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Original Research Article

Antianemic Activity of *Anamirta cocculus* Hydro-Ethanol Extract against Sodium Nitrite-Induced Anemia in Rats

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ABSTRACT

Anamirta cocculus is a plant endemic to Papua, Indonesia, which is used empirically by indigenous Papuans to treat various health problems, including anemia. This investigation aims to ascertain whether the hydro-ethanol extract from *Anamirta cocculus* stems can prevent anemia in rats induced by sodium nitrite ($NaNO_2$). Five groups of five rats each were designed from 25 rats. Aquadestillata 5 mg/kg was given to the control group in Group I. Sangobion 1.465 mg/kg, po was given to the control positive group II. *Anamirta cocculus* (400, 550, and 700 mg/kg, po) was given to groups III, IV, and V, respectively. All rats were first orally administered $NaNO_2$ at a dose of 187.5 mg/kg once daily for 1 week to obtain anemic rats. Thereafter, the various treatments (groups I to V) were done once daily for 15 days. All animals were given as food standard rodent pellets and water during treatment. Blood specimens were collected before and during treatment on days 0, 3, 9, and 15 via venipuncture to assess blood hemoglobin (Hb) levels using a UV-visible spectrophotometer. Data analysis using SPSS paired-sample and independent-sample t-test. The findings showed that $NaNO_2$ caused a statistically significant ($p<0.05$) decrease in Hb level as seen in the control group, which indicated anemia. According to the findings, oral administration of 550 and 700 mg/kg of *Anamirta cocculus* extract significantly reduced anemia in rats that had been induced by $NaNO_2$ ($p <0.05$). Indeed, these observations may be useful for managing anemia.

Keywords: Anemia, *Anamirta cocculus*, Antianemic activity, Rats, Hemoglobin (Hb)

Introduction

Anemia is a disorder that arises when the blood's hemoglobin (Hb) level is below normal. The three main causes of anemia are decreased production of red blood cells, increased destruction of red blood cells, and bleeding. [1,2], Low hemoglobin levels in red blood cells, which hinder the cells' ability to transport oxygen to other tissues, are the hallmark of iron deficiency, the primary cause of anemia. Anemia is one of the most common medical conditions in the world. When a woman's hemoglobin level is less than 12 g/dL and a man's is less than 13 g/dL, the World Health Organization (WHO) defines anemia. Every age group is vulnerable to anemia. [3,4] Globally, the prevalence of anemia was 36.5% in pregnant women, 29.6% in non-pregnant women, and 39.8% in children aged 6 to 59 months. [5] In Indonesia, the highest prevalence of anemia is found in pregnant women (48.9%), followed by toddlers (38.5%), and teenage girls (32%). [6] Anemia is a global public health concern that can impact individuals of any age; however, it is more common in children and expectant mothers. [7] The body's response to counteract free radicals is to produce endogenous antioxidants such as superoxide dismutase (SOD), catalase The effects of anemia include decreased cognitive, motor, and verbal function, cardiovascular disorders, insomnia, and low immune function. It may increase the risk of prolonged hospitalization and inhibit linear growth in children. [8,9]Hypoxia, or low oxygen levels in the body, is brought on by insufficient iron consumption, which also lowers levels of insulin-like growth factor-1 (IGF-1) because of elevated IGF-1 binding protein levels.

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Together, these two effects prevent protein synthesis from occurring. A child at risk of stunting is characterized by stunted growth due to low levels of IGF-1 in the body. [8,10]

Anemia and stunting are related, according to a study on anemia in children aged 2 to 6 years in Sorong Regency, West Papua Province, Indonesia. Children with anemia are 5.4 times more likely to experience stunting than children who do not suffer from anemia. [11] The fact that the risk factors for stunting are related to the hematological profile, especially iron deficiency anemia, it is a challenge for health workers to emphasize the need to focus on improving postnatal services to prevent stunting. [12] Indeed, it is crucial to keep developing anemia prevention and treatment programs. Many Indonesians are more trusting and think that using traditional medicine is safer and more effective than using synthetic medications that are already in the market, thus anemia can be treated pharmacologically, specifically by using blood supplement tablets (Fe), or non-pharmacologically, using natural resources.[13]

One of the plants that can raise hemoglobin levels in the blood is the endemic *Anamirta cocculus*, which is Indigenous Papuans in Indonesia and used extensively as an antimalarial medication.[14,15] Research on *Anamirta cocculus* has shown that its primary bioactive components, flavonoids and alkaloids, have very substantial antioxidant activity in the ethyl acetate fraction of the plant.[16] The systemic regulation of iron metabolism and iron absorption by flavonoid bioactive compounds is thought to control protein expression and activity, which holds therapeutic promise in the prevention and treatment of anemia or iron overload diseases. [17,18], Furthermore, by decreasing lipid peroxidation, flavonoids can shield bodily cells from harm brought on by oxidative stress and free radicals. [19,20] The purpose of this investigation is to ascertain whether the extract from *Anamirta cocculus* can prevent anemia in rats that have been caused by sodium nitrite ($NaNO_2$).

