



EFFECT OF INFUSION OF A COMBINATION OF TEMU IRENG, TEMULAWAK, KENCUR, GINGER ON ERYTHROCYTE COUNT AND HEMATOCRIT LEVELS IN ANEMIA WHITE RATS

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Abstract

Anemia is a condition in which the body experiences a decrease in hemoglobin levels, erythrocyte count, and hematocrit levels. The rhizomes of temu black, temulawak, kencur and ginger, contain compounds that have antioxidant and anti-inflammatory activities that can prevent and repair damage from oxidative stress in the body that causes anemia. This study aims to determine the effect and optimal frequency of infusion administration of a combination of temu ireng, temulawak, kencur, and ginger on erythrocyte count and hematocrit levels in white rats of the wistar strain anemia. The method used is experimental research with a *pre-post-test control design*. The four rhizomes used in this study contribute to the treatment of anemia. The isoflavone content in black temu and curcumin in curcumin in curcumin can prevent damage to erythrocytes due to oxidative stress of sodium nitrite exposure. Other ingredients such as Ethyl p-methoxycinnamate in ginger act as an anti-inflammatory by reducing the production of cytokines IL-1 and TNF- α . Then the content of 6-gingerol in ginger can stop the formation of hepcidin which inhibits the absorption of iron, so that the process of forming red blood cells can continue in the bone marrow. It can be concluded that the combination of infusion of temu black, temulawak, kencur and ginger can increase the number of erythrocytes and hematocrit levels in anemia white rats. Meanwhile, the optimal frequency in increasing the number of erythrocytes and hematocrit levels occurred in the P2 group with a frequency of administration 2 times a day.

Keywords: Anemia, Eritrosit, Hematokrit, Infusa.

INTRODUCTION

According to the WHO in 2011, anemia occurs when the concentration of hemoglobin, erythrocytes and hematocrit in the blood drops below normal limits. Hemoglobin and erythrocytes have an important role in maintaining oxygen saturation and supplying oxygen to all parts of the body, including muscles and brain, so that their functions can run as they should (Ministry of Health of the Republic of Indonesia, 2022). The prevalence of anemia in Indonesia is based on basic health research in 2018, stating that 26.8% of anemia occurs at the age level of 5-14 years and 32% occurs at the age level of 15-24 years. Based on this data, it can be seen that anemia occurs most often among adolescents. The incidence of anemia in adolescents tends to be greater in adolescent girls by 22.7% because they experience a menstrual cycle every month. Anemia in adolescents has the potential to be long until pregnancy which can interfere with fetal growth and development, resulting in low birth weight (BBLR), causing bleeding during childbirth, and even causing maternal and child death (Ministry of Health of the Republic of Indonesia, 2022).

Many Indonesian cultures have traditionally used herbal medicine or medicinal plants as a component of their medical practices and cultures. Its existence as an alternative treatment provides solutions for many health conditions that affect society, including anemia. One of the plants that has the potential as an antianemia is the black temu (*Curcuma aeruginosa* Roxb.), with the content of flavonoids of the isoflavone group which has antioxidant activity by playing a role in oxidative stress (Yumita *et al.*, 2022). Antioxidants in flavonoids also have the property of retaining *heme ions* in the form of iron which plays a role in the formation of methemoglobin, so that it can be used effectively in the treatment of anemia (Ardiansyah *et al.*, 2022).

Another medicinal plant that has the potential to be an antianemia is curcumin with its main ingredient in the form of curcumin. Curcumin content from curcumin rhizomes (*Curcumin Xanthorrhiza* Roxb.) also has antioxidant activity (Fadila *et al.*, 2024). The curcumin content in curcumin, especially the phenolic hydroxy group and β diketone, has the ability to protect hemoglobin and oxihemoglobin by inhibiting the action of free radicals in red blood cells (Fadila *et al.*, 2024). In black temu, there are also curcumin compounds that play a greater role in increasing immunity. The role of curcumin in increasing immunity is by working in increasing the production of antibodies and cytotoxicity of cells (Puspita *et al.*, 2023).

According to Danarsih *et al.* (2023) In addition to oxidative stress that can cause damage to hemoglobin cells, a decrease in hemoglobin levels can be caused by inflammation or inflammation that interferes with the ability of the bone marrow to be able to produce red blood cells normally. One of the plants that has anti-inflammatory activity is kencur (*Kaempferia galanga* L.). Ethyl p-methoxycinnamate is the most abundant ingredient found in kencur rhizomes with anti-inflammatory activity (Sandy and Susilawati, 2021). Apart from these three plants, ginger rhizomes (*Zingiber officinale* Rosc.) also have the potential to be antianemia with the content of 6-gingerol which is the most pharmacologically active compound with antioxidant and anti-inflammatory activities. Ginger has also been reported to improve iron bioaccessibility (Ooi *et al.*, 2022).

