The Influence of Fermented and Non-Fermented *Sauropus Androgynus* (L.) Merr. Leaves Extract on The Hematopoiesis in The Postnatal Mice

Pengaruh Fermentasi dan Non-Fermentasi Ekstrak Daun *Sauropus Androgynus* (L.) Merr. pada Hematopoiesis pada Tikus Postnatal

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Abstrak

Penelitian ini bertujuan untuk mengetahui pengaruh dua bentuk ekstrak daun *Sauropus androgynus* (SA) yaitu bentuk fermentasi dan non-fermentasi terhadap hematopoesis pada 36 tikus betina postnatal. Sampel dibagi menjadi 3 kelompok, masing-masing kelompok terdiri dari 12 tikus. Kelompok pertama adalah control diberikan air suling, kelompok kedua diberikan ekstrak fermentasi SA (Fermentasi SA extract,FSA) , dan kelompok tiga diberikan ekstrak non fermentasi (non Fermentrasi SA extract, nFSA). Setiap grup diberikan minuman cairan 0,6% selama 21 hari sebelum partus dan dilanjutkan hingga 1 minggu postnatal dengan dosis 1.68 g/kg BB/hari. Pengambilan darah dilakukan intrakardiak dengan anastesi inhalasi pada hari ke-1 hingga hari ke-7 postnatal kemudian dilakukan pemeriksaan hematologis yaitu jumlah eritrosit, leukosit, hematokrit (Ht) dan hemoglobin (Hb) yang dianalisa dengan menggunakan hemocitometer-neubauer, Sahli, metode tabung kapiler. Hasil menunjukkan respon hematologis positif terhadap kedua periakuan. Jumlah eritrosit, leukosit, Ht dan Hb meningkat secara progresif dari hari pertama hingga hari ke-7 postnatal, dengan kemompok nFSA menunjukkan peningkatan yang bermakna terutama pada Ht, Hb dan leukosit dibandingkan dengan kelompok FSA. Hal ini menunjukkan bahwa bentuk non fermentasi ekstrak daun SA memiliki respon hematopoeisis yang lebih baik dibandingkan dengan bentuk fermentasinya.

Kata Kunci: *Sauropus androgynus*, hematopoiesis, postnatal, tikus

INTRODUCTION

Sauropus androgynus (SA), a member of *Euphorbiaceae* family, is a leafy shrub found in Malaysia, Indonesia, Southwest China and Vietnam. The habitat altitude is 3–1300 meters above sea level (Padmavathi and Rao, 1990). Indonesian believes that *Sauropus androgynus* plant supports lactation in human beings. Mothers eat or drink SA leaves and preparations respectively, in order to increase their breast feeding capacity (Soeparto, 1994). Even as a traditional medicine, SA-extract tablet has been produced as a food supplement by an Indonesian pharmaceutical companies bearing caplet, tablet and steeping for human. For ruminants, SA powder and SA extract administration could enhance milk yield in lactating ewes. The enhancement of milk yield in the cellular level of mammary gland could have been caused by two importance factors such as, first there were increasing of population of secretory cells and the synthetic activities in the secretory cells and seconds, the increase in nutrient supply to the lactating mammary gland (Suprayogi et al., 2001). For poultry production, supplementation of SA meal 30 g per day to the broiler diet was effective to improve feed conversion ratio without reducing body weight, besides the supplementation could reduce fat accumulation in broiler chickens. The

supplementation also reduced feed intake, it was caused by anti-palatability effects of saponin, alkaloid and tannin that might be mediated in part by a neurological effect (Santoso and Sartini, 2001). The inhibition of calcium and phosphorus absorption in the digestive tract occurred due to the administration of SA leaves powder suspension, however this negative effect could be reversible since after 35 days of administration (Suprayogi and ter Meulen, 2006). SA leaves administration also showed the increasing of glucose absorption in the digestive tract and liver glucose metabolism in rabbits (Suprayogi, 2006). The other hand, studied using female sheep showed that propionic acid, n-butyric acid, isobutyric acid, and total VFAs production in rumen liquor were significantly improved by dried SA leaves and SA leaves extract inclusion (Suprayogi, 2005).

One of the active compounds of SA was identified as alkaloid papaverine (Bender and Ismail, 1974). The alkaloid is known as a vasodilator, relaxant, and a spasmolyticum for various smooth muscle and cardiac tissue. Consequently it has some general effects on the physiological function, particularly on the gastrointestinal tract, cardiovascular system and on the metabolism.

