https://doi.org/10.48048/tis.2025.9332

Teratogen and Hematology Effects of Ethanol Extract from Unripe Pisang Kayu (*Musa paradisiaca* L. var. Kayu) on Mice (*Mus musculus*)

Awik Puji Dyah Nurhayati[1,*], Nurlita Abdulgani[1], Endry Nugroho Prasetyo[1], Noor Nailis Saadah[1], Maya Shovitri[1], Arista Wahyu Ningsih[2], Fajri Malik[1] and Kamila Islamiati[1]

[1]*Department of Biology, Faculty of Science and Data Analytics, Institut Teknologi Sepuluh Nopember, Surabaya 60111, Indonesia*
[2]*Faculty of Health Sciences, Pharmacy Study Program, Anwar Medika University, East Java 61262, Indonesia*

([*]Corresponding author's e-mail: awiknurhayati@gmail.com)

Received: 15 November 2024, Revised: 18 December 2024, Accepted: 25 December 2024, Published: 5 March 2025

Abstract

Unripe Pisang Kayu (*Musa paradisiaca* L. var. Kayu) has been traditionally used in Senduro Village, Lumajang, East Java, as a remedy for diarrhea, with the unripe fruit typically prepared by roasting, steaming or boiling. This traditional use highlights the need to develop it into standardized herbal medicine for broader healthcare applications, necessitating rigorous safety and efficacy evaluations, including preclinical studies. This study aimed to assess the teratogenic and hematological effects of its ethanol extract on female mice (*Mus musculus*) as part of these evaluations. Mice were administered varying doses (50 - 800 mg/kg BW) of the extract for 14 days, with hematological parameters, including erythrocyte and leukocyte counts, hemoglobin levels and differential leukocyte profiles, measured alongside teratogenicity assessments of fetal length, weight, skeletal structure and organ formation. Observations revealed no significant differences in blood parameters or fetal development between treated and control groups, with no malformations or abnormalities detected. These results suggest that the ethanol extract of unripe Pisang Kayu is non-toxic at tested doses and safe for use, supporting its potential as a standardized herbal antidiarrheal remedy. Further studies, including chronic toxicity and clinical trials, are recommended to ensure its safety and efficacy for human application.

Keywords: Anti-diarrheal, Differential leukocytes, Hematology, Malformation, Pisang Kayu, Teratogen

Introduction

Modern medicine has largely replaced traditional medicine as the primary therapeutic approach for treating human diseases globally. However, the use of medicinal plants for health promotion and disease prevention has seen a resurgence in recent decades [1]. In Indonesia, the unripe fruit of *Musa paradisiaca* L. var. Kayu, locally called "Pisang Kayu", has been traditionally utilized by the people of Senduro Village in Lumajang, East Java, as a remedy for diarrhea. Traditionally, the unripe fruit is utilized by roasting, steaming or boiling and then consumed directly. This traditional use of Pisang Kayu as an anti-diarrheal remedy highlights the need to develop it into a standardized herbal medicine, suitable for broader application in healthcare. Standardizing traditional medicines is crucial for their development into recognized herbal medicines and phytopharmaceuticals. This process involves establishing standards for raw materials (simplisia), refining preparation techniques, and ensuring consistency in final products. It is followed by comprehensive preclinical safety and efficacy evaluations, encompassing *in vitro* and *in vivo* studies, followed by progression to clinical trials.

Earlier studies show that the unripe Pisang Kayu fruit provides the highest extract yield, reaching 9 %, with a moisture level under 10 %, aligning with quality standards [2]. This finding suggests that the unripe fruit of Pisang Kayu is the most viable plant part for extract preparation, making it a strong candidate for herbal medicine development. Research by Ningsih [3] has demonstrated the anti-diarrheal properties of Pisang Kayu, revealing that the ethanol extract from unripe Pisang Kayu (*M. paradisiaca* L. var. Kayu) contains tannins with anti-diarrheal activity at a dosage of 100 mg/kg body weight. Further research by Ningsih *et al.* [4] confirmed that this dosage effectively solidifies stool consistency, reduces defecation frequency and decreases stool weight, suggesting its potential as a standardized herbal medicine. Moreover, subchronic toxicity testing of unripe Pisang Kayu ethanol extract on the gastric histopathology of mice revealed no toxic effects across a range of doses, including 50, 100, 200, 400 and 800 mg/kg of body weight [5].

Ningsih *et al.* [10] found that the extract from unripe Pisang Kayu fruit (*Musa paradisiaca* L. var. Kayu) includes alkaloids, saponins, tannins and flavonoids, which collectively enhance its antidiarrheal effects. Tannins, particularly the hydrolyzed and condensed types, play crucial roles in treating diarrhea. Hydrolyzed tannins act as strong astringents, while condensed tannins form protective barriers on mucous membranes, reducing fluid loss and promoting the balance of intestinal flora by enhancing gamma-delta T cells in the gut [6,7]. The antidiarrheal mechanism of this extract is further supported by its ability to target μ opioid receptors in the gut, reducing peristalsis and increasing fluid absorption, thereby drying and slowing the movement of feces [8]. Flavonoids in the extract inhibit intestinal motility and prolong transit time, saponins enhance fluid absorption by inhibiting histamine release, and tannins provide chelating and spasmolytic effects that reduce peristalsis and disrupt bacterial cell walls, inhibiting bacterial growth [9].

Moreover, alkaloids demonstrate antibacterial effects by disrupting the formation of peptidoglycan cross-links in bacterial cell walls, resulting in the death of bacterial cells. This comprehensive action of multiple phytochemicals underlines the potential of wooden pisang extract as an effective antidiarrheal agent, offering a natural remedy that targets various aspects of diarrhea pathology. Ningsih *et al.* [10] reveals the antibacterial potential of compounds from unripe Kayu banana fruit (*Musa paradisiaca* L. var. Kayu), with LCMS-MS analysis identifying 2-HoTrE ((9Z,12Z,15Z)-2-hydroxyoctadeca-9,12,15-trienoic acid) as the most effective. Molecular docking shows its superior binding to penicillin-binding protein and E. coli MurB protein compared to a reference ligand. Toxicity analysis using Protox II classifies 2-HoTrE as toxicity class 5, indicating a low toxicity level based on the Globally Harmonized System (GHS) and Hodge and Sterner’s classification.

The preclinical testing phase is vital for potential drug candidates, providing information on pharmacological effects, pharmacokinetic profiles and toxicity, which are essential for predicting human outcomes. Toxicity testing includes acute, sub chronic and chronic toxicity evaluations, as well as specific tests for teratogenicity, mutagenicity and carcinogenicity [11]. Testing on humans without prior animal testing is both dangerous and unethical due to the health risks involved. Mice (*Mus musculus*) are frequently utilized in these studies because of their physiological, behavioral and pathological resemblances to humans, with approximately 99 % of mouse genes having corresponding human homologs. Their small size, low cost, ease of maintenance, docile nature and rapid reproductive cycle make them ideal for observing multiple generations within a relatively short period [12].

Testing for teratogenicity is crucial to detect fetal abnormalities during the organogenesis phase, accomplished by administering teratogenic substances to pregnant mice (*M. musculus*) following OECD guidelines and FDA regulations, which set the oral dose limit at 1 mL per 100 g of body weight. This test evaluates the impact of substances, like ethanol extract of Pisang Kayu (*M. paradisiaca* L. var. Kayu), on fetal development by observing the effects on organogenesis - a critical phase occurring after the formation of the germ layers (ectoderm, endoderm and mesoderm) and involving complex processes like cell proliferation and differentiation [13,14]. Proper teratogenic testing is crucial as exposure to harmful compounds can cause malformations in the fetus [15]. Moreover, hematological analysis is essential for diagnosing and managing diseases, as it involves the examination of blood’s cellular components, which include red blood cells (erythrocytes), white blood cells (leukocytes) and platelets [16,17]. Hematology helps assess overall health, detect infections and monitor toxicological effects. Recommended core hematological tests for laboratory animals include total leukocyte count, differential leukocyte count, hemoglobin concentration and blood coagulation [18]. These analyses are fundamental for ensuring the safety and efficacy of therapeutic agents and understanding their impact on both fetal development and overall health.

