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Leucocyte profile and offspring production of guinea pig (*Cavia cobaya*) given *Anredera cordifolia* leaf extract

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ABSTRAK

Tujuan penelitian ini adalah mengetahui profil leukosit dan jumlah anak dari marmut (*Cavia cobaya*) akibat pemberian ekstrak daun binahong (*Anredera cordifolia*). Materi yang digunakan adalah marmut betina masing-masing berjumlah 16 ekor dengan bobot badan 425 g. Ekstrak daun binahong dengan dosis 0, 10, 50 dan 90 mg, masing-masing ditetapkan sebagai T0, T1, T2 dan T3. Pemberian dilakukan secara oral, dari 0 hari prepartum sampai 10 hari postpartum. Pengambilan darah dilakukan 10 hari prepartum, pasca beranak, 10 hari postpartum, dan juga dilakukan pengamatan jumlah kelahiran dan kematian fetus pasca beranak. Data dianalisis menggunakan analisis ragam dilanjutkan analisis beda rata-rata dengan Duncan Multiple Range Test untuk tampilan leukosit dan Chi-Square untuk produksi anak yang diberi ekstrak daun *A. cordifolia* dengan kontrol. Hasil menunjukkan bahwa kelompok perlakuan 10 hari prepartum (control) tidak berbeda nyata, kelompok perlakuan postpartum pada monosit total terjadi kenaikan tertinggi pada level 50 mg/ekor dibandingkan dengan level 0, 10, dan 90 mg/ekor. Kelompok perlakuan 10 hari postpartum pada leukosit total dan monosit total terjadi kenaikan tertinggi pada level 50 dan 90 mg/ekor dibandingkan 10 mg/ekor. Limfosit total terjadi kenaikan tertinggi pada level 90 mg/ekor dibandingkan dengan level 10 dan 50 mg/ekor. Neutrofil total kenaikan tertinggi pada level 50 mg/ekor dibandingkan dengan level 0 dan 10 mg/ekor. Pemberian ekstrak daun *A. cordifolia* dapat menaikkan jumlah anak yang dilahirkan ($P<0,05$; $\chi^2=9,267$) dan dapat menurunkan jumlah anak yang mati ($P<0,05$; $\chi^2=6,4$). Kesimpulannya adalah pemberian ekstrak daun *A. cordifolia* 50 mg/ekor dapat meningkatkan profil leukosit dan produksi keturunan.

Kata kunci: *Anredera cordifolia*, total leukosit, differensial leukosit, *Cavia cobaya*, produksi keturunan

ABSTRACT

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The objective of this study was to determine leucocyte and offspring production of guinea pig (*Cavia cobaya*) giving *Anredera cordifolia* leaf extract. Materials used were female 16 heads of guinea pig with body weight of 425g. The treatments were an extract of *A. cordifolia* leaf at doses of 0, 10, 50 and 90 mg/head, designated as T0, T1, T2 and T3, respectively. *A. cordifolia* leaf extract was administered orally from 10 days prepartum to 10 days postpartum. Blood was taken at 10 days prepartum and 10 days postpartum. Total birth of the offspring was observed. Data were analyzed by analysis of variance and if there was effect of treatment, then continued with Duncan multiple range test. Chi-Square test for fetal production between the given *A. cordifolia* leaf extract and control. The result showed that there was no significant difference for 10 days prepartum after addition of *A.*

cordifolia leaf extract treatment. The postpartum treated showed a total 50 mg/head level increased for monocytes than that of level 0, 10 and 90 mg/head. Ten days postpartum treatment showed the total increase for leucocyte and monocytes total were 50 and 90 mg/head, respectively compared to 10 mg/head level. Total lymphocyte of 90 mg/head increased compared to level 10 and 50 mg/head. The highest total neutrophil as found at level of 50 mg/head which increased compared to the level of 0 and 10 mg/head. Provisioning *A. cordifolia* leaf extract at doses level of 50 and 90 mg/head could increase litter size ($P<0.05$; $\chi^2=9.267$) and decreased offspring mortality ($P<0.05$; $\chi^2=6.4$). In conclusion, by giving 50 mg/head *A. cordifolia* leaf extract could increase leucocyte profile and offspring production of guinea pig.

