

Effect of Combined Kelakai (*Stenochlaena palustris*) Leaves Extract and Stingless Bee Honey on Hemoglobin Levels in Mice (*Mus musculus*)

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ABSTRACT

Anemia is a hematological condition characterized by decreased hemoglobin (Hb) levels, leading to impaired oxygen transport and reduced physiological function. Natural products such as kelakai leaves (*Stenochlaena palustris*) extract and stingless bee honey have potential as complementary therapies for anemia due to their iron content and bioactive compounds, including flavonoids and antioxidants that may support erythropoiesis. This study aimed to evaluate the effect of a combination of kelakai leaves extract and stingless bee honey on hemoglobin levels in mice (*Mus musculus*). A true experimental study with a pretest–posttest control group design was conducted using 30 male mice divided into six groups: normal control, untreated anemic control, anemic group treated with iron supplementation, and three treatment groups receiving combinations of kelakai leaves extract and stingless bee honey at ratios of 30:70, 50:50, and 70:30. Anemia was induced using sodium nitrite, and hemoglobin levels were measured before and after treatment. Data were analyzed using one-way analysis of variance (ANOVA) followed by post hoc tests. The results demonstrated that sodium nitrite induction successfully reduced hemoglobin levels below normal, while all treatment groups showed a significant increase in hemoglobin levels compared to the untreated anemic group ($p < 0.05$). The highest mean hemoglobin level was observed in the 70:30 combination group (16.36 g/dL), whereas the lowest was found in the untreated anemic group (10.22 g/dL). In conclusion, the combination of kelakai leaves extract and stingless bee honey effectively increased hemoglobin levels in mice, with the 70:30 ratio identified as the most effective formulation.

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1. Introduction

Anemia remains a critical global health disparity, characterized by a reduction in hemoglobin (Hb) levels or red blood cell (RBC) mass, which compromises the blood's oxygen-carrying capacity to vital tissues (Astuti, 2023; Suryawan et al., 2023). Pathophysiologically, anemia occurs when there is an imbalance between erythrocyte production in the bone marrow and the loss or destruction of these cells. Hemoglobin deficiency causes disruption in the complex process of red blood cell formation or erythropoiesis (Rahayu et al., 2019). Globally, anemia affects approximately 32.9% of the population, with adolescent girls and pregnant women exhibiting the highest vulnerability due to increased physiological demands (Chaparro et al., 2019).

The etiology of anemia is multifactorial, but iron deficiency anemia (IDA) accounts for the majority of cases (Pamela et al., 2022). Iron (Fe) is the central atom of the heme group, which is essential for oxygen binding. However, successful hemoglobin synthesis requires more than just iron. It requires the synergistic interaction of micronutrients, including vitamin B12, folate, and vitamin C, which act as cofactors in DNA synthesis and mineral absorption. Although conventional iron supplementation (such as ferrous sulfate) is widely available, its effectiveness is often limited by poor patient compliance due to adverse gastrointestinal side effects and low bioavailability.

Kelakai (*Stenochlaena palustris*), a medicinal fern endemic to Kalimantan, Indonesia, has been used ethnobotanically by the Dayak tribes for generations to treat anemia and fatigue (Nafisah et al., 2022). Scientific analysis reveals that *S. palustris* possesses an exceptionally high iron content, approximately 291.32 mg per 100g. That alongside significant levels of plant based protein (11.48%), flavonoids, and steroids (Agustina D, 2017; Syamsul et al., 2019).

Another natural ingredient frequently consumed by the people of East Kalimantan is stingless bee honey (*Heterotrigona itama*). This honey functions as a potent therapeutic agent rich in carbohydrates, organic acids, and essential vitamins (Kustiawan et al., 2023). Honey is rich in carbohydrates, amino acids, vitamins, and minerals. The secondary metabolite components in honey act as antioxidants (Rizky et al., 2025). Importantly, the vitamin C content in stingless bee honey can increase the bioavailability of non-heme iron from plant sources by reducing ferric iron to the more soluble ferrous form in the intestinal lumen (Dahlansyah & Petrika, 2020; Wijianto et al., 2025).

