HISTOPATHOLOGICAL OVERVIEW OF KIDNEY AND LIVER FEMALE WHITE RAT ADMINISTERED (Ananas comosus var. microstachys L.) EXTRACT AS HERBAL PLANT FOR DYSMENORREA

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Abstract
Dysmenorrhea is an important clinical as well as social problem affecting more than 50% of menstruating women. Nanas bongsai (Ananas comosus var. microstachys L.) is commonly used as a medical plant, which local people believe of Riau Province Indonesia, as medicine to reduce pain while the menstruation period (dysmenorrhea). This study was aim to find histopathologic changes in the kidney and liver after being treated with nanas bongsai extract in the female white rat. Design experimental of this research is complete randomized design with 5 treatments. Each treatment was composed of two control (zero control given with water, positive control given with mfenamic acid) and nanas bongsai extract with 3 different dosage serials. Histology preparations were made by paraffin method and Hematoxylin-Eosin staining. The results showed that given nanas bongsai extract with three serial dosages towards the kidney show a picnosis in the nucleus. However, this damage did not affect the glomerulus structure. While observation towards the liver shows some injury, namely hydropic degeneration, lipid degeneration, and necrosis. Both of these damaged less than 25%. As a result, this percentage did not affect the structure of the kidney and liver. The results of this study indicate that there is no damage to the kidneys and liver due to the use of nanas bongsai so it is safe to use in herbal medicine and can be developed as a dysmenorrhea drug.

Keywords: Dysmenorrhea; Kidney; Liver; Nanas bongsai; Women

Abstrak

Kata kunci: Dismenore; Ginjal; Hati; Nanas Bongsai; Wanita

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INTRODUCTION

Dysmenorrhea is a condition experienced by women during menstruation. Symptoms of dysmenorrhea are sometimes accompanied by systemic symptoms such as nausea, vomiting, diarrhea, headache, and dizziness. In relieving the pain, women will consume drugs such as painkillers and contraceptive pills, but some women who can't stand the side effects (Alsaleem, 2018; Bernardi et al., 2017). Therefore, it seems necessary to find a new and simple treatment for dysmenorrhea, like plant herbs.

Nanas bongsai (Ananas comosus var. microstachys L.) is used by local people at Muara Lembu village, Kuantan Singing District, Riau, Indonesia to reduce pain while the menstruation period (dysmenorrhea) and has already been consumed for generations. Nanas bongsai has been clinically tested to be safe for consumption and not harmful to organs. Preliminary research has proven that there is no toxic effect on uterine and lymphatic function after administration of different doses of nanas bongsai extract to female white rats (Rattus norvegicus) (Fitmawati et al., 2018). However, preclinical testing is needed for the function of other organs such as liver and kidneys. Traditional medicine can be prevented by correct utilization and a barrage of preclinical and clinical tests and chemical drugs (Arsad et al., 2014; Chen et al., 2011).

The kidneys are organ that functions to remove metabolism waste and toxins from the body through the urinary system (Rubenstein & David, 2012). In addition, kidneys have a role in maintaining salinity, homeostasis, and electrolytes in the human body (Dhondup & Qian, 2017). The kidneys are vulnerable organs towards foreign particles because this organ accepts 25–30% blood circulation to be cleaned. As a consequence, a tremendous pathologic change would probably happen (Tomino, 2014). The kidneys were often facing problems with toxicity if the human body was highly exposed to an anti-nutrition substance (Ibrahim et al., 2018; Morgado & Neves, 2012).

The liver is the biggest organ in mammals, which has a tender texture, is limber, and is located above cavities abdominals under the diaphragm (Diffaa, 2021; Muhammad et al., 2019). The liver's primary function in mammals is metabolizing carbohydrates, lipids, and protein (Niranjan, 2016; Ozougwu, 2017). In addition, the liver plays a role as a filter towards foreign substances in the human body. The liver is the first organ that responds to strange materials. As a result, cells in its tissue occurring histology structures changes (Gasmi & Kleiner, 2020; Ozougwu, 2017).

Local people have indigenous knowledge about nanas bongsai as anti-pain during the menstruation period. However, there is no information about dosage standardization and toxic effects on organisms (based on histopathology of the liver and kidney) of nanas bongsai. The aim of this research is a preclinical test of nanas bongsai extract effects to the kidneys and liver histology structure in female white rats at different serial dosages. Add 1–2 sentences of advantages this research for the future.

