

RESEARCH ARTICLES

## Administration of Red Mushroom Rice (Angkak) Affects the Increase in Platelet Count in Chloramphenicol-Induced Rats

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**Abstract:** Thrombocytopenia is a condition where platelet levels in the blood decrease. The isoflavone and lovastatin content in Angkak has the potential to be an additional therapy to increase the number of platelets. This study aims to determine the effect of Red Mushroom Rice Administration (Angkak) in increasing the Number of Platelets in Chloramphenicol-Induced Rats. This research is experimental research using a pretest and posttest with a control group design. The results of the paired t-test show a significance value of  $0.027 < 0.05$ . This shows that between day 0 and day 12 there are significant differences in each group. The ANOVA test results show a significance value for day 12 of 0.002. The significance value shows a value  $< 0.05$ . This shows that administering Angkak on day 12 has a significant effect on platelet counts. In this study, the positive control was significantly different from the other groups. This positive control group also showed the lowest average platelet count on day 0. Treatment Group I also showed significantly different results with the highest average platelet count on day 12. Conclusion: From the results of this study, it can be concluded that the administration of boiled water, increasing the doses of 2 g/KgBW, 1 g/KgBW, and 0.5 g/KgBW, had an effect in increasing the number of platelets in male rats induced by chloramphenicol.

**Keywords:** Angkak, chloramphenicol, platelets.

## INTRODUCTION

Thrombocytopenia is a condition in which the platelet count in the blood decreases.<sup>1,2</sup> It is a clinical condition that constitutes one of the contributing factors to mortality.<sup>3,4</sup> Thrombocytopenia is characterised by a reduction in platelet levels from the normal range of 150,000–400,000/ $\mu$ L.<sup>1,5</sup> Bleeding may occur when platelet levels fall below 50,000/ $\mu$ L and can become fatal if they drop below 10,000/ $\mu$ L without prompt management.<sup>6,7</sup> Diseases such as aplastic anaemia, leukaemia, and the side effects of radiation therapy or chemotherapy are among the causes of thrombocytopenia.<sup>2,8,9</sup> One of the major health problems in Indonesia is dengue hemorrhagic fever (DHF). The clinical condition in DHF worsens when complications such as bleeding from the nose, mouth, gums, and bruising of the skin occur as a result of thrombocytopenia.<sup>3,4,10,11,12</sup>

Based on World Health Organisation (WHO) data in 2020, the estimated number of cases reaches around 390 million annually.<sup>5,13,14</sup> In Indonesia, the incidence of DHF tends to fluctuate, which is thought to be influenced by climatic factors.<sup>6,15,16</sup> In 2015, DHF cases increased to 126,675 cases. Mortality due to DHF can occur in both children and adults, with incidence reaching 94,893 cases per year.<sup>7,17,18</sup>

The isoflavone and lovastatin content in Angkak has the potential to serve as an adjunct therapy for DHF patients because it can increase platelet counts. However,

research on the use of Angkak as an anti-thrombocytopenic agent is still limited. Therefore, further investigation is needed to explore the potential of Angkak as a therapeutic intervention to increase platelet counts in individuals suffering from dengue fever.<sup>11,19,20</sup>

In a study by Gunawan (2007), administration of Angkak infusion at a dose of 1.3 g/kg body weight and isolated yellow Angkak metabolite at a dose of 6.6 mg/kg body weight resulted in a significant increase in platelet count ( $P < 0.05$ ) compared to the control group in rats.<sup>12,21,22</sup> Meanwhile, a study conducted by Marisa in 2008 showed that administration of red rice at a dose of 0.25  $\mu$ g/g body weight after induction with chloramphenicol at 500 mg/kg body weight did not produce a statistically significant effect ( $P > 0.05$ ).<sup>13,23,24</sup>

Setiawan (2015) found that administration of Angkak at a dose of 2 g/kg body weight in mice for 5 days in treatment group I resulted in an increase in platelet count of 0.72 g/kg body weight compared to animals induced with chloramphenicol for 7 days.<sup>25,26,27</sup> The platelet counts at the end of interventions II and III did not show a significant difference ( $P < 0.05$ ). This indicates that doses of 1 g/kg body weight and 0.5 g/kg body weight are effective in increasing platelet counts in male rats induced by chloramphenicol, reaching levels comparable to the negative control.<sup>14,28,29</sup>

Based on Schalm's study in 1965, platelet counts in rats generally range between 500,000 and 1,000,000, with an

average value of approximately 850,000. Due to the large variation in platelet counts in rats, further research is needed to determine clinically significant differences in platelet levels.<sup>15</sup>

## METHOD

This study is an experimental study using a pretest–posttest design with a control group. It specifically evaluates treatment samples before and after induction with chloramphenicol and administration of red rice boiled water.