Keywords : *Anredera cordifolia*, leucocyte total, leucocyte differential, *Cavia cobaya*, offspring production

INTRODUCTION

Decreasing in livestock productivity may lead to decrease animal reproduction. Abortion was one of the causes of decreasing reproduction. Nowadays, vitamins and antibodies were widely used for animals. However, the drugs leave a dangerous residue in the body of pregnant animals. Research conducted by Bahri *et al.* (2005) found many antibiotic residues and penicillin and tetracycline type hormones in beef and its liver. Herbs were plants containing active substances that were safe to be used. Binahong leaf (*A. cordifolia*) was one of the herbs that contain flavonoids, alkaloids, saponins and vitamin C that could be used as an alternative for commercial antibiotics.

Flavonoids have the ability to recover damaged body cells. Flavonoids and terpenoids were isometric hydrocarbon compounds that help the process of organic synthesis and recovery of body cells (Astuti *et al.*, 2011). The *A. cordifolia* leaf extract was containing an antioxidant that increases energy for stamina of the body. According to Vanhees *et al.* (2011), the work system of the flavonoid is by releasing hydrogen atoms from its hydroxyl groups.

Flavonoids were one of the active substances functioning as antifungal and antimicrobial. Microbial cells were damaged by the activity of the flavonoid. Damaged microbial cells could reduce the appearance of increasingly severe inflammation. According to the research of Clara *et al.* (2014), the flavonoid contained of 1-4 mg/mL that derived from *Monanthotaxis littoralis* could inhibit the toxic microorganisms of mycotoxin producers. Plants contained by phenol and flavonoid compounds were more potent as antibacterial, antimicrobial, increase resistance to infection, accelerate healing and immunostimulant. Flavonoids could improve

body health and reduce the risk of death. *A. cordifolia* also containing phytoestrogens could increase fertility in the females (Todaka, 2005). According to Beazley and Nurminskaya (2016), flavonoids also serve to keep the condition of the uterus when pregnant, so there was no abortion resulting in death on the offspring.

The purpose of this study was to determine leucocyte and fetal production of guinea pig (*C. cobaya*) by giving *A. cordifolia* leaf extract. Provisioning of *A. cordifolia* leaf extract was expected to increase endurance through animal experimental of *C. cobaya* during postpartum and on offspring production.

MATERIALS AND METHODS

Materials used were 16 female of *C. cobaya* with body weight 425 g.

Determination of Total Flavonoid and *A. cordifolia* Leaf Extraction Preparation. The total of flavonoid content was determined by visible spectrophotometry as described by Wijayanti *et al.* (2017). Fresh leaves of *A. cordifolia* weighed 500 g then finely ground to maceration with ethanol (1:10) at room temperature for 5 days (Wijayanti *et al.*, 2017). After maceration, filtration was done to obtain the filtrate. The filtrate was evaporated with a temperature of 70°C and produced a viscous extract. The extract was stored in the refrigerator (Djamil *et al.*, 2012).

Experimental Procedure. The dose of *A. cordifolia* leaf extract was administrated based on animal weight body by converting human weight to *C. cobaya* (Kusumawati, 2004). Completely Randomized Design was used in this study with 4 treatments and 4 replications for leucocyte profile and Chi-Square test for offspring production. The

doses of *A. cordifolia* leaf extract administered was 0, 10, 50 and 90 mg/head, designated as T0, T1, T2 and T3 respectively. *A. cordifolia* leaf extract was diluted with 0.5 mL of aquadest for each provisioning. *A. cordifolia* leaf extract was administered orally from 10 days of prepartum to 10 days postpartum.

Blood Collection. Blood collection was done three times, i.e. firstly at before giving *A. cordifolia* leaf extract, secondly at 10 day prepartum and thirdly at 10 days postpartum according to Wijayanti *et al.* (2016).

