Histology Structure of Lymph and Uterus of White Rat (*Rattus norvegicus*) Given with *Nanas Bongsai* (*Ananas comosus var. microstachys* L.)

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Abstract

*Nanas Bongsai* (*Ananas comosus var. microstachys* L.) is an amazing herb which is used in traditional medicine by local people of Muara Lembu district as a potion to reduce pain while menstruation period for a women. The present work was designed to investigate its probable side effects on the histopathologic changes in lymph and uterus tissues after treated with *A. comosus var. microstachys* L extract in female white rats. This is an experimental research consisted of five treatments and three repetitions. Treatment composed of two controls (P₀ and P₃) and given *Nanas Bongsai* extract with three different dosages. Histology result of lymph show that all treatments given with *Nanas Bongsai* with three different dosages have different diameter average of white pulp and there is no significant changes with normal control. While observation towards histology structure of endometrium thickness with dosage P₁, dosage P₂, dosage P₃ is 206.333±33.486; 215.667±33.486; 197.667±60.871 respectively. The most thin endometrium layer found in treatment with dosage P₃ compared to control P₀. From this study showed that there is no toxic effect on uterus and lymph function of *Nanas Bongsai* (*A. comosus var. microstachys* L.) at different doses, so the plant is secure for consumption by the community.

How to Cite


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INTRODUCTION

Utilization of medicinal plants has long been known by Indonesians called as _jamu_. Knowledge about utilization this medicine has inherited from generation to generation both oral and lettering. Utilization of this medicine have widely used by local people. One noticeable reason to use this plant is high price of synthetic medicine (Cottencin et al., 2014). According to World Conservation Monitoring Center claimed that Indonesia was a region that rich of many medicinal plant. Medicinal plant that has been utilized about 2,518 kinds of medicinal plant (EISAI, 1995). In Indonesia at least 3000 kinds of medicinal plant have been identified (Zuhud & Hikmat, 1998). Knowledge of herbal medicine and mixed herbal potions originally came from the knowledge of local community gained inadvertently as indigenous knowledge and developed by generations (Fitmawati et al., 2017)

In Riau Province, There is a lot of traditional medicinal knowledge specifically in _Muara Lembu_ village sungigi hilir subdistrict, Kuantan singi district. The societies have been used to _Nanas Bonsai_ (Ananas comosus var. microstachys L.) from family Bromiliaceae which used for commercial fruit production in Indonesia as medical plants. This plant was believed by local community able to reduce pain during menstruation period and have been consumed for generations. Empirical verification is highly required in order to improvement of this plant. According to Ekor (2014) the accuracy of consuming traditional medicine correctly and have been tested both of preclinical and clinic test able to prevent adverse effect from traditional medicine itself.

An effort to commercialization of _jamu_ required a standardization and preclinical test towards the simplicial. Simplicial standardization is a whole effort begins with the selection of certain medicinal plants. This is a preclinical test using _Nenas Bongsai_ extract for limph and uterus organs. The lymphatic system may control the ultimate immune response including antigens coming outside from the body (Fitmawati et al, 2017; Liao et al., 2016, Prakash et al., 2013), and plays an important role in homeostasis by controlling extracellular fluid volume and in combating infection (Margaris et al., 2012). The uterus is the major female reproductive organ of mammals, including humans. The female rabbit has a bicornuate duplex uterus. This type of uterus has two separate uteri. Each uterus has its own cervix, and the two cervices open into a single vagina. Many of fertility problems in mammals are of uterine origin (Alkareem, 2017). As this information is traditional and there is a dearth of publications on the effects of this plant on the limph and uterus tissues, this research was aimed to investigate its probable side effects on the histopathologic changes in limph and uterus tissues after treated with _Nenas Bongsai_ extract in female white rats at different serial dosage. This research supports the importance knowledge and of comprehensive data from medicinal plants _Nanas Bongsai_ that is ancestral heritage community of _Muara Lembu_ village Singigi hilir subdistrict, Kuantan singi district, Riau Province.

