VOL. 3, NO. 5, May 2013 ISSN 2225-7217
ARPN Journal of Science and Technology

http://www.ejournalofscience.org

Flavonoids of *Phylanthus Niruri* as Immunomodulators A Prospect to Animal Disease Control

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ABSTRACT

Previously researchers have examined that an arabinogalactan (AG) obtained from *Phylanthus* spp act as immunomodulator because it appears to stimulate immune system. In this research, we want to examine the potential of flavonoids of *P niruri* as immunomodulators. Fifteen rats were divided into 5 groups; Group 1 without flavonoids; Group 2 had 1% flavonoids; Group 3 had 2% flavonoids, Group 4 had 3% flavonoids and Group 5 had 4% of flavonoid of body weight of rats. Flavonoids were given for fourteen (14) consecutive days. The results showed *P. niruri* flavonoids can be as immunomodulators especially as immunostimulators because it can increase the activity and capacity of the phagocytosis. Flavonoids of *P.niruri* also can increase antibody titer level. Flavonoid of *P.niruri* does not affect animals' performance because it does not affect the levels of hemoglobin, erythrocyte total and hematocrit.

Keywords: *P.niruri, immunomodulators,activity and capacity of phagocytosis, antibody titre level*

1. INTRODUCTION

Studies on immunomodulators compounds that can enhance animal immunity against infectious microorganism need to be done. Antibiotics are not effective against viral diseases such as Avian Influenza (AI) that had led millions of poultry in the world death. Immunomodulators has been expected to boost the animals' body defense against infectious microorganisms like the virus.

Phyllathus niruri (*P. niruri*) contains chemical compounds with pharmacological effect. Active phytochemical compounds such as flavonoids, alkaloids, tannins, and saponins have been identified from *P.niruri*[4]. Extract of this plant has been shown to have the effect of treatment on several clinical studies. *P.niruri* has the effect of treatment on the number of conditions such as dysentery, influenza, vaginitis, tumors, diabetes, diuretic, jaundice, kidney stones, dyspepsia, anti-hepatoxic, anti-hepatitis B, as well as antiviral and antihiperglicemic[7].*P.niruri* had antivirus activity, also against human immunodeficiency virus [2]. *P.niruri* extract exhibits significant anti-tumor activity [8,9]. *P niruri* also mentioned as immunomodulators[1].

Several researchers previously examined that extract of *Phylanthus* spp act as immunomodulator because it appears to stimulate immune system. An arabinogalactan (AG) obtained from tea preparations of *P. niruri* was investigated and presented immunological properties when tested with peritoneal mice macrophages. The substance can promote macrophage (to phagocyte) by an increase of superoxide anion production [5]. This recent research wants to examine the potential of flavonoids of *P niruri* as immunomodulators.

2. MATERIAL AND METHODS

2.1 Animals and Material Experiment

Fifteen (15) male Wistar rats , 8 weeks old, weighing 400 grams. Material trial n-hexane extract of flavonoids from *P niruri*, antigen M of *S. aureus*, Giemsa dye, *P.niruri* plants in this study have been examined in the laboratory of Agronomy University of Muhammadiyah Malang.

2.2. The design of experiments

This research method is experimental. This study examined flavonoids compounds as immunomodulators. Experimental design used is completely randomized design (CRD) with five (5) treatment and three (3) replications.

2.3. Flavonoids of *P.niruri* as Immunomodulators

2.3.1. Non-Specific Immune Responds: Activity and Phagocytosis Capacity

Extraction and identification of flavonoids compound is according to the methods [4]. Flavonoids of *P.niruri* are then given to fifteen (15) male Wistar rats. Fifteen rats were divided in 5 groups; Group 1 without flavonoids; Group 2 had 1% flavonoids; Group 3 had 2% flavonoids, Group 4 had 3% flavonoids and Group 5 had 4% of flavonoid of body weight of rats.

