Effect of *Musa paradisiaca* L. var. Kayu Unripe Fruit Ethanol Extract on Leukocyte Differentiation and Skin Histology of *Mus musculus*

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Abstract

The unripe fruit of the pisang kayu (*Musa paradisiaca* L. Var. Kayu) has been utilized to treat diarrheal illnesses traditionally by the people of Senduro Village, Lumajang, East Java, Indonesia. Previous studies have shown that ethanol extracts of unripe banana fruit have antidiarrheal effects. The opportunity to utilize the ethanol extract of unripe fruit of pisang kayu as a herbal medicine is quite large, but this herbal medicine must be developed further to meet safety requirements. Allergy tests are conducted to determine the safety of drug candidates because allergies can cause angioedema on the skin as well as an increase in eosinophils and basophils in differential leukocyte counts. Therefore, this study aims to conduct an allergy test by looking at the effect of unripe fruit ethanol extract of pisang kayu (*M. paradisiaca* L. Var. Kayu) on skin histology and differential leukocytes of Mus musculus. The allergy test was conducted acutely and subchronically. The dorsal skin was then made into histological preparations. Mice blood was taken from the tail, and the leukocyte differential test was conducted. The results showed that unripe fruit ethanol extract of pisang kayu (*M. paradisiaca* L. Var. Kayu) did not cause allergy. This can be seen from the absence of signs of reddish rash and angioedema in the observation of the morphology of the skin of *Mus musculus*. In addition, the absence of allergies was also seen from the absence of significant differences in all treatment groups ($p > 0.05$) seen from the thickness of the epidermis, the thickness of the dermis, and the presence or absence of hyperplasia. Furthermore, the lack of significant differences between treatment groups ($p > 0.05$) indicated the absence of allergy and the number of differential leukocytes, which were in the normal category as an indicator of allergy in all treatment groups.

Keywords: Allergy, Skin histology, Leukocytes differential, Pisang kayu

Introduction

Bananas are a primary fruit crop in tropical and subtropical climates [1]. Indonesia, as a location of origin for *Musa*, exhibits significant germplasm variety [2]. One type of banana plant is pisang kayu (*Musa paradisiaca* L. Var. Kayu), that was popular among locals in Senduro Village, Lumajang, Indonesia, as remedy for digestive tract infection [3]. Empirically, the Senduro people steam, boil and burn the unripe fruit of the pisang kayu (*Musa paradisiaca* L. var. Kayu) [4]. In previous studies, the ethanol extract of pisang kayu (EPPK) fruit has an antidiarrheal effect on *Escherichia coli*-induced *Mus musculus*. The antidiarrheal effect is produced in the form of changes in the consistency of feces to become denser and a decrease in the frequency of defecation and weight [5]. Based on the phytochemical screening test, ethanol extract of unripe pisang kayu (EEUPK) contains alkaloids, tannins, saponins, and flavonoids [6]. Tannin content, which

exhibited antidiarrheal activity induced by *Oleum ricini* at a dose of 200 mg/kg body weight, also demonstrated inhibitory and bactericidal effects on *Escherichia coli* in media concentrations of 25 and 50 % [4]. In addition, the results of previous studies [4] show that the content of phenolic compounds in pisang kayu fruit such as tannins, flavonoids and saponins has antibacterial activity against *Escherichia coli* so that it can be developed into topical drugs.

Herbal medicines are currently popular and acknowledged to varying extents across different countries as significant complementary and alternative treatments for chronic diseases [7]. Herbal medicines comprise any part of plants, including herbs and herbal materials, as well as their combinations, extracts, and purified products, all of which contain active ingredients known as phytochemicals [8]. The historical utilization of herbal medicines highlights their potential benefits; however, the modern scientific community emphasizes the necessity for rigorous evaluations of their safety and efficacy. Unlike pharmaceutical drugs, herbal products frequently lack standardized dosages and may interact with other medications [9]. Besides, comprehensive data concerning the quality, safety, and efficacy of numerous plants, their extracts, preparations, and active compounds remain limited [10]. Therefore, ensuring their quality is of paramount importance to guarantee their safety and effectiveness [9].

In the realm of drug development, achieving safety and efficacy is a crucial objective. This necessitates a thorough understanding of the complex interactions between drugs and biological systems [11]. The process of developing new drugs and obtaining approval from regulatory agencies necessitates extensive research and rigorous testing to confirm their safety and efficacy [12]. Drug hypersensitivity reactions (DHR) are characterized as immune-mediated responses to a drug, whether it is a small molecule or a protein, resulting in an inflammatory reaction [13]. Even though various drug classes, such as antibiotics, nonsteroidal anti-inflammatory drugs (NSAIDs), and anesthetics, can generate potentially immunogenic epitopes, only a small proportion of patients exhibit allergic reactions. However, when these reactions do occur, they can result in severe outcomes, including significant organ damage and potentially fatal anaphylaxis. Additionally, while the skin, liver, lungs, and kidneys are the most frequently affected tissues, any organ or tissue can potentially be harmed through immunological mechanisms [14].

Various medicinal herbs possess pharmacological properties that can induce allergic reactions in sensitive individuals [15]. Allergic reactions are distinguished by swift inflammatory immune responses to antigens that are otherwise innocuous or harmless. The defining feature of these reactions is hypersensitivity upon re-exposure to the antigen, usually occurring at the sites of antigen contact. This swift response is driven by antigen-specific immunoglobulin E (IgE) [16]. The generation of antigen-specific IgE antibodies can trigger the degranulation of mast cells, leading to a range of symptoms [17]. Allergies can also give a reaction in the form of an increased number of leukocyte type counts [18]. An increase in the number of eosinophils can occur due to an excessive immune response to allergens, infection, or inflammation, as well as other conditions such as parasitic infections or certain drug reactions involved in leukocytosis [19]. An increase in basophils can also indicate an allergic mechanism in the body because basophils play an important role in allergic response [20]. This increase is due to eosinophils and basophils playing an important role as effector cells in allergic inflammation mechanisms, as well as in adaptive and innate immune responses [15].

In addition to leukocytes, allergies can manifest through a variety of symptoms, including skin reactions like urticaria, angioedema, atopic eczema, or contact dermatitis. Additionally, individuals may experience skin sensations that occur independently of visible skin lesions, such as pruritus, flushing, burning, and pain [21]. Angioedema is characterized by pale or erythematous swelling, primarily impacting areas such as the face, lips, tongue, oral mucosa, male genitalia, hands, and feet, due to deeper edema within the dermis [22]. Skin reactions caused by allergies can have a substantial and significant impact on the quality of life [21,23]. In addition, allergy testing also includes a series of *in vivo* nonclinical toxicity tests required by the Indonesian Food and Drug Administration (BPOM) [24]. Therefore, an allergy assessment was conducted to determine the safety of prospective drugs by looking at the effect of EEUPK on skin histology and differential leukocytes of *M. musculus*.

Materials and methods

Pisang kayu extraction

Pisang kayu fruits, 3 months after flowering, were washed and cleaned of foreign particles and then drained. A food dehydrator with the temperature of 50 °C was then used to dry the fruit after it had been cut into small pieces. Drying is done with the aim of opening the cell walls of plants to be extracted so that the content of phenolic compounds can come out. After drying, the sample was mashed using a blender then stored in a tightly closed container [4]. Extraction was carried out using the maceration method. This method was chosen because of several advantages, such as simple methods and tools, and it does not damage the content of natural ingredients because it does not use heat [25]. This method was carried out by weighing 500 g of pisang kayu unripe fruit powder and adding 96 % ethanol solvent, totaling 2.5 L. The powder was put into a glass jar, and the solvent was added little by little. The pisang kayu unripe fruit powder was macerated with 96 % ethanol solvent for 2 days with stirring and changing solvents every day. The maceration period lasts 24 h with 2 stirrings; following this, the solvent is changed, and soaking occurs for an additional 24 h. The soaking procedure is reiterated until the solvent's color becomes transparent [4]. To create a thick extract, the solution was filtered using a Buchner funnel, and the macerate was then evaporated using a rotary evaporator with a temperature of 50 °C, adjusting the boiling point of the ethanol solvent to avoid damaging the content in the extract.