Previous studies indicate that secondary metabolites like tannins can bind to proteins and amino acids, impairing nutrient absorption in the maternal intestine and potentially depriving the fetus of essential nutrients needed for cell division during organogenesis. Alkaloids are thought to enhance uterine muscle activity, disrupting nutrient transfer through the placenta and hindering cell division by affecting mitotic spindle function, leading to chromosomal abnormalities and cell death. Saponins have the potential to induce cell cycle arrest in osteoblasts during the G1 phase, hindering their advancement to later phases and consequently interfering with mitosis. Flavonoids, similarly, can interfere with nutrient distribution by increasing uterine muscle activity. Additionally, hydrolyzed tannins, such as tannic acid, have been shown to reduce iron absorption without affecting the uptake of other essential nutrients. Afsana *et al.* [42] demonstrated that diets containing over 10 g of tannic acid/kg feed led to decreased iron absorption, lowering hemoglobin and hematocrit levels in Sprague-Dawley rats. Given these findings, it is crucial to investigate the effects of the ethanol extract of unripe Pisang Kayu fruit (*M. paradisiaca* L. var. Kayu) on *M. musculus* fetuses by assessing fetal growth metrics, organ development and maternal hematological profile. This research will help elucidate the impact of such extracts on fetal development and maternal health.

Materials and methods

Research ethics

The research received approval from the Ethics Committee for Health Research, Faculty of Dental Medicine, Universitas Airlangga. (Research ethics number: 0061/HREEC.FODM//II/2024).

Animal treatment

The study utilized female white mice (*Mus musculus*) aged between 2 to 3 months, with weights ranging from 20 to 30 g, which had not previously received any medication. Before the experiment, the mice underwent a 7-day acclimatization period to adapt to their new surroundings, ensure their health and standardize their food intake. They were kept in specially designed cages, with 2 mice per cage. The experimental subjects were divided into 7 groups, each containing 4 mice. The use of 4 female mice per group was determined based on a formula to calculate the number of individuals required for each dose replication: $df = (T - 1)\times(n - 1)$, where T represents the number of treatment groups or levels, and n denotes the number of replications per treatment group. This approach ensures adequate replication for statistical analysis while adhering to ethical considerations for the use of laboratory animals.

The first group served as the control and was administered 0.5 mL of distilled water; the second group received 0.5 % CMC-Na. Groups 3, 4, 5, 6 and 7 were given ethanol extract from unripe Pisang Kayu (*Musa paradisiaca L. var. Kayu*) at doses of 50 100, 200, 400 and 800 mg/kg BW, respectively, via oral administration for 14 days. The dose groups used in this study were selected based on the results of the acute toxicity test of the ethanol extract, which yielded an LD50 value of 2,535.128 mg/kg BW, categorizing it as mildly toxic. To ensure safety, doses of 50, 100, 200, 400 and 800 mg/kg BW were chosen for this test. The test substance was administered daily, starting from implantation (day 5 post-mating) until 1 day before the scheduled caesarean section, in accordance with the guidelines of the Organization for Environmental Control Development (OECD) Test No. 414: Prenatal Developmental Toxicity Study. On the 15^{th} day, the mice were euthanized for further analysis.

Mating and determination of pregnancy period

Mating of test animals is done by determining the estrus period of the test animals. Determination of the estrus cycle is done by observing vaginal smears in mice. Vaginal smear observations are used to identify the estrous cycle, which consists of estrus, metestrus, diestrus and proestrus. The density and ratio of epithelial cells and leukocytes can be analyzed through vaginal cytology to identify each phase of the cycle.

The first step is to wipe the mice’s vagina using a cotton bud moistened with NaCl 0.9 %. The smear is then scratched onto a glass slide and stained using 1 %

methylene blue with a few drops until the entire surface is evenly colored, followed by a 5-minute wait. After 5 min, distilled water is used to remove excess stain, and the slide is dried. The dried glass slide is then observed under a light microscope at 10× magnification to analyze the cells. If microscopic observation confirms the estrus phase, the female mice are mated with male mice by placing them together in the male's cage for approximately 1 day.

On the day the estrus cycle is completed, female mice are observed for changes, especially the presence of a vaginal plug, which indicates copulation has occurred and marks the Day 0 of pregnancy. The pregnancy period in mice can also be identified by the presence of a vaginal plug, characterized by the closure of the female mouse's vagina along with whitish-yellow fluid consisting of residual sperm from the male and reproductive secretions from the female. Additionally, pregnancy can be confirmed by a significant increase in body weight as the fetus develops. If no vaginal plug is observed and body weight does not increase, the test animal will be removed and reintroduced for mating.

All test animals in the experimental groups successfully conceived after repeated cycles of observation and mating. While the onset of conception varied among individuals, the ethanol extract was administered to all animals for a consistent duration of 14 days. The study utilized 5 dams per experimental group to generate fetuses, which were the same animals used for hematological analysis. For fetal observations, 1 fetus from each dam was selected for evaluation in each experimental group.

Fetus observation

The fetuses obtained were weighed individually and measured from caput to caudal length. The fetuses were then divided into 2 groups for observation, namely the bone observation group and the organ observation group. The ossification fetus group was first put into absolute ethanol for 2 days. Absolute ethanol was replaced every 24 h. Then, after the fetus began to look transparent, it was stained with alizarin red S dye for 10 min. After that, it was rinsed using 1 % KOH. Then remove the remaining alizarin red S dye, soaking with a solution of KOH: Glycerin in levels of 3:1, 1:1 and 1:3. After completion, the fetus was observed using a light microscope to see malformations in the bones. Groups of organ fetuses were directly sliced at the caput with a transverse cut. Then the fetus was rinsed with 0.9 % NaCl. After rinsing, the fetus organs were observed with a stereo microscope to see any organ malformations. Then random sampling of fetuses from each treatment group of the test preparation was done in as many as 4 fetuses.

Hemoglobin level calculation

The hemoglobin level in mice (*M. musculus*) was measured using a Hemoglobin Meter. A drop of blood was placed on an HB test strip, which was then inserted into the HB meter. After a blood drop symbol appeared on the screen, the blood sample was applied to the reagent area on the strip. The hemoglobin level was then displayed on the meter.

Erythrocyte count calculation

Blood is drawn up to the "0.5" mark, and any excess is wiped off. Hayem's solution is then drawn up to the "101" mark. The pipette is homogenized by swirling for 2 min, and the first 3 drops are discarded. A drop of the mixture is placed on the counting chamber. Erythrocytes are counted under a microscope in the R squares of 5 fields. The total erythrocyte count per mm^3 is calculated using the formula:

$$\text{Total erythrocyte} = \frac{\text{nE} \times \text{P}}{\text{V}}$$

where nE is the number of erythrocytes in 5 R square, P is the dilution factor, and V is the R-grid volume.

Leukocyte count calculation

A Thoma leukocyte pipette was filled with fresh blood up to the "0.5" mark, and then Turk's solution was added until reaching the "11" mark. After sealing the pipette, it was gently shaken, and a drop of the mixture was placed on the counting chamber after discarding the initial drops. The sample is allowed to settle for 2 - 3 min before counting leukocytes under a microscope in the W-grid. The leukocyte count per cubic millimeter (mm^3) is calculated as:

$$\text{Total leukocytes} = \frac{\text{nL} \times \text{P}}{\text{V}}$$

where nL is the leukocytes counted in 4 W-grids, P is the dilution factor, and V is the W-grid volume.

Percentage of leukocyte types calculation

To prepare a blood smear, a drop of blood was placed on a glass slide and spread using another slide held at a 30 - 40 ° angle. The second slide was moved forward to form a thin layer of blood. After allowing it to dry, the smear was fixed with methanol for 3 - 5 min and then left to air dry. Subsequently, the preparation was stained with a 1:9 dilution of Giemsa solution for 30 min, rinsed with distilled water, and air-dried. Leukocyte percentage was determined by counting 100 leukocytes using a microscope with OptiLab at 100× magnification, starting from the edge of the blood smear. The percentage of each leukocyte type - neutrophils, eosinophils, basophils, lymphocytes and monocytes - was calculated using the formula:

$$\%L = \frac{N}{100} \times 100\ \%$$

where %L represents the percentage of the counted leukocytes, N is the number of counted leukocytes, and 100 is the total number of leukocytes counted.

Data analysis

The research data were gathered and compiled into a table. The Shapiro-Wilk test was utilized to evaluate normality, while the Levene Test was employed to check for homogeneity, considering a significance level (alpha) greater than 0.05 as acceptable. For data that met the criteria of normality and homogeneity, analysis was conducted using 1-way ANOVA. In cases where ANOVA indicated significant differences, Tukey's Honestly Significant Difference (HSD) test was employed to determine specific differences between groups, with a significance level (Sig.) of less than 0.05 indicating statistically significant differences. For data that did not meet normality or homogeneity assumptions, the Kruskal-Wallis test was applied. All analyses and visual representations of the data were conducted using IBM SPSS version 27.

Results and discussion

Litter size

The results presented in **Figure 1** indicate that there are no significant differences in litter length across the different groups. This conclusion was drawn from the 1-way ANOVA test results, which revealed a *p*-value of 0.951 for all fetal lengths ($p > 0.05$). This result indicates that there are no significant differences in litter length across all treatment groups. Since the 1-way ANOVA test results are not significant, no further post hoc Tukey test was conducted.