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Materials and Methods

The chemicals used in this research were sodium nitrite ($NaNO_2$) for the induction of anemia, obtained from Merck & Co. (Germany), hemoglobin CIR (Onemed, Indonesia), and 70% ethanol (Onemed, Indonesia).

Plant Collection and Identification

The plant material used is the stem of Tali Kuning (*Anamirta cocculus*) collected from Folley village, East Misool, Raja Ampat Regency, Southwest Papua Province, Indonesia in August 2024. The location of Folley village is at the coordinates: -1.7899350105285654º (Latitude), 130.3293199034863º (Longitude). Mr. Djitmau from the Manokwarienses Herbarium, Papua State University, identified the original specimen of this plant, which matches the collection of the New Guinea Forestry Department (NGF) No. 12272.

Experimental Animals

For seven days, 25 healthy adult female rats weighing between 155 and 316 grammes were placed in cages with natural light to aid in their adjustment to the new surroundings. During the acclimatization period, test animals were given standard feed with an adequate supply of clean water that changed periodically. This research has received ethical permission for health research with test animal subjects from the Research Ethics Committee of the Makassar College of Pharmaceutical Sciences with ethical approval number: 339/EC.1.1.B/VII/KEPK/2024 on June 10, 2024.

Preparation of Extracts

The collected *Anamirta cocculus* stems were dried using an oven at 50 °C. The dried samples were then powdered and then extracted via maceration using 70% ethanol solvent in a ratio of 1:4, left for 72 hours in a closed vessel and protected from direct light, with occasional stirring, and then filtered. After the extraction process is complete, the extract is then evaporated using a water bath at a temperature of 50 °C until a thick residue is obtained. The percentage yield was calculated and recorded.

Induction of Anemia

Anemia was induced by giving $NaNO_2$ suspension orally at a dose of 187.5 mg/kg to rats for 1 week. Anemia was confirmed by a decrease in Hb levels measured using a UV-visible spectrophotometer.

Hematinic Screening

A total of 25 rats were used in this study, and all rats suffered from anemia due to the influence of oral $NaNO_2$ suspension induction at a dose of 187.5 mg/kg for 1 week. 25 anaemic rats were divided into 5 groups, each consisting of 5 rats. Group I: negative control (aquadestillata 5mL/kg, po), Group II: positive control (sangobion 1,465 mg/kg, po), Group III: *Anamirta cocculus* (400 mg/kg, po), Group IV: *Anamirta cocculus* (550mg/kg, po), Group V: *Anamirta cocculus* (700 mg/kg, po). The treatment was given once daily for 15 days. During the treatment period, all animals had access to food (standard pellets) for rodents and water. Blood specimens were taken before and during treatment via venipuncture on days 0, 3, 9, and 15 to evaluate blood Hb levels using a UV-visible spectrophotometer.

Statistical analysis

All study data were analyzed using IBM SPSS version 27 for IOS (iPhone Operating System). Bivariate analysis was employed to compare the data between the control and intervention groups using an independent-sample t-test. A paired-sample t-test was used to compare the pre- and post-test data for each group in order to perform statistical analysis. The results were then displayed as mean±SD. A p-value of less than 0.05 indicates that the result is statistically significant.

Results and Discussion

Haemoglobin (Hb) levels was significantly decreased in rats given sodium nitrite ($NaNO_2$) for seven days, leading to anemia induction ($p < 0.001$) as exemplified in group I. The mean data for $NaNO_2$ induction are shown in Table 1. Considering that $NaNO_2$ might bind hemoglobinand result in anemia, Reactive oxygen species (ROS) generation causes oxidative stress in the erythrocyte membrane, which makes the erythrocytes unable to retain their flexibility and results in hemolysis. [21,22] In addition, using $NaNO_2$ can result in severe methemoglobinemia, a disorder where hemoglobin loses its capacity to bind and transport oxygen after being oxidized to methemoglobin. [23]

Table 1: Effect of sodium nitrite ($NaNO_2$) administration on Hemoglobin (Hb) Levels in Rats

Groups	Pre-induction Hb (mg/dl)	Post-Induction Hb (mg/dl)	Δ (mg/dl)	P-Value
Group-I (Negatif control)	19.23±2.31	10.52±0.82	9.56±0.95	<0.001*
Group-II (Positive control)	19.72±1.78	10.16±0.88	10.06±1.04	<0.001*
Group-III (AC 400 mg/kg Treatment)	19.02±3.15	10.72±0.80	8.30±2.79	<0.001*
Group-IV (AC 550 mg/kg Treatment)	17.34±1.46	10.28±0.83	7.06±0.71	<0.001*
Group-V (AC 700 mg/kg Treatment)	19.70±2.63	11.12±0.58	8.58±2.29	0.001*

Values are expressed as Mean ± SD, **Δ** = The difference between pre-induction with $NaNO_2$ and post-induction with $NaNO_2$, *p < 0.05 paired-samples t-test.
AC = *Anamirta cocculus*

A paired-sample t-test statistical analysis comparing each group's pre- and post-treatment Hb levels revealed a substantial rise ($p<0.05$) in Hb levels across all test groups (Table 2).

