The effect of administration of *Pasak bumi* (*Eurycoma longifolia* Jack.) roots to haematological profile of lactating mice

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The roots of *Pasak bumi* have been used as a traditional medicine as tonic after childbirth in Indonesia. However, the usefulness of its is still empiric. The aim of this research is to evaluate the effect of *pasak bumi* roots methanol extract and its derived fraction to haematological profile of lactating mice. Each mouse was administered methanol extract and its derived fractions (*n*-hexane fraction, chloroform fraction, ethyl acetate fraction, and methanol-water fraction) at dose 500 mg/kg rat body weight for 21 consecutive days in lactation period. Positive control group received Moloco+B12 at dose 0.13 gm/kg rat body weight, negative control (placebo) group received 2 mL/kg rat body weight of aquadest daily, and normal control group. There were no significant differences in erythrocytes count, haemoglobin, and haematocrit value among the groups during the study (*p* >0.05). On the contrary, leukocytes count of methanol extract was higher than normal control, placebo, positive control, and derived fractions of methanol extract (*p* <0.05), but the differentiation of leukocytes (neutrophils, eosinophils, basophils, lymphocytes, and monocytes) were not significantly different (*p* >0.05). It can be concluded that the methanol extract haematological profile is more similar to physiological value.

**Keywords:** *Eurycoma longifolia* Jack., Lactating mice, Haematological profile

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People usually consume medicinal plants to maintain their health such as *Eurycoma longifolia* Jack. which is found in the forests of Indonesia, Malaysia, Thailand, Philippines, Vietnam, and Burma. Empirically, all parts of this plant are usefulness in the treatment. For instance, the roots of *pasak bumi* are used as a tonic as well a medicine to treat fever, malaria, and dysentery. Stems bark and stems are used to treat fever, mouth sores, stomach worms, and bone pain, and also to support after childbirth. The leaves are used to treat itching. Furthermore, the flowers and fruits are used to treat headaches, abdominal pain, and bone pain. According to Bunyapraphatsara (1987) cit. Satayavivad et al. (1998)³, Thai people use *pasak bumi* to treat febrifuge and malaria. Furthermore, Malaysian and Singaporean use decoction of roots, roots bark, and stems bark of *pasak bumi* to treat diarrhea, fever, chronic cough, swollen glands, dropsy, haemorrhage, hypertension, bone pain, aphrodisiac, and tonic. However, the roots of *pasak bumi* are more popular as aphrodisiac and it has been proven by laboratory testing. So far, it is reported that the side effect caused by the consumption of the roots of *pasak bumi* are difficult to sleep, but until now the proper dose to this effect occurred is unknown. It was also reported that the roots of *pasak bumi* consumption at large amounts can lead the increase body temperature and restless.

Lactation is anabolic processes controlled by hormones that distribute nutrients to highly specialized maternal issues, such as mammary glands. In the lactation period, a mother is not only to breastfeeding her baby but also to recover her health in order to care for her baby. During lactation period, health and nutritional qualities of maternal need to be considered because the healthy mother will produce breast milk which is qualified.

Associated with the maintenance of health, the postpartum mothers usually consume a traditional herb. Empirically, the roots of *pasak bumi* are used as a tonic for postpartum mothers. However, usefulness of the roots of *pasak bumi* is only based on empirical experience, so it is necessary to determine the effects
of *pasak bumi* roots on lactation period seen from the haematological profile. Hence, through the measurement of the haematological profile can be known by physiological condition of an individual\textsuperscript{10-11}. Thus, the aim of this study is to evaluate the effect of *pasak bumi* roots of the haematological profile of lactating mice.

**Methodology**

**Animals**

DDY mice (30 ± 7 gm) were purchased from the Faculty of Husbandry, Bogor Agricultural University, Indonesia. Animals were provided with standard rodent pellet diet, the food and water were allowed *ad libitum*. Before the experiment, female mice mated with male mice with a ratio of one male to four females. As an indicator of pregnancy vaginal plug was found after 18 hrs mated. Treatment was started since the mice pups were born.

**Collection of plant material**

The fresh roots of *E. longifolia* Jack. were collected from National Park in West Kalimantan, Indonesia. The plant specimen was authenticated by Herbarium Bogoriensis LIPI Bogor, Indonesia.