MATERIALS AND METHODS

The processing extract of nanas bongsai fruit extract refers to the way of consumption by the community. The extract was made as much as 100 mL. Plant materials washed with distilled water. Scrapped nanas bongsai (Ananas comosus var. microstachys L.) fruit that had already rinsed and squeezed to obtain the water made the extract. The extract was filtered through a Wattman ≠1 paper. Add water gradually to help in the process of taking the nanas bongsai extract. This nanas bongsai drink extract is consumed by women suffering from dysmenorrhea once a day and consumed for eight days to optimize menstrual pain relief. This extract will be tested directly on female white rats.

This research was used 15 female white rats aged 3 months and weighing 160–200g. Design experimental of this research is complete randomized design with 5 treatments and 3 repetitions. Two control groups were zero control giving water, and positive control by giving mefenamic acid. The nanas bongsai extracts were varied with three different dosage treatments and conducted orally once a day for eight days using 1 mL split disposable without pin. On the eight day, all white rats were euthanasia using chloroform.
Dosage Determination of Experimental Animal

Dosage determination based on Laurence and 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 dosage was graded 0.5x, 1x and 1.5x; 0.9 mL/200g BB, 1.8 mL/200g BB, 2.7 mL/200g BB respectively.

Making Process of Preserved Preparations

The preparation process of tissue sample at kidney using paraffin method. The organ was checked and fixed using Buffered Neutral Formalin (BNF) liquid 10% for 24 hours. The following stage is the organ cut transverse, arranged in tissue cassette, 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 followed by infiltration using paraffin. Embedding process into mould block and poured using paraffin liquid for a night. After solidified sectioning process using microtome with thickness about 6–7 micron. 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 and documented using light microscopy (Fitmawati et al., 2018).

Statistical Analysis

Observation towards histology was analyzed descriptively. Microscopic data were analyzed by observing damaged cells on the kidney and liver of white rats by observing five viewing fields. Obtained data were then analyzed using ANOVA and followed by Duncan Multiple Range Test (DMRT). Data analysis for kidney and liver histology scores the percentage of damaged cells based on Baldatina et al. (2008) (Table 1).

<table>
<thead>
<tr>
<th>Score value</th>
<th>Histopathological changes</th>
</tr>
</thead>
<tbody>
<tr>
<td>0; if &lt;25%</td>
<td>Liver undergoes hydropic degeneration, parenchymal degeneration and apoptosis around the centrolobular (central vein)</td>
</tr>
<tr>
<td>1; if 25–50%</td>
<td>Liver experience hydropic degeneration, parenchymal degeneration and apoptosis that extends to the center (midzone)</td>
</tr>
<tr>
<td>2; if 50–75%</td>
<td>Liver Experience hydropic degeneration, parenchymal degeneration and apoptosis that extends to the periporta (perilobular)</td>
</tr>
<tr>
<td>3; if &gt;75%</td>
<td>Liver Experience hydropic degeneration, parenchymal degeneration and apoptosis that extends to the periporta (perilobular) zone</td>
</tr>
</tbody>
</table>

Ethical Clearance

This study has received ethical review board for medicine and health researcher of Universitas Riau No. 174/UN.19.5.1.1.8/UEPKK.2016.

RESULTS

Macroscopic Observation of White Rats Kidney

Macroscopic observation of white rats kidney including damaged tissue. Based on the data, the average presentation occurs in the glomerulus of white rats. Changing hepatology observed is intumescence at the glomerulus. Percentage data and damage score were presented in Table 2.

<table>
<thead>
<tr>
<th>Table 2. Percentage and scoring table of damaged glomerulus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
</tr>
<tr>
<td>-----------</td>
</tr>
<tr>
<td>P0</td>
</tr>
<tr>
<td>P+</td>
</tr>
<tr>
<td>P1</td>
</tr>
<tr>
<td>P2</td>
</tr>
<tr>
<td>P3</td>
</tr>
</tbody>
</table>
Notes: P0= control 0, P+= positive control, P1= nanas bongsai dosage 1, P2= nanas bongsai dosage 2, P3= nanas bongsai dosage 3. Numbers followed by different alphabet at same coloumn is significantly different at $\alpha$ 5%. Damage score (0) based on Baldatina (2008)