METHOD

Acclimatization of Test Animals

Test animals are first acclimatized before being given treatment to help test animals make adjustments to environmental conditions or new residences. This acclimatization was carried out at the STIKes Ibnu Sina Ajibarang Pharmacology Laboratory. This stage is carried out for 7 days with the aim of avoiding stress in test animals. The test animals were fed in the form of a pallet mixed with water and a hanging drinking bottle. The test animals were placed in cages made of plastic, with sufficient air retention, equipped with a base in the form of wood fibers that were regularly replaced.

Sample Preparation

As much as 1kg of each rhizome is cleaned of residual dirt that sticks and stored in a dry container.

Infusion Preparation Formulation

The weight of each rhizome used, is taken based on the reference of the Use of Indonesian Traditional Medicine (Indonesian Traditional Medicine Herb Formulary, (Ministry of Health, 2017) and has been converted from human dose to rat dose with the formula:

$$Dose = \frac{BB\ Indonesia\ (kg)}{BB\ adults\ Indonesia\ (kg)} \times rhizome\ dose\ (gr) \times Human\ to\ mouse\ conversion\ factor$$

After conversion, then the calculation of the dose of the combination infusion and the volume of the combination infusion to be used with the formula:

$$Combination\ infusion\ dosage = \frac{BB\ mouse\ (gr)}{rat\ conversion\ weight\ (gr)} \times (conversion\ dose\ (gr))$$

Combination infusion volume administration

$$= \frac{Combined\ infusion\ dose\ (mg)}{Combination\ infusion\ stock\ solution\ levels\ (mg)} \times (Stock\ solution\ volume\ (ml))$$

Infusion Manufacturing

The rhizomes to be used are first cleaned from the remaining dirt that is carried, then cut and weighed according to the stock solution to be made, which is 5.4 grams and heated using a water solvent of 10ml. Heating is carried out for 15 minutes with a water temperature of 90°C. When heating takes place, stirring is carried out occasionally to facilitate the dissolution of the compounds contained in the rhizomes. Next, the infusion is filtered with a flannel cloth, and a hot water solvent is added through the pulp until the desired volume is met.

Examination of erythrocyte count and hematocrit levels

Blood is taken at the tail through a lateral vein by cutting using a scalpel knife and blood is stored in an EDTA tube. Before blood collection, the feathers on the tail are shaved first using a razor and cleaned using alcohol to make it easier to take blood samples (BPOM, 2021). Blood tests were carried out using a hematology analyzer and were taken 3 times at different times (Acclimatization (Day 0) blood samples were taken before being induced by sodium nitrite; Pretest (Day 16) blood samples are taken after being induced sodium nitrite for 15 days; Posttest (Day 24) blood samples were taken after being given a combination of temu ireng, ginger, temulawak, and kencur infuca for 7 days).

Sodium Nitrite Manufacturing (NaNO₂)

Sodium nitrite of 3mg/200gBB is dissolved in 3ml of aquadest and administered orally for 15 days.

Test Animal Treatment

The rats were grouped into 4 groups consisting of 5 rats in each group and 2 reserve rats. So that the total number of rats used is 28. After the rats were acclimatized, they were then given sodium nitrite for 15 days and continued with combination infusions with different frequencies (P0: aquadest; P1: combination infusion with a frequency of administration 1 time a day; P2: combination infusion with a frequency of administration 2 times a day; P3: combination infusion with a frequency of administration 3 times a day).

RESULTS AND DISCUSSION

The results of blood measurements at the acclimatization stage showed that all groups of test animals were in good health and did not experience anemia. This is shown by measuring the number of erythrocytes and hematocrit levels are in normal conditions. Meanwhile, the results of measurements at the pretest stage or administration of sodium nitrite as much as 3mg/200gBB for 15 days showed a decrease in the number of erythrocytes and hematocrit levels (Figure 1).