**Preparation of plant extract and partitions**

The first process, the air dried roots (12.5 kg) of *E. longifolia* Jack. were made into a coarse powder. The second, the powdered material was dissolved in methanol and subjected to maceration process. The extract was filtrated with evaporated under reduce pressure and vacuum-dried (2.75%). Finally, 95% of methanol extract was partitioned with *n*-hexane, chloroform, and ethyl acetate. The fractions obtained were as follows *n*-hexane fraction (4.34%), chloroform fraction (28.79%), ethyl acetate fraction (7.22%), and residue (methanol water fraction) (53.74%)\textsuperscript{12}.

**Evaluation of the administration of *pasak bumi* roots on lactating mice**

First, mice were divided into 6 groups that each group has three mice. Group I (normal control), group II animals were administered aquadest (2 ml/ kg rat body weight, per oral), group III animals were administered Moloco+B12 (0.13 gm/ kg rat body weight, per oral). Moloco+B12 is utilized by postpartum mothers to increase milk production. Group IV to VIII animals were administered *pasak bumi* roots methanol extract and its derived fractions (*n*-hexane, chloroform, ethyl acetate, and methanol-water) at dose 500 mg/ kg rat body weight per oral. Second, treatment was given every day during lactation period (21 days). Third, the blood samples from mice heart were collected on the 22\textsuperscript{nd} day to evaluate the haematological profile (erythrocytes count, leukocytes count, haemoglobin, haematocrit, and the differentiation of leukocytes (neutrophils, eosinophils, basophils, lymphocytes, and monocytes). The method used to analyze haematological profile was based on Preet & Prakash (2011)\textsuperscript{13}.

**Statistical analysis**

The data were expressed as mean (*n*=3). The haematological profile was analyzed statistically by one-way ANOVA followed by Tukey’s multiple comparison using SPSS 11.5 version for Windows. The difference was considered significant at *p*<0.05.

**Results**

The assessment of haematological profile can be used to determine the extent of deleterious effect of foreign compounds including plant extracts on the blood constituents of an animal. It can be also used to explain blood relating functions of chemical compounds from plant extracts. The results show that there are no difference among the administration of aquadest, Moloco+B12, *pasak bumi* roots methanol extract, and derived fraction of *pasak bumi* roots methanol extract on the erythrocytes count, haemoglobin, and haematocrit (*p*>0.05). In contrast, the administration *pasak bumi* roots methanol extract increased the leukocytes count (*p*<0.05) (Table 1). But the differentiation of leukocytes among normal control, aquadest, Moloco+B12, and methanol extract and its derived fractions is not different (*p*>0.05) (Table 2).

**Discussion**

Lactating period is the period when a mother acts as the only one provider energy for her baby. During the lactation period, a baby only gets intake from her mother, therefore it is important to observe the nutritional status of mother during lactating. The nutrient quality of mother does not affect the volume and the macronutrient content of breast milk in the first week of lactation period, but a mother who is malnourished will not be able to maintain the same amount of nutrients in their breast milk for the long term\textsuperscript{14-16}. Malnutrition can affect physiological condition of mother\textsuperscript{11,15} and many researchers reported that through measurement of haematological profile, physiological condition can be known\textsuperscript{17-23}.
Blood is composed of plasma (blood fluid) and cellular blood (blood cells including erythrocytes, leukocytes, and platelets). Blood is responsible for transporting various substances in the body, to bring nutrients and hormones to various tissues, and to transport metabolic waste products. Through the measurement of the haematological profile including erythrocytes count, haemoglobin, haematocrit level, leukocytes count, and differentiation of leukocytes, an individual's physiological condition can be known.

Erythrocytes are blood cells which transport the respiratory gases, therefore erythrocytes are equipped with a protein molecule called haemoglobin. Haemoglobin consists of protoporphyrin, globin, and ferro. Measurement of haemoglobin in the blood indicates Fe content. Meanwhile, the haematocrit value is used as an indicator of the number of erythrocytes in the blood. In normal condition, based on the haematological analysis, it is known that the erythrocytes count, haemoglobin, and haematocrit value of 8–12 month old non-pregnant and 1–2 yrs old pregnant goats were higher than adult pregnant and lactating goats of over 2 yrs of age. Erythrocytes count, haemoglobin, and haematocrit value decreased in late pregnancy and early lactation.