Table 1 Fetal skeletal observations after the treatment with ethanol extract from unripe Pisang Kayu (*M. paradisiaca* L. var. Kayu) (mean ± SD).

Group	N	Litter length (cm)	Birth weight (g)
Group 1 (Aquadest control)	4	3.48 ± 0.1500[a]	1.27 ± 0.0829[a]
Group 2 (CMC-Na 0.5 %)	4	3.53 ± 0.2062[a]	1.26 ± 0.0263[a]
Group 3 (50 mg/kg BB)	4	3.50 ± 0.1826[a]	1.27 ± 0.0467[a]
Group 4 (100 mg/kg BB)	4	3.40 ± 0.1414[a]	1.26 ± 0.0250[a]
Group 5 (200 mg/kg BB)	4	3.45 ± 0.1291[a]	1.28 ± 0.0480[a]
Group 6 (400 mg/kg BB)	4	3.40 ± 0.0817[a]	1.26 ± 0.0271[a]
Group 7 (800 mg/kg BB)	4	3.43 ± 0.1259[a]	1.26 ± 0.0289[a]

Identical letters in the same columns indicate that there is no significant difference at $p \leq 0.05$ according to ANOVA. [a]values are expressed as means ± standard error.

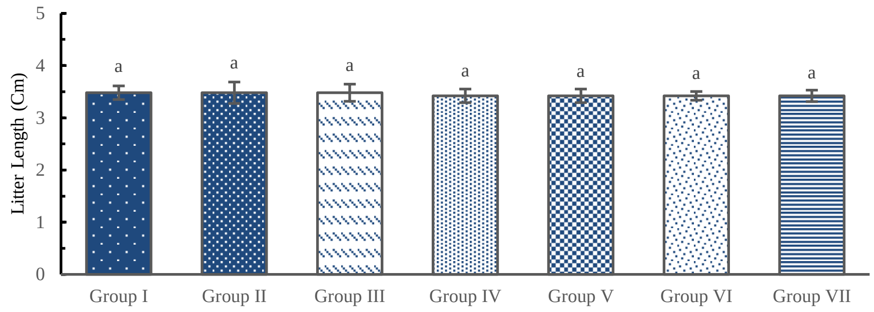

Figure 1 Graph of litter length after administration of Pisang Kayu extract (*M. paradisiaca* L. var. Kayu) in gestation period (day 6 - 15). Group I (Aquadest control); Group II (CMC-Na 0.5 %); Group III (50 mg/kg BW); Group IV (100 mg/kg BW); Group V (200 mg/kg BW); Group VI (400 mg/kg BW); Group VII (800 mg/BW). The same notation shows no difference results.

Based on the data obtained, there are no significant differences in fetal length across the treatments. This indicates that the ethanol extract of raw pisang fruit (*M. paradisiaca* L. var. Kayu) has no significant impact on fetal length. Measuring fetal length is important because it is one of the minor effects caused by teratogenic agents [19]. Fetal length reflects fetal development and nutrient absorption during pregnancy. If one or a group of cells is damaged by a toxic compound, the surrounding normal cells will divide and take over the role of the damaged cells. This replacement of damaged cells is maintained during organogenesis to ensure the formation of normal fetal morphology. However, if the damaged cells cannot be repaired, it can lead to malformations or abnormalities, resulting in a fetus with normal morphology but smaller size [20]. Fetuses with malformations are generally smaller compared to normal fetuses. Therefore, before declaring any abnormalities in an individual, the length of the fetus under the given treatment should be compared with the control fetus to ensure that any observed growth restriction in a specific body part reflects general growth retardation [21].

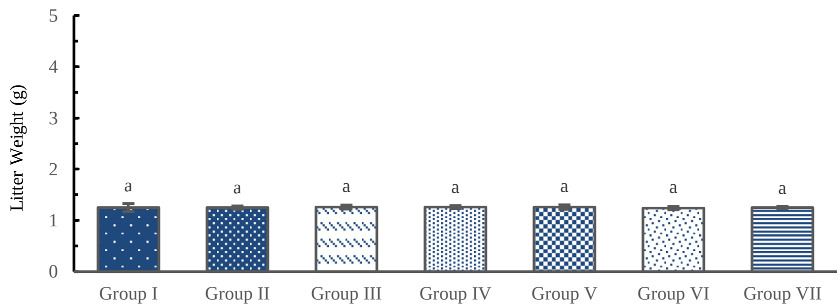

Figure 2 Graph of birth weight after administration of Pisang Kayu extract (*M. paradisiaca* L. var. Kayu) in gestation period (day 6 - 15). Group I (Aquadest control); Group II (CMC-Na 0.5 %); Group III (50 mg/kg BW); Group IV (100 mg/kg BW); Group V (200 mg/kg BW); Group VI (400 mg/kg BW); Group VII (800 mg/BW). The same notation shows there is no difference in results.

According to **Figure 2**, there are no notable differences in fetal weight across the groups. This conclusion is supported by the findings from a 1-way ANOVA test, which yielded a *p*-value of 0.985 ($p > 0.05$) for all fetal weights. This suggests that fetal weight remains consistent among all treatment groups. Given the absence of significant results from the 1-way ANOVA, a post hoc Tukey test was not performed.

Typically, individuals with malformations or abnormalities exhibit smaller sizes than their normal counterparts. Consequently, assessing fetal length and weight is essential for detecting any growth irregularities in the fetus. This assessment involves comparing the lengths and weights of fetuses in the experimental groups with those in the control group to ensure that any observed organ growth inhibition corresponds to overall growth. The anticipated hypothesis for evaluating the ethanol extract of unripe pisang fruit (*M. paradisiaca* L. var. Kayu) has no significant impact the length and weight of fetal mice (*M. musculus*).

Rates of fetal growth and development can vary among offspring sizes, with a reduction in fetal body weight serving as a subtle indicator of teratogenic effects and a more sensitive parameter for teratogenic testing. Abnormalities such as atypical birth weights can arise from developmental disturbances within the uterus, with decreased fetal weight suggesting growth inhibition. This inhibition occurs when a substance impacts cell proliferation, cellular interactions and decreases biosynthesis rates related to the suppression of nucleic acid, protein or mucopolysaccharide synthesis. Body weight is a crucial metric for evaluating the effects of foreign substances on the fetus, as indicated by reduced fetal weight. The rates of growth and development contribute to differences in offspring size. The data indicates that there are no significant differences in fetal weight across the various treatments, suggesting that the ethanol extract of unripe Pisang Kayu (*M. paradisiaca* L. var. Kayu) has no significant impact on fetal weight. Assessing fetal weight is vital as it represents one of the minor effects caused by teratogenic agents. Additionally, statistical analyses of fetal length measurements reveal no significant differences among treatment groups ($p > 0.05$), indicating that the administration of the ethanol extract of unripe pisang fruit to female mice (*M. musculus*) at various doses (50, 100, 200, 400 and 800 mg/kg BW) for anti-diarrheal purposes has no significant impact on fetal length and weight.

The lack of significant decreases in fetal length and weight could be due to several factors, such as the administered dosage. According to the Indonesian Food and Drug Authority (BPOM) (2017), the dosage for testing herbal medicines ranges from 50 to 1,000 mg/kg body weight. The dosages administered in this study fall within the safe range recommended by BPOM. Observations of fetal length and weight can serve as supporting data for morphological assessments of bone and organ development, demonstrating that no malformations occurred, as evidenced by the absence of abnormalities in fetal length. This is done with the expectation that the ethanol extract of unripe Pisang Kayu (*M. paradisiaca* L. var. Kayu), as a candidate for Standardized Herbal Medicine (OHT), must meet one of the criteria set by BPOM. BPOM states that a drug with market authorization must have adequate safety, as demonstrated by non-clinical and clinical tests or other evidence following the level of scientific knowledge [22].

Skeletal evaluation

Following the initial evaluation of the impacts of the ethanol extract from unripe Pisang Kayu (*M. paradisiaca* L. var. Kayu) on fetal length and weight, the results indicated no significant differences between the control group and the various treatment groups. Further observations are needed, specifically concerning skeletal development. Observations of internal malformations, including the development of the fetal skeleton, should be conducted next.

Santoso [21] stated that teratogenic agents can affect the thickness of the cells of the fetal femur. Accumulation of teratogenic agents in some organs, for example in calcified organs, will result in developmental abnormalities in the fetus. This is because the fetus still does not have enzymes that can completely metabolize the toxic agent. The function of the observation of fetal ossification is to determine whether ethanol extract of unripe fruit of Pisang Kayu (*M. paradisiaca* L. var. Kayu) has teratogenic effects on bone structure and ossification in fetuses. The results of the effect of giving ethanol extract of unripe Pisang Kayu (*M. paradisiaca* L. var. Kayu) on fetal ossification can be seen in **Table 2**.