METHODS

Preparation of the Extract of _Nanas Bongsai_

Extraction method was made by local people habit by making the extraction for 100 ml. _Nanas Bongsai_ that already rinsed and squeezed to obtain the water made the extract. Add water gradually, the extract were used for treatments process. This extracts only use once in a day for eight days.

In Vivo Assay

This research is an experiment research that conducted in laboratory using 15 female white rats aged 3 months and weighing 160g-200g. This research using completed randomized design with five treatments and three repetitions that consisted of two controls; zero control giving water (P0), positive control by giving mfenamait acid (P1) and extract of _Nanas Bongsai_ were varied with three different dosages. Treatments were conducted orally once in a day for eight day using 1 ml spuit disposable without pin. At 8th day, all white rats were euthanasia using chloroform.

Dosage Determination of Experimental Animal

Dosage determination based on Lawrance & Bacharach (1964) by converting common dosage consume by human (100 ml) with white rats conversion factor 0.018 and obtained conversion factor 1.8 ml/200g BB that have been set as two route dosage given orally. Then the dosages were graded 0.5x, 1x and 1.5. 0.9 ml/200g BB, 1.8 ml/200g BB, 2.7 ml/200g BB respectively.

Making Process of Preserved Preparations

Preparation process of tissue sample at lymph and uterus using paraffin method. The organ were checked and fixed using BNF liquid (Buffered Neutral Formalin) 10% for 24 hours. The...
next following stage is the organ were cut transverse and arrange in tissue cassette and then soaked in BNF 10% for 15 minutes and rinsed using water. Dehydration process using alcohol 30%, 50%, 70%, 90% and ethanol for 45 minutes and clearing process using xylol I and II for 45 minutes and followed by infiltration using paraffin. Embedding process into mold block and poured using paraffin liquid for a night. After solified sectioning process using microtom with thickness about 6-7 mikron. The next step is deflation using ethanol I and II, graded alcohol 96%, 80%, dan 70% for two minutes. Staining process including Hematoxylin-Eosin (HE). Observation using microscope and documented.

Data Analysis

Observation towards histology were analysed descriptively. Microscopic data were analysed by observing damaged cells on kidney and liver of white rats by observing five viewing field. Obtained data then analyzed using ANOVA and followed by Duncan Multiple Range Test (DMRT) (Assiam et al., 2014). Data analysis for Lymph and uterus histology by scoring the percentage of damage cells based on Baldatina (2008).

\[
\text{Percentage of damage cell} = \frac{\text{Damage cell}}{\text{All cells}} \times 100\%
\]

Table 1. Diameter average of lymph white pulp of white rat.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Diameter Average (μm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P₀</td>
<td>155.6±22.517ab</td>
</tr>
<tr>
<td>P₁</td>
<td>166.333±15.358b</td>
</tr>
<tr>
<td>P₂</td>
<td>136.933±3.349a</td>
</tr>
<tr>
<td>P₃</td>
<td>153.267±13.927ab</td>
</tr>
</tbody>
</table>

Notes: P₀: zero control, P₂: positive control, P₁: Nanas Bongsai Dosage 1, P₂: Nanas Bongsai Dosage 2, P₃: Nanas Bongsai Dosage 3. Numbers followed by different alphabet at same column is significantly different at α 5%.

Table 1. shows the average of pulp diameter of white rat lymph after given treatment. Based on the table differences were shown between pulp diameter in every treatment. Based on ANOVA result for white pulp lymph P value is 0.038. P<0.05 that there is a significant different of pulp diameter of white rat lymph in every treatment. White pulp diameter in control P₀ is 155.6±22.517. While the average in treatment and given Nanas Bongsai with three serial dosage P₁, dosage P₂, dosage P₃ is 136.933±3.349; 153.267±13.927 and 136.067±3.7 respective. Real differences from all treatments found in positive control (Mefenamat Acid) which is different from given Nanas Bongsai extract with three different dosage serial 166.333±15.358. According to Affah et al (2014), the observed effect from Ginger (Zingiber officinale), is dose dependent as greater effects were seen in animals that were administered high doses. This effect may be due to the highlighted phytochemicals contained in the plant.