Research ethics approval

The Health Research Ethics Committee of the Faculty of Dentistry at Airlangga University has reviewed and approved this study. (Research ethics number: 0061/HREEC.FODM//II/2024).

Acute allergy test

An acute allergy test was conducted on 30 male *M. musculus* that had been acclimatized for 2 weeks. The number of animals has been adjusted to the Organization for Environmental Control Development (OECD) Guidelines for the Testing of Chemicals no.406 regarding Skin Sensitization where a minimum of 10 animals for the treatment group and 5 animals for the control group are required [26]. The test animals were then grouped into 6 groups with one control group and 5 treatment groups. *M. musculus* had its back hair shaved, then punctured to the dermis layer and given treatment according to the existing group. The test is carried out using the skin prick test method by puncturing the dermis layer that contains dendritic cells [27]. The EEUPK that will be given has been mixed with 0.5 % CMC-Na solution. Grouping of test animals for treatment can be seen in **Table 1**. The dose group used in this study refers to the results of the acute toxicity test of EEUPK, which received an LD50 value of 2,535.128 mg/KgBW and is included in the mild toxic category [28]. Regarding the adjustment of safe doses from the previous toxicity test, doses of 50, 100, 200, 400 and 800 mg/KgBW were selected for this test. After 30, 60 and 90 min post-application, signs of allergy, such as redness and rash on the skin were observed. The data obtained from skin assessment then converted into numbers according to the Magnusson and Kligman scale in **Table 2**.

Table 1 Group assignment of test animals.

Groups	Treatment
Control	CMC Na 0.5 %
Group II	Ethanol extract pisang kayu 50 mg/kg BW
Group III	Ethanol extract pisang kayu 100 mg/kg BW
Group IV	Ethanol extract pisang kayu 200 mg/kg BW
Group V	Ethanol extract pisang kayu 400 mg/kg BW
Group VI	Ethanol extract pisang kayu 800 mg/kg BW

Table 2 Magnusson and Kligman scale.

Topical reaction	Grade
No visible changes	0
Light erythema	1
Moderate erythema	2
Severe erythema and edema	3

Subchronic allergy test

A subchronic sensitization test was carried out on 30 male mice that had been acclimatized and used the same dose groups as the acute allergy testing. The test is divided into 3 stages, namely preliminary tests using the Buehler test, topical induction tests, and challenge tests. In the preliminary test, samples of varying concentrations were applied to the flank region. The test preparation, as much as 0.5 mL for the liquid material, was placed on the chamber, and added filter paper (2×4 cm) was then applied to the shaved area, followed by an elastic bandage and an occlusive dressing. After 6 h the patch was removed, and observations were made 24 and 48 h following the removal of the patch. The results were observed using the Magnusson and Kligman scales in **Table 2**. Entering the next stage, the topical induction test, the test substance was administered to the shaved flank region on day 0. The test preparation (weighing 0.5 g for semi-solid materials or 0.5 mL for liquid materials) was placed on the chamber; for liquid materials, after applying filter paper, the skin was coated with an occlusive dressing and wrapped in an elastic bandage. After (6 ± 0.5) h, the patch was opened, and observations were made. The same procedure was repeated on the same test area (if necessary, the hair was shaved again) on day (7 ± 1) and day (14 ± 1). The control group underwent the identical procedure, except a solvent was used in place of the test preparation. All test groups and control groups underwent the challenge test 14 days following the last topical induction, with the flanks being shaved 24 h beforehand. The topical induction and test preparation exposure should not take place at the same time. Mice's shaved flanks were topically treated with the test preparation at the greatest concentration that did not cause erythema, followed by an occlusive dressing and an elastic bandage. After (6 ± 0.5) h, the patch was opened. Approximately 21 h after the patch was removed, the test area was cleaned and shaved if necessary. Observations were made on the appearance of the skin in the challenge area at 24th and 48th hours after the patches were removed, and the presence of edema and erythema was recorded according to Magnusson and Kligman scale ratings. The Magnusson and Kligman scales (**Table 2**) are used to characterize and classify skin reactions in relation to erythema and edema. Additional notes may be made if unusual responses are found. In addition, the body weight of the test animals before and after testing should be recorded [26].

Leukocytes differential

After the allergy test, *M. musculus* was anesthetized by administering ketamine 0.04 mg/KgBW and xylazine 0.02 mL/KgBW by injecting liquid into the vein. Blood was taken from the lateral vein of the tail [29]. Blood smears were fixed for 5 min by immersing the slides in methanol. After being taken out of the methanol, the smears were dried. After 30 min of staining with 1 % Giemsa solution, blood smears were rinsed with distilled water and left to dry. At 1,000× magnification, the stained blood smears were quantified as 100 leukocytes [30].

Skin histology

The backs of mice that have been shaved are anesthetized with ketamine 0.04 mg/KgBW and xylazine 0.02 mL/KgBW by injecting liquid in the muscularis. The skin was cut with a thickness of ± 3 mm to reach dWAT (dermal white adipose tissue) and 2 cm long. The animals were sacrificed after skin collection was completed. The skin was then preserved using a 10 % formalin solution and allowed to come to room temperature for ± 48 h [31]. Tissue samples were dehydrated with graded alcohol (70, 80, 90 %, and absolute alcohol) twice for 2 h each. In the clearing stage, the samples were put into xylol 3 times for 1 h each. After that, it was continued with infiltration in paraffin at 60 °C for 3 times for 1 h each. Then the

samples were embedded in paraffin and tissue blocks. Using a microtome, tissue blocks that were 5 μm thick were cut and put on a piece of object glass that had been coated with entellan [32].

Tissue staining begins with the process of deparaffinization using xylol for 3 repetitions, each for 2 min, followed by rehydration using alcohol solution with decreasing concentration (absolute, 90, 80 and 70 %), each for 5 min, then rinsed with distilled water and dried. Next, the tissue was stained with hematoxylin for 5 min and rinsed again with distilled water and waited to dry. The tissue was then stained again with eosin for 2 min and followed by graded alcohol solutions (70, 80, 90 % and absolute), clearing with xylol, and ending with slides covered with a glass cover (mounting process) using entellan [32]. The skin thickness was subsequently observed and measured utilizing the Optilab® system. Additionally, the ImageRaster® provided 400× photo magnification when attached to the ocular lens of the light microscope [33].

Data analysis

Results from skin thickness measurements and differential leucocyte tests were expressed as averages. Prior to analysis, a normality test was performed using the saphiro-wilk test. After that, the data were statistically analyzed using Kruskal-Wallis for non-normally distributed data and one-way analysis of variance (ANOVA) for normally distributed data. Tukey's post hoc test was used to determine whether the differences between treatment groups were significant. Meanwhile, data from the observation of rash size or redness and edema were analyzed descriptively and quantitatively.

Results and discussion

The resulting unripe fruit extract of pisang kayu (*M. paradisaca* L. Var. Kayu) is a thick, blackish-green extract with a distinctive odor. The extract obtained by the maceration method produced a yield (% b/v) of 6.45. Based on the previous studies, this extract contains secondary metabolites such as tannins, alkaloids, saponins, and flavonoids, according to phytochemical screening [6]. In the acute test, a puncture was made on the skin as a pathway for the entry of EEUPK into the body of *M. musculus*. While the treatment in the subchronic test was carried out by attaching filter paper containing liquid to the skin of the flank area. The administration of EEUPK is expected not to cause allergic reactions indicated by rash and edema. The results of the effect of the administration of EEUPK on the skin can be seen in **Tables 3** and **4**.