The urgency of this research is to address the need for a more easily applicable alternative to synthetic treatments. Although *S. palustris* has a high mineral density, plant-derived iron is typically poorly absorbed, requiring a biochemical catalyst. By integrating stingless bee honey, this research is expected to address the critical challenge of iron absorption by utilizing synergistic mechanisms to optimize hemoglobin synthesis. Furthermore, as a preliminary study, this research serves as a vital scientific basis for validating the efficacy of this herbal combination. This is to support the future development of locally sourced standardized phytomedicines and to pave the way for further clinical trials. Therefore, this study aims to evaluate the synergistic effect of a combination of kelakai (*Stenochlaena palustris*) extract and stingless bee (*Heterotrigona itama*) honey on hemoglobin levels in mice (*Mus musculus*). This combination is expected to provide a more optimal and holistic strategy for anemia management.

2. Methods

2.1. Plant Determination

Determination was carried out to identify the plants used in the research according to the intended species. The determination process was carried out at the Tropical Forest Ecology and Biodiversity Conservation Laboratory, Faculty of Forestry, Mulawarman University, Samarinda. The plant material used in this study was taxonomically authenticated by a qualified botanist, with an official identification certificate issued under number 93/UN.17.4.08/LL/2025.

2.2. Preparation of animals

The test animals used in the experiment were 30 male mice aged 2-4 months and weighing 20-25 grams. The animals were acclimatized for one week in cages provided with standard mouse food and distilled water. The cages were provided with adequate ventilation and indirect light, and the cages were cleaned three times a week. This experiment received ethical approval for the use of experimental animals from the Health Research Ethics Committee, Universitas Muhammadiyah Surakarta, under approval number 6093/A.1/KEPK-FKUMS/XII/2025.

2.3. Division of treatment groups

After acclimatization for one week, the mice were randomly divided into six groups of five mice each.

Group 1: normal control without any treatment.

Group 2: negative control induced by sodium nitrite but without any treatment.

Group 3: positive control induced by sodium nitrite and given blood-boosting medication.

Group 4: induced by sodium nitrite and given a combination of kelakai and stingless bee honey in a 30:70 ratio.

Group 5: induced by sodium nitrite and given a combination of kelakai extract and stingless bee honey in a 50:50 ratio.

Group 6: induced by sodium nitrite and given a combination of kelakai extract and stingless bee honey in a 70:30 ratio.

2.4. Preparation of kelakai extract

The kelakai is cleaned and weighed, after that it is ground using a blender and mixed with water in a 1:1 ratio, then filtered and squeezed with a clean cloth, then the juice is taken.

2.5. Phytochemical screening of extract

Identification of secondary metabolites was carried out by referring to Demiati et al. (2024). This screening was carried out with qualitative identification using related reagents. The screening included alkaloids, flavonoids, saponins, and tannins.

2.6. Administration of sodium nitrite

Sodium Nitrite (NaNO_2) administration is used for pathological anemia treatment in mice by determining LD50, where the average oral dose of Sodium Nitrite in mice is 250 mg/kg. In this study, the body weight of mice was 20 g, so the dose of Sodium Nitrite per mouse was 2.5 mg/20 g. Combination extract treatment was carried out after the Hb of the mice fell below normal Hb after induction.

2.7. Provision of blood-boosting supplements

The positive control used was blood-boosting syrup. The standard dose of blood-boosting syrup for adult humans is 30 mL per day. Using a dose conversion factor from humans to mice of 0.0026, the dose for mice is $30 \text{ mL} \times 0.0026 = 0.078 \text{ mL}$. Thus, the dose of blood-boosting syrup given to mice weighing 20 g is 0.078 mL/20 g body weight.

2.8. Blood sampling

The tail of the mouse was cleaned with 70% alcohol before sampling. Blood was drawn from the lateral vein using a lancet in the mouse's tail, then the tip of the Hb test strip was touched to the blood that came out.

2.9. Examination and measurement of hemoglobin levels

After taking a blood sample, hemoglobin levels are measured using the Easy Touch GCHb device. Blood is smeared onto a strip and counted by the device.

2.10. Data Analysis

The research design used was a pretest-posttest with control group design of a pure experimental type. The data obtained were analyzed using a one-way ANOVA test to determine the difference in hemoglobin (Hb) levels in mice in each treatment group. If the data were not normally distributed, the analysis was continued using a nonparametric test, namely the Kruskal-Wallis test. Furthermore, if significant differences were found, a Post Hoc test was conducted to determine the significant differences between treatment groups.

3. Results and Discussion

Identification of secondary metabolites was carried out to screen the compound groups present in the samples. The results are shown in Table 1.