Suprayogi et al., (2001) reported that SA leaves contain 7 major substances, which have an important role to its biological effects. These substances are five substances as polyunsaturated fatty acids groups, as precursors in the eicosanoids biosynthesis such as: prostaglandin, prostacycline, thromboxane, lipoxines dan leukotrienes. One substance is a 17-ketosteroid, *androstan-17-one,3-ethyl-3-hydroxy-5 alpha* which could be involved in the steroid hormone biosynthesis such as: progesterone, estradiol, testosteron dan glucocorticoid. Another substance, *3,4-dimethyl-2-oxocyclopent-3-enylacetic acid* could be hydrolyzed to acetate and participate in the citric acid cycle to produce ATP.

Concerning to the properties of SA leaves as stimulating milk yield in the postnatal period, and related to the health of the mother during pregnancy and parturition, it high susceptible for anemic possibility. SA leaves active substances might be play an important role to ovoid the anemic postnatal occurred. Unfortunately, it has not been established whether SA leaves could improve hematopoiesis in the postnatal condition. The other hands, processes technology of the SA leaves for health food and drink through a fermentation process of the leaves have been known as an attempt to enhancing the palatability and taste (Suprayogi, 2002). The fermented form of SA leaves need also to be studied according to the possible effect on the improvement of hematopoiesis in the postnatal condition. This experiment was conducted to elucidate the influence of two forms of *Sauropus androgynus* (SA) leaves extract (Fermented and Non-Fermented) administration on the hematopoiesis in the postnatal mice.

RESEARCH METHODS

The study was executed in Department Anatomy, Physiology and Pharmacology, Faculty of Veterinary Medicine, Bogor Agriculture University, Bogor-Indonesia. Thirty-six female mice (*Mus musculus*) with a mean body weight of (25.00 ± 3.03) g and age of 8 months were fed with commercial pellet fed (HI-PRO-VITE 789®), were placed in plastic individual cages of 3 mice each in size of (40X30X15) cm^3. Nutrient composition of the fed was crude protein of 26-28 %, crude fat of 3-5 %, crude fiber of 4-6 %, ash of 5-8 % and water contain of 11-13 %. All animal were fed *ad libitum* and water available freely. Average calculated fed and water consuming a day was (6.09 ± 0.15) g and (7.93 ± 0.07) ml. Climatic condition in the animal cages room were air temperature of (26.88 ± 2.75) ^0C and humidity of (78.20 ± 11.21) %.

Fresh SA leaves from the local markets around Bogor-Indonesia was processed in the aerobic incubator for 18-20 hours using oxidation enzymatic fermentation method (Suprayogi, 2002), after processing the leaves were used as fermented SA leaves. Otherwise, fresh SA leaves without any processing used as non-fermented SA leaves. Both fresh materials form were dried in an automatic oven at 60°C overnight. The dry leaves were ground to be powder, and the powder was extracted using ethanol 70 % (1:4) to produce the thick fermented SA leaf extract (FSA) and non-fermented SA leaf extract (nFSA). Both thick extract, each 6 g

was diluted with water to be 0.6 % solution, was prepared as (FSA) and (nFSA) drinking water.

Adaptation period on the cages environment and food needed to be executed for 10 days in all animal. After adaptation period was breeding period, all mice were divided into three groups of 12 animals each. Three solutions 0.6 % were prepared as a drinking water, and placed in the individual cages a day to each group such as, distilled water to the control group, (FSA) solution to the FSA-group, and (nFSA) solution to the nFSA-group. Pregnancy mice, approximately 2 weeks, had to be removed to the other individual cage.

Administration of the solution in each group from breeding up to parturition spend approximately 21 days and continued up to a week after parturition (postnatal). Calculated doses known was 1.68 g/kg BW a day. Intracardiac blood collection were conducted to gain blood serum for each group using inhalant anesthetic, first approximately 24 hours (1st day), and continued to the 48 hours (2nd day), 120 hours (5th day), and 148 hours (7th day) after parturition respectively of 3 mice each. Hematopoiesis could be presented by obtained experiment parameters such as amount of red blood cells (RBC), white blood cells (WBC), hemoglobin (Hb), and packed cells volume (PCV), which analyzed by using hemocitometer-neubauer, Sahli, and capillary tube method respectively. Analysis of variance (ANOVA) was used to determine the difference between the treatment means (Snedecor and Cochran, 1982). A probability (P) value less than 0.05 was accepted as significantly different. Duncan's multiple range test (Steel & Torrie, 1980) was used to determine differences between the treatment means.

