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Effect of *Premna pubescens* Ethanol Extract on Erythrocyte Count and Kidney Histology in *Rattus norvegicus* L

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Abstract

Premna pubescens (wild leaves) has a rich history of traditional medicinal use, including as an anti-inflammatory, antioxidant, and anticancer agent. This study aimed to investigate the potential therapeutic effects of the ethanol extract of Premna pubescens leaves on erythrocyte count and kidney histology in *Rattus norvegicus L*. Twenty-four male Wistar rats were divided into four groups: control, ethanol extract of wild leaves, Sheep Red Blood Cell (SRBC) antigen, and combined ethanol extract with SRBC. The experiment involved administering wild leaf ethanol extract at 250 mg/kg BW for 30 days, followed by SRBC injection on days 8 and 15. Erythrocyte counts were measured, and kidney histological changes were observed.The results showed a significant increase in erythrocyte count in the A1 (7.42 ± 0.35 million cells/µl) and A3 groups (7.77 ± 0.23 million cells/µl) compared to the control group (7.05 ± 1.07 million cells/µl) and SRBC-treated rats (6.61 ± 0.18 million cells/µl). Histological analysis of the kidneys revealed clearer glomeruli and tubules, with reduced signs of inflammation and bleeding compared to the SRBC-treated group. These findings suggest that *Premna pubescens* extract has potential therapeutic effects on erythrocyte count and renal tissue, likely due to its antioxidant and anti-inflammatory properties. In conclusion, the ethanol extract of *Premna pubescens* shows promise in positively affecting erythrocyte count and mitigating kidney damage, demonstrating its potential as a therapeutic agent.

Keywords: *Premna pubescens, Ethanol extract, Erythrocyte count, Kidney histology, Rattus norvegicus*

1. Introduction

1.1 Sub Introduction

Premna pubescens, also known as savage plant or wild leaf, is a herbal plant that has long been traditionally utilized in Southeast Asia, particularly among Malay communities. The plant has several scientific synonyms, including *Premna obtusifolia*, *Premna integrifolia* L., *Premna corymbosa*, and *Premna serratifolia* L., reflecting its wide distribution and varied usage across different regions. In Malaysia, the leaves of this plant are commonly added to traditional dishes such as spicy porridge, a special meal during the fasting month. The inclusion of *Premna pubescens* in this dish is believed to boost endurance during fasting, a traditional practice considered essential for maintaining vitality and stamina.

Beyond its culinary applications, *Premna pubescens* is highly regarded for its medicinal properties, which have been recognized and passed down through generations. Traditionally, the leaves have been used to treat a variety of health complaints, including colds and worm infections, and as a galactagogue, a substance that stimulates milk production in breastfeeding mothers. Furthermore, the plant is commonly used to aid postpartum recovery, making it essential in caring for women after childbirth. In this context, *Premna pubescens* is valued for its physical health benefits and ability to restore energy and strength to a fatigued body.

From a scientific perspective, the plant exhibits critical pharmacological properties, including anti-inflammatory, antioxidant, anticancer, antidiabetic,

and blood-clotting effects. These properties make *Premna pubescens* valuable in traditional medicine for addressing chronic diseases and inflammatory conditions. However, despite its widespread use in traditional remedies, there remains limited scientific research on its effects on red blood cells (erythrocytes) and kidney histology. Erythrocytes are crucial in oxygen transport throughout the body and are vital indicators of circulatory system health. Investigating how *Premna pubescens* affects these cells will provide deeper insights into its potential role in maintaining hematological health.

2. Section headings

As vital organs for filtering blood and regulating fluid and electrolyte balance, the kidneys also require special attention in studying the plant's effects. Kidney histology, which examines the microscopic structure of kidney tissue, can reveal potential damage or functional disturbances. Previous studies have shown that ethanol extract from *Premna pubescens* can enhance immune response by increasing lymphocyte, monocyte, and granulocyte counts and boosting antibody titers in animal models. While these findings suggest the plant's potential to improve immune function, few studies have examined its effects on erythrocytes and organ histology, including the kidneys.