The ANOVA test showed an expansion of the glomerulus found $P$ 0.038. $P < 0.05$ value means a real difference in damage percentage of white rats kidneys in every treatment. DMRT shows a real difference between zero control and positive control (mefenamic Acid) and given nanas bongsai extract at dosage 1 and 2. While treatment with given extract at dosage 3 did not show any significant difference with the other treatments 0 control (feed and drink in adlibitum) positive control (mefenamic Acid) as well as given nanas bongsai extract at dosage 1 and dosage 2. Observation towards histology structure of white rat by counting abnormal glomerulus cell. In table 1, the lowest percentage of damaged glomerulus found in zero control (drink and feed in adlibum) was $7.488 \pm 3.055$, while the average rate of damaged glomerulus found in the positive control (mefenamic Acid) accounts for $9.847 \pm 0.577$.

Table 1 illustrates the scoring result of damaged glomerulus in every treatment at 0–25%. Damaged glomerulus found in given nanas bongsai extract with three different dosages serials $11.120 \pm 1.155$; $10.401 \pm 1$; $9.650 \pm 6.245$ respectively. Damaged glomerulus found in every treatment has a score of 0 which means that the damaged grade is still in normal condition.

**Microscopic Observation of White Rat Liver**

In this results, observations were conducted towards microscopic histology of white rats liver after being treated with control (zero control and positive control) and given nanas bongsai extract with 3 different dosage serial. The data are presented in Table 3. Hepatocytes changes entailing hydropic degeneration, lipid degeneration, and necrosis.

**Table 3.** Percentage of damaged hepatocytes of white rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Hydropic degeneration</th>
<th>Lipid degeneration</th>
<th>Necrosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>P0</td>
<td>$8.1 \pm 2.887$</td>
<td>$3.935 \pm 1.594$</td>
<td>$4.684 \pm 2.092$</td>
</tr>
<tr>
<td>P+</td>
<td>$6.667 \pm 1.261$</td>
<td>$5.447 \pm 1.353$</td>
<td>$3.169 \pm 0.226$</td>
</tr>
<tr>
<td>P1</td>
<td>$7.648 \pm 2.874$</td>
<td>$4.416 \pm 0.635$</td>
<td>$3.453 \pm 1.227$</td>
</tr>
<tr>
<td>P2</td>
<td>$9.745 \pm 1.454$</td>
<td>$3.426 \pm 1.334$</td>
<td>$3.88 \pm 0.225$</td>
</tr>
<tr>
<td>P3</td>
<td>$8.227 \pm 0.386$</td>
<td>$3.607 \pm 2.618$</td>
<td>$4.872 \pm 2.670$</td>
</tr>
</tbody>
</table>

Notes: P0= control 0, P+= positive control, P1= nanas bongsai dosage 1, P2= nanas bongsai dosage 2, P3: nanas bongsai dosage 3. Numbers followed by different alphabet at same coloumn is significantly different at $\alpha$ 5%. Damage score (0) based on Baldatina (2008)

Based on ANOVA for lipid degeneration occur in hepatocytes obtained $P$ equal 0.493. $P$-value $> 0.05$ means that there is no real difference in damaged percentage in every treatment. While based on ANOVA for lipid degeneration and necrosis obtained $P$ values were 0.578 and 0.647, respectively.

**Table 4.** Damaged scoring of white rats hepatocytes

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Hydropic degeneration</th>
<th>Lipid degeneration</th>
<th>Necrosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>P0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>P+</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>P1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>P2</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>P3</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Notes: P0= control 0, P+= positive control, P1= nanas bongsai dosage 1, P2= nanas bongsai dosage 2, P3= nanas bongsai dosage 3. Numbers followed by different alphabet at same coloumn is significantly different at $\alpha$ 5%. Damage score (0) based on Baldatina (2008)
Based on Table 4, further test towards hepatocytes in every treatment. The result shows that damaged, including hydropic degeneration, did not give the real difference in every treatment. The highest hydropic found in P2 (given nanas bongsai extract dosage 2) is 9.745 ± 1.454, while the lowest hydropic degeneration occurs in P+ treatment (given mefenamic Acid) 6.667 ± 1.261. In histology preparations, the hepatocytes underwent lipid degeneration in all treatments did not show any significant difference. The lowest lipid degeneration found in hepatocytes at P2 treatment (nanas bongsai dosage 2) 3.426 ± 1.334. The percentage of lipid degeneration in nanas bongsai dosage 2 was fewer than two kinds of given extract at different dosage serial. While hepatocytes underwent the lowest necrosis found in P+ treatment (given mefenamic Acid) 3.169 ± 0.226.