Flavonoids were given for fourteen (14) consecutive days. Beside control group, mice are infected with the bacterium *S. aureus* at a dose of 10^8 cells /ml. After two (2) hours, blood was taken from the tail end to make preparations blood smear and stained with Giemsa. Blood smear had been taken to be examined under microscope about phagocytic activity (numbers of phagocyte cells that active to phagocytes bacteria) and phagocytic capacity (numbers of bacterial cells that phagocyted by 50 phagocyte cells [3].

2.3.2. Specific Immune Response

The rats infected with the bacterium *S. aureus* (beside control group) at a dose of 10^8 cells / ml for seven (7) consecutive days. Bacteria *S. aureus* grown in THB medium for 18-24 hours, then centrifuged for 10 min at 10,000 g, the supernatant was discarded and the pellet was washed with a solution of Nacl fisiologis 2-5 ml depending on the number of pellets, and then centrifuged again for 10 minutes. Then 10.000g pellets supplemented with 0.335 ml physiological saline (depending on the number of pellets), homogenized, and then added a drop of phenol red indicator / phenolphtalein. If the suspension has been given a phenol red is yellow, then it must be neutralized with NaOH or KOH until the suspension is red then autoclaved for 15 min. The suspension was cooled, and then centrifuged for 10 minutes on 10.000g. Pellet discarded, the supernatant was antigen. Measurement of antibodies following the OIE procedure Indonesian method (pusvetma).

2.4. Effect of Flavonoid P.niruri to the performan of Animals

Hemoglobin, hematocrit (PCV) and the number of erythrocytes measured by using automatic blood cell count tool, ABX Hematology PENTRA 60.

2.5.Data Analysis:

All the values of data were analyzed by using ANOVA and Duncan Test. Differences between groups were considered significant at $P < 0.05$.

3. RESULTS

3.1.Influence of Flavonoids *of P.niruri* to the activity and Capacitation of Phagocytosis

Phagocytosis activity of leucocyte cells in Group 1,2,3,4 and 5 respectively are 30.66; 50.33; 62.66; 83.66 and 95.66.Duncan test showed, the highest activity phagocytosis in group with had highest dose of flavonoids *P.niruri* (Table 1).

Phagocytosis capacity value in Group 1,2,3,4 and 5 respectively are 234.00; 260.33; 310.00 ; 346.66 and 462.33. Duncan test showed highest capacity of phagocytosis in group that had highest dose of flavonoids *P.niruri* (Table 1).

Table 1: Effect of Flavonoids *P niruri* to Activities and phagocytic capacity

Variables	Flavonoids *P niruri*				
	0%	1%	2%	3%	4%
Phagocytosis Activity	30.66^a	50.33^b	62.66^c	83.66^d	95.66^e
Phagocytosis Capacity	234.00^a	260.33^b	310.00^c	346.66^d	462.33^e

Note: different superscripts in the same column shows very significant difference $p<0.01$

The result showed that flavonoids of *P.niruri* can stimulate immune reponse, because can increase activity and capacity of phagocytosis. Higher dose of flavonoids make leucocyte (phagocyte) cells more active to phagocyte bacteria cells, and more bacteria can destruction and digestion with leucocyte cells. Flavonoids have potency work to limphokine that product of T-cells to increase of phagocytocis response [3]. *P niruri* is as immunomodulators [1]. The immunomodulators increased activation of effector cells such as lymphocyte, macrophages that produce and release cytokines, interleukins, (IL) -1; IL_6; IL-12; tumor necrosis factor alpha (TNF alpha) [10].

The process of phagocytosis by macrophages takes place in 5 phases in sequence, the stage chemotaxis, recognition and attachment, engulfment, phagosom maturation and phagolysosome formation, destruction and digestion [6].

3.2. Influence of Flavonoids *of P. niruri* on Antibody Titre Level

Humoral antibodies are antibodies that circulate in the blood circulation. After three (3) weeks post injection antibody titers reached protective titers.