Therefore, this study aims to investigate further how ethanol extract from *Premna pubescens* leaves affects erythrocyte count and induces histological changes in the kidneys, particularly in the experimental model of *Rattus norvegicus* L. By studying its impact on hematological conditions and kidney function. This research hopes to provide new insights into *Premna pubescens'* therapeutic potential as a traditional medicinal agent and a potential candidate for modern drug development targeting hematological and renal disorders

3. Equations

Materials

Samples

The research sample was 24 male white rats with an average weight of 100-250 grams .

The materials used in this study were 24 male white rats of the Wistar strain (mother rats obtained from the Faculty of Pharmacy, University of North Sumatra with an average weight of 150-250 grams, PC 05 pellet form feed, drinking water given ad libitum, wooden husks as a mat for rat cages, ethanol extract of wild leaves, SRBC obtained from the Medan Veterinary Laboratory, CMC, formalin 10% to preserve the spleen before being used as preparation, BNF solution 10%, alcohol 70, 80, 90, 95% and absolute alcohol), xylol I, xylol II as well as paraffin I and II, Mayers dye hematoxin, Lithium Carbonate solution.

Cage Provision and Acclimatization of White Rats

In the study, the number of cages provided was eight pieces. The cage is made of plastic material (40x20x15) with a cover of fine wire on the top to prevent rats from entering the cage 13. The cage's base is lined with wooden husks with a 0.5-1 cm thickness and replaced once every two days to keep the cage clean and free of ammonia that can interfere with the respiratory system. Each cage consists of 3 rats. Each cage that contains experimental rats is then placed on a wooden shelf that has been provided. The rats were acclimatized for two weeks by feeding them PC 05 pellets and drinking water.

The manufacture of Leaf Ethanol

The extract was picked in the morning at around 10.00 WIB, as much as 3420 grams of wet weight. The leaves are located from the 3rd order from the base of the twig to the 4th leaf from the shoot. After weighing, the leaves are washed, drained, and aerated for one night. Then, it is finely sliced and dried in the sun until dry (crumbly). After drying (crumbly), blend until smooth. The weight of the leaf flour obtained is 1050 grams. Then, the flour of the refined wild leaves was soaked using 70% alcohol in a dark bottle and left for three days. During the soaking process, the bottle is stirred every day. After three days, it is then extracted using Soxhlet 15.

Determination of Dosage of Savage Extracts

The dosage of wild water extract for rats was determined based on previous research on Premna13 with a dose of 250 mg/kg BB of rats. The ethanol extract of wild leaves was mixed with 1% CMC. The treatment was given for 30 days. On the 8th and 15th days, SRBC was injected into mice intramuscularly as much as 0.1 ml 16.

Parameter Measurement

Before the staining process, deparaffinization is carried out using xylol I and II solutions. Next, dehydration is carried out gradually in a solution of absolute alcohol (2 minutes), 95% alcohol (1 minute), and 80% alcohol (1 minute). The preparation is then washed under running water and dried. The staining process begins with soaking the preparation in Mayer's hematoxin dye (8 minutes), then washing it down under running water for 10 minutes.

The preparation is then dipped in a Lithium Carbonate solution (10-15 minutes) and soaked in tap water for 2 minutes. The preparation is then dipped in eosin dye (2-3 minutes) and washed with tap water (30-60 seconds) to remove excess dye. Furthermore, rehydration was carried out with ten dips of 95% alcohol solution, ten dips of absolute alcohol I, absolute alcohol II (2 minutes), xylol I (1 minute), and xylol II (2 minutes). Then, the preparation is dried and covered with a cover glass using per mount adhesive 18. The variables in this research are categorized into independent and dependent variables. The independent variable in this study is the ethanol extract derived from wild leaves (*Premna pubescens*). The dependent variables include the number of erythrocytes and the histological appearance of the kidneys in the experimental mice (*Rattus norvegicus*). These variables are crucial for assessing the potential effects of *Premna pubescens* on blood cell counts and kidney tissue structure.

4. Figures and Tables

4.1 General

Effect of EEDBB on Erythrocyte Count in White Rats. The total number of erythrocytes in this study was obtained by calculating the total number of erythrocytes using the ABX Micros 60 tool. After being treated, the total erythrocyte value data was obtained, as shown in this table.