Changes occur in hepatocytes, including hydropic degeneration, lipid degeneration, as well as necrosis. Damage found in hepatocytes cells found in P0 treatment can arise caused by the ageing process or the death of hepatocytes itself. This condition is supported by Fitmawati et al. (2017) that stated cells in the body constantly undergo an ageing process followed by the death of the cells. This death cell will replace by new cells through the regeneration process.

The scoring value found of damaged hepatocytes of white rats in every treatment entail hydropic degeneration, lipid degeneration, and necrosis. The scoring result of damaged hepatocytes called hydropic degeneration is 0 and lipid degeneration 0 and necrosis in each treatment. Based on scoring result, damaged stages in each treatment are still below 25%, and the kidney can be stated in normal condition (Table 3).

Figure 1 showed the microscopic structure of kidney histology of white rats in every treatment. It can be seen clearly that cells of the glomerulus in every treatment in normal condition. Figure 2 showed a histology description of white rats livers after treatment. Based on the figure, in every treatment, some damaged cells of hepatocytes can be found, namely hydropic degeneration, lipid degeneration, and necrosis. Damaged cells found in hepatocytes did not harm hepatocytes because still under 25%.

**Figure 1.** Histology structure of *Rattus novergicus* kidney. Hematoxylin-Eosin staining, 640x magnification. Zero control (a), positive control (b), nanas bongsai dosage 1 (c), nanas bongsai dosage 2 (d), and nanas bongsai dosage 3 (e). Glomelurus (1), bowman capsule (2), and piknosis nucleus (3)
DISCUSSION

Traditional medicine plays an essential role in the health care of tribal, rural, and urban people for all ailments. As herbal use becomes more common worldwide, ethnobotanical researches can provide insight into other medical systems that differ from the biomedical model. Knowledge of herbal medicine and mixed herbal potions originally came from the local community, gained inadvertently as indigenous knowledge, and developed by generations (Agyeman et al., 2020; Fitmawati et al., 2017; Teixeira et al., 2016).

The average of the damaged glomerulus in zero control (drink and feed in adlibitium) should be 0%. Several factors can cause this damage. The external factors such as the initial condition of white rats kidney before treatment, physiology condition could affect the kidney, and enhancement of cytosol enzyme activity could trigger stress condition that gives adverse effect on kidney and heart cells (Sánchez et al., 2002; Tomino, 2014).

This condition characterized with glomerulus cells of white rats have polyhedral shaped and nucleus. In addition, the bowman capsule covering the glomerulus and borders between pars visceralis epithelium and pars parietal epithelium can be seen clearly. Normal glomerulus marked by whole bowman capsule surrounding it and shaped like a bowl (Monfared, 2013). In the preparations, it can be seen some damaged glomerulus noticeably in every treatment. Damaged cells found underwent necrosis, including the nucleus looks darker and more condensed. Damage found in every treatment is still in a small percentage and within normal limits. This small percentage did not affect the shape of the glomerulus.

The glomerulus has a function as a blood filter that consists of a blood capillary. When filtration and enhancement of capillary permeability can cause plasm protein leakage and red blood cell, this leakage cause destruction in glomerulus filtration membrane expansion and edema in bowman capsule. Contraction in the bowman capsule can cause accumulation of glomerulus, which is marked by the improvement of glomerulus volume. Damage that occurs can disturb the production of filtrate and filtrate control. In addition, the glomerulus is vulnerable to be exposed to low circulation toxins compared to the other tissue (Radi, 2019; Tomino, 2014).

Hydropic degeneration occurs in hepatocytes found in every treatment. This condition can be seen with more extensive vacuoles in the cytoplasm, cell expansion, and pale nucleus (Figure 2).
Hydropic degeneration occurs due to ischemia, anemia, abnormal metabolism, and toxic chemicals (Yudhani et al., 2020). This condition caused a damaged cell membrane and disturbed the process of outflow and inflow of K⁺, N⁺, Ca⁺, and water. These will stimulate an increasing volume of water in cells. This condition is known as hydropic degeneration (Arsad et al., 2014; Cheville, 1999).

In Figure 2, lipid degeneration is found in every treatment. This circumstance is characterized by a lipid spot that can be seen clearly. According to Westbrook et al. (2