Proceedings of
The 3rd Annual International Conference Syiah Kuala University (AIC Unsyiah) 2013
In conjunction with
The 2nd International Conference on Multidisciplinary Research (ICMR) 2013
October 2-4, 2013, Banda Aceh, Indonesia

Asses humoral immunity of wistar strains rats (*Rattus norvegicus* L.) which given ethanol extract-buas buas (*Premna pubescens* Blume) leaves

Martina Restuati and Restianna Eliawaty Simanjuntak

Department of Biology, State University of Medan, Medan, 20221, Indonesia.
Coorresponding Author: t.restuati@gmail.com

Abstract. The aim of the research was to to assess the effect of ethanol extract *Premna pubescens* leaves on humoral immunity and the body weight of rats (*Rattus norvegicus* L.). The white rats use 24 tails and devided into 4 groups with each group replicates 6 times. There are A0 as control which give with aquadest, A1 give with ethanol extract of *Premna pubescens* leaves, A2 give with ethanol extract of *Premna pubescens* leaves and Sheep Red Blood Cells (SRBC), and A3 give with SRBC only. White Wistar rats aged 3 months with body weight 100-300 grams. Aquadest and ethanol extracts of *Premna pubescens* given orally every day and SRBC given by injections on the 8th and 15th day . The rats blood taken by decapitation on 31th day. Measurements antibody titer of the rat as humoral immunity used hemagglutination method using a V microplate 96. The results were analyzed statistically with ANOVA. The result showed that the antibody titer at A2 increased significantly , where t (65,78) > t table (4,94). This suggests that the ethanol extract of *Premna pubescens* leaves influence the humoral imunnity of Wistar strains rats using SRBC, The result of body weight statistically, t_{count} 0.23 < t_{table} 3.10. It means that ethanol extract of *Premna pubescens* leaves did not influence the body weigh.
Keywords: Haemagglutination, *Premna pubescens* Blume, SRBC, antibody titers

Introduction

The efforts to improve body immunity system against specific antigen is needed to be concern. Medicinal plants are source of important therapeutic aids for alleviating human ailments. It is also good for self medication because it could not occur any side effects. The utilization of medicinal plants is less expensive dan easy to be found compared with synthetic medicinal. Thus natural products have been a major source of drugs for centuries.

In tune with this effort, the objective set for the present investigation is to identify the leaves of *Premna* in order to determine the nature of the principle component responsible for its medicinal property. *Premna pubescens* Blume is the one of genus of *Premna* that can be found in Indonesia but not familiar to be used as medicinal plant. It has reported that *Premna pubescens* has a biological activity. The leaves are anti-inflammatory, antibacterial, antitumour (Patel,2007). Similarly, Thiruvenkatasubramaniam (2010) defined that the extracts of *P. corymbosa* on blood glucose levels and serum lipid profile possesses a hypoglycemic effect. There was significant reduction in total cholesterol, LDL cholesterol, VLDL cholesterol and improvement in HDL cholesterol in diabetic rats.

These biological activity is along with the chemical constituent in *Premna pubescens*. This plant has an apigenin from flavonoid. Apigenin is a nontoxic dietary flavonoid that has been shown to possess anti-tumor propertie and therefore poses special interest for the development of a novel chemopreventive and/or chemotherapeutic agent for cancer. Fang (2005) demonstrated that apigenin inhibits expression of vascular endothelial growth factor (VEGF) in human ovarian cancer cells. VEGF plays an important role in tumor angiogenesis and growth. It was found that apigenin inhibited VEGF expression at the transcriptional level through expression of hypoxia-inducible facto 1 (HIF-1).. These findings reveal a novel role of apigenin in inhibiting HIF-1 and VEGF expression that is important for tumor angiogenesis and growth.

Gupta (2001) also defined that apigenin is capable of selectively inhibiting cell growth and inducing apoptosis in cancer cells without affecting normal cell. Apigenin has

been shown to suppress angiogenesis in melanoma and carcinoma of the breast, skin, and colon (Liu, 2005). Regarding apoptosis, apigenin decreased in the protein expression of Bcl-2 protein and this anti-apoptotic factor might be also participated in the apoptotic process (Zheng, 2005)

Based on these recent studies, it need to investigate the utilization of *Premna pubescens* Blume as immunomodulator by measurements antibody titer of the rat (*Rattus norvegicus* L.) as humoral immunity.