Table 3 Results of back morphological observations of acute allergy test of *M. musculus*.

Groups	Observation time (min)		
	30	**60**	**90**
Control	0	0	0
50 mg/KgBW	0	0	0
100 mg/KgBW	0	0	0
200 mg/KgBW	0	0	0
400 mg/KgBW	0	0	0
800 mg/KgBW	0	0	0

Table 4 Results of back morphological observations of subchronic allergy test of *M. musculus*.

Groups	Observation time (Day)				
	D1	D7	D14	Challenge test 24 h	Challenge test 48 h
Control	0	0	0	0	0
50 mg/KgBW	0	0	0	0	0
100 mg/KgBW	0	0	0	0	0
200 mg/KgBW	0	0	0	0	0
400 mg/KgBW	0	0	0	0	0
800 mg/KgBW	0	0	0	0	0

Note: D1 is Day 1, D7 is Day 7, and D14 is Day 14.

Skin testing has long been a fundamental procedure in diagnosing allergies. It provides the benefit of immediate results, allowing for prompt discussions with patients. This approach eliminates the need to wait for laboratory results or schedule a follow-up appointment to discuss the clinical significance of the allergy tests [34]. As the primary interface between the body and the environment, the skin serves as a potential route for allergen penetration and plays a crucial role in allergy development. An intact epidermal barrier protects against exposure to external allergens, while any physical or functional compromise of this barrier facilitates sensitization [21]. The skin also serves as a crucial pathway for drug delivery, enabling topically administered drugs to permeate through the stratum corneum (SC) and reach deeper skin layers, potentially entering systemic circulation [35].

Based on the involvement of the immune system and the specific cells implicated, drug hypersensitivity reactions (DHRs) are categorized into allergic drug hypersensitivity reactions (ADHRs) and non-allergic drug hypersensitivity reactions (NADHRs), with type I reactions being mediated by IgE antibodies [36]. This type of hypersensitivity can cause life-threatening conditions, such as angioedema [37]. Angioedema is a swelling reaction that is not followed by itching with a soft consistency that occurs quickly after contact with an allergen [38]. It results from increased vascular permeability and can persist from a few hours to several days. This condition may manifest as non-pitting, non-pruritic skin edema or swollen mucosa, which has the potential to obstruct the airway [37].

In various forms of angioedema, there are 2 main mediators, histamine and bradykinin [37]. Both of these inflammatory mediators have been demonstrated to cause a transient opening of the endothelial barrier. Histamine and bradykinin facilitate the formation of intercellular gaps and leakage in the endothelium by promoting VE-cadherin internalization through its phosphorylation at Tyr658 and Tyr685 [39]. In bradykinin-mediated angioedema, bradykinin acts as a vasoactive peptide that causes edema to occur by increasing vascular permeability and vasodilation [40]. Meanwhile, the most prevalent type of angioedema is histamine-mediated, which is frequently encountered in emergency departments, accounting for approximately 40 - 50 % of angioedema cases. It frequently appears as an immediate type I hypersensitivity reaction, impacting the tongue, lips, throat, and lips, often accompanied by hives or urticaria [41]. Urticaria, an inflammatory disorder, manifests with temporary wheals and angioedema, either individually or together, and does not involve systemic symptoms. It arises from mast cell degranulation, which can be spontaneous or induced by various factors [42]. This condition may last for a few minutes to less than 24 h [43].

Based on observations in **Tables 3** and **4** regarding post-treatment skin morphology, it can be seen that all treatment groups in the acute and subchronic tests received a score of 0, which means that there was no rash and edema or swelling in all treatment groups and the control group. This indicates that EEUPK does not cause allergies in all treatment groups seen from the skin morphology of the test animals. The absence of allergic reactions to the skin is also indicated by the findings of the average thickness measurements of the dermis and epidermis, which are displayed in **Table 5**. However, this absence of edema may also be due to the flavonoid content in the extract. It is said that flavonoids such as quercetin and morin, exhibit lipoxygenase inhibition.

Flavonoids, as potent inhibitors of arachidonic acid, phospholipase A2, cyclooxygenase, and nitric oxide synthase (NOS), can reduce the synthesis of prostaglandins, leukotrienes, and nitric oxide (NO) as important inflammatory substances. Flavonoids also lessen the release of chemokines and metabolites of arachidonic acid, which lowers leukocyte infiltration and edema [44].

Table 5 Average thickness of epidermis and dermis of *M. musculus* in each treatment in acute and subchronic test.

Groups	Thickness average (µm)	
	Epidermis	Dermis
Acute test result		
Control	23.46 ± 5.46[a]	267.6 ± 26.17[a]
50 mg/KgBW	23.25 ± 5.72[a]	266.2 ± 12[a]
100 mg/KgBW	17.79 ± 3.85[a]	232 ± 50.8[a]
200 mg/KgBW	20.28 ± 1.49[a]	272.3 ± 24.95[a]
400 mg/KgBW	21.45 ± 4[a]	240.6 ± 21.9[a]
800 mg/KgBW	15.97 ±2.8[a]	242.7 ± 20.41[a]
Subchronic test result		
Control	22.82 ± 6.14[a]	258.6 ± 31.7[a]
50 mg/KgBW	22.83 ± 3.96[a]	254.9 ± 19.51[a]
100 mg/KgBW	17.21± 3[a]	266.6 ± 56.22[a]
200 mg/KgBW	20.33 ± 1.53[a]	229.9 ± 29.93[a]
400 mg/KgBW	20.27 ± 4.73[a]	230.4 ± 31.65[a]
800 mg/KgBW	20.48 ± 5[a]	185.6 ± 59.7[a]

Note: All skin layers in all treatment groups in both acute and subchronic test have the same letter notation, and the *p*-value obtained in all skin layers is above 0.05 ($p > 0.05$) indicating non-significant difference in all treatment groups.

The observation of skin thickness in histological preparations acts as supporting data from observations of skin morphology, which states that there is no allergic reaction as evidenced by the absence of skin thickening or hyperplasia. **Figures 1** and **2** illustrate how the thickness of the epidermis differs among individuals. Even within the same species, the thickness of the epidermis will change in various body parts and within a single area according to physiological factors, including gender and hair cycle stage [45]. Epidermis in mice has a size of < 25 µm [46]. Based on this, it can be said that the epidermis in the treatment group given EEUPK does not have thickening because it has a thickness of < 25 µm. These results also indicate that the epidermis in the test animals is normal and there is no thickening or hyperplasia in both the epidermis and dermis layers. Hyperplasia refers to the increase in the number of cells typically found in a tissue or organ. In many instances, this condition is attributed to enhanced cellular replication. Drugs and chemicals can induce hyperplasia both directly and indirectly. A direct example of hyperplasia is the increased cell proliferation observed after administering agents that function as ligands for nuclear receptors [47]. This condition, as well as inflammation, is a feature of various skin conditions. IgE through FcεRI signalling on basophils will potentially promote epidermal hyperplasia [48].