Table 1. Secondary metabolite screening of the sample

Phytochemical Screening	Kelakai Extract	Stingless Bee Honey
Alkaloid	+	-
Flavonoid	+	+
Saponin	+	+
Tannin	+	+

Flavonoids are polyphenolic compounds that function as free radical scavengers for antioxidants (Rao & Zheng, 2025). Flavonoids work by donating hydrogen atoms to unstable free radicals, thereby preventing cell damage at the DNA level. In addition to flavonoids, kelakai contains alkaloids. Alkaloids are often associated with antimicrobial and analgesic activity. Meanwhile, the saponin and

tannin content in kelakai gives it its distinctive astringent taste. That can aid in wound healing and act as antibacterial (Cosme et al., 2025; Sharma et al., 2023).

Iron is a core component of the hemoglobin molecule, which carries oxygen throughout the body. Iron deficiency causes anemia, which is characterized by symptoms of fatigue, weakness, and lethargy. The kelakai is popular in traditional medicine and proven by the high levels of iron (Fe) in its leaves tissue. Ramadhanti et al. (2023) showed that dietary interventions based on ferns (such as kelakai and jukut fern) have a significant effect on increasing hemoglobin levels in adolescent girls. Biochemically, iron from plants (non heme iron) does have a lower absorption rate than iron from meat (heme iron). However, kelakai naturally contains vitamin C and organic acids that help increase the bioavailability of iron in the human intestine. The addition of lime acid in making kelakai product was used to eliminate unpleasant odors, but intelligently improve iron solubility through a decrease in pH (Wijiniindyah et al., 2025).

Stingless bee honey has a characteristic sour taste. Phytochemical results indicate flavonoids, saponins, and tannins, but no alkaloids. The sour taste of stingless bee honey indicates the presence of vitamin C (Agus et al., 2019). This vitamin C plays a crucial role when combined, creating synergy with the iron content in the kelakai leaves extract, increasing its activity.

The Hb levels from the treatment were recorded and analyzed using univariate and bivariate tests. The results of the pre- and post-test hemoglobin levels are shown in Figure 1.

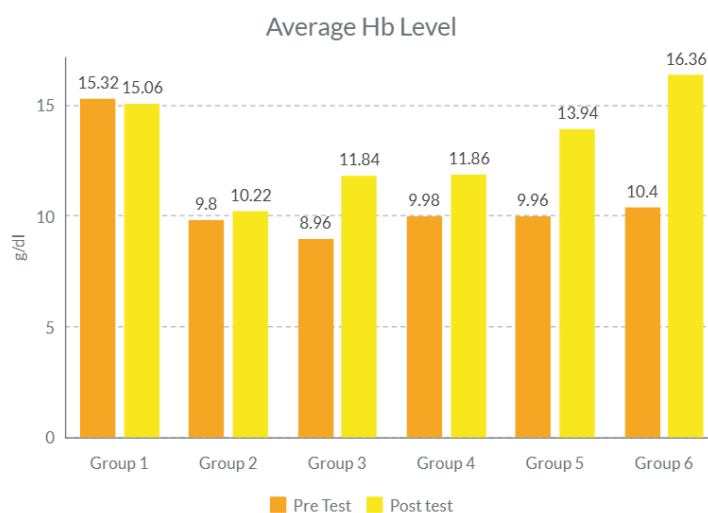


Figure 1. Hb level of mice blood before (pre) and after (post) treatment with extract combination. Orange color showed pre value and yellow color showed post value of Hb level on mice. Group 1/Normal control: without any treatment; Group 2: Negative control: induced by sodium nitrite but without any treatment; Group 3/Positive control: induced by sodium nitrite and given blood-boosting medication.; Group 4: Honey 70%: Kelakai 30%; Group 5: Honey 50%: Kelakai 50%; Group 6: Honey 30%: Kelakai 70%.

Overall, the measurement results show that sodium nitrite induction successfully lowered hemoglobin levels in groups 2 to Group 6 compared to the normal control group. In the pre-test stage, group Positive Control had the lowest average Hb of 8.96 g/dL, while the normal control group remained at a normal level of 15.32 g/dL. After 7 days of intervention, there was a varying increase in hemoglobin levels in all treatment groups with the most significant increase seen in group 6.

The results of normality testing using the Shapiro-Wilk method show that hemoglobin level data are normally distributed. A significance value of $p = 0.160$ ($p > 0.05$) was obtained, then it can be concluded that the sample is normally distributed. The significant value of the pretest is $p = 10.399$ and the posttest $p = 0.195$, which means that the variance is homogeneous because “if the significance value (P) is equal to or greater than (\geq) 0.05, then the variance of two or more groups of measured data is homogeneous and can proceed to One Way ANOVA parametric test.