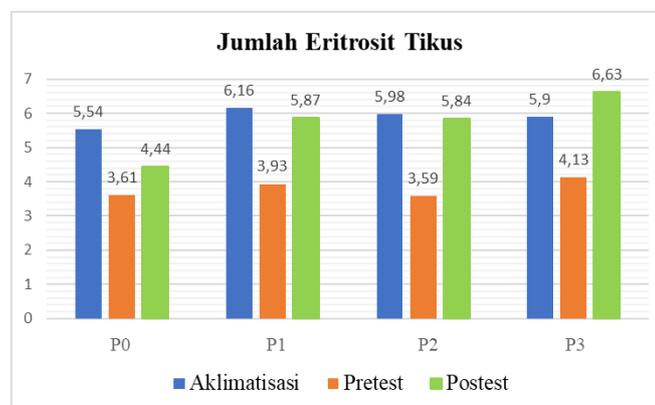


Figure 1. Diagram of the average number of erythrocytes in mice

Administration of sodium nitrite can lead to the formation of *Oxygen Reactive Species* (ROS) clusters on hemoglobin. Sodium nitrite will enter red blood cells through transport anions and others through diffusion, resulting in decreased membrane function and oxidation of hemoglobin. So that oxidative stress can increase and facilitate the oxidation process from hemoglobin to methemoglobin (Setyarini *et al.*, 2023). The formation of methemoglobin in the blood will certainly reduce normal hemoglobin levels. Methemoglobin in the form of ferric Fe³⁺ can no longer bind oxygen, so it cannot supply oxygen needs in the body properly. Where methemoglobin that is formed excessively can cause erythrocytes to become stiff until lysis occurs in erythrocytes (Setyarini *et al.*, 2023)

In addition to occurring in hemoglobin, sodium nitrite that forms the ROS group can also cause oxidative stress in the erythrocyte membrane. Thus, erythrocytes will experience a decrease in function by being able to carry only a small amount of oxygen to the rest of the body and erythrocytes will easily undergo premature hemolysis (Setyarini *et al.*, 2023). With early hemolysis, erythrocytes will be destroyed faster than they should be and cause the number of erythrocytes to decrease in the

blood. In addition to being caused by oxidative stress, a decrease in erythrocyte levels can also occur due to excessive formation of methemoglobin. These results are in line with research conducted by Setyarini *et al.*, (2023) where each group of test animals induced sodium nitrite at the same dose, experienced a decrease in the number of erythrocytes after 14 days of administration. A similar thing was found during the research process, where the test animals induced by sodium nitrite experienced a change in condition from active and healthy to weak, rarely moving and breathing conditions quickly or panting.

Normal erythrocyte levels in white rats ranged from $5.16 \times 10^6/\mu\text{L}$ - $6.92 \times 10^6/\mu\text{L}$. The same thing also happened with hematocrit, where hematocrit itself is the percentage of red blood cells or erythrocytes contained in the blood. When erythrocytes undergo premature hemolysis and decrease erythrocyte levels, indirectly the percentage of hematocrit levels will also decrease (Figure 2). The percentage of normal hematocrit levels in white rats ranged from 31.80%-38.06% (Fitria *et al.*, 2020).

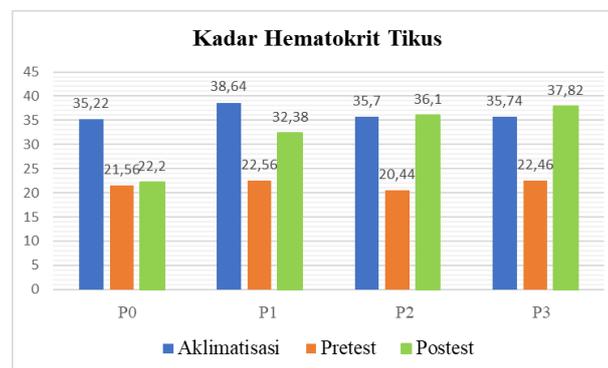


Figure 2. Diagram of the average hematocrit levels of rats

At the posttest stage, it can be seen that the group that was given a combination infusion for 7 days experienced an increase in erythrocyte count and hematocrit levels. Infusions are given for 7 consecutive days with several treatment models. At P0 as a negative control, test animals were only given aquadest. In P1, test animals were given an infusion once a day at 7 a.m. Then in P2, test animals are given infusions twice a day at 7 a.m. and 2 p.m. Meanwhile, in P3, test animals are given infusions three times a day, namely at 7 am, 2 pm, and 9 pm.

The antioxidant activity in black meet played by flavonoids of the isoflavone group is by keeping *heme ions* in the form of ferro, which contributes to the production of methemoglobin (Ardiansyah *et al.*, 2022). Thus, hemoglobin can still be maintained in the form of ferro (Fe^{2+}) which is involved in the normal function of hemoglobin. Flavonoids are antioxidants that can also stimulate the immune system and increase the formation of red blood cells in the spinal cord (erythropoiesis) (Ardiansyah *et al.*, 2022). With the activity of these flavonoids, it can prevent the excessive formation of methemoglobin which can cause premature hemolysis and help the spinal cord in producing new red blood cells. Curcumin compounds in black meeting also play a role in increasing immunity. The role of curcumin in increasing immunity is by working to increase antibody production and cell

cytotoxicity (Puspita *et al.*, 2022). Temu ireng itself does not produce antibodies, but the content of active compounds in temu ireng can play a role in supporting the immune system. So that the curcumin content in black meeting supports the immune system and helps the body in the production of antibodies needed to fight infections due to damage from free radical compounds caused by sodium nitrite.