RESULTS AND DISCUSSION

The influence of FSA and nFSA administration on the hematopoiesis in postnatal mice which indicated parameters such as amount of RBC, WBC, Hb, and PCV during breeding period (21 days prenatal) up to 7 days postnatal could be presented in the Table 1.

Generally, the hematological values obtained in this study still in the normal values. This study reveals that hematological values had a positive response to the both treatment of FSA and nFSA for 21 days and continued 7 days postnatal administration. It could be seen that the amount of RBC, PCV, Hb and WBC tend to progressive enhanced in the 1st day up to 7th day postnatal period. The administration of nFSA presented more significant increase especially on the PCV, Hb, and WBC values than the FSA administration obtained. Tendency increase of the hematological values also occurred on the FSA administration in the postnatal period compared to the values of the control-group. it could be supposed that nFSA solution showed better physiological response on the hematopoiesis in the postnatal mice than FSA solution administration.

Many factors could be involved in the hematopoiesis process, such as oxygen consumption, altitude, and also as a response of the releasing hormones such as: prostaglandins, androgen, thyroid stimulating hormone (TSH), prolactin, cortisone, thyroxin. epinephrine, norepinephrine, and angiotensine, all those hormones could play an important role in the eritropoietin release from kidney and liver (Jain and AH, 1993). Nutrional, species, sex, age, climate. pregnancy, parturation, estrus, and lactation are other factors which also involved in the hematopoiesis (Cunningham, 2002). According to the positive response on the increasing hematological values of mice induced FSA and nFSA administration, it could be assumed that active compounds contained in SA leaves plays an important role. Besides, SA leaves also has a positive effect on the nutrient absorption (Suprayogi. 2005; Suprayogi, 2006), it also other factor to maintain the hematopoiesis processes.

Papaverine contained in the SA leaves (Bender and Ismail, 1974) and Polyunsaturated fatty acids groups, as precursors in the eicosanoids biosynthesis such as: prostaglandin, prostacycline. thromboxane, lipoxines dan leukotrienes. Besides, 17-ketosteroid, *androstan-17-one,3-ethyl-3-hydroxy-5 alpha* which could be involved in the steroid hormone biosynthesis such as: progesterone, estradiol, testosteron dan glucocorticoid (Suprayogi et al., 2001) .

Table 1. Postnatal RBC, PCV, Hb, and WBC values in mice after administration of FSA and nFSA during 21 days prenatal up to 7 days postnatal

Treatment	Postnatal RBC (million/mm^3)			
	(1st day)	(2nd day)	(5th day)	(7th day)
Control group	7.16±1.61^{bc}	7.81±0.60^{bc}	7.22±0.93^{bc}	8.57±1.66^{abc}
nFSA-Group	8.54±1.49^{abc}	8.68±0.76^{abc}	8.89±0.94^{abc}	10.90±2.60^{a}
FSA-Group	6.48±0.46^{c}	8.31±0.61^{bc}	7.60±1.39^{bc}	9.47±0.30^{ab}
Treatment	**Postnatal PCV (%)**			
	(1st day)	(2nd day)	(5th day)	(7th day)
Control group	30.33±4.04^{cd}	33.67±0.58^{bcd}	29.67±5.51^{d}	35.67±0.58^{ab}
nFSA-Group	36.00±4.58^{ab}	37.33±1.53^{ab}	37.33±1.53^{ab}	40.33±2.52^{a}
FSA-Group	30.00±1.00^{d}	35.00±0.00^{bc}	33.33±2.08^{bcd}	37.0±1.00^{ab}
Treatment	**Postnatal Hb (g%)**			
	(1st day)	(2nd day)	(5th day)	(7th day)
Control group	7.80±0.20^{d}	8.80±0.20^{bc}	7.73±0.31^{d}	8.13±0.23^{dc}
nFSA-Group	9.47±0.95^{ab}	9.93±0.76^{a}	9.67±0.61^{a}	9.47±0.31^{ab}
FSA-Group	8.77±0.15^{bc}	8.00±0.20^{dc}	8.40±0.35^{dc}	8.27±0.23^{dc}
Treatment	**Postnatal WBC (thousand/mm^3)**			
	(1st day)	(2nd day)	(5th day)	(7th day)
Control group	6.81 ± 1.07^{e}	7.70 ± 0.56^{de}	8.38 ± 0.58^{bcd}	8.68 ± 0.55^{bcd}
nFSA-Group	9.28 ± 0.58^{abc}	9.48 ± 0.46^{ab}	9.58 ± 1.10^{ab}	10.45 ± 0.59^{a}
FSA-Group	6.82 ± 1.07^{e}	8.15 ± 0.36^{cd}	8.38 ± 0.58^{bcd}	8.70 ± 0.56^{bcd}