Materials and Methods

Animals
Male and female Wistar rats (*Rattus norvegicus*) weighing 100-300 g. were collected from Chemistry Laboratory in State University of Medan. They were housed under standard laboratory conditions of light, temperature and relative humidity. The animals were given standard rat pellets and tap water *ad libitum*. The rats were randomly divided into four experimental groups: A_0 (control), A_1 (Etanol extract of *Premna pubescens*), A_2 (Etanol extract of *Premna pubescens* + SRBCs), A_3 (SRBCs).

Antigen
The antigen that used in thus research was sheep red blood cells (SRBCs) Fresh blood was collected from sheep sacrified in the local slaughter house, in Veterinary Laboratory, Medan.

The design of research
The white rats use 24 tails and devided into 4 groups with each group replicates 6 times. There are A_0 as control which give with aquadest, A_1 give with ethanol extract of *Premna pubescens* leaves, A_2 give with ethanol extract of *Premna pubescens* leaves and Sheep Red Blood Cells (SRBC), and A_3 give with SRBC only. Aquadest and ethanol extracts of *Premna pubescens* given orally every day and SRBC given by injections on the 8th and 15th day . The rats blood taken by decapitation on 31^{th} day.

Materials and Methods

Preparation of premna pubescens leaf aqueous extract

3450 *Premna pubescens* fresh leaves were air-dried at room temperature. 1250 gr of the air-dried leaves of the plant was milled into fine powder. The powdered leaf was macerated in etanol and extracted twice, on each occasion with 12.5 1itre of etanol at room temperature for 5 days. The combined aqueous extract solubles were concentrated to dryness in a rotary evaporator. The resulting aqueous extract was freeze-dried, powdery crude aqueous leaf extract of *Premna pubescens*. Aliquot portions of the crude plant extract residue were weighed for use on each day of experiments.

Determining dose of premna pubescens

Doses of the test formulation were determined based on Vadivu (2009), 250 mg/kg p.o. It was administered orally to Wistar rats for 31 days. The etanol extract mixed CMC 1 %.

Determining antibody levels by the haemagglutination technique

a) Preparation serum

Blood was collected by decapitation process on day 30^{th}. Then the blood centrifuged and serum was obtained.

b) Hemaglutination reaction

The antibody titer was determined by a two-fold serial dilution of one volume (100 µl) of serum and one volume (50 µl) of physiological saline (reagent). Quantitation of serum antibodies were carried out by antibody titre plate technique containing respective antigens. 50µl of physiological saline was added into all wells of microtitre plate, and then 100µl of antiserum added into the first well of microtitre plate, the antiserum was serially diluted in the well of the first row till the 11th well of the microtitre plate leaving the 12th well as positive control. Then 25µl of 1% test antigen (SRBCs) in saline was added to all the wells of the microtitre plate. The plate was were mixed thoroughly for the effective mixing of reagents and incubated for an hour at 37° C for about 1 day until the control tube showed a negative pattern (a small button formation). The highest serum dilution showing visible hemagglutination was taken as the antibody titer .

Statistical analysis

The data was analysed using one-way analysis of variance (ANOVA), $P<0.05$ was considered significant.

Results and Discussion

Antibody titer

Antibody titer in this research was found by calculated total antibody titer. Measurement antibody titer of the rat as humoral immunity used hemagglutination method using a V microplate 96

Table 4.1. The average humoral immunity of *Rattus norvegicus* by antibody titre after treating.

No.	Treatment	Antibody Titer
1.	A_0	1
2.	A_1	1,67
3.	A_2	7,17
4.	A_3	6,67

Antibody titre by HA Test and weight of *Rattus norvegicus* for each treatment had a various result after giving ethanol extract of *Premna pubescens*. The highest level was A_2 with 7,17 of the average antibody titre. The data was tested by ANOVA one way test.

Table 4.2 The analysis of ANOVA one way, the effect of ethanol extract of *Premna pubescens* treatment toward humoral immunity at *Rattus norvegicus.*

Variance	**Df**	**JK**	**KT**	**F_{count}**	**F table**	
					0,05	0,01
Treatment	3	189,13	37,82	65,78	3,10	4,94
Error	20	11,5	0,575	-	-	-
Total	23	200,63	-	-	-	-

Based on the statistic by using ANOVA one way, it was found that F_{count} = 65,78> F_{table} (0,01) = 4,94. This result indicated that there was an effect of ethanol extract of *Premna pubescens* toward humoral immunity at *Rattus norvegicus*.

Body weight

Tabel 4.3. The average of *Rattus norvegicus* weight after administered the treatment.

No.	Experiment	Weight
1.	A_0	12,33
2.	A_1	8,5
3.	A_2	13,17
4.	A_3	18,5

To investigate the difference of the average for each group, data was tested statistically with ANOVA one way (Table 4.5).