Table 2 Fetus skeletal observations after the treatment with ethanol extract from unripe Pisang Kayu (*M. paradisiaca* L. var. Kayu) (mean ± SD).

Group	N	Skeletal evaluation on malformations				
		Cranium	Vertebrae	Costae	Forelimb	Hindlimb
Group 1 (Aquadest control)	4	0	0	0	0	0
Group 2 (CMC-Na 0.5 %)	4	0	0	0	0	0
Group 3 (50 mg/kg BB)	4	0	0	0	0	0
Group 4 (100 mg/kg BB)	4	0	0	0	0	0
Group 5 (200 mg/kg BB)	4	0	0	0	0	0
Group 6 (400 mg/kg BB)	4	0	0	0	0	0
Group 7 (800 mg/kg BB)	4	0	0	0	0	0

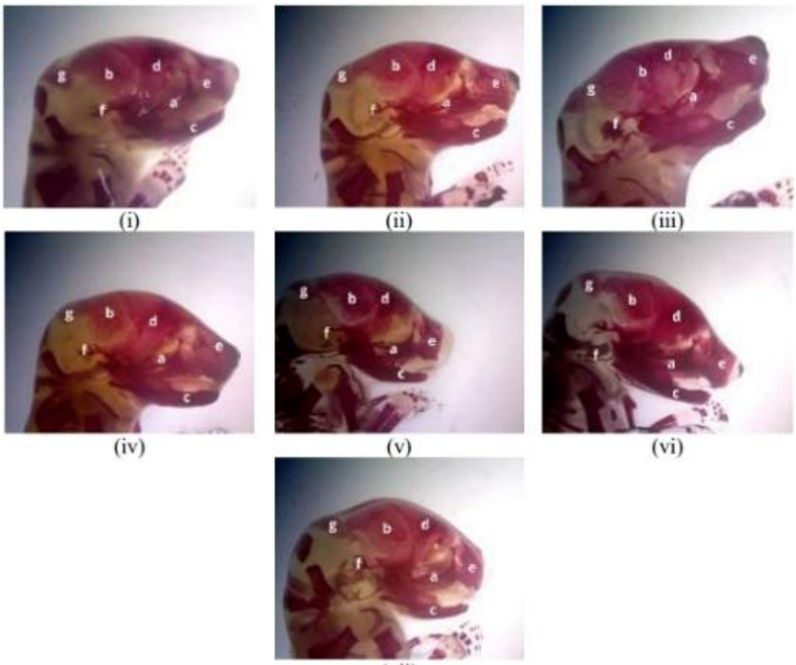

Figure 3 Observation of fetal cranium in all treatment groups; (i) Control I, (ii) Control II, (iii) Dose I, (iv) Dose II, (v) Dose III, (vi) Dose IV and (vii) Dose V. (Description: a) maxilla, b) parietal, c) mandible, d) frontal, e) nasal, f) tympanic bulb and g) occipital).

Based on observations of the cranium in all treatment groups, it appears that all fetuses have a fully formed skull. The morphological characteristics of a perfect cranium are that it has a tight arrangement, there are eye and nose holes, cheekbones that connect the lower jaw to the upper jaw and ear holes [23].

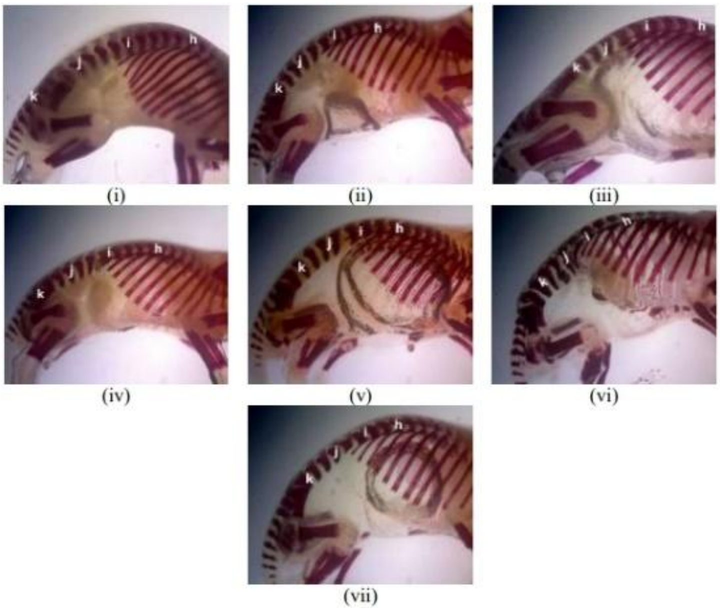

Figure 4 Observation of fetal vertebrae in all treatment groups; (i) Control I, (ii) Control II, (iii) Dose I, (iv) Dose II, (v) Dose III, (vi) Dose IV and (vii) Dose V. (Notes: h) 12^{th} thoracic vertebra, i) 2^{nd} lumbar vertebra, j) 7^{th} lumbar vertebra and k) 3^{rd} sacral vertebra).

Based on observations of the vertebrae in all treatment groups, there were no abnormalities in the shape and number of vertebrae. In general, mice have 7 cervical vertebrae, 13 thoracic vertebrae, 6 - 7 lumbar vertebrae, 3 - 5 sacral vertebrae and 16 - 23 caudal vertebrae. Vertebrae in mice have a connection distance between vertebrae that tends to be short [21].

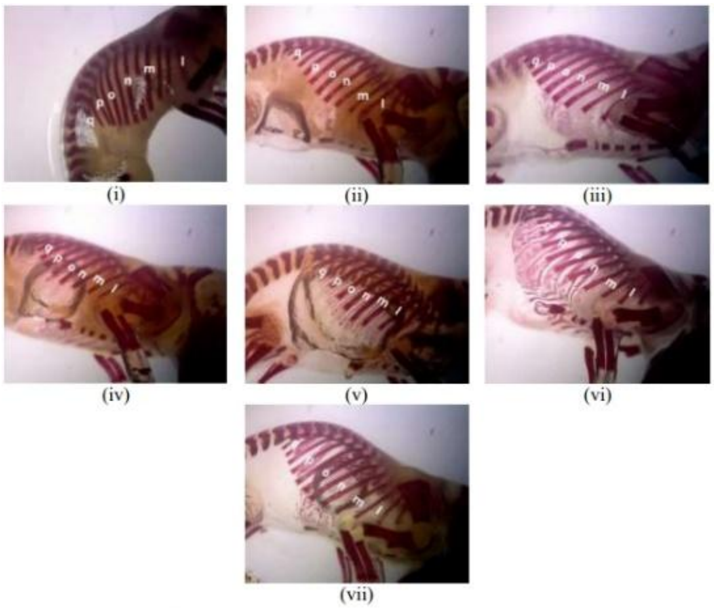

Figure 5 Observation of fetal costae in all treatment groups; (i) Control I, (ii) Control II, (iii) Dose I, (iv) Dose II, (v) Dose III, (vi) Dose IV and (vii) Dose V. (Caption: l) 2^{nd} rib, m) 5^{th} rib, n) 7^{th} rib, o) 9^{th} rib, p) 11^{th} rib and q) 13^{th} rib). Based on observations of costae in all treatment groups, there were no abnormalities in the shape and number of vertebrae. In general, mice have 13 costae [21]. It was also seen that there was no attachment or fused costae, and no broken costae were found.

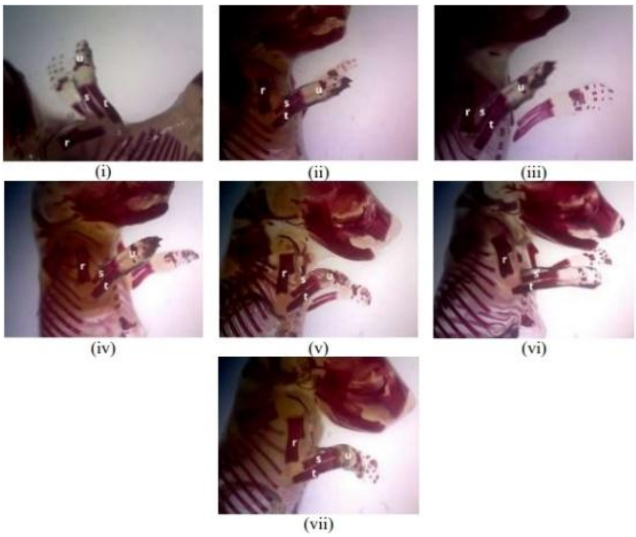

Figure 6 Observation of anterior limb of fetus in all treatment groups; (i) Control I, (ii) Control II, (iii) Dose I, (iv) Dose II, (v) Dose III, (vi) Dose IV and (vii) Dose V. (Description: r) humerus, s) ulna, t) radius and u) finger bone).