Curcumin in curcumin can act as an antioxidant, where there are two chemical groups in curcumin that act as antioxidants. The first group is phenolic hydroxy and the second group is β diketon. Both have the same mechanism, namely capturing free radicals in the phase of the antioxidant mechanism and acting in different phases (Fadila *et al.*, 2024). Thus, the curcumin compound in curcumin acts as an antioxidant and captures free radicals in red blood cells in the antioxidant phase and inhibits damage to erythrocytes, so that the oxygen needs in the body can still be met.

Oxidative stress occurs due to an imbalance between oxygen and antioxidants, and can lead to redox signaling disruption and molecular damage control. When oxidative stress increases, it can cause damage to cell and tissue structures and potentially inflammation (Ramos-González *et al.*, 2024). When inflammation occurs, it can interfere with the production of red blood cells in the bone marrow, so it can reduce the number of erythrocytes (Danarsih *et al.*, 2023). When macrophages recognize the presence of inflammatory receptors, they can trigger the production of cytokines such as pro-inflammatory cytokines and anti-inflammatory cytokines. The production of cytokines can directly affect the absorption of iron from erythrocytes (Taherkhani *et al.*, 2020). Given that iron is needed for the production of red blood cells in the bone marrow, it can disrupt the iron balance in the body.

Kencur is a plant that has anti-inflammatory activity with the main content of its essential oil in the form of Ethyl p-methoxycinnamate. The mechanism of Ethyl p-methoxycinnamate as an anti-inflammatory is by reducing the production of inflammatory cytokines such as *Interleukin-1* (IL-1) and *Tumor Necrosis Factor alpha* (TNF- α) (Sandy and Susilawati, 2021). The cytokines IL-1 and TNF- α are cytokines that trigger inflammation in the adaptive immune system, so by suppressing the production of these cytokines and Ethyl p-methoxycinnamate can reduce the inflammatory response that occurs. So that the iron balance in the body can be maintained and the formation of new red blood cells can take place. 6-gingerol is a pharmacologically active phenolic compound found in ginger rhizomes with antioxidant and anti-inflammatory activities. Ginger is also reported to increase iron bioaccessibility in people with anemia. 6-gingerol can suppress NF- κ B activity by inhibiting pro-inflammatory cytokines, as well as can stop the formation of hepcidin. Hepcidin is an acute phase protein, which functions to inhibit iron absorption (Ooi *et al.*, 2022). Thus, 6-gingerol can also prevent the impaired activity of iron transporters in the body caused by pro-inflammatory cytokines and prevent the formation of hepcidin.

Ginger has a lot of polyphenol content, the polyphenol content is a probiotic for intestinal microbes, as well as improving intestinal health. Ginger has also been reported to improve iron bioaccessibility (Ooi *et al.*, 2022). In other words, ginger bioactive polyphenols can function to

maintain good bacteria in the gut and improve gut health. Ginger polyphenols can indirectly increase the absorption of iron in the body through food in the intestines more efficiently. So, the need for iron in the body will be maintained. Meanwhile, the antioxidant mechanism in ginger is by inhibiting the production of free radicals. Compounds that have antioxidant activity will convert protons to free radicals by taking H-phenol atoms directly and through electron transfer, so that they can inactivate the properties of free radicals (Sari and Nasuha, 2021).

Thus, the mechanism owned by each of the four rhizomes can repair or prevent more severe damage to cells in the body due to oxidative stress. So that the cells in the body can improve their function and restore their function normally. The results of blood measurements obtained from the group of test animals given combination infusions showed that there was a difference between the average number of erythrocytes and hematocrit levels before and after the infusion, with the *paired t-test* value ($p < 0.05$). The result was obtained with a value of $p = 0.000$ ($p < 0.05$) in the *ANOVA one-way test*. The results showed that there was a significant difference in mean values between the test animal groups that were given combination infusions. When viewed from the frequency of administration and the average increase in the number of erythrocytes and hematocrit levels in each group of test animals, the more often the combination infusion is given, the higher the increase that occurs. The average increase in the number of erythrocytes and hematocrit levels was highest in the P2 group with a frequency of administration 2 times a day and the P3 group with a frequency of administration 3 times a day. However, the results of the *Post-hoc LSD* test that has been carried out, the P2 and P3 groups have no significant difference ($p > 0.05$). Where this shows that the increase that occurred between the P2 and P3 groups is not much different. So, it can be said that the optimal frequency of combination infusion administration in the number of erythrocytes and hematocrit levels occurs in the P2 group. Because in the P2 group with a frequency of administration only 2 times a day, it can have an optimal effect in raising levels to normal limits.

CONCLUSION

It can be concluded that the combination infusion of temu black, temulawak, kencur and ginger can increase the number of erythrocytes and hematocrit levels in anemia white rats with a value of $p = 0.00$ ($p < 0.05$). Meanwhile, the optimal frequency in increasing erythrocyte counts and hematocrit levels occurred in the P2 group with a frequency of administration 2 times a day.

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