This study involves two types of variables: the independent variable, which is the administration of red rice boiled water at doses adjusted to the body weight of the test subjects, and the dependent variable, which is the effectiveness of red rice boiled water in increasing platelet counts in rats after chloramphenicol induction. The study population consisted of adult male Wistar rats (*Rattus norvegicus* L), white strain, that met the inclusion and exclusion criteria. A total of 30 male Wistar rats were used as samples.

1. Group 1: Negative control group (–) received 1% CMC-Na orally for 7 consecutive days, followed by distilled water for 5 days. Blood samples were collected on day 0 and day 12.
2. Group 2: Positive control group (+) received oral chloramphenicol at a dose of 0.72 g/kg body weight for 7 days, followed by distilled water orally for 5 days. Blood samples were collected on day 0 and day 12.

3. Group 3: Treatment I group received oral chloramphenicol at a dose of 0.72 g/kg body weight for 7 days. On day 7, blood samples were collected, followed by administration of red rice boiled water orally at a dose of 2 g/kg body weight for 5 days. Additional blood samples were collected on day 12.
4. Group 4: The treatment II group received oral chloramphenicol at a dose of 0.72 g/kg body weight for 7 days. On day 7, blood samples were collected, followed by administration of red rice boiled water orally at a dose of 1 g/kg body weight for 5 days. Additional blood samples were collected on day 12.
5. Group 5: The treatment III group received oral chloramphenicol at a dose of 0.72 g/kg body weight for 7 days. On day 7, blood samples were collected, followed by administration of red rice boiled water orally at a dose of 0.5 g/kg body weight for 5 days. Additional blood samples were collected on day 12.

Platelet counts were measured on day 0 and day 12. On day 0, blood samples were collected to determine the baseline platelet count of the rats before the administration of chloramphenicol and Angkak boiled water. On day 12, comparisons were made among the negative control group, positive control group, and treatment groups I, II, and III after chloramphenicol induction and administration of Angkak boiled water to assess whether there was an increase in platelet counts in the rats<sup>14</sup>. Furthermore, the responses of each experimental group of

animals induced with chloramphenicol were compared to evaluate the effect of Angkak in increasing platelet counts.

If platelet count data obtained from the normality test showed a value of ( $p > 0.05$ ), it indicated that the data were normally distributed. The Levene's test for homogeneity showed a value of ( $p > 0.05$ ), indicating that the data had homogeneous variances. If  $p > 0.05$ , the analysis was continued using the paired t-test (paired t-test). For normally distributed data, analysis was performed using One-Way ANOVA, and if homogeneity was met, it was followed by the LSD (Least Significant Difference) test to determine differences between groups in pre- and post-testing conditions. Results were considered statistically significant if ( $p < 0.05$ ) and not significant if ( $p > 0.05$ ).

## RESULT

### Univariate Analysis

#### 1. Pretest Platelet Count in Rats (Day 0)

Based on the findings of the pre-induction assessment conducted on rats on day 0, platelet counts were measured and yielded the following results.

**Table 1 Mean Platelet Count of Rats on Day 0**

Group	Perlakuan	Average Trombosit ( $10^3$ ) $\mu$ l $\pm$ std
I	Negative Control	968 $\pm$ 41.473
II	Positive Control	832 $\pm$ 113.666
III	Treatment I	1002 $\pm$ 101.094
IV	Treatment II	976 $\pm$ 26.077
V	Treatment III	956 $\pm$ 100.399

Based on Table 1, it can be observed that the highest mean platelet count among the five groups of rats in the pretest (day 0) was 1,002,000, found in Treatment Group I. This group consisted of rats induced with chloramphenicol at a dose of 0.72 g/kg body weight orally for 7 days; on day 7, blood samples were collected after administration of Angkak boiled water at a dose of 2 g/kg body weight orally for 5 days, and additional blood samples were collected on day 12. The highest standard deviation was observed in the positive control group.