Based on observations of the anterior extremities in all treatment groups, it appears to have 3 vertebrae in the metacarpals, indicating that there are no abnormalities in the shape and amount of reinforcement in the anterior extremities. The bone structure of the anterior extremities as a whole looks normal, this is seen and compared to the Atlas of Sectional Anatomy of the Mouse [24].

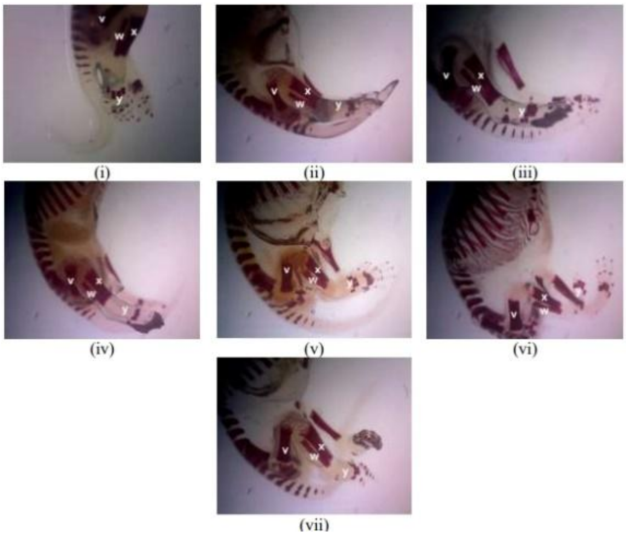

Figure 7. Observation of posterior limb of fetus in all treatment groups (i) Control I, (ii) Control II, (iii) Dose I, (iv) Dose II, (v) Dose III, (vi) Dose IV, (vii) Dose V (Description: v. femur, w. fibula, x. tibia, y. finger bone).

Based on observations of the posterior extremities in all treatment groups, it appears to have 3 segments in the metatarsals, indicating no deformities and the amount of reinforcement in the anterior extremities. The bone structure of the anterior extremities looks normal, this is seen and compared to the Atlas of Sectional Anatomy of the Mouse [24].

From the overall data obtained, the administration of ethanol extract of the unripe fruit of Pisang Kayu (*M. paradisiaca* L. var. Kayu) did not cause malformations in the fetus of mice (*M. musculus*). Secondary metabolite compounds in ethanol extract of unripe pisang wood fruit are tannins, alkaloids, saponin and flavonoids. Tannins can bind to essential minerals like calcium, zinc and iron, leading to a decrease in the bioavailability of these minerals, which are crucial for the process of bone mineralization [25]. Some alkaloids can affect the cell cycle by interfering with certain phases of cell division. For example, alkaloids such as vincristine, used in chemotherapy, affect microtubules and inhibit cell division [26]. This disruption in the cell cycle can affect the proliferation of bone progenitor cells that are essential for fetal bone formation and growth [20]. Saponins could form complexes with lipids and proteins in cell membranes, which can increase cell membrane permeability [27]. This increased permeability can compromise cell integrity and allow the entry of harmful substances or the loss of important components from within the cell. This may affect the health and function of bone cells, as well as the bone formation process. Some flavonoids can inhibit cell proliferation by affecting the cell cycle. For example, flavonoids such as quercetin can interfere with certain phases of the cell cycle, inhibiting the growth and division of bone progenitor cells necessary for bone formation and maintenance. Thus, in consuming compounds containing tannins, alkaloids, saponins and flavonoids, care needs to be taken and in amounts that are not too large. In this study, no malformations were found in the fetal ossification, this is because the secondary metabolite compounds contained in the ethanol extract of unripe fruit of Pisang Kayu (*M. paradisiaca* L. var. Kayu) tend to be harmless and in concentrations that are not excessive.

Visceral morphology

The findings from this study indicated that all fetuses, regardless of whether they were in the control or experimental groups, exhibited no external malformations. External malformations such as hydrocephalus, spina bifida, anencephaly and others. Observation of the brain images in the fetuses revealed that the brain appeared normal in both size and color. All fetuses are born with a normal brain shape. Rapid brain formation occurs starting from the early neonatal phase where brain volume increases and maturation of cognitive abilities occurs. Prenatal exposure to teratogenic substances can hinder brain development and significantly affect a child's cognitive growth. Each brain part develops at its own specific time and pace under normal conditions. When exposed to possible teratogens, the maturation rate and functionality of neurons may be disrupted, potentially leading to cognitive delays or impairments [28]. This indicates that ethanol extract of unripe fruit of Pisang Kayu (*M. paradisiaca* L. var. Kayu) does not cause malformations in the brain of the fetus, which is reviewed from the visual aspect and compared to the control treatment.

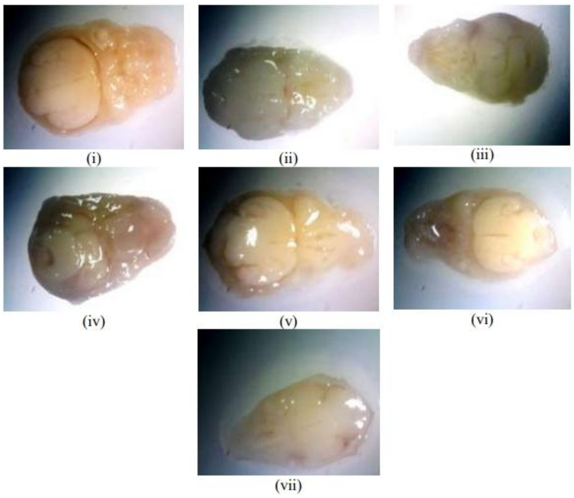

Figure 8 Observation of fetal brain organ in all treatment groups; (i) Control I, (ii) Control II, (iii) Dose I, (iv) Dose II, (v) Dose III, (vi) Dose IV and (vii) Dose V.

A study by Ningsih *et al.* [10] found that the extract of unripe Pisang Kayu fruit flesh contains various phytochemical compounds, including saponins, tannins, alkaloids and flavonoids. Tannin is divided into 2 types, namely hydrolyzed tannin and condensed tannin. Hydrolyzed tannin has strong astringent properties against diarrhea caused by infection. Meanwhile, condensed tannin plays a protective role with its ability to bind to mucous membranes and other tissues, forming a barrier against microbial reactions. Due to these properties, condensed tannin can be used in the treatment of diarrhea to reduce fluid loss from the digestive tract. In addition, condensed tannin helps restore the balance of gut flora by stimulating the expansion of gamma-delta T cells in the gut, which boosts the immune system to fight pathogenic bacteria [6,7].

Antidiarrheal medications primarily function by interacting with μ receptors located in the gut. The gut contains both μ and δ opioid receptors, each playing distinct roles in intestinal absorption; δ receptors facilitate intestinal secretion, while μ receptors are involved in mediating intestinal peristalsis. When drugs act as μ agonists, they effectively reduce peristalsis in the colon, leading to a significant delay in the passage of feces. This delay enhances the absorption of fluid from the feces, resulting in drier stool and a slowdown in its transit. This mechanism is also present in the ethanol extract of unripe Pisang Kayu (*M. paradisiaca* L. var. Kayu), which is utilized as an antidiarrheal agent due to its content of secondary metabolites such as flavonoids, tannins, saponins and alkaloids. Flavonoids can inhibit intestinal motility, reduce water secretion and increase transit time, thereby enhancing the absorption of bodily fluids. Additionally, saponins in the extract assist fluid absorption by inhibiting histamine release, which leads to reduced fluid absorption. Tannins possess chelating properties and spasmolytic effects that can induce intestinal contractions, ultimately decreasing peristalsis. These spasmolytic properties may also cause contractions in the bacterial cell wall or membrane, disrupting cell permeability and inhibiting bacterial growth or causing bacterial death. Furthermore, alkaloid compounds exhibit antibacterial properties by interfering with the formation of cross-bridges in peptidoglycan components within the bacterial cell wall, preventing proper cell wall formation and resulting in bacterial cell death [29].

Teratogenic test is a test that must be done when clinical use of drugs is given during organogenesis during pregnancy. Teratogenic effects of traditional medicines are known to occur in many cases, this is because the chemicals contained in traditional medicines are transported through the placenta which can cause toxic effects on the growth of sensitive fetuses. This test is important considering the possibility that the consumer is a pregnant woman who uses the plant as a solution to health problems [30]. This research aims to evaluate the impact of administering ethanol extract from the unripe fruit of Pisang Kayu (*M. paradisiaca* L. var. Kayu) on the fetal development of test subjects (*M. musculus*). The resulting data can serve as a foundation for developing the ethanol extract from unripe Pisang Kayu into Standardized Herbal Medicine (OHT). Understanding the effects of this extract on the fetal development of test animals (*M. musculus*) will further contribute to its potential use as a standardized herbal remedy for treating diarrhea.