Table 2. Results of One Way ANOVA

Variabel	F	Sig
Pretest	8.370	0.000
Posttest	7.753	0.000

The results of One Way ANOVA for both groups showed a P value of 0.000 ($p < 0.05$), which means that there was a significant difference between the treatment groups.

The administration of sodium nitrite was effective in reducing the hemoglobin levels of mice compared to the control group. This can be seen from the relatively low average hemoglobin levels of the mice. The decrease in hemoglobin levels after administration of sodium nitrite may be caused by the oxidative properties of the compound, which trigger the formation of methemoglobin. Sodium nitrite can oxidize some hemoglobin, forming ferric ions in the bloodstream. When nitrite is absorbed into the blood and interacts with erythrocytes, this compound oxidizes the Fe^{2+} iron ions in hemoglobin to Fe^{3+} , which then forms methemoglobin (Azkiyah et al., 2021).

After treatment with a combination of kelakai leaves extract and stingless bee honey, an increase in average hemoglobin levels was observed. This increase indicates that treatment with a combination of kelakai leaves extract and stingless bee honey had a positive response. The standard deviation, which is relatively comparable to the condition after the administration of sodium nitrite, shows that the increase in hemoglobin levels occurred relatively consistently in most mice, not just in certain individuals. The synergistic effect of these two natural ingredients can have beneficial effects. This is supported by previous research supporting the potential of these natural ingredients. The administering kelakai extract showed a rapid increase in hemoglobin levels compared to the group that did not receive kelakai extract, which only experienced an increase within normal limits (Negara, 2017). The kelakai leaves tea was have effectiveness in increasing hemoglobin levels in pregnant women (Risyaad et al., 2022). The results of the intervention showed an average increase in hemoglobin levels in pregnant women of 0.82 g/dL after consuming kelakai vegetables and The results showed a significant difference between the two groups. In the intervention group, the average increase in hemoglobin levels reached 2.11 g/dL, while in the control group it was 1.57 g/dL (Putri et al., 2023).

Rista & Yuziani (2014) also found that a dose of 0.25 ml was effective in increasing Hb in white rats, resulting in an increase of 2 g/dl, a dose of 0.5 ml resulted in an increase of 1.17 g/dl, and a dose of 0.75 ml resulted in an increase of 1 g/dl. A study (Harjuna et al., 2019) has proven that honey intervention is effective in increasing hemoglobin levels in a group of female workers at PT. Maruki International Indonesia. There was an average increase of 0.83 g/dl and (Rianti et al., 2022) The results of this study indicate that the average Hb level before honey was administered was 9.04 (mild anemia), while the average Hb level after honey was administered was 11.16 (no anemia).

The highest average hemoglobin level after testing was found in Group 6, at 16.36 g/dL. Meanwhile, the lowest average value was found in Group 2, at 9.80 g/dL. This pattern shows that as the concentration of kelakai leaves extract increases, so does the hemoglobin level. This increase is assumed to be due to the fact that stingless bee honey contains vitamin C as an antioxidant compound (Syamsul et al., 2022). Vitamin C acts as a reducing agent, converting Fe^{3+} to Fe^{2+} , which is more easily absorbed by the body. Iron is difficult for the body to absorb, so vitamin C is needed for iron to be absorbed properly. Additionally, non-heme iron is absorbed four times more effectively, and kelakai has the highest iron content at 291.32 mg/100 g (Hayati et al., 2024).

Kelakai serves as the main source of iron, which is an important part of the blood production process, where excess iron is stored as ferritin protein, hemosiderin in the liver, bone marrow, spleen, and muscles. If iron stores are sufficient, the need for red blood cell formation in the bone marrow will be met (Rusmiati, 2019). Kelakai is also supported by honey, which contains vitamin C that aids in the absorption of iron. These results indicate that the treatment administered had an effect on increasing hemoglobin levels in mice that had previously experienced a decrease in hemoglobin levels due to sodium nitrite induction. The combination of kelakai and stingless bee honey increased hemoglobin in mice. This demonstrates the synergistic effect of the two natural ingredients and could serve as a reference for their potential development into pharmaceutical products.

4. Conclusion

This study confirms a synergistic interaction between kelakai extract and stingless bee honey in elevating hemoglobin levels ($p < 0.05$). This combined treatment successfully counteracted the effects of sodium nitrite-induced anemia, with Group 6 showing the highest efficacy. The results suggest that the two natural ingredients work together more effectively, providing a potent intervention for restoring hemoglobin concentrations.

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