil-activating peptide 2 (NAP2), and epithelial neutrophil-activating peptide 78 (ENA 78) with low affinity. Many types of cells, such as

fibroblasts, keratinocytes, endothelial cells, neutrophils, mast cells, monocytes, macrophages, and megakaryocytes, produce IL 8 in vitro, which performs various biological activities.

IL 8 plays an important role in acute inflammation because it acts as a potential chemotactic agent and neutrophil activator by activating CXCR1 and CXCR2 (Emadi et al., 2005). The reduced IL 8 response induced by LPS in neutrophils has been confirmed in human whole blood translational and non-animal models. This was achieved by exposing neutrophils in vitro to a pro-inflammatory microenvironment containing N-formyl Met Leu Phe, LPS, or IL 8, resulting in a response accompanied by an increase in cell size and pH that leads to a decrease. The detailed kinetics of this degradation can be shown almost in real time by measuring flow cytometry. Overall, we provide a new mechanistic overview of the interaction between IL 8 and neutrophils and explain how IL 8 changes during systemic inflammation.

Meanwhile, MKK6 phosphorylates another MAPK, p38, which leads to the phosphorylation of MK 2 and stabilizes IL 8 mRNA. The activation of the C/EBP signaling pathway is largely still unknown (Matsushima et al., 2022).

## CHAPTER III

## RESEARCH METHOD

A. Type of ResearchThe type of research used is Clinical Experimental, designed with a Randomized Controlled Trial double blind, with pre and post test design on samples of patients with persistent allergic rhinitis.

B. Place and Time of ResearchThe research is conducted at the Outpatient Clinic of Ear, Nose, Throat Health Science Surgery at the Sebelas Maret University Hospital and the biomedical laboratory of the Faculty of Medicine UNS for the examination of MDA, NF kB, TNF α, Interleukin 6, Interleukin 8. The research will be carried out after obtaining research approval from the dissertation proposal examination board and the Research Ethics Commission.

C. Subjects/Sample of ResearchOutpatient patients who have undergone anamnesis and physical examination of ENT.

1. Restriction CriteriaInclusion Criteria:

   1) Patients aged 18 – 60 years who have signed informed consent

   2) Diagnosed with persistent allergic rhinitis with Skin Prick Test or Positive IgE Examination.

Exclusion Criteria:

1) The presence of complications in the nose such as nasal septum deviation, polyps, rhinosinusitis

2) Have undergone specific immunotherapy for 2 years

3) Use of medications that affect allergy test results: antihistamines, corticosteroids, antihypertensive medications, and decongestants

4) Pregnancy and Breastfeeding.

Sample Size

The sample size uses the Frederer formula.

Frederer Formula = (n - 1) (t - 1) ≥ 15

Explanation: n: sample size of each groupt: number of groups

According to the Frederer formula, the number of samples required:

(n - 1) (t - 1) ≥ 15(n - 1) (3 - 1) ≥ 15(n - 1) 2 ≥ 152n = 17, n = 8.5

The number of samples used must be greater than or equal to 9 samples per group. In this study, 10 test samples will be used in each group. This is done to facilitate the author in data analysis calculations, so the total number of samples used in this study is 40 samples.

Sampling Technique

Randomized Control Trial pre and post-test design. Eligible participants/patients who meet the inclusion and exclusion criteria will be randomized. Participants will be grouped into four groups, namely the control group and the treatment group, and then the outcomes will be observed.

## F. Implementation/Research Procedure

1. Recruitment of research subjects and randomizationAll patients with persistent allergic rhinitis, Randomization with closed envelopes, Therapy according to the persistent allergic rhinitis algorithm

2. Source of apple fruit extractApple fruit extract is made in the Pharmacy Laboratory of Muhammadiyah University of Surakarta.

3. Preparation of apple fruit extract Polyphenol extract is derived from the fruit of Mallus sylvestris mill obtained from apple farmers in Malang. Dried apples are extracted, and re-extracted twice with 50% ethanol. The obtained material is concentrated using a rotary evaporator (Rotavapor, Buchi Labortechnik AG, Switzerland) in a water bath at 40 °C. Furthermore, the extract is adsorbed using Amberlite XAD 16 resin (Brentag, Essen, Germany), and after the evaporation of ethanol, the collected fraction is dried in a vacuum oven SPT 200 (Zeamil, Krakow, Poland) (Mihailović et al., 2018).

4. Measurement of Malondialdehyde (MDA)Measurement of MDA using the Enzyme Linked techniqueItchy noseClinical symptoms in allergic rhinitis.TNSS Checked on the First day and the 14th dayOrdinalRhinorrheaClinical symptoms in allergic rhinitis.TNSS Checked on the First day and the 14th dayOrdinal.

## G. Statistical Analysis

Statistical analysis was conducted using IBM SPSS Statistics for Windows, version 25.0 (IBM Corp., Armonk, NY, USA). Data normality was evaluated using the Shapiro–Wilk test. For normally distributed and homogeneous data, one-way analysis of variance (ANOVA) was applied. When the data were not normally distributed but homogeneous, the Kruskal–Wallis test was used. Comparisons across three time points were performed using repeated-measures ANOVA, with the Friedman test employed as the nonparametric alternative.

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