Blood hematology

The research findings on the effects of ethanol extract from the unripe Pisang Kayu (*M. paradisiaca* L. var. Kayu) on various blood parameters in mice (*M. musculus*) reveal important insights into the extract's safety and efficacy. The study observed that the average total erythrocyte count ranged from 7.08 to $9.37\times10^6/mm^3$ across all treatment groups (**Figure 9**), which falls within the normal range of 7.0 to $12.5\times10^6/mm^3$ as referenced by Harkness *et al.* [31]. The statistical evaluation conducted through 1-way ANOVA revealed no significant differences between the groups ($p > 0.05$). This suggests that the administration of the ethanol extract at doses between 50 and 800 mg/kg BW had no impact on the erythrocyte count in female *M. musculus*. This suggests that the extract does not exert toxic effects on erythrocytes, supporting its safety as an antidiarrheal agent. However, to ensure human safety, preclinical toxicity testing should be conducted *in vivo* to examine potential cumulative dose effects that could lead to adverse outcomes. Any deviation in erythrocyte count beyond the normal range could indicate pathological activity in the blood, such as anemia, which impairs oxygen transport, or an excessively high erythrocyte count, which increases blood viscosity and thrombosis risk, potentially leading to severe cardiovascular events [32].

In addition to erythrocytes, the study also assessed hemoglobin (Hb) levels, a crucial protein responsible for oxygen delivery to tissues. The findings depicted in **Figure 10**, showed that the average hemoglobin levels across all treatment groups ranged from 15.05 to 16.43 g/dL, within the normal range of 10.2 to 16.6 g/dL (102 - 166 g/L) as reported by Harkness *et al.* [31]. The 1-way ANOVA assessment indicated has no differences between the groups (p > 0.05), indicating that the ethanol extract did not impact hemoglobin levels in the blood of *M. musculus*. Maintaining normal hemoglobin levels is critical, as fluctuations outside the normal range could lead to pathological conditions such as anemia, which disrupts oxygen transport in the body [33].

Furthermore, the effects of the ethanol extract on the total leukocyte count were examined, with results presented in **Figure 11**. The study found that the average leukocyte counts in Groups I, II, III, IV and VI were within the normal range from 6 to 15×10^3/mm³ [31]. However, Groups V and VII exhibited leukocyte counts below the normal range. Nonetheless, the 1-way ANOVA analysis revealed no significant differences among the treatment groups (p > 0.05), indicating that the extract does not influence the overall leukocyte count in *M. musculus*. Leukocytes are crucial for the body's immune response to foreign agents, and alterations in their counts beyond the normal range may indicate the presence of underlying inflammation or infection [34].

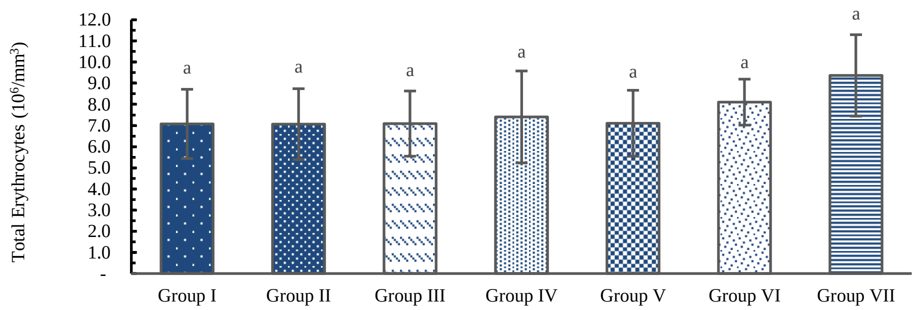

Figure 9 Graph of total erythrocytes of female white mice after administration of Pisang Kayu extract (*M. paradisiaca* L. var. Kayu) for 14 days. Group I (Aquadest control); Group II (CMC-Na 0.5 %); Group III (50 mg/kg BW); Group IV (100 mg/kg BW); Group V (200 mg/kg BW); Group VI (400 mg/kg BW); Group VII (800 mg/kg BW). The same notation shows there is no difference result.

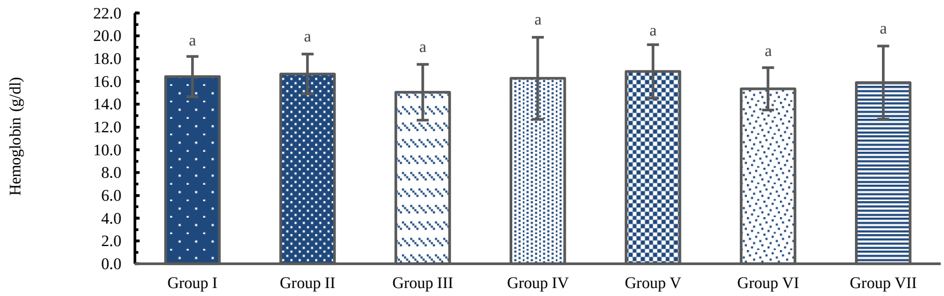

Figure 10 Graph of hemoglobin level of female white mice after administration of Pisang Kayu extract (*M. paradisiaca* L. var. Kayu) for 14 days. Group I (Aquadest control); Group II (CMC-Na 0.5 %); Group III (50 mg/kg BW); Group IV (100 mg/kg BW); Grup V (200 mg/kg BW); Group VI (400 mg/kg BW); Group VII (800 mg/kg BW). The same notation shows there is no difference result.

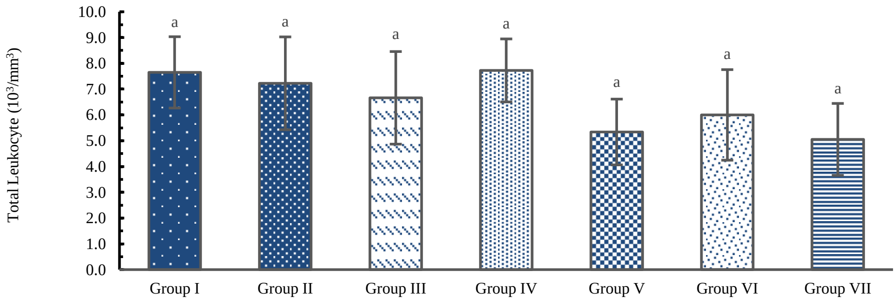

Figure 11 Graph of total leukocytes of female white mice after administration of Pisang Kayu extract (*M. paradisiaca* L. var. Kayu) for 14 days. Group I (Aquadest control); Group II (CMC-Na 0.5 %); Group III (50 mg/kg BW); Group IV (100 mg/kg BW); Grup V (200 mg/kg BW); Group VI (400 mg/kg BW); Group VII (800 mg/kg BW). The same notation shows there is no difference among groups.

In hematological testing, leukocytes are typically categorized according to their cell types. To obtain the total leukocyte count, the percentage of each cell type is multiplied by the overall leukocyte count. Leukocytes in most strains of rats and mice consist primarily of neutrophils, lymphocytes, monocytes and eosinophils, with basophils being present in very small numbers [35]. The research findings on the effects of ethanol extract from unripe Pisang Kayu fruit on the differential leukocyte percentage in *M. musculus* are shown in **Table 3**.

Table 3 The average differential leukocytes percentage in mice.

Treatment	Percentage (%) X ± D				
	Neutrophil	**Lymphocyte**	**Eosinophil**	**Monocyte**	**Basophil**
Group I (Aquadest control)	34.50 ± 10.63	61.25 ± 11.09	4.00 ± 0.82	2.75 ± 0.96	0.00 ± 0.00
Group II (0.5 % CMC-Na)	24.50 ± 13.68	70.50 ± 14.73	2.25 ± 1.26	2.75 ± 1.71	0.00 ± 0.00
Group III (50 mg/kg BW)	37.50 ± 4.80	58.25 ± 2.06	1.75 ± 0.96	3.25 ± 2.22	0.00 ± 0.00
Group IV (100 mg/kg BW)	39.50 ± 6.46	58.75 ± 4.79	1.75 ± 1.50	3.00 ± 2.16	0.00 ± 0.00
Group V (200 mg/kg BW)	29.50 ± 4.44	68.00 ± 6.33	2.00 ± 1.15	2.00 ± 0.00	0.00 ± 0.00
Group VI (400 mg/kg BW)	39.75 ± 8.18	57.25 ± 8.96	1.00 ± 0.82	2.00 ± 1.41	0.00 ± 0.00
Group VII (800 mg/kg BW)	36.25 ± 2.76	58.00 ± 2.45	2.75 ± 0.96	3.00 ± 0.82	0.00 ± 0.00
Normal percentage in *M. musculus* [31]	10 - 40 %	55 - 95 %	0 - 4 %	0.1 - 3.5 %	0 - 0.3 %

Statistical significance was determined based on *p*-values: $p < 0.05$ indicates statistically significant differences between groups. The results of the statistical tests are shown in the table.