#### 2. Posttest Platelet Count in Rats (Day 12)

Based on the findings of the study conducted on rats on day 12, the rats were exposed to chloramphenicol and administered Angkak boiled water, resulting in the following outcomes.

**Table 2 Mean Platelet Count of Rats on Day 12**

Group	Perlakuan	Average Trombosit ( $10^3$ ) $\mu$ l $\pm$ std
I	Negative Control	1.040 $\pm$ 114.018
II	Positive Control	924 $\pm$ 88.769
III	Treatment I	1.196 $\pm$ 66.933
IV	Treatment II	1.164 $\pm$ 110.815
V	Treatment III	980 $\pm$ 126.491

Based on Table 2, the highest mean platelet count on day 12 after the experiment among the five groups of rats reached 1,196,000. The mean platelet count was observed in Treatment Group I, which consisted of rats administered oral chloramphenicol at a dose of 0.72 g/kg body weight for 7 days. On day 7, blood samples

were collected after the rats were given Angkak boiled water orally at a dose of 2 g/kg body weight for 5 days. Additional blood samples were collected on day 12. Treatment Group III had the largest standard deviation.

**Bivariate Analysis**

**1. Differences in Mean Platelet Count on Day 0 and Day 12**

This study involved conducting tests to compare the mean platelet counts of rats on day 0 and day 12 in each group.

**Table 3 Paired T-Test**

		t	df,	Sig. (2-tailed)
Pair 1	Posttest - Pretest	3,420	4	,027

Table 3 shows that the results of the paired t-test yielded a significance value of 0.027, which is less than 0.05. This finding indicates that there is a statistically significant difference within each group between day 0 and day 12.

**2. Effect of Angkak Administration on Increasing Platelet Count**

**Table 4 ANOVA Test**

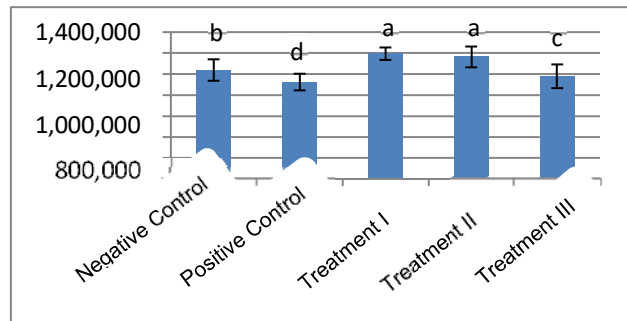
	Sum of Squares	df	Mean Square	F	Taraf Sig.
Posttest between groups	27302 40000 00.000	4	68256 00000 0.000	6.362	.002
Within group	21456 00000 00.000	20	10728 00000 0.000		
<b>Total</b>	<b>48758</b>	<b>24</b>			

40000
00.000

Based on the table above, the ANOVA test results show a significance value of 0.002 for day 12. The p-value on day 12 indicates a significant effect on platelet counts.

**Table 5 Mean Platelet Count on Day 0 and Day 12**

Rat	Platelet Count				
	Negative Control	Positive Control	Treatment I	Treatment II	Treatment III
1	1.000.000	920.000	1.220.000	1.220.000	960.000
2	1.200.000	1.020.000	1.300.000	1.300.000	1.180.000
3	1.100.000	940.000	1.140.000	1.000.000	1.000.000
4	900.000	780.000	1.180.000	1.140.000	840.000
5	1.000.000	960.000	1.140.000	1.160.000	920.000
Rata-rata	1.040.000	924.000	1.196.000	1.164.000	980.000
SD	101.980	79.398	59.867	99.116	113.137



From the graph above, it can be seen that Angkak has an effect on increasing platelet counts in chloramphenicol-induced subjects. The values indicate an increase in platelet counts in each treatment group (Treatment I, Treatment II, and Treatment III).