RESULT AND DISCUSSION

Microscopic Observation of Lymph

Lymph is the biggest lymphoid organ in body that design as organ for immune response towards foreign substance that enters to body (Aaspelund et al., 2016; El-Sherief et al., 2017). In this research observation towards microscopic structure changes towards white rats lymph that have been given five treatments entailing control (P₀ and P₁) as well as three different dosage serial of Nanas Bongsai extract. One of parameter need to observe changes in diameter size of white pulp in every treatment. The data were presented in Table 1.

Figure 2. Histology structure lymph of Rattus norvegicus. Hematoxylin-Eosin staining. 640x magnification. A: P₀ control, B: P₁ control, C: Nanas Bongsai Dosage 1, D: Nanas Bongsai Dosage 2, E: Nanas Bongsai Dosage 3. () white pulp

Based on result the average of white pulp treatment with given Nanas Bongsai extract dosage P₁ has the smallest white pulp diameter (Figure 2). Different size of white pulp diameter caused by activity enhancement of white pulp cells. This is a form of stimulation of defense system towards foreign substances that enter to the body. This circumstance can be seen from white pulp diameter and become widened indicates that immune system in lymph become increased (Abdelhafiz et al., 2015; Omar, 2012). In addition dif-
different size of white pulp in given Nanas Bongsai extract with three different dosage serial can caused by phytochemistry compound in Nanas Bongsai Extract. According to Parasuraman (2011), every active substance has pharmacology effect and potentially has toxic effect.

Microscopic Observation of Uterus

The uterus is the major female reproductive organ of most mammals. Uterus is organ for zygote implementation. The female rabbit has a bicornate duplex uterus. Each uterus has its own cervix, and the two cervices open into a single vagina. Many of fertility problems in mammals are of uterine origin (Abd-Elkareem, 2017). This organ consisted of the endometrium; the mucosa, the myometrium, the thick muscular layer and the perimetrium; the serosa. The endometrium was equipped with gland to supply nutrition to fertilized ovum. Endometrium and myometrium were part of tissue that plays a role in implementation to determine individual fertility that was consisted of lamina epithelialis of simple columnar type and connective tissue lamina propria containing leucocytic infiltration and endometrial glands (Brosens et al., 2013; Faria et al., 2015). The myometrium was formed of an inner circular (IC) and outer longitudinal (OL) smooth muscle fibers separated by tunica vascularize (layer of loose connective tissue containing blood vessels). The perimetrium was formed of a mesothelium and submesothelial loose connective tissue (Abd-Elkareem, 2017).

In this research, observation towards microscopic structure of white rat uterus after given treatment (P₀, P₁, and P₂ control) and given Nanas Bongsai extract with three different dosage serial. Observed result including the thickness of endometrium and myometrium. Data analysis of endometrium and myometrium were presented in Table 2.

Table 2. Thickness average of endometrium and myometrium of white rat.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Endometrium</th>
<th>Myometrium</th>
</tr>
</thead>
<tbody>
<tr>
<td>P₀</td>
<td>254±57.236</td>
<td>104±27.622</td>
</tr>
<tr>
<td>P₁</td>
<td>284.667±64.933</td>
<td>84±36.592</td>
</tr>
<tr>
<td>P₂</td>
<td>206.333±33.486</td>
<td>97.667±6.351</td>
</tr>
<tr>
<td>P₃</td>
<td>215.667±33.486</td>
<td>60.333±14.434</td>
</tr>
</tbody>
</table>

Notes: P₀: zero control, P₁: positive control, P₃: Nanas Bongsai Dosage 1, P₁: Nanas Bongsai Dosage 2, P₃: Nanas Bongsai Dosage 3. Numbers followed by different alphabet at same column is significantly different at α 5%