Determination of Total Leucocytes, Leucocyte Differential and Examination of Blood Smear. Determination of total leucocytes was done according to Astuti *et al.* (2011) and smear preparation for leucocyte differential according to Irianti and Ardinata (2008). The cells was counted at least 100 cells and calculated as percentage of leucocyte types (neutrophils, eosinophils, lymphocytes and monocytes).

Litter size and Offspring Mortality. Data of litter size and offspring mortality were taken at postpartum by counting the litter size, average of offspring per female, offspring mortality and percentage of the offspring mortality on *C. cobaya* postpartum per female.

Data Analysis

Data were analysed by analysis of variance and if there was an effect of treatment, then continued to Duncan Multiple Range Test for leucocyte at 5% significances. Chi-Square test was performed for statistical analysis of offspring production (steel and Torrie, 1995).

RESULT AND DISCUSSION

Total Leucocyte and Differential Leucocyte 10 Days Prepartum of *Cavia cobaya*

Based on Table 1, total leucocyte and differential leucocyte was not significantly different ($P>0.05$). The *C. cobaya* condition was indicated in normal condition before being given *A. cordifolia* leaf extract. The normal total leucocyte, lymphocytes, monocytes, neutrophils, and eosinophil in guinea pigs ranged from $6.0-17.0 \times 10^3/\text{mm}^3$, $2.4-13.6 \times 10^3/\text{mm}^3$, $0.06-2.04 \times 10^3/\text{mm}^3$, $1.2-9.25 \times 10^3/\text{mm}^3$ and $0.018-0.68 \times 10^3/\text{mm}^3$ (Smith and Mangkoewidjojo, 1988).

Total Leucocyte and Differential Leucocyte Postpartum of *Cavia cobaya*

Table 2 shows that total leucocyte, lymphocytes, neutrophils, and eosinophil total was no significantly different. Monocytes total was significantly different ($P<0.05$). *A. cordifolia* leaf extract in doses level of 50 mg/head could increase total monocyte comparing to level of 0, 10 and 90 mg/head. Monocyte was increased due to a process of macrophage phagocytosis from inflammation postpartum in *C. cobaya* postpartum. Total monocytes in blood was showed as an indicator ability of *C. cobaya* to destroy microorganisms. Flavonoids in *A. cordifolia* have an important role to stimulate monocytes, macrophages, and other cells in the inflammatory and proliferative stages healing so that monocyte production was doubled (Sukrama *et al.*, 2017; Sumartiningsih, 2011).

The flavonoids content in *A. cordifolia* leaf extract could increase total monocyte *C. cobaya* postpartum. Monocytes increased because flavonoids activate lymph nodes to increase monocyte production. Flavonoids have water soluble properties and serve as antimicrobials and immunostimulants (Astuti, 2016; Hu *et al.*, 2016). Monocyte stimulates the body immunity so that cells do better. Immune levels increased when helped postpartum the healing process of uterine wounds, the body's cells would be more active to work and stay healthy when attacked by viruses, bacteria and microbes. The process of phagocytosis would be very active when total monocytes increases. According to Sukandar *et al.* (2016), monocytes increased because of the influence of flavonoids that work against lymphokines produced by T cells that would

Table 1. The Average of Total Leucocyte and Differential Leucocyte 10 Days Prepartum of Guinea Pig (*C. cobaya*)

Parameters	Total
Total Leucocyte ($\times 10^3/\text{mm}^3$)	11.11 \pm 1.77
Lymphocytes ($\times 10^3/\text{mm}^3$)	7.11 \pm 1.40
Monocytes ($\times 10^3/\text{mm}^3$)	0.84 \pm 0.42
Neutrophils ($\times 10^3/\text{mm}^3$)	2.89 \pm 0.78
Eosinophils ($\times 10^3/\text{mm}^3$)	0.27 \pm 0.19

Table 2. The Average of Total Leucocyte and Differential Leucocyte Postpartum of Guinea Pig (*C. cobaya*)