In addition, erythrocytes count, haemoglobin, and haematocrit value of non pregnant women were higher than pregnant women. Furthermore, on people treatment, mothers who got multivitamin and multimineral supplements had higher haemoglobin than mothers who did not. The treatment was carried out on the 7th, 11th, and 15th postpartum week. According to Malole & Pramono (1989), the results of this study showed that there is a variation in the erythrocytes count, haemoglobin, and haematocrit value in each treatment group. However, statistically, the average of erythrocytes count, haemoglobin, and haematocrit

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<th>Table 1—Haematological profile of lactating female after the administration of pasak bumi roots methanol extract and its derived fraction during lactation period. Blood sampling on the 22nd day in lactating period (n = 3)</th>
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Values are mean±S.E.M. of three animals; symbols represent statistical significance (p < 0.05).

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<th>Table 2—The differentiation of leukocytes of lactating female after the administration of pasak bumi roots methanol extract and its derived fraction during lactation period. Blood sampling on the 22nd day in lactating period (n = 3)</th>
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Values are mean±S.E.M. of three animals; no differences between groups (p > 0.05).
value in the whole experimental group were not different. Thus, it is assumed that the administration of *pasak bumi* roots during lactation did not affect the role of erythrocytes in transporting of respiratory gases, which means that the energy metabolism in animal cells was not disturbed. According to Mehta & Hoffbrand (2008)\textsuperscript{11}, the low number of erythrocytes indicates anemia problem. Anemia is a condition in which the number of red blood cells or hemoglobin is low.

In human body, leukocytes or white blood cells have a role has immune system in our body\textsuperscript{3}. Mehta & Hoffbrand (2008)\textsuperscript{11} stated that the leukocytes have big size, but the number is only 1% of the total blood volume. Furthermore, leukocytes count may be an indicator of inflammation in the body. The high number of leukocytes is the body’s attempt to defend itself\textsuperscript{2}. Mbassa & Poulsen (1991)\textsuperscript{22} said that leukocytes count in 8–12 month old non-pregnant and 1–2 yrs old pregnant goats were higher than in adult pregnant and lactating goats of over 2 yrs of age. In addition, leukocytes count, eosinophils, and neutrophils were significantly higher in pregnant women than non pregnant women. However, lymphocytes and monocytes count were significantly lower in pregnant women than non pregnant women\textsuperscript{33}. According Malole & Pramono (1989)\textsuperscript{34}, leukocytes count of mice is 6-15x 10\textsuperscript{3} /mm\textsuperscript{3}, the percentage of neutrophils, eosinophils, basophils, lymphocytes, and monocytes respectively 10-40\%, 0-4\%, 0-0.3\%, 55-95\%, and 0.1-3.5\%. Mehta & Hoffbrand (2008)\textsuperscript{11} and Ward et al. (2009)\textsuperscript{25} stated that neutrophils are white blood cells, the fore in the body's defense system, to protect the body from microorganisms. Eosinophils are important in response to parasitic diseases and allergic diseases. Basophils, associate with mast cells, play an important role in hypersensitivity reactions and in defense against allergens and parasitic pathogens. Lymphocytes have the main role the immune system, as well as in monocyte phagocytosis. In this research, leukocytes count in all groups were relatively low, but statistically leukocytes count among group the root of *pasak bumi* methanol extract with aquadest and n-hexane fraction, chloroform fraction, also methanol-water fraction are not different (p>0.05). In contrast, statistically the average number of leukocytes among group the root of *pasak bumi* methanol extract with the normal control group, Moloco+B12, and the ethyl acetate fraction are different (p<0.05). However, statistically average leukocyte differentiation in the overall experimental group did not differ.

As reported by researchers, the roots of *Eurycoma longifolia* Jack. contains a series of quassinoids\textsuperscript{8-26,32}, canthin-6-one alkaloids, β-carboline alkaloids\textsuperscript{31,32}, tirucallane-type triterpenes\textsuperscript{32,33}, squalene derivatives\textsuperscript{32,33}, and biphenylneolignans\textsuperscript{32}. Moreover, base on previous research, the roots of *pasak bumi* have many activities to support human health\textsuperscript{34,41} such as hepatoprotector\textsuperscript{12}. We assumed that administration of the root of *pasak bumi* methanol extract and its derived fractions does not affect the haematological profile of lactating mice. Related to Al Salahi et al. (2012)\textsuperscript{22} the administration of crude methanol extract of roots of *pasak bumi* for 16 days on male mice does not affect haematological profile (haemoglobin, red blood cells, hematocrit, white blood cells, platelets count, mean cell volume, and mean cell corpuscular). It can be concluded that the haematological profile after given of methanol extract similar with physiological value.

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**References**

9. Correia-Santos AM, Pereira KB, Santelli RE, Boaventura GT, Blondet de Azeredo V, Dietary supplements for...