In $NaNO_2$-induced anemic rats, Group II (sangobion/ positive control treatment dose 1.465 mg/kg, po), Group III, IV, and V (*Anamirta cocculus* treatment doses 400, 550, and 700 mg/kg, po), respectively, were compared with Group I (negative control). The results of the statistical analysis using an independent-samples t-test revealed a significant increase in Hb levels ($p <0.05$) in Groups II, IV, and V. A noteworthy rise in hemoglobin levels ($p <0.05$) was observed in all treatment groups (groups II, III, IV, and V) on the ninth and fifteenth testing days.Test data comparing groups III, IV, and V (*Anamirta cocculus* treatment doses 400, 550, and 700 mg/kg, po, respectively) with group II (sangobion treatment dose 1.465 mg/kg, po) (Table 2) shows that on the 3rd day of testing, group V (*Anamirta cocculus* treatment dose 700 mg/kg, po) was significantly ($p <0.05$) more effective in increasing Hb levels compared to group II and extract groups. Meanwhile, testing on days 9 and 15 showed that group II (sangobion/ positive control treatment dose 1.465 mg/kg, po) was more effective ($p <0.05$) in increasing Hb levels compared to group III (*Anamirta cocculus* treatment dose 400 mg/kg, po), while group IV and V (*Anamirta cocculus* treatment doses 550 and 700 mg/kg, po), had the same effectiveness (not significant $p > 0.05$) with group II (sangobion treatment dose 1.465 mg/kg, po).

In this investigation, Hb levels in the blood following after the administration of *Anamirta cocculus* extract (doses of 400, 550, and 700 mg/kg, po) demonstrated a significant increase in comparison to the negative control group. However, of the three-dose variations, only the doses of 550 and 700 mg/kg, po of *Anamirta cocculus* are as effective as the dose of 1,465 mg/kg, po of sangobion (positive control group). Consequently, it is thought that *Anamirta cocculus*, when administered in the presence of flavonoids and alkaloids, can enhance the hemoglobin profile in rats with $NaNO_2$-induced anemia. Treatment and prevention of anemia and iron overload disorders are thought to be possible with flavonoids, an important phytochemical present in *Anamirta cocculus.* [17,18] Flavonoids help the body's defenses by lowering lipid peroxidation,

which helps to stop oxidative stress and free radical damage to body cells. The mechanism for protecting the balance of Hb levels is very influential and has a very important role in human health because a body that experiences a deficiency or excess of Hb will have serious consequences for the body. The systemic regulation of iron metabolism and iron absorption is known to be influenced by flavonoids in terms of protein expression and activity. [19,20]

Table 2: Effect of Administration of *Anamirta cocculus* Stem Extract on Hemoglobin (Hb) Levels in $NaNO_2$-Induced Anemic Rats

Variable	Group-I (Negatif control)	Group-II (Positive control)	Group-III (AC 400mg/kg Treatment)	Group-IV (AC 550mg/kg Treatment)	Group-V (AC 700mg/kg Treatment)
Pre-treatment Hb (mg/dl)	10.42±0.58	10.24±0.86	10.90±0.70	10.24±0.83	11.26±0.47
Post-treatment Hb (mg/dl)					
Day-3	11.00±0.47*	11.89±0.55*	11.20±0.56	12.00±0.20*	14.25±0.29*
Day-9	11.08±0.49*	14.28±0.25*	12.35±0.17*	14.15±0.27*	15.14±0.22*
Day-15	11.31±0.39*	16.16±0.39*	14.92±0.85*	15.07±0.21*	18.22±0.59*
Δ (mg/dl)					
Day 3	0.58±0.30b**	1.65±0.51a**	0.30±0.67b**	1.76±1.01a**	2.99±0.63ab**
Day 9	0.66±0.30b**	4.05±1.05a**	1.45±0.67ab**	3.91±1.07a**	3.88± 0.65a**
Day 15	0.89±0.32b**	5.92±0.98a**	4.02±0.64ab**	4.83±0.70a**	6.96 ± 0.87a**

Values are expressed Mean ± SD, n = 4, Δ = The difference between pre-treatment and post-treatment, *p < 0.05 paired-samples t-test, **p < 0.05 independent-samples t-test. [a]vs. Negative control, [b]vs. Positive control.
AC = *Anamirta cocculus*

Conclusion

The study reveals that rats with sodium nitrite-induced anemia, oral treatment with *Anamirta cocculus* extract at doses of 550 and 700 mg/kg demonstrated considerable anti-anemic efficacy. An increase in hemoglobin levels by *Anamirta cocculus* extract suggests that the *Anamirta cocculus* extract has anti-anemic properties and can be used to treat anemia. Therefore, extract from *Anamirta cocculus* is useful for low hemoglobin levels. Futhermore, the potential toxicity of *Anamirta cocculus* extracts, needs more investigation into the extract's phytochemical compounds and elucidation of the phytochemicals that responsible for the anti-anemic actions of *Anamirta cocculus* extract.

Conflict of Interest

Authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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