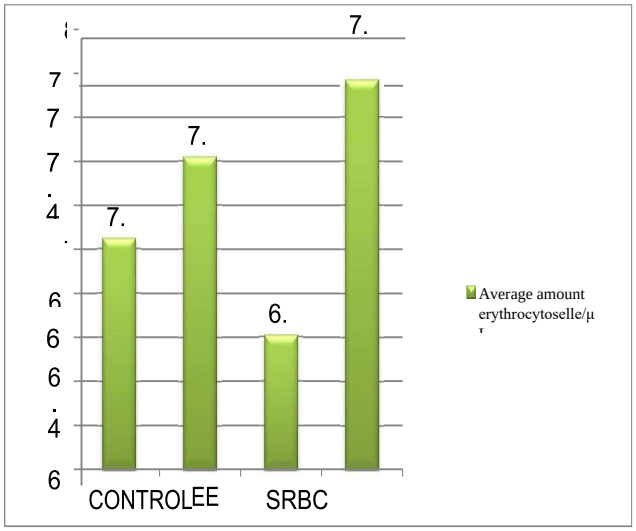

Fig. 1 Average Value of Erythrocyte Count at Each Treatment

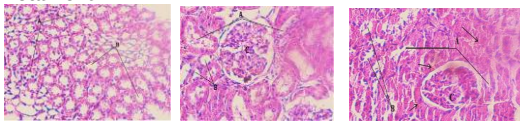

Fig. 2. Effect of EEDBB on the Histology of White Rat's Kidneys

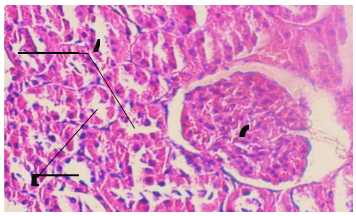

Fig.-3. Renal histology of proximal (A) distal (B), and glomerular (C) contortus tubules in SRBC group mice

Effect of Ethanol Extract Administration of Wild Leaves on the Number of Erythrocytes in White Rats. The number of erythrocytes is one of the important parameters for assessing health, considering its massive role in the body. *Erythrocytes* are blood cells that do not have a nucleus, mitochondria, or ribosomes. Most erythrocytes in mammals are described as biconcave discs without a nucleus. The shape of the erythrocyte biconcave provides a large volume surface ratio, making it easy to exchange gas. Erythrocytes are flexible, allowing erythrocytes to adapt to their regular shape and small capillary centerline. Based on the data, it can be seen that the average number of white *ichthyrocytes* after treatment experienced an increase and a decrease when compared to the control. The SRBC treatment showed a reduction in the number of erythrocytes. This situation causes mice to experience anemia because the number of erythrocytes is below the normal range. The decrease in the number of erythrocytes is suspected to have occurred due to the death of the stem due to exposure to antigens given to mice twice, resulting in erythrocytes being easily ruptured or hemolysis. If there is no intake of antioxidants in the body, there is a possibility of a decrease in erythrocytes; this is proven in the treatment, which has a reduction in erythrocytes to below average. This is by a study conducted in which the number of white erythrocytes after being given SRBC decreased due to stress experienced by rats.
The EEDBB treatment group experienced an increase in erythrocytes. The higher number of erythrocytes evidences this compared to the CONTROL treatment. This increase in erythrocytes is thought to be due to the activity of flavonoids in wild leaves. Wild leaves were detected to contain bioactive components, one of which is flavonoids responsible for antioxidant activity—previous research conducted.

In addition, the increase in the number of erythrocytes is also suspected to be due to the content of saponins in the leaves of wild beasts. Saponins have a bitter and bitter taste when dissolved. Foaming saponins will increase the membrane's permeability, making it easier for large molecules to be absorbed into the body so that the absorption of nutrients increases. Based on the description above, it can be seen that administering wild leaf extract to white rats can stimulate the formation of erythrocytes with the content of flavonoids, saponins, and iron with the help of vitamins C and A contained in wild leaves. Effect of Ethanol Extract of Wild Leaves on the Histology of White Rats' Kidneys The kidneys are one of the most important excretory organs and are integral to the body's homeostasis, which involves maintaining balance, including physical and chemical balance. In the observation of the histology of the rat kidneys with treatment, it can be seen that the glomerulus, proximal tubules, and distal tubules are visible, both in size and between their cells. In addition, no changes indicated bleeding and necrosis in the kidneys; this showed that the wild leaf extract did not cause toxicity to the kidney tissue of white rats, so it could be said to be safe for the kidneys.