Rats that have been injected with antigen *S. aureus* and treated by per oral flavonoids had measured for antibody titer. In PO (control group), obtained negative results, meaning that precipitation occurs in RBC, because this group had no antigen M of *S.*aures and no flavonoids, so no binding of antigen and antibodies that marked on the tube agglutination. In treatment P1, P2, P3 and P4 occur agglutination indicating there is interaction of antigens and antibodies that reacted in tube according to the test given.

Table 2: Effect of Flavonoids *P niruri* to Antibody Titre Level

Flavonoid of *P.niruri*	Replication	Antibody titre level
0%	1	0
	2	0
	3	0
1%	1	2^1
	2	2^4
	3	2^4
2%	1	2^4
	2	2^6
	3	2^6
3%	1	2^6
	2	2^7
	3	2^6
4%	1	2^6
	2	2^6
	3	2^7

Antibody titre level in group that had 1% of flavonoids lower than had 2%,3% and 4%. There is no significant differences of antibody titre level between Group 2%, 3% and 4% of flavonoids (Table 3).

Table 3: Average of antibody titer

Flavonoids treatment	Average of Antibody Titer
without flavonoid	0^a
1%	$11,33\pm1,73^a$
2%	$48,15\pm1,15^b$
3%	$85.33\pm0,57^b$
4%	$85,33\pm0,58^b$

Note: different superscripts in the same column shows significant difference ($p<0.05$)

Antibodies are protein molecules produced by plasma cells as a result of the interaction between antigen and B lymphocyte antigen that have sensitized. Antibody having an ability to bind to the antigen as well as accelerating the destruction and removal. In this study, the antibodies bind to *S. aureus* antigens and destroy the bacteria.

Flavonoids of *P.niruri* with up to four (4)% in this study is still acting as immunomodulators especially as imunostimulators because it can increase the antibody titer. It need onother research to measured antibody titer in higher level concentrations of flavonoids *P.niruri.*

3.3. Influence of Flavonoids of *P.niruri* to Animals Performances

Average value of Hb, PCV and number of erythrocytes mice listed in Table 4.Average Hb (g / dl) in Group 1,2,4,4 and 5 respectively are 14.20; 13.97; 15; 13.20 and 14.77. Mean PCV values (%) in in Group 1,2,4,4 and 5 respectively are respectively are 14.20; 44.70; 47.70; 41.90 and 47.47. Mean while, the number of erythrocytes (millions/mm3) at P0 , P1, P2, P3 and P4 respectively 7.79; 8:10; 8:41; 6.95 and 8:44 (Table 4).

Table 4: Effect of Flavonoids *P niruri* on Performance Indicators

Variable	Flavonoids of *P niruri* (% of body weight)				
	0	1	2	3	4
Hb (g/dl)	14.20[a]	13.97[a]	15[a]	13.20[a]	14.77[a]
Hematocrite/PCV (%)	45.70[a]	44.70[a]	47.70[a]	41.90[a]	47.47[a]
Eritrocyte (juta/mm^3)	7.79[a]	8.10[a]	8.41[a]	6.95[a]	8.44[a]

The results showed flavonoids *P.niruri* does not affect the health of rats. All values indicators of fitness / performance of animals used in this study showed no differences between the groups that had not been given flavonoids *P.niruri* with the group that had been given the flavonoids. The number of erythrocytes cells, the percentage of cells erythrocytes (hematocrite) and blood pigment hemoglobin (which binds oxygen) is not affected by the administration of *P.niruri* flavonoids. This means that flavonoid of the *P.niruri* is potential to be applied to animals / humans.

4.CONCLUSION

1. Flavonoids of *P.niruri* act as immunomodulators especially immunostimulators because it can increase activity and phagocytic capacity and antibody titers.
2. Flavonoids of *P.niruri* did not affect the health of animals that seems of no consequences for hemoglobin, total erythrocyte and hematocrite.

ACKNOWLEDGEMENTS

Thanks to the Directorate of Higher Education, which has provided this research's funding. This study is one part of the Research Featured of Higher Educations (RIP) UMM in fiscal year 2012.

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