The size of the dermis obtained is also normal, where the dermis on the back of *M. musculus* should have a size of less than 280 µm (275 - 280 µm) [49]. The dermis is a layer of skin that originates from the mesoderm and is a place for hair follicles, sweat glands, nerves, and oil glands [50]. The dermis predominantly consists of an acellular component known as the extracellular matrix (ECM). Collagen fibers are a key element of the ECM, comprising 75 % of the skin's dry weight and offering tensile strength and elasticity [51]. Dermis is divided into 2 distinct regions: The superficial

papillary layer and the deeper reticular layer [52]. In the reticular dermis, the fibers are typically thicker and

often bundled together, whereas the papillary dermis is much thinner in comparison [53].
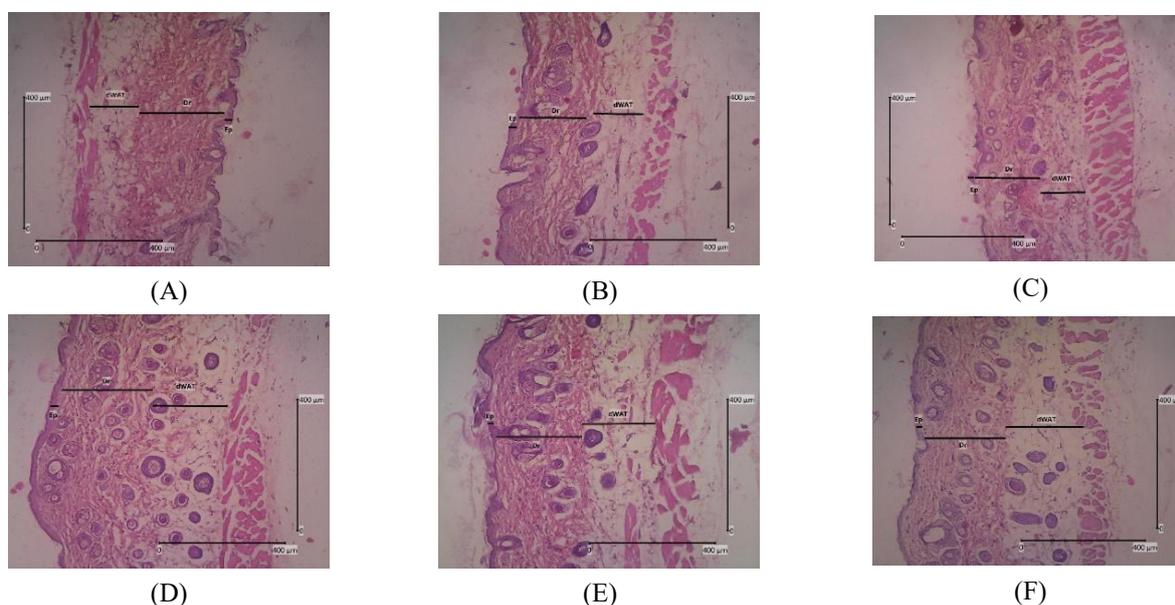
Note: (A) Control, (B) 50 mg/KgBW, (C) 100 mg/KgBW, (D) 200 mg/KgBW, (E) 400 mg/KgBW and (F) 800 mg/KgBW. The difference in thickness in dWAT (Dermal White Adipose Tissue) is due to differences in the growth phase of hair follicles in each individual.

Figure 1 *M musculus* skin histology after treatment in acute allergy test with magnifition 400× and hematoxylyn-eosin dye.

Ensuring drug safety is an important step to prevent patients or prospective patients from various negative reactions, especially drug allergy reactions. Drug hypersensitivity reactions (DHRs) are a form of adverse drug reaction that can occur across various drug classes, affecting multiple organ systems and a wide range of patient populations [14]. Drug hypersensitivity reactions are type B drug interactions and represent one-3rd of all drug interactions [54]. According to the World Health Organization (WHO) consensus, adverse drug reactions (ADRs) are classified into 2 categories: Predictable (type A) and unpredictable (type B) reactions [36]. Type B reactions are rare but tend to be severe in nature and can potentially lead to mortality [55]. This is due to type B reactions are more challenging to identify and detect during preclinical and clinical trials prior to a drug's market approval [36].

In this study, drug allergy testing was carried out through the skin prick test (SPT) method. In the United States, diagnostic tests for drug allergies predominantly rely on immediate skin testing and drug challenges [56].

SPT and IDT with direct reading are used to investigate hypersensitivity reactions directly. Reactions tend to occur 1 h after drug administration. Self-onset symptoms occur on the skin and mucous membranes, such as rash/urticaria and angio-edema, and can occasionally develop into more severe anaphylactic shock, hypotension, and bronchospasm symptoms [54]. Large molecular weight drugs can be directly identified by immune cells and antibodies. In contrast, most drugs function as haptens because they are too small (less than 1,000 Da) to trigger an immune response on their own and must bind covalently to a protein to form an antigen [57]. These hapten-self protein complexes are then either taken up by or produced from drug pro-haptens within antigen-presenting cells (APCs). They are processed and presented on major histocompatibility complex (MHC) I or II molecules, which may lead to the activation of drug-specific T cells [14]. Tumor necrosis factor alpha (TNF-α), mast cell protease (MCP), interleukin-9 (IL-9), and other inflammatory mediators are released when IgE binds to certain

receptors on the surface of mast cells in response to allergen recognition. In the intestinal mucosa, Th-2 cytokines like IL-5 and IL-13, which cause mast cell hyperplasia and B lymphocytes to produce allergen-specific IgE, dominate the ensuing inflammatory response [58].

Differential leukocyte data were also obtained from male *M. musculus* used as test animals. White blood cell differential analysis is a commonly employed diagnostic method in both medical practice and research. It provides valuable insights into the immune system's condition and offers crucial information regarding an organism's overall health [59]. Differential leukocytes need to be known because the increase in the number of leukocyte types can be caused by allergic reactions [18]. Male animals were chosen because

female animals have estrogen hormones that rise and fall according to the estrus phase. Estrogen has an influence on the immune system because estrogen will stimulate the production of Th2 cytokines, which will increase the growth and proliferation of Th2 cells to elevate IgE production and eosinophil function related to inflammatory reactions due to allergies and bacterial infections [60]. The test is carried out by piercing the skin to the dermis layer so that the ethanol extract can be recognized by the immune system. There is a more varied population of mononuclear phagocytes in the dermis layer, such as dermal macrophages and dermal dendritic cells, or DC cells [61]. Macrophages are antigen-presenting cells present in the dermis that are able to identify pathogens and cause damage by triggering the proper immune response [62].
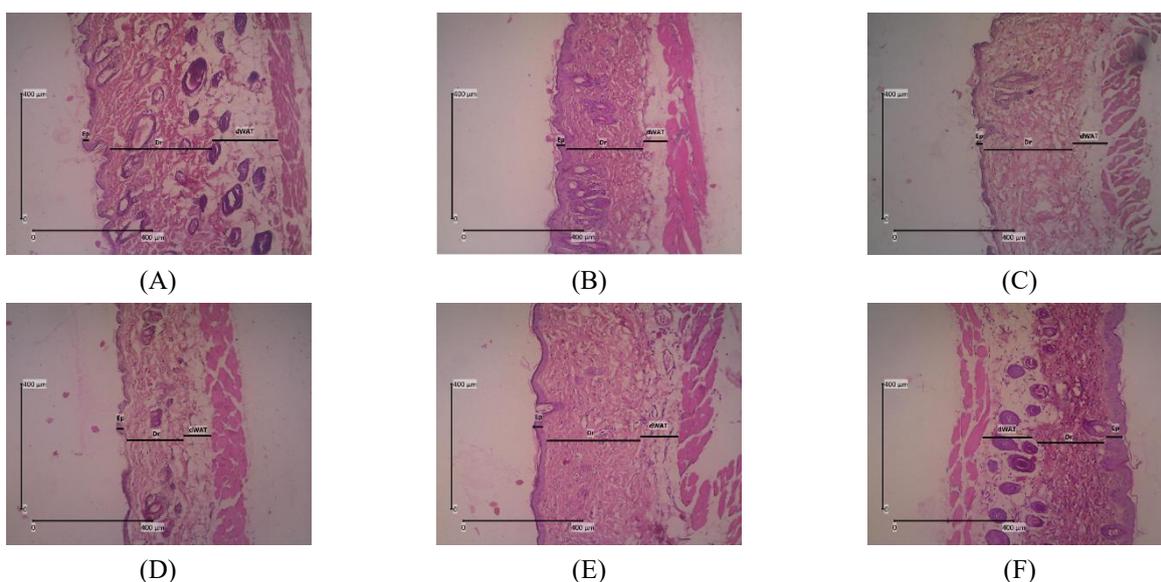
(A)　　　　　　　　　　(B)　　　　　　　　　　(C)

(D)　　　　　　　　　　(E)　　　　　　　　　　(F)

Note: (A) Control, (B) 50 mg/KgBW, (C) 100 mg/KgBW, (D) 200 mg/KgBW, (E) 400 mg/KgBW and (F) 800 mg/KgBW. The difference in thickness in dWAT (Dermal White Adipose Tissue) is due to differences in the growth phase of hair follicles in each individual.