Parameters	Doses of <i>A. cordifolia</i> Leaf Extract (mg/head)			
	0	10	50	90
Total Leucocyte ($\times 10^3/\text{mm}^3$)	7.98 \pm 4.70	7.85 \pm 2.99	10.77 \pm 1.42	8.58 \pm 2.99
Lymphocytes ($\times 10^3/\text{mm}^3$)	5.10 \pm 3.61	4.93 \pm 1.59	5.68 \pm 0.15	4.87 \pm 1.91
Monocytes ($\times 10^3/\text{mm}^3$)	0.48 \pm 0.37 ^b	0.63 \pm 0.28 ^b	1.25 \pm 0.21 ^a	0.72 \pm 0.38 ^b
Neutrophils ($\times 10^3/\text{mm}^3$)	2.18 \pm 1.21	2.08 \pm 1.26	3.29 \pm 0.99	2.79 \pm 1.33
Eosinophils ($\times 10^3/\text{mm}^3$)	0.21 \pm 0.19	0.20 \pm 0.16	0.08 \pm 0.01	0.19 \pm 0.13

Different superscript in the same row indicates significantly different ($P < 0.05$)

stimulate phagocyte cells to respond to phagocytosis.

Total Leucocyte and Differential Leucocyte 10 Days Postpartum of *Cavia cobaya*

Total leucocyte and monocyte at 10 days postpartum treatment (Table 3) with 50 mg/ head of *A. cordifolia* leaf extract showed significant difference ($P < 0.05$) with 0 and 10 mg /head. Level of 50 mg /head was not significantly different from levels of 90 mg/head. Treatment of 10 days postpartum *C. cobaya* was a period of wound healing in the womb and an increase in total of leucocytes and monocytes. The total leucocyte would become an infected wound defence agent. Vinothapooshan *et al.* (2010) showed that leucocytes were the active unit of the body's defence system by providing rapid and robust defence against every infectious agent. The antioxidant compounds of *A. cordifolia* extract could minimize free radical attack on blood cells and the process of blood cell formation could increase so that leucocytes could be maintained or even increased (within normal limits) when the condition of the body was attacked by disease (Kumar and Pandey, 2013; Paju *et al.*, 2013). Total saponin from *A. cordifolia* leaf extract would increase the number of monocytes *C. Cobaya*. The saponin of content in *A. cordifolia* leaf extract as antimicrobial in accelerating the process of proliferation in wound healing. According to Astuti *et al.* (2011) that saponins could increase the number of macrophages as monocyte phagocytes in order to secrete growth factors that could attract many fibroblasts and

synthesize collagen.

A. cordifolia leaf extract was given 90 mg / head level was significantly different of lymphocytes total ($P < 0.05$) with levels of 10 and 50 mg/head (Table 3). The lymphocyte was the highly increased at the level of 90 mg/head proved that the process of wound healing and endurance increases in attacking the stronger bacteria. Neutrophils total (Table 3) at 50 mg/head level was significantly different ($P < 0.05$) with 0 and 10 mg / head, but not significantly different with 90 mg / head. The flavonoids of content in *A. cordifolia* leaf extract would increase the neutrophils total as infectious agent attacks. Sukma *et al.* (2014) and Harikrishnan *et al.* (2010) stated that the main function of neutrophil cells was as bacterial cell phagocytosis, so that if swelling occurs in the limbs a large neutrophil total cells would be released. Chuang *et al.* (2007) indicates that neutrophils in the blood would increase in the event of infection and act as the first in the body and recovery of body cells and enhance immunoreactivity.