## DISCUSSION

The findings of this study indicate that the administration of Angkak on day 12 has a significant effect on platelet counts. The chloramphenicol solution in 1% CMC-Na was prepared by suspending 24 capsules of chloramphenicol containing 6.0 g of chloramphenicol in 100 mL of 1% CMC-Na suspension.<sup>30</sup> When converted to the rat dosage, the value obtained was 0.36 g/kg body weight. This dose was selected because previous studies showed that a dose of 0.36 g/kg body weight did not affect platelet levels in rats. To achieve a higher effect, the dose was doubled to 0.72 g/kg body weight.<sup>31</sup>

Furthermore, subsequent analysis showed a substantial difference between the positive control group and Treatment I compared to the other groups. The positive control group consisted of samples receiving oral chloramphenicol at a dose of 0.72 g/kg body weight for 7 days, followed by oral administration of distilled water for 5 days. Blood samples were collected on day 0 and day 12. Meanwhile, Treatment I involved administration of chloramphenicol at a dose of 0.72 g/kg body weight orally for 7 days. On day 7, blood samples were collected after the subjects were given Angkak boiled water orally at a dose of 2 g/kg body weight for 5 days, followed by another blood sample collection on day 12.<sup>32</sup>

The findings of this study are consistent with those of Setiawan (2015), which demonstrated that the administration of Angkak boiled water at doses of 2 g/kg body weight, 1 g/kg body weight, and 0.5 g/kg

body weight resulted in an increase in platelet counts in male rats induced with chloramphenicol. The effect of Angkak on platelet levels in chloramphenicol-treated male rats depends on whether there is an increase in platelet counts following Angkak therapy compared to the negative control group.<sup>14,33,34</sup>

In Setiawan's study (2015), the maximum daily dose of 4000 mg listed in the Drug Information Handbook (DIH) was converted to a rat dose of 0.36 g/kg body weight. The results showed that administration of  $2 \times 0.36$  g/kg body weight orally for 7 days led to a decrease in platelet counts in mice. Administration of red rice at a dose of 2 g/kg body weight for 5 days in Treatment Group I resulted in an increase in platelet counts in chloramphenicol-induced rats, equivalent to 0.72 g/kg body weight over 7 days. This finding indicates that a dose of 2 g/kg body weight can increase platelet counts in male rats treated with chloramphenicol, with an increase of 18.78% compared to baseline platelet levels.<sup>35</sup>

Administration of red rice at a dose of 1 g/kg body weight for 5 days in Treatment Group II resulted in an increase in platelet counts equivalent to 0.72 g/kg body weight in rats induced with chloramphenicol for 7 days. This finding suggests that a dose of 1 g/kg body weight can increase platelet counts, resulting in a 14.02% increase compared to baseline. Meanwhile, administration of 0.5 g/kg body weight of red rice for 5 days in Treatment Group III also resulted in an increase in platelet counts in

chloramphenicol-induced rats. This indicates that a dose of 0.5 g/kg body weight is effective in restoring platelet levels in male rats depleted by chloramphenicol. Statistical analysis revealed that the only significant difference observed was between platelet counts before treatment in the positive control group compared to the negative control group. The normal range of platelet counts in rats may vary considerably, indicating that normal platelet levels are influenced by the specific pathophysiological conditions of the animals.<sup>14,36</sup>

A study conducted by Hosea in 2018 utilized red guava (*Psidium guajava* L.) as the research material. Guava pulp was mixed with distilled water to produce concentrations of 5 g/mL and 10 g/mL. The mixture was then blended and filtered through cloth to obtain the juice extract. Thrombocytopenia was induced by administering chloramphenicol at a dose equivalent to that for rats, determined as 30 mg per 30 g of body weight. A total of 24 male mice that had undergone a one-week adaptation period were divided into four groups: healthy control, negative control, Treatment I, and Treatment II. The treatment duration was 12 days. Before administration of chloramphenicol and guava juice orally, the rats were fasted for approximately 3 hours to ensure an empty stomach. The results showed that administration of red guava juice at concentrations of 5 g/mL and 10 g/mL was effective in increasing platelet counts in rats. Furthermore, administration of guava juice at a concentration of 10 g/mL for 5 consecutive days resulted in the most significant increase

in platelet counts compared to other treatments.<sup>37</sup>

## CONCLUSION

The findings of this study indicate that the administration of red rice boiled water at doses of 2 g/kg body weight, 1 g/kg body weight, and 0.5 g/kg body weight resulted in an increase in platelet counts in male rats induced with chloramphenicol.

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