Note: Numbers followed by the same letter are not significantly different ($p < 0.05$)

All the active compounds could stimulate erythropoietin to initiate the hematopoiesis activity; therefore the increasing hematological values caused by SA leaves administration are really reasonable. The administration of FSA showed the less response on the hematopoiesis activity than nFSA administration in this study, might be the fermentation process on the SA leaves could influence bioactivity of the active compounds contained in the SA leaves. However, there is no clear information whether fermentation process could affect to the bioactivities changes of the active substances in SA leaves.

CONCLUSION

The administration of fermented SA leaves extract (FSA) and non-fermented SA leaves extract (nFSA) to the female mice for 21 days prenatal and continued to the 7 days postnatal administration reveals that hematological values showed a positive response. The amount of RBC, PCV, Hb and WBC values tend to progressive enhance in the 1st day up to 7th day postnatal period.

The administration of nFSA present more significant increase especially on the PCV, Hb, and WBC values than the FSA administration obtained hematological values. It could be supposed that nFSA solution showed better physiological response on the hematopoiesis in the postnatal mice than FSA solution administration.

The active compounds contained in the SA leaves might be playing an important role in the increasing hematopoiesis in both forms of extract solutions.

REFERENCES

Bender, A.E. dan K.S. Ismail. 1973. Nutritive Values and Toxicity of *Sauropus androgynus*, 79A-80A. Proceedings of The Nutrition Society Vol. 32. Cambridge.

Cunningham, J.G. 2002. Texbook of Veterinary Physiology. 3th-edition, W.B. Saunders Company, Philadelphia, London.

Jain, N.C. and AH. 1993. Essentials of Veterinary Hematology. Lea and Febiger. Philadelphia.Padmavathi. P., and M.P. Rao. 1990. Nutritive value of *Sauropus androgynus* leaves. Plant Foods for Human Nutrition, 40, pp.107-113.

Santoso dan Sartini. 2001. Reduction of Fat Acumulation in Broiler Chickens by *Sauropus androgynus* (Katuk) Leaf Meal Supplementation. Asian-Aust.J. Anim. Sci. 14 : 346-350.

Snedecor, G.W., and W.C. Cochran. 1982. Statistical Methods. Edn. Iowa State University Press, Ames, IA, pp.234-235.

Soeparto, S.. 1994. Jamu Jawa Asli, p. 54. Pustaka Sinar Harapan, Jakarta.

Steel, R.G.D., and J.H. Torrie. 1980. Principles and Procedures of Statistics. A Biometrical Approach. Second edition. McGraw Hill Book Co., New York.

Suprayogi, A. 2002. Proses Produksi dan Formulasi Daun Katuk Sebagai Bahan minuman Berkhasiat. Laporan Penelitian Teknologi Proses. Pusat Studi Ilmu Hayati, Lembaga Penelitian Institut Pertanian Bogor. Bogor. Tidak Dipublikasikan.

Suprayogi, A., U. ter Meulen, T. Ungerer, and W. Manalu. 2001. Population of secretory cells and synthetic activities in mammary gland of lactating sheep after consuming *Sauropus androgynus (L.) Merr.* leaves. Indon. J. Trop. Agric. 10(1):1-3.

Suprayogi A. and U. ter Meulen. 2006. The influence of *sauropus androgynus* (L.) merr. Leaves on the body weight changes and calcium - phosphorus absorption in lactating sheep. Gakuryoku XII(3):50-55.

Suprayogi A. 2005. Blood serum volatile fatty acids (VFAs) in lactating sheep and VFAs production under *in-vitro* conditions using Sauropus androgynus (L.) Merr. Leaves. Gakuryoku XI(3):57-60

Suprayogi A. 2006. The influence of *sauropus androgynus* (L.) merr. leaves and papaverine on the glucose absorption and glucose metabolism in the liver. Proceeding of 1st International Asia Association Veterinary School (AAVS) Scientific Conference, July 2006, Jakarta-Indonesia.