Leukocytes in most strains of rats and mice consist primarily of neutrophils, lymphocytes, monocytes and eosinophils, with basophils being present in very small numbers [35]. The research findings on the effects of ethanol extract from unripe *M. paradisiaca* L. var. Kayu fruit on the differential leukocyte percentage in *M. musculus* are shown in **Table 3**.

The results presented in **Table 1** indicate that the average neutrophil percentage across all treatment groups remained within the normal range of 24.5 to 39.75 %, consistent with the reference range of 10 to 40 % [31]. One-way ANOVA analysis showed no significant differences among the groups ($p > 0.05$), suggesting that the ethanol extract of unripe Pisang Kayu has no significant impact on neutrophil percentage in *M. musculus*. Neutrophils are the initial type of leukocytes that react to acute inflammation by engaging in phagocytosis and eliminating microorganisms. They utilize free radicals, including superoxide and hydrogen peroxide, along with various inflammatory mediators to perform this function. Although flavonoids in the extract are believed to influence neutrophil levels, the study found no inflammatory effects, as evidenced by the lack of increased neutrophil levels in any treatment group [36]. Similarly, the lymphocyte percentage across all groups ranged from 57.2 to 70.5 %, within the normal range of 55 to 95 % [31]. The 1-way ANOVA results ($p > 0.05$) confirmed that the extract did not significantly alter lymphocyte percentages. Lymphocytes, key players in the humoral, cellular and cytotoxic immune responses, interact with antigens in the body, with T cells providing cellular immunity and B cells producing antibodies for humoral immunity [37]. Fluctuations in lymphocyte levels may indicate viral or bacterial infections, metabolic diseases or chronic inflammatory conditions, but no such effects were observed in this study.

The study also examined eosinophil and monocyte percentages, finding that both remained within normal ranges across all treatment groups. The average eosinophil percentage was 2 to 3 %, aligning with the normal range of 0 to 4 % [31]. Despite non-normal data distribution and subsequent Kruskal-Wallis testing, no significant differences were detected among the groups ($p > 0.05$). This indicates that the ethanol extract has no significant impact on eosinophil levels, which are typically involved in responses to infections and allergies [38]. Normal eosinophil levels suggest no infection response due to the extract, supporting its anti-inflammatory properties. Monocyte percentages were also normal, ranging from 2 to 3 %, with 1 way ANOVA analysis ($p > 0.05$) showing no significant effect from the extract. Monocytes contribute to inflammation by producing inflammatory mediators such as TNF and IL-1, which can activate lymphocytes and further drive chronic inflammation [39]. Nevertheless, the research revealed no signs of chronic inflammation, which may be attributed to the anti-inflammatory properties of the ethanol extract that inhibited neutrophil activation during acute inflammation. Notably, no basophils were observed in this study, likely due to their typically low presence in circulation (0 to 0.3 %) [31]. Basophils, precursors to mast cells, release heparin at inflammation sites to prevent blood and lymph stasis, but their absence here further suggests a lack of inflammatory response.

The unripe Pisang Kayu fruit used in this study contains various secondary metabolites. Phytochemical screening by Ningsih *et al.* [40] revealed that the unripe fruit extract of Pisang Kayu includes alkaloids, saponins, tannins, triterpenoids and flavonoids. Flavonoids, a class of polyphenols, act as antioxidants within blood cells, scavenging hydroxyl and superoxide radicals, thereby protecting membrane lipids. These antioxidants prevent oxidative damage, including free radicals. Additionally, flavonoids have demonstrated the ability to promote erythropoiesis, which is the process of red blood cell production in the bone marrow, and they also possess immunostimulatory properties [41]. The antioxidant properties of flavonoids maintain the heme ion in its ferrous (Fe^{2+}) state, which is essential for preventing the formation of methemoglobin. This was supported by the findings in this study, where average hemoglobin levels remained within the normal range of 10.2 - 16.6 g/dL (102 - 166 g/L) [31].

Tannins, another significant secondary metabolite in the ethanol extract of unripe wooden pisang fruit, were also identified. Tannins are classified into 2 categories: Hydrolyzed tannins and condensed tannins. The identification of tannins performed by Ningsih *et al.* [40] utilizing ferric chloride ($FeCl_3$) reagent yielded a positive result for condensed tannins, as evidenced by the appearance of a dark green color. This color change occurs due to the formation of a complex between hydroxyl groups and Fe^{3+} ions. Condensed tannins, being polar due to their hydroxyl groups, are distinct from hydrolyzed tannins like tannic acid, which have been shown to affect iron absorption negatively. Afsana *et al.* [42] demonstrated that dietary tannic acid at levels above 10 g/kg reduced iron absorption in male Sprague-Dawley rats, leading to decreased hemoglobin, hematocrit and serum iron levels. However, in this study, the ethanol extract of unripe Pisang Kayu fruit did not significantly affect hemoglobin levels, likely because the tannins present were condensed rather than hydrolyzed, thus avoiding a reduction in hemoglobin concentration in the blood of mice.

Diarrhea occurs when there is an overproduction of fluids or a reduced absorption of fluids and electrolytes in the intestinal lining. This imbalance is influenced by the active transport of ions such as Na^+, Cl^-, HCO_3^- and K^+, as well as solutes like glucose. The condition can lead to dehydration, electrolyte imbalances, malnutrition and deficiencies in essential vitamins and minerals, which, in severe cases, may result in fatal outcomes. Dehydration arises when fluid loss exceeds intake, reducing plasma volume and concentrating blood components. This phenomenon, known as hemoconcentration, increases blood viscosity and elevates the proportion of red blood cells in circulation. Diarrhea often results in liquid stool consistency, sometimes accompanied by blood or mucus, further exacerbating fluid and nutrient loss. Normal erythrocyte levels typically range from 7.0 to $12.5\times10^6/mm^3$, with increases in hematocrit often linked to reduced plasma volume without a corresponding rise in total red blood cells, as seen in dehydration and diarrhea. In this study, the average total erythrocyte count ranged from 7.08 to $9.37\times10^6/mm^3$, indicating normal levels across all treatment groups of *Mus musculus*. Statistical analysis confirmed that the ethanol extract of unripe Pisang Kayu fruit did not significantly affect total erythrocyte or hemoglobin levels, suggesting its safety in this regard.

Leukocytes play a crucial role in defending the body against foreign agents. An increase in leukocyte count can indicate an inflammatory response to infection or injury [34]. In this study, statistical analysis revealed that administering ethanol extract of unripe Pisang Kayu (*M. paradisiaca* L. var. Kayu) to female *M. musculus* as an antidiarrheal agent did not affect total blood leukocyte levels. Any significant changes in leukocyte count outside the normal range would suggest inflammation. In research by Asnilawati *et al.* [43], leukocyte levels in *M. musculus* infected with Staphylococcus aureus showed a significant increase in the positive control group, which was infected without additional treatment, reaching approximately $26.18\times10^3/mm^3$, well above the normal range of 6 - $15\times10^3/mm^3$ [31,43]. This comparison highlights that the extract did not induce an inflammatory response, as the leukocyte levels remained within the normal range.

Conclusions

The study on ethanol extract of unripe Pisang Kayu (*Musa paradisiaca* L. var. Kayu) shows that this extract has no significant impact on hematological parameters in test mice (*Mus musculus*). No significant changes were observed in total erythrocyte count, hemoglobin levels, total leukocyte count or differential leukocytes (neutrophils, lymphocytes, monocytes, eosinophils and basophils) among the treatment groups. All average blood parameter values were within normal ranges, indicating that the ethanol extract of unripe Pisang Kayu has no significant impact on hematology of mice. Additionally, in the teratogenic study, ethanol extract did not cause malformations in fetal mice. The study observed fetal length, weight and the development of bones and organs, finding no significant decreases or differences between the dose groups (50, 100, 200, 400 and 800 mg/kg BW) and control groups (aquades and 0.5 % CMC-Na). These results suggest that the ethanol extract of unripe Pisang Kayu does not cause abnormalities or malformations in fetuses, and meets safety criteria for use as a herbal medicine.

Acknowledgements

This study received financial support from the Institut Teknologi Sepuluh Nopember, Surabaya, Indonesia. The authors express their gratitude to the Department of Biology, Faculty of Science and Data Analytics at the same institution for offering laboratory facilities for conducting this research.

References

[1] DJ Newman and GM Cragg. Natural products as sources of new drugs over the nearly four decades from 01/1981 to 09/2019. *Journal of Natural Products* 2020; **83(3)**, 770-803.

[2] ICS Klau, AW Ningsih and WFI Putra. Profil rendemen ekstrak dan fraksi kulit buah, daging buah dan buah pisang mentah (*Musa paradisiaca* L.) (*in Indonesian*). *Journal of Pharmacy Science and Technology* 2022; **3(1)**, 181-190.