Figure 2 *M musculus* skin histology after treatment in subchronic allergy test with magnifition 400× and hematoxylyn-eosin dye.

Table 6 Differential leukocyte results in acute and subchronic testing.

Groups	Leukocyte differential results in acute testing				
	Mean acute test leukocyte differential (%)				
	Neutrophils	Lymphocytes	Eosinophils	Monocytes	Basophils
Control	47 ± 7^a	47.4 ± 7.8^a	3.2 ± 0.7^a	2 ± 1^a	0^a
50 mg/KgBW	44.5 ± 4.5^a	49 ± 4.58^a	4 ± 1.22^a	2.5 ± 1.12^a	0^a
100 mg/KgBW	42.25 ± 7.6^a	52.5 ± 7.57^a	2.75 ± 1.3^a	2.5 ± 0.5^a	0^a

Groups	Leukocyte differential results in acute testing				
	Mean acute test leukocyte differential (%)				
	Neutrophils	Lymphocytes	Eosinophils	Monocytes	Basophils
200 mg/KgBW	37.75 ± 4.06[a]	56 ± 4[a]	3.75 ± 1.09[a]	2.5 ± 0.5[a]	0[a]
400 mg/KgBW	48.25 ± 8.06[a]	47.25 ± 9[a]	2.75 ± 1.26[a]	1.75 ± 0.55[a]	0[a]
800 mg/KgBW	41.75 ± 8.88[a]	53 ± 8.62[a]	3.25 ± 0.96[a]	1.25 ± 0.5[a]	0[a]
Control	26.75 ± 5.17[a]	66.75 ± 5.45[a]	4.5 ± 0.5[a]	2 ± 0[a]	0[a]
50 mg/KgBW	31.75 ± 1.92[a]	63.25 ± 1.48[a]	3.25 ± 0.83[a]	1.75 ± 0.43[a]	0[a]
100 mg/KgBW	23.5 ± 4.39[a]	73 ± 4.12[a]	2.25 ± 0.83[a]	1.25 ± 0.43[a]	0[a]
200 mg/KgBW	31.5 ± 4.27[a]	62.75 ± 4.26[a]	3.75 ± 0.43[a]	2 ± 0[a]	0[a]
400 mg/KgBW	32 ± 13.34[a]	63.5 ± 12.92[a]	2.5 ± 1.29[a]	2 ± 0.81[a]	0[a]
800 mg/KgBW	27.5 ± 8.27[a]	68.5 ± 8.81[a]	2.5 ± 0.58[a]	1.5 ± 0.58[a]	0[a]
Reference	10 - 40 [63]	> 50 [59]	1 - 5 [64]	< 2 [65]	0.5 - 1 [66]

Note: All types of leukocytes in all treatment groups in both acute and subchronic testing have the same letter notation and show that the *p*-value obtained is above 0.05 ($p > 0.05$) showing no significant differences in all treatment groups.

The differential leukocyte result in **Table 6** is derived from the calculation formula: Leukocyte type/100×%. The number 100 represents the total leukocytes counted. This calculation can be done due to the 1 % Giemsa dye applied. Giemsa dye contains eosin, which stains basic cell component like cytoplasm granules, and methylene blue which stains the nucleus as an acidic component of cell [67]. Based on the leukocyte differential data in **Table 6**, there are differences in the results in each treatment group for each number and type of leukocytes present. The results in the **Table 6** show that the average value of lymphocytes is higher than the average value of neutrophils except in the 400 mg/kgBW dose group. The average number of lymphocytes that is higher than neutrophils is in accordance with the existing literature where *M. musculus* has a high number of lymphocytes, which is > 50 % [59] with only 10 - 40 % neutrophils [63]. This contrasts with humans, who have 30 - 50 % lymphocytes and 50 - 70 % neutrophils [68]. However, the number of neutrophils in all treatment groups in acute testing had values above 30 % even though it is still within the normal range. This can be caused by stab wounds that trigger inflammation. Wounds are the result of physical trauma that breaks the skin tissue [69].

The impact of the wound increasing the number of neutrophils will be seen in the comparison of differential

leukocyte results in acute testing with subchronic testing results. The neutrophil results in the subchronic test were lower in the range of 23.5 - 32 %. This is due to the absence of wounds made as a pathway for extract entry. In the healing process, wounds undergo 4 phases, namely homeostasis, inflammation, proliferation, and remodelling [70]. Neutrophils are the primary circulating leukocytes in the blood and are regarded as the 1st responders of the immune system [68]. The early stages of inflammation are dominated by neutrophils [71]. Neutrophils are typically the 1st cells to gather at sites of injury and microbial infection. Their departure from the blood into tissues is a multi-step process, which includes the activation of polymorphonuclear leukocytes (PMNs), rolling and adhering to the activated endothelium, trans endothelial migration, and subsequent chemotactic movement toward the site of inflammation [72]. Activated neutrophils mediate inflammation by secreting and synthesizing leukotrienes, cytokines, prostaglandins, and chemokines. In particular, neutrophils have been shown to secrete and synthesize the chemokine CXCL8, which will attract more neutrophils [73].

Table 6 shows that the mean value of eosinophils in all treatment groups varied in the range of 2.75 - 4 % for acute testing and 2.5 - 4.5 % for subchronic testing. These results are still included in the normal category

because in healthy individuals eosinophils will be found with a small percentage of 1 - 5 % of total white blood cells count [64]. Eosinophils are known to contribute to allergic reactions. It has been documented that an increase in eosinophil infiltration in tissues is a characteristic feature of allergic diseases [74]. Increased eosinophil counts are caused by parasites, atopic disorders, and allergic illnesses [75]. Eosinophil levels rise considerably in certain pathological conditions, affecting both blood and tissue compartments. This increase is primarily due to a multi-immune response involving type 2 (T2) cytokines and other pro-inflammatory mediators [76]. Eosinophils promote allergic inflammation through the release of pro-inflammatory mediators such as leukotrienes C4 (LTC4), major basic protein (MBP), eosinophil cationic protein (ECP), IL-4, and IL-13 [64]. Therefore, the mean value of eosinophils that is less than 5 % in the test results indicates that no hypersensitivity or allergic response occurs.

The mean value of monocytes as displayed in **Table 6** shows that monocytes are in the range of 1.25 - 2.5 for acute testing and 1.25 - 2 for subchronic. Monocytes make up less than 2 % of the total leukocyte count in mice [65]. Monocytes are an essential part of the mononuclear phagocytic system, which breaks down foreign objects and microbes in a variety of tissues. By interacting with lymphocytes and developing into dendritic cells, these blood cells also serve as antigen-presenting cells, controlling immunological and inflammatory responses [77]. Based on the test results, there is an average value for both tests where the results of the acute test show a higher upper limit average value of 2.5 % compared to the subchronic test, which has a value of 2 %. This difference is again due to the presence of puncture wounds on the back in acute individuals. The increase in monocytes can be attributed to the tissue damage caused by the puncture wound on the dorsal skin of *M. musculus*. Following an injury, circulating monocytes rapidly exit the peripheral blood and migrate into tissues, where they play a crucial role in initiating, coordinating, and resolving inflammation [78]. The rupture/damaged tissue can release a danger signal called Damaged-Associated Molecular Patterns (DAMPSs) [79]. Furthermore, monocytes that have cellular receptors called Pattern Recognition Receptors (PRRs) will recognize DAMPs. Pro-inflammatory

cytokines will activate and attract inflammatory cells such as monocytes from the blood circulation to the location where DAMPs are located, which will increase monocyte cells [80].