Litter Size

Provisioning *A. cordifolia* leaf extract at dose level of 10, 50 and 90 mg/head extract significantly different on litter size ($P < 0.05$) with value $\chi^2 = 9.267$ with 0 mg/head (Table 4). This suggests that administration of *A. cordifolia* leaf extract could increase the total of litter size. The flavonoids or phytoestrogens of content helping the body's defence process with a rise in total leucocytes postpartum there by increasing the ability of the offspring to survive. Accumulating

Table 3. The Average of Total Leucocyte and Differential Leucocyte 10 Days Postpartum of Guinea Pig (*C. cobaya*)

Parameters	Doses of <i>A. cordifolia</i> Leaf Extract (mg/head)			
	0	10	50	90
Total Leucocytes ($\times 10^3/\text{mm}^3$)	6.64 \pm 2.31 ^{ab}	4.30 \pm 0.97 ^b	9.51 \pm 0.40 ^a	8.95 \pm 2.60 ^a
Lymphocytes ($\times 10^3/\text{mm}^3$)	3.95 \pm 1.34 ^{ab}	2.57 \pm 0.57 ^b	2.44 \pm 0.92 ^b	5.33 \pm 1.51 ^a
Monocytes ($\times 10^3/\text{mm}^3$)	0.50 \pm 0.28 ^{ab}	0.25 \pm 0.06 ^b	0.72 \pm 0.07 ^a	0.81 \pm 0.26 ^a
Neutrophils ($\times 10^3/\text{mm}^3$)	1.99 \pm 0.80 ^b	1.38 \pm 0.44 ^b	3.54 \pm 0.53 ^a	2.66 \pm 1.38 ^{ab}
Eosinophils ($\times 10^3/\text{mm}^3$)	0.15 \pm 0.14	0.10 \pm 0.05	0.14 \pm 0.04	0.14 \pm 0.10

Different superscript in the same row shows significantly different ($P < 0.05$)

Table 4. The Litter Size, Average of Offspring Per Female, Offspring Mortality and Percentage of the Offspring Mortality on Guinea Pig (*Cavia cobaya*) Postpartum

Giving <i>A. cordifolia</i> Leaf Extract (mg)	Litter size (head)	Average of offspring Per Female (head)	Offspring Mortality (head)	Percentage of the Offspring Mortality
0	4	1.00	1	25
10	6	1.50	1	16.67
50	4	1.00	2	50
90	11	2.75	3	27.27

evidence that dietary bioflavonoids were beneficial for health and longevity (Beazley and Nurminskaya, 2016). Provisioning phytoestrogens during pregnancy could actually increase the immunity of the fetus in the uterus so that there was no early birth. This was in accordance with the opinion of Todaka (2005) that provision phytoestrogens to the female during pregnancy would transmit phytoestrogens from the female to the offspring. According to Tshikalange *et al.* (2005), the process transfer of phytoestrogens from female to offspring through the placenta was influenced by blood pressure, placental type, molecular size, pH, flow rate (blood flow rate).

Offspring Mortality

Provisioning of *A. cordifolia* leaf extract was significantly different with no treatment ($P < 0.05$) with value $\chi^2 = 6.400$. The higher provisioning of *A. cordifolia* leaf extract could decrease offspring

mortality (Table 4). The ability of the decrease offspring mortality happens because *A. cordifolia* leaf extract in the form of flavonoids and phytoestrogens stimulates the female body to always maintain the condition of the body during the pregnancy. Flavonoids have antioxidant functions that inhibit the presence of free radicals that could interfere with the development of children while they still in the womb. The ability of children to survive the free radical attacks then the offspring mortality rate during the birth process was getting less. Free radical was one of the causes of abortion. Flavonoids as antioxidants overcome the emergence of free radicals that damage fetal growth cells during pregnancy (Vanhees *et al.*, 2011). Free radicals could be able to make offspring with birth defects or stillbirth. The *A. cordifolia* leaf extract content has a role in strengthening the uterine wall and maintaining the hormone progesterone so as not to experience

abortion. This is in accordance with the research of Fan *et al.* (2015) using cows fed 200 mc and 300 mc of quercetin per kg diet showed higher rates of living and healthy offspring than that of controls.

CONCLUSION

Provisioning *A. cordifolia* leaf extract at 50 mg/head could increase leucocyte profile and offspring production of guinea pig (*C. cobaya*).

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