Table 4.4. Analysis of Variance (Anova) of the effect ethanol extract of *Premna pubescens* toward *Rattus norvegicus* body weight.

Variance	Df	JK	KT	F_{count}	F table	
					0,05	0,01
Treatment	3	305,46	61,09	0,23	3,10	4,94
Error	20	5221,18	261,06	-	-	-
Total	23	5526,62	-	-	-	-

Based on statistic test by using Anova one way, it was found that F_{count} = 0,23 > F_{table} (0,01) = 4,94. This result indicated that H_0 accepted and H_a rejected. It means that the effect of ethanol extract of *Premna pubescens* has not an effect toward body weight of *Rattus norvegicus*.

The effect of ethanol extract of Premna pubescens toward humoral immunity of Rattus norvegicus

Positive reaction show a positive effect if SRBCs deposition dispersed covering wells of microtitre plate. Deposition proceed from a webbing between antibody with SRBCs until cover much of the wells of microtitre plate. Whereas the negative reaction show SRBCs deposition collected among the wells of microtitre plate because there has not antibody and antigent is excessive. Regarding weight effect of antigent, so it will collected among the wells of microtitre plate construct an deposition like a pink button (Achyat, dkk. 2008).

Regarding the result of this research, the average of antibody titer for each treatments had a different value. The group that had a highest value was A_2. This caused the imunization with SRBCs for twice. This result was along with A_3 that antibody titer had a high value. It caused A_3 already experienced with SRBCs. Group of A_0 and A_1 also formed an imunity humoral although these group never had experienced with SRBCs.

Serum of A_0 had an hemaglutination reaction but in low value about 0-1. Meanwhile A_1 had a higher value between 1-3. This caused by serum contain anti antigent of SRBCs although it never experience with SRBCs. Similar with the previous research, Achyat, et al (2008) reported that the antigent is natural antibody. In A_3 was found that the antibody titer was high. It caused by A_3 had an imunization with SRBCs for twice. The antibody titer of A_3 was higher than A_0 and A_1 because the group had produced antibody in large scale to dissolved the antigent when imunization.

The effect of ethanol extract of Premna pubescens toward the alteration of Rattus norvegicus body weight

The average of *Rattus norvegicus* body weight A_1 was lower than A_0. The average of *Rattus norvegicus* body weight A_0 had lower than A_2 and A_3. This caused by the difference of disire

for each day. Based on statistic test by using Anova one way, it was found that ethanol extract of *Premna pubescens* has not an effect toward body weight of *Rattus norvegicus*.

Conclusions

1. Ethanol extract of *Premna pubescens* has a significant effect in increasing humoral imunity by using antibody titer at *Rattus norvegicus*.
2. Giving ethanol extract of *Premna pubescens* toward body weight of *Rattus norvegicus* has not a significant effect.

References

Achyat S R, Sadikin M, dkk., (2008), *Pengaruh Pemberian Minyak Buah Merah (Pandanus conoideus Lam.) terhadap Imunitas Humoral Tikus (Rattus norvegicus L.) Galur Wistar Melalui Pengamatan Titer Antibodi Anti-SDMD*, Jurnal Bahan Alam Indonesia, Vol. 6, No.4.

Fang, J. et al. 2005. Apigenin inhibits VEGF and HIF-1 expression via PI3K/AKT/p70S6K1 and HDM2/p53 pathways. *FASEB J*. 19. 342–353.

Gupta S., et al. 2001. Selective growth-inhibitory, cell-cycle deregulatory and apoptotic response of apigenin in normal versus human prostate carcinoma cells. *Biochem Biophys Res Commun*. 287:914–920.

Liu LZ,. Et al. 2005. Apigenin inhibits expression of vascular endothelial growth factor and angiogenesis in human lung cancer cells: implication of chemoprevention of lung cancer. *Mol Pharmacol*. 68. 635–643

Sharp, P E., Regina, M C L., (1998), *The Laboratory Rat*, CRC Press LLC, Florida.

Thiruvenkatasubramaniam, R., Jayakar, B., (2010), Anti-hyperglycemic and Anti-hyperlipidemic activities of Premna corymbosa (Burm.F) Rottl on Streptozotocin induced diabetic rats*, Der Pharmacia Lettre*, 2 (1) 505-509.

Vadivu R., et al., 2009. Evaluation of Hepatoprotective and In-vitro Cytotoxic Activity of Leaves of Premna serratifolia Linn*, Journal of Scientific Research*, 1 (1), 145-152.

Zheng, PW., et al. 2005. Apigenin induced apoptosis through p53-dependent pathway in human cervical carcinoma cells. *Life Science*. Volume 76, pages 1367-1379.