[3] AW Ningsih. 2010, Uji efek antidiare ekstrak buah pisang (*Musa paradisiaca*) pada mencit (*Mus Muculus*) jantan galur Balb-C (*in Indonesian*). Bachelor’s Thesis. Universitas Jember, Jember, Indonesia.

[4] 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 Indonesiana* 2021; **56(3)**, 208-215.

[5] TL Zafirah. 2023, Uji toksistas subkronis ekstrak etanol buah mentah pisang kayu (*Musa paradisiaca* L. var. Kayu) terhadap histopatologi lambung mencit (*in Indonesian*). Ph. D. Dissertation. Universitas Anwar Medika, Jawa Timur, Indonesia.

[6] C Clinton. Plant tannins: A novel approach to the treatment of ulcerative colitis. *Natural Medicine Journal* 2015; **2**, 1-4.

[7] E Aas. Traditional tannin-treatment against intestinal parasites in sheep and cattle. *Ethnobotany Research and Applications* 2008; **1**, 31-38.

[8] SK Bardal, JE Waechter and DS Martin. *Applied pharmacology*. Elsevier Health Sciences, Amsterdam, Netherlands, 2011.

[9] KI Astuti, LD Wulandari and EF Susiani. Antidiarrheal activity of ethanolic extract of *Vernonia amygdalina* Del leaves against male mice induced by oleum ricini. *Borneo Journal of Pharmacy* 2019; **2(1)**, 10-14.

[10] 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 (*in Indonesian*). *Pharmacy Education* 2024; **24(3)**, 329-335.

[11] ER Yuslianti, BM Bachtiar, DF Suniarti and AB Sudjiatmo. Standardisasi farmasetikal bahan alam menuju fitofarmaka untuk pengembangan obat tradisional Indonesia (*in Indonesian*). *Dentika Dental Journal* 2016; **19(2)**, 179-185.

[12] JG Fox, SW Barthold, MT Davisson, CE Newcomer, FW Quimby and AL Smith. *The mouse in biomedical research*. Elsevier, Amsterdam, Netherlands, 2007.

[13] SF Gilbert and MJ Barresi. *Developmental biology*. Sinauer Associates, Sunderland, England, 2016.

[14] LJ Pillitteri, X Guo and J Dong. Asymmetric cell division in plants: Mechanisms of symmetry breaking and cell fate determination. *Cellular and Molecular Life Sciences* 2016; **73(22)**, 4213-4229.

[15] K Enjelika, PD Pratiwi, Yuliawati and MR Efendi. Uji efek teratogenik ekstrak etanol daun andong merah (*Cordyline fruticosa* L) pada fetus mencit putih (*Mus musculus*. L) (*in Indonesian*). *Indonesian Journal of Pharma Science* 2022; **4(2)**, 132-142.

[16] AP Bolliger and N Everds. *Haematology of the mouse*. *In*: MA Suckow, S Hashway and KR Pritchett-Corning (Eds.). The laboratory mouse. Academic Press, Cambridge, 2012, p. 133-170.

[17] P Wexler. *Encyclopedia of toxicology*. Academic Press, Cambridge, 2014.

[18] IM Washington and GV Hoosier. *Clinical biochemistry and hematology*. *In*: MA Suckow, KA Stevens and RP Wilson (Eds.). The laboratory rabbit, guinea pig, hamster, and other rodents. Academic Press, Cambridge, 2012, p. 57-116.

[19] JG Wilson and J Warkany. *Teratology: Principles and techniques*. University of Chicago Press, Chicago, 1993.

[20] L Muna, OP Astirin and Sugiyarto. Teratogenic test of *Pandanus conoideus* var. yellow fruit extract to development of rat embryo (*Rattus norvegicus*). *Nusantara Bioscience* 2010; **2(3)**, 126-134.

[21] HB Santoso. Kelainan struktur anatomi skeleton fetus mencit akibat kafein (*in Indonesian*). *Bioscientiae* 2004; **1(2)**, 23-30.

[22] Badan Pengawas Obat dan Makanan RI. *Peraturan badan pengawas obat dan makanan nomor 24 Tahun 2017 tentang kriteria dan tata laksana registrasi obat*. Badan Pengawas Obat dan Makanan RI, Jakarta, Indonesia, 2017.

[23] HE White, A Goswami and AS Tucker. The intertwined evolution and development of sutures and cranial morphology. *Frontiers in Cell and Developmental Biology* 2021; **9**, 653579.

[24] T Iwaki. *A color atlas of sectional anatomy of the mouse*. Braintree Scientific, Massachusetts, 2001.

[25] OE Nelsen. *Comparative embryology of the vertebrates*. Literary Licensing, Montana, 2012.

[26] MIH Mondal and MS Yeasmin. Toxicity study of food-grade carboxymethyl cellulose synthesized from maize husk in Swiss albino mice. *International Journal of Biological Macromolecules* 2016; **92**, 965-971.

[27] K Naka, T Muraguchi, T Hoshii and A Hirao. Regulation of reactive oxygen species and genomic stability in hematopoietic stem cells. *Antioxidants & Redox Signaling* 2008; **10(11)**, 1883-1894

[28] B Little, N Sud, Z Nobile and D Bhattacharya. Teratogenic effects of maternal drug abuse on developing brain and underlying neurotransmitter mechanisms. *Neurotoxicology* 2021; **86**, 172-179.

[29] Y Fratiwi. The potential of guava leaf (*Psidium guajava* L.) for diarrhea. *Eureka Herba Indonesia* 2022; **3(1)**, 131-135.

[30] S Rahimah, M Salampe and N Rahmania. Uji toksisitas teratogenik ekstrak etanol daun asam jawa (*Tamarindus indica* Linn) terhadap tikus (*Rattus norvegicus*) (*in Indonesian*). *Jurnal Ilmiah As-Syifaa* 2020; **12(1)**, 29-35.

[31] JE Harkness, PV Turner, SV Woude and CL Wheler. *Harkness and Wagner's biology and medicine of rabbits and rodents*. 5th ed. John Wiley & Sons, New Jersey, 2010.

[32] F Lang and M Föller. *Erythrocytes: Physiology and pathophysiology*. Imperial College Press, London, 2012.

[33] NN Etim, ME Williams, U Akpabio and EEA Offiong. Haematological parameters and factors affecting their values agricultural science haematological parameters and factors affecting their values. *Agricultural Science* 2014; **2(1)**, 37-47.

[34] PP Chmielewski and B Strzelec. Elevated leukocyte count as a harbinger of systemic inflammation, disease progression, and poor prognosis: A review. *Folia Morphologica* 2018; **77(2)**, 171-178.

[35] NE Everds. *Hematology of the laboratory mouse*. *In*: JG Fox, MT Davisson, FW Quimby, SW Barthold, CE Newcomer and AL Smith (Eds.). The mouse in biomedical research: History, wild mice, and genetics. 2nd ed. Academic Press, Cambridge, 2007.

[36] MH Pan, CS Lai and CT Ho. Anti-inflammatory activity of natural dietary flavonoids. *Food & Function* 2010; **1(1)**, 15-31.

[37] K Tietze. *Clinical skills for pharmacists*. Elsevier, Amsterdam, Netherlands, 2012.

[38] RD Septianto, IBK Ardana, IW Sudira and OAAG Dharmayudha. Profil hematologi mencit pasca pemberian jamu temulawak secara oral (*in Indonesian*). *Buletin Veteriner Udayana* 2015; **7(1)**, 34-40.

[39] F Ummah, M Belladonna and R Retnaningsih. Rasio neutrofil limfosit darah tepi sebagai indikator outcome pada stroke iskemik akut (*in Indonesian*). *Diponegoro Medical Journal* 2016; **5(4)**, 827-841.

[40] 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.

[41] A Sundaryono. Uji aktivitas senyawa flavonoid total dari gynura segetum (Lour) terhadap peningkatan eritrosit dan penurunan leukosit pada mencit (*Mus musculus*) (*in Indonesian*). *Jurnal Exacta* 2011; **9(2)**, 8-16.

[42] K Afsana, K Shiga, S Ishizuka and H Hara. Reducing effect of ingesting tannic acid on the absorption of iron, but not of zinc, copper and manganese by rats. *Bioscience, Biotechnology, and Biochemistry* 2004; **68(3)**, 584-592.

[43] Asnilawati, E Restia, N Tadulako and D Miranda. Pengaruh ekstrak daun sungkai (*Peronema canescens* Jack) terhadap kadar leukosit, LED dan hematokrit pada mencit jantan (*Mus musculus* L.) yang terpapar *Staphylococcus aureus* (*in Indonesian*). *Jurnal Aisyiyah Medika* 2024; **9(1)**, 338-359.