White blood cells called basophils are important in a number of allergy diseases. Following exposure to allergens, mast cells and basophils release vasoactive mediators that later will cause anaphylaxis, which is an allergic reaction that occurs rapidly and is potentially life threatening [81]. Considering the information in **Table 6**, it can be seen that basophils in all test models and all treatment groups have a value of 0. The value is very small when compared to other white blood cells. This is due to the fact that when measuring white blood cells (WBCs), basophils are the least prevalent type of leukocyte that representing only 0.5 - 1 % [82]. The absence of basophils in this calculation does not mean that there are no basophils in the blood circulation of the test animals. This because basophils are only 0.5 - 1 % of all leukocytes, making them the least common granulocytes. The expression of IgE receptors, where basophils contain a high-affinity FcεRI on the cell surface that may bind directly to IgE, is the primary characteristic that distinguishes these cells from mast cells. Crosslinking between FcεRI and allergen-induced IgE causes the release of effector and immunoregulatory mediators, including leukotriene-4 (LTC-4), histamine, and Th2 cytokines like IL-4 and IL-13. Where there is allergic irritation in the skin and airways, basophils and eosinophils are found [66].

Conclusions

The result of this study concludes that ethanol extract of unripe pisang kayu (EEUPK) does not have an allergic effect as seen from the absence of notable differences ($p > 0.05$) in the epidermis and dermis thickness in all treatment groups, which indicates the absence of hyperplasia. The absence of allergy is also evident from the absence of reddish rash and edema in the morphological observation of the back of *M. musculus*. The extract also did not have an allergic effect as seen from the absence of significant differences in the average results of differential leukocytes. In addition, the number of eosinophils and basophils used as an indicator of allergy is also still in the normal category in all treatment groups, indicating the absence of allergy in *M. musculus*.

References

[1] J Zhou, S Liang, Y Cui, Y Rong, J Song and D Lv. Study on environmental behaviour of fluopyram in different banana planting soil. *Scientific Reports* 2021; **11(1)**, 15346.

[2] RF Rahayuniati, S Subandiyah, S Hartono, S Somowiyarjo, REK Kurniawan, AB Prakoso, K Crew, ME Vance, JD Ray and JE Thomas. Recent distribution and diversity analysis on banana bunchy top virus of banana and alternative host in Indonesia. *Tropical Plant Pathology* 2021; **46(5)**, 506-517.

[3] AW Ningsih, A Syahrani, AW Septama and Sukardiman. Predicting toxicity and conducting molecular docking analysis of compounds from unripe kayu banana fruit (*Musa paradisiaca* L. var. Kayu) against 3mzd and 2q85 proteins in *Escherichia coli* for antibacterial activity. *Pharmacy Education* 2024; **24(3)**, 329-335.

[4] AW Ningsih, A Syahrani, AW Septama and Sukardiman. Metabolit profilling and antibacterial activity of ethanol extract of the unripe fruit peel of kayu banana (*Musa paradisiaca* L. Var. Kayu). *Trends In Sciences* 2024; **21(2)**, 7154.

[5] ICS Klau, AW Ningsih and WFI Putra. Profil *Rendemen Ekstrak dan Fraksi Kulit Buah, Daging Buah, dan Buah Pisang Mentah* (*Musa paradisiaca* L.). *Journal of Pharmacy Science and Technology* 2021; **3(1)**, 181-190.

[6] AW. Ningsih, M Rochmanti and A Basori. Effectiveness of antidiarrheal unripe wooden banana (*Musa paradisiaca* L.) in male Balb-c/mice induced with *Escherichia coli*. *Folia Medica Indones* 2021; **56(3)**, 208.

[7] J Zhang, K Hu, L Di, P Wang, Z Liu, J Zhang, P Yue, W, Song, J Zhang, T Chen, Z Wang, Y Zhang, X Wang, C Zhan, YC CHeng, X Li, Q Li, JY Fan, Y Shen, JY Han and H Qiao. Traditional herbal medicine and nanomedicine: Converging disciplines to improve therapeutic efficacy and human health. *Advanced Drug Delivery Reviews* 2021; **178**, 113964.

[8] P Monika, MN Chandraprabha, A Rangarajan, PV Waiker and KNC Murthy. Challenges in healing wound: Role of complementary and alternative medicine. *Frontiers in Nutrition* 2022; **8**, 791899.

[9] A Balkrishna, N Sharma, D Srivastava, A Kukreti, S Srivastava and V Arya. Exploring the safety, efficacy, and bioactivity of herbal medicines: Bridging traditional wisdom and modern science in healthcare. *Future Integrative Medicine* 2024; **3(1)**, 35-49.

[10] D Dori, N Méda, FA Gbaguidi, J Quetin-Leclercq and R Semdé. Wholesale distribution and delivery of plant-based medicinal products through the pharmaceutical system of Burkina Faso. *Annales Pharmaceutiques Françaises* 2020; **78(2)**, 179-188.

[11] AMB Amorim, LF Piochi, AT Gaspar, AJ Preto, N Rosário-Ferreira and IS Moreira. Advancing drug safety in drug development: Bridging computational predictions for enhanced toxicity prediction. *Chemical Research in Toxicology* 2024; **37(6)**, 827-849.

[12] YM Al-Worafi. *Technology for drug safety: Current tatus and future developments*. Springer, Switzerland, 2023.

[13] WJ Pichler. The important role of non-covalent drug-protein interactions in drug hypersensitivity reactions. *European Journal of Allergy and Clinical Immunology* 2022; **77(2)**, 404-415.

[14] M Pallardy, R Bechara, J Whritenour, S Mitchell-Ryan, D Herzyk, H Lebrec, H Merk, I Gourley, WJ Komocsar, JR Piccotti, M Balaze, A Sharma, DB Walker and D Weinstock. Drug hypersensitivity reactions: Review of the state of the science for prediction and diagnosis. *Toxicological Sciences* 2024; **200(1)**, 11-30.

[15] S Bose, PP Das, S Banerjee and P Chakraborty. A comprehensive review on natural products caused allergy and its mechanism. *Journal of Herbal Medicine* 2023; **42**, 100778.

[16] PF Cañete and D Yu. Follicular T cells and the control of IgE responses. *Allergology International* 2024; **74(1)**, 13-19.

[17] EY Yim, AC Zhou, YC Yim, X Wang and T Xia. Antigen-specific mRNA lipid nanoparticle

platforms for the prevention and treatment of allergy and autoimmune diseases. *BMEMat* 2024; **2(2)**, e12060.

[18] PM Wiguna, IGA Sukarya and ME Suryani. Efek pemberian ekstrak daun tabat barito (*Ficus deltoidea*) terhadap hitung jenis leukosit pada tikus putih (*Rattus norvegicus*) alergi yang diinduksi ovalbumin. *Mahakam Medical Laboratory Technology Journal* 2023; **3(1)**, 13-21.

[19] SA Bennett, N Harris, J Chaffin and S Beal. Educational case: Hematologic and immunologic response to allergic rhinitis with other causes of leukocytosis. *Academic Pathology* 2020; **7**, 2374289520953568.

[20] H Shah, S Eisenbarth, CA Tormey and AJ Siddon. Behind the scenes with basophils: An emerging therapeutic target. *Immunotherapy Advances* 2021; **1(1)**, ltab008.

[21] S Salah, C Taieb, AL Demessant and M Haftek. Prevalence of skin reactions and self-reported allergies in 5 countries with their social impact measured through quality of life impairment. *International Journal of Environmental Research and Public Health* 2021; **18(9)**, 4501.