Based on histology observation from the thickness of endometrium and myometrium that ANOVA test resulting P for 0.55. P value less than 0.05 and presumed that there is no significant changes between P₀ control (feed and drink in adlibitum) P₁ control (Mefenamat Acid) and given Nanas Bongsai extract with three different dosage serial. Towards the thickness of white rat endometrium. Based on table 2 show the average of endometrium thickness at three different dosage serial did not show any significant changes of endometrium thickness. Same result with positive control (Mefenamat Acid). The average of endometrium thickness with three different dosage serial are 206.333±33.486; 215.667±33.486; 197.667±60.871 respectively. The lowest thickness of endometrium layer found in treatment of dosage P₃. The result of this study revealed no pathological effects in the uterus of the rats. Based on Adkar et al (2014), the histology of uterus of mice in control group found normal whereas mice treated with of Trapa bispinosa Roxb. leaves with lower and higher dose (200 and 400 mg/kg body weight respectively), did not change in the histology of uterus as compared to control group. This is in line with Amabe et al (2010) who reported normal uterine morphologies, and functions with the seed oil extract of Azadirachta indica.

Based on the table endometrium thickness of endometrium layer at positive control occurs an enhancement while treatment with given Nanas Bongsai extract underwent a declining along with the improvement of given dosage towards the rats. ANOVA result did not show any significant changes in every treatment. However, based on counting result of endometrium thickness at the table show that given extract of Nanas Bongsai at three different dosages serial affect the thickness of endometrium layer of white rat uterus. Table 2 show the average of myometrium thickness of white rats after given treatment. Based on the table there is significant difference of myometrium thickness from each treatment. ANOVA test result show that P value is 0.371. P less than 0.05 it means that there is no significant difference of white pulp diameter of white rat lymph in every treatment.

Table 2 shows that thickness in myometrium at positive control and given extract of Nanas Bongsai compared to normal condition.
Based on table 2 myometrium at normal control is 104±27.622 while given extract of Nanas Bongsai at dosage P₁, P₂, and P₃ shows a decline in myometrium thickness. However, given extract at dosage 3 (73.333±40.104) is higher rather than dosage P₁ (73.333±40.104). Declining in myometrium thickness at given extract of Nanas Bongsai with three different dosage serial can presume in normal condition showed by same notation in each treatment.


Figure 3 is the description of microscopic histology of white rat uterus in all treatment including (normal control and positive control) as well as three varied dosage of Nanas Bongsai. Based on data analysis there is no significant effect towards endometrium layer as well as myometrium. In this figure, endometrium layer after given treatment of Nanas Bongsai extract. It can be seen clearly that given extract of Nanas Bongsai at dosage P₁ and dosage P₂, become thicker rather than dosage P₃. While myometrium after given treatment of Nanas Bongsai extract show that myometrium layer in dosage P₁ show a decline compared to dosage P₂, and dosage P₃. According Al-Qudsi & Linjawi (2012), there was a slight insignificant increase in the thickness of endometrium between the treated and the control groups and this increase was noticeable in G2 (10 mg/kg) agree with the anatomical measurements.

Plant medicinal is widely perceived by the public as being natural, healthful and free from side effects. The local community believe that Plant medicinal has no side effects or any potential risks due to their natural origins and are often considered as food supplements and not drugs (Arsad et al., 2014). All this time, pineapple was presumed able to prevent pregnancy. Consequently, this fruits is frequently used to overcome late period of menstruation and potential as an abortivum the existence of bromelain enzyme in pineapple cause the thickness of endometrium and myometrium in uterus. In addition, Nanas Bongsai has high activity of bromelain enzyme and cause contraction of uterus.

From this study showed that there is no toxic effect on uterus and lymph function of of Nanas Bongsai (Ananas comosus var. microstachys L.) at different doses, so the plant is secure for consumption by the community and will not cause toxicity effect on the limph and uterus function.

CONCLUSION

Given extract of Nanas Bongsai with three different dosage serial did not give significant changes can be seen from ANOVA result that have been obtained. Given Nanas Bongsai extract towards R. norvegicus can decrease white pulp diameter and damage in lymph organ compared to control treatment. Same result also obtained with given Nanas Bongsai extract towards R. norvegicus can decrease thickness endometrium layer as well as myometrium compared to control treatment. Base on this research was obtained that there is no toxic effect on uterus and lymph function of of Nanas Bongsai (A. comosus var. microstachys L.) at different doses.

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