Meanwhile, in the A2 treatment group, it can be seen that the picture of the glomerulus, proximal tubules, and distal tubules is not very clear; besides that, there is also bleeding around the outside of the glomerulus. The size of the proximal and distal tubules is also getting smaller compared to other treatments, and the boundaries between cells are also fading. In the treatment group, a clearer picture of the glomerulus, proximal and distal tubules was seen compared to the treatment. In addition, it is also more significant, the boundaries between cells are more visible, and the bleeding around the glomerulus is reduced. This is due to the bioactive content of wild leaves, which can recover the damage caused by the antigen. Based on the above description, it can be concluded that

SRBCs causes histological changes which is significant in the kidneys and can damage the kidneys. However, this damage can be reversed with the content of flavonoids and vitamin C possessed by wild leaves which function as antioxidants that are able to lysize antigens so that they do not damage parts of the kidneys and with the help of saponins that help the kidneys work more actively.

4.2 Tables

Table-1: Average number of erythrocytes (Million cells/µl)

No	Treatment	Average
1	K (A_0)	7,05 ± 1,07
2	EEDBB (A_1)	7,42 ± 0,35
3	SRBC (A_2)	6,61 ± 0,18
4	EEDBB + SRBC (A_3)	7,77 ± 0,23
TOTAL		28,85

K = control; EEDBB = Ethanol Ester of Wild Leaves; EEDBB + SRBC = Savage Leaf Ethanol Extract + Sheep Red Blood Cell, and SRBC = Sheep Red Blood Cell

Table 1 shows that the average number of erythrocytes in mice treated with EEDBB+SRBC is 7.77 million cells/µl, while in the EEDBB treatment, it is 7.42 million cells/µl. Mice with CONTROL treatment , which is 7.05 million cells/µl, are quite different from those with SRBC treatment , which is 6.61 million cells/µl. For more details, the average value of erythrocytes can be seen in this picture. The data is statistically tested with a one-way ANOVA test to determine whether the average difference of each treatment has an effect (Fig-1).

	Average	SRBC	Control	EEDBB	EEDBB+SRBC
SRBC (A_2)	6,61	-	-	-	-
Control (A_0)	7,05	$O,44^{tn}$	-	-	-
EEDBB (A_1)	7,42	$0,81^{*}$	$0,37^{tn}$	-	-
EEDBB +SRBC (A_3)	7,77	1,16*	0,72	$0,35^{tn}$	-

Table 2. Table-2: Erythrocyte Count BNT Test
Information: BNT (0.05) =1.11 * = Real Difference, BNT (0.01) = 2.32 tn = unreal

CONCLUSION

Based on the results of the research that has been obtained, the following conclusions can be drawn: There was an effect of ethanol extract administration of wild leaves on the number of erythrocytes in white rats, which was with an average number of 7.42 million cells/µl. There was an effect of the administration of wild leaf ethanol extract on the number of erythrocytes in white rats with the administration of SRBC as an antigen with an average number of 7.77 million cells/µl. The findings of this study demonstrate that the administration of ethanol extract from wild leaves (*Premna pubescens*) significantly affects the number of erythrocytes in white rats (*Rattus norvegicus*). The average erythrocyte count in rats treated with the extract was 7.42 million cells/µl, indicating a positive influence on red blood cell production. Additionally, when *Premna pubescens* ethanol extract was administered alongside sheep red blood cells (SRBC) as an antigen, the erythrocyte count further increased to an average of 7.77 million cells/µl. This suggests that the extract may enhance erythropoiesis and potentially boost the immune response when combined with an antigen, highlighting its possible therapeutic benefits for hematological conditions.

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