[22] K Brockow. *Drug allergy: Definitions and phenotypes*. *In*: DA Khan and A Banerji (Eds.). Drug allergy testing. Elsevier, Missouri, 2017, p. 19-26.

[23] ED Agosta, L Salvati, M Corazza, I Balardini, F Ambrogio, L Angileri, E Antonelli, F Belluzzo, D Bonamonte, L Bonzano, R Brancaccio, P Custurone, AD Marco, A Detoraki, AD Guilda, ED Leo, M Fanto, F Fassio, SM Ferrucci, C Foti, …, and O Rossi. Quality of life in patients with allergic and immunologic skin diseases: In the eye of the beholder. *Clinical and Molecular Allergy* 2021; **19(1)**, 26.

[24] Badan Pengawas Obat dan Makanan. *Peraturan badan pengawas obat dan makanan nomor 10 tahun 2022*. Peraturan Badan Pengawas Obat dan Makanan (Peraturan BPOM), Central Jakarta, Indonesia, 2022.

[25] AW Ningsih, Sukardiman, A Syahrani, AM Charisma and KI Wahyuni. Study of drying methods and extraction methods on phenolic contenct. *In*: Proceedings of the 2nd International Conference on Government Education Management and Tourism (ICoGEMT), Bandung, Indodnesia. 2022.

[26] OECD. *Test guideline no.406 skin sensitisation*. OECD, Paris, France, 2022.

[27] ES Chambers and M Vukmanovic-Stejic. Skin barrier immunity and ageing. *Immunology* 2020; **160(2)**, 116-125.

[28] DW Hikmal. 2023, Uji toksisitas akut ekstrak etanol buah mentah pisang kayu (*Musa paradisiaca* l. var. kayu) secara *in vivo* pada mencit (*Mus musculus*). Bachelor Degrees. Anwar Medika University, Sidoardjo, Indonesia.

[29] AL Whittaker and TH Barker. The impact of common recovery blood sampling methods, in mice (*Mus musculus*), on well-being and sample quality: A systematic review. *Animals* 2020; **10(6)**, 989.

[30] AM Halim, AB Cahyanurani and AA Aonullah. The effect of *Ocimum sanctum* L. crude extract on haematology of *Cyprinus carpio* infected by *Aeromonas hydrophila*. *IOP Conference Series: Earth and Environmental Science* 2022; **1036(1)**, 012086.

[31] B Febram, I Wientarsih and DB Pontjo. Activity of ambon banana (*Musa paradisiaca* var. sapientum) stem extract in ointment formulation on the wound healing process of mice skin (*Mus musculus* albinus). *Traditional Medicine Jornal* 2010; **15(3)**, 121-137.

[32] AL Mescher. *Junqueira's basic histology: Text and atlas*. McGraw Hill Education, New York, 2021.

[33] I Yudhika, M Jailani and Dasrul. Histopathological overview of wound healing process in white rats (*Rattus norvegicus*) using *Chromolaena odorata* leaf jelly extract. *Journal of International Surgery and Clinical Medicine* 2021; **1(2)**, 21-28.

[34] T Gureczny, B Heindl, L Klug, F Wantke, W Hemmer and S Wöhrl. Allergy screening with extract-based skin prick tests demonstrates higher sensitivity over *in vitro* molecular allergy testing. *Clinical and Translational Allergy* 2023; **13(2)**, e12220.

[35] YQ Yu, X Yang, XF Wu and YB Fan. Enhancing permeation of drug molecules across the skin via

delivery in nanocarriers: Novel strategies for effective transdermal applications. *Frontiers in Bioengineering and Biotechnology* 2021; **9**, 646554.

[36] J Han, C Pan, X Tang, Q Li, Y Zhu, Y Zhang and A Liang. Hypersensitivity reactions to small molecule drugs. *Frontiers in Immunology* 2022; **13**, 1016730.

[37] AM Eraky, A Wright and D McDonald. Pseudo-Allergies in the Emergency department: A common misdiagnosis of hypersensitivity type 1 allergic reaction. *Cureus* 2023; **15(10)**, e46536.

[38] S Lelyana. Hypersensitivity in dentistry. *Sound of Dentistry* 2020; **5(2)**, 22-31.

[39] R Wakasugi, K Suzuki and T Kaneko-Kawano. Molecular mechanisms regulating vascular endothelial permeability. *International Journal of Molecular Sciences* 2024; **25(12)**, 6415.

[40] M Lepelley, C Bernardeau, F Defendi, J Crochet, M Mallaret and L Bouillet. Update on bradykinin-mediated angioedema in 2020. *Therapies* 2020; **75(2)**, 195-205.

[41] H Lima, J Zheng, D Wong, S Waserman and GL Sussman. Pathophysiology of bradykinin and histamine mediated angioedema. *Frontiers in Allergy* 2023; **4**, 1263432.

[42] N Schettini, M Corazza, C Schenetti, L Pacetti and A Borghi. Urticaria: A narrative overview of differential diagnosis. *Biomedicines* 2023; **11(4)**, 1096.

[43] E Macy. Practical management of new-onset urticaria and angioedema presenting in primary care, urgent care, and the emergency department. *The Permanente Journal* 2021; **25(4)**, 21.058.

[44] JM Al-Khayri, GR. Sahana, P Nagella, BV Joseph, FM Alessa and MQ Al-Mssallem. Flavonoids as potential anti-inflammatory molecules: A review. *Molecules* 2022; **27(9)**, 2901.

[45] L Mecklenburg, D Kusewiti, C Kolly, S treuman, ET Adams, K Diegel, J Yamate, W Kaufmann, S Muller, D Danilenko and A Bradley. Proliferative and non-proliferative lesions of the rat and mouse integument. *Journal of Toxicologic Pathology* 2013; **26(3)**, 27S-57S.

[46] E Zingkou, G Pampalakis and G Sotiropoulou. Keratinocyte differentiation and proteolytic pathways in skin (patho) physiology. *The International Journal of Developmental Biology* 2021; **66(1-2-3)**, 269-275.

[47] RC Cattley. *Modes of chemically induced cell proliferation. In*: CA McQueen (Ed.). Comprehensive toxicology. Elsevier, Amsterdam, Netherlands, 2018.

[48] MD Hayes, S Ward, G Crawfoed, RC Seoane, WD Jackson, D Kipling, D Voehringer, D Dunn-Walters and J Strid. Inflammation-induced IgE promotes epithelial hyperplasia and tumour growth. *Elife* 2020; **9**, e51862.

[49] W Zhiqiang, L Jieshu and L Ji. Hair regeneration after epidermal peeling. *European Journal of Dermatology* 2020; **30(2)**, 203-205.

[50] GMCT Pratama, IGNBRM Hartawan, IGAT Indriani, MU Yusrika, SAA Suryantari and PSS Sudarsa. Potency of *Spirulina platensis* extract as sunscreen on ultraviolet b exposure. *Journal of Medicine & Health* 2020; **2(6)**, 205-217.

[51] JW Shin, SH Kwon, JY Choi, JI Na, CH Huh, HR Choi and KC Park. Molecular mechanisms of dermal aging and antiaging approaches. *International Journal of Molecular Sciences* 2019; **20(9)**, 2126.

[52] Z Lotfollahi. The anatomy, physiology and function of all skin layers and the impact of ageing on the skin. *Wound Practice and Research* 2024; **32(1)**, 6-10.

[53] B Lynch, H Pageon, HL Blay, S Brizion, P Bastien, T Vornschlogl and Y Domanov. A mechanistic view on the aging human skin through *ex vivo* layer-by-layer analysis of mechanics and microstructure of facial and mammary dermis. *Scientific Reports* 2022; **12(1)**, 849.

[54] K Brockow, H Garvey, W Aberer, M Atanaskovic-Markovic, A Barbaud, MB Bilo, A Bircher, M Blanca, B Bonadonna, P Campi, E Castro, JR Cernadas, AM Chiriac, P Demoly, M Grosber, J Gooi, C lombardo, PM Mertes, H Mosbech, S Nasser, …, and I Terreehorst. Skin test concentrations for systemically administered drugs - an ENDA/EAACI drug allergy interest group position paper. *European Journal of Allergy and Clinical Immunology* 2013; **68(6)**, 702-712.

[55] P Thomson, S Hammond and DJ Naisbitt. Pathology of drug hypersensitivity reactions and

mechanisms of immune tolerance. *Clinical & Experimental Allergy* 2022; **52(12)**, 1379-1390.

[56] DA Khan, A Banerji, KG Blumenthai, EJ Philips, R Solensky, AA White, JA Bernstein, DK Chu, AK Ellis, DBJ Golde, MJ Greenhawt, CC Horner, D Ledford, JA Lieberman, J Oppenheimer, MA Rank, MS Shaker, DR Stukus, D Wallace and J Wang. Drug allergy: A 2022 practice parameter update. *The Journal of Allergy and Clinical Immunology* 2022; **150(6)**, 1333-1393.

[57] RG Wilkerson. Drug hypersensitivity reactions. *Emergency Medicine Clinics of North America* 2022; **40(1)**, 39-55.

[58] E Salinas, D Reyes-Pavon, NG Cortes-Perez, E Torres-Maravilla, OK Bitzer-Quintero, P Langella and LG Bermudez-Humaran. Bioactive compounds in food as a current therapeutic approach to maintain a healthy intestinal epithelium. *Microorganisms* 2021; **9(8)**, 1634.

[59] E Arlt, A Kindermann, AK Fritsche, A Navarrete Santos, H Kielstein and I Bazwinsky-Wutschke. A flow cytometry-based examination of the mouse white blood cell differential in the context of age and sex. *Cells* 2024; **13(18)**, 1583.

[60] Z Fan, H Che, S Yang and C Chen. Estrogen and estrogen receptor signaling promotes allergic immune responses: Effects on immune cells, cytokines, and inflammatory factors involved in allergy. *Allergologia et Immunopathologia* 2019; **47(5)**, 506-512.

[61] M Collin and V Bigley. Human dendritic cell subsets: An update. *Immunology* 2018; **154(1)**, 3-20.

[62] J Kolter, R Feuerstein, P Zeis, N Hagemyer, N Paterson, P d-Errico, S Baasch, L Amann, T Masauda, A Losslein, K Gharun, M Meyer-Luehmann, C Waskow, CW Franzke, D Grun, T Lammermann, M Prinz and P Henneke. A subset of skin macrophages contributes to the surveillance and regeneration of local nerves. *Immunity* 2019; **50(6)**, 1482-1497.

[63] FZ Kelishomi, F Nikkahi, S Amereh, F Ghayyaz, SMA Marashi, A Javadi, G Shahbazi and M Khakpour. Evaluation of the therapeutic effect of a novel bacteriophage in the healing process of infected wounds with *Klebsiella pneumoniae* in

mice. *Journal of Global Antimicrobial Resistance* 2024; **36**, 371-378.

[64] L Gigon, T Fettrelet, S Yousefi, D Simon and HU Simon. Eosinophils from A to Z. *European Journal of Allergy and Clinical Immunology* 2023; **78(7)**, 1810-1846.

[65] KE O'Connell, AM Mikkola, AM Stepanek, A Vernet, CD Hall, CC Sun, E Yildirim, JF Staropoli, JT Leey and DE Brown. Practical murine hematopathology: A comparative review and implications for research. *Comparative Medicine* 2015; **65(2)**, 96-113.

[66] J Iype and M Fux. Basophils orchestrating eosinophils' chemotaxis and function in allergic inflammation. *Cells* 2021; **10(4)**, 895.

[67] KB Megha, S Aneeta, X Joseph, J Abey, GN Baiju and PV Mohanan. Insights into cellular interactions of characterized Mg-Al layered double hydroxide on L929 cells. *Materials Chemistry and Physics* 2024; **323**, 129631.

[68] HE Conley and MK Sheats. Targeting neutrophil β2-integrins: A review of relevant resources, tools, and methods. *Biomolecules* 2023; **13(6)**, 892.

[69] A Sungkar, D Widyatmoko, KY Yarso and B Wasita. The effect of duration of wound skin tissue on mda, tnf-α, il-6, caspase 3, vegf levels, and granulation tissue thickness in the white rat (*Rattus novergicus*). *Bali Medical Journal* 2020; **9(3)**, 918-923.

[70] A Hassanshahi, M Moradzad, S Ghalamkari, M Fadaei, AJ Cowin and M Hassanshahi. Macrophage-mediated inflammation in skin wound healing. *Cells* 2022; **11(19)**, 2953.

[71] LG Ng, R Ostuni and A Hidalgo. Heterogeneity of neutrophils. *Nature Reviews Immunology* 2019; **19(4)**, 255-265.

[72] JG Filep and A Ariel. Neutrophil heterogeneity and fate in inflamed tissues: Implications for the resolution of inflammation. *American Journal of Physiology-Cell Physiology* 2020; **319(3)**, C510-C532.

[73] HK Lehman and BH Segal. The role of neutrophils in host defense and disease. *Journal of Allergy and Clinical Immunology* 2020; **145(6)**, 1535-1544.

[74] SA Gwalabe, A Adamu, AM Kirfi, JA Dunga and IM Maigari. Serum immunoglobulin e level and its relationship with eosinophil count among patients

with allergic rhinitis in tertiary hospital in bauchi, northeastern nigeria: A cross-sectional study. *Nigerian Journal of Clinical Practice* 2023; **27(3)**, 389-393.

[75] H Kutlu, E Avci and F Özyurt. White blood cells detection and classification based on regional convolutional neural networks. *Medical Hypotheses* 2019; **135**, 109472.

[76] S Quirce, BG Cosio, A Espana, R Blanco, J Mullol, C Santander and VD Pozo. Management of eosinophil-associated inflammatory diseases: The importance of a multidisciplinary approach. *Frontiers in Immunology* 2023; **14**, 1192284.

[77] AA Mangaonkar, AJ Tande and DI Bekele. Differential diagnosis and workup of monocytosis: A systematic approach to a common hematologic finding. *Current Hematologic Malignancy Reports* 2021; **16(3)**, 267-275.

[78] NR Chan, B Hwang, BD Ratner and JD Bryers. Monocytes contribute to a pro-healing response in 40 μm diameter uniform-pore, precision-templated scaffolds. *Journal of Tissue Engineering and Regenerative Medicine* 2022; **16(3)**, 297-310.

[79] MJ Caballero-Herrero, E Jumilla, M Buitrago-Ruiz, G Valero-Navarro and S Cuevas. Role of damage-associated molecular patterns (DAMPS) in the postoperative period after colorectal surgery. *International Journal of Molecular Sciences* 2023; **24(4)**, 3862.

[80] RTA Lebang, VZ Latuconsina, H Rahawarin, I Hutagalung, PY Silalahi and S Maruanaya. The effect of ethanol extract of brown algae (*Sargassum* Sp.) on the calculation of leukocyte type of mice (*Mus Musculus*) after acute stress induction. *PAMERI Pattimura Medical Review* 2021; **3(2)**, 8-24.

[81] R Poto, AR Gambadella, G Marone, JT Schroeder, F Matteni, G Schiavoni and G Varrichi. Basophils from allergy to cancer. *Frontiers in Immunology* 2022; **13**, 1056838.

[82] P Chopra, S Bhardwaj and A Arora. Comparison of basophil count by Beckman Coulter UniCel DxH 800, Sysmex XN-1000, and manual microscopy in cases of suspected chronic myeloid leukemia. *Iraqi Journal of Hematology* 2021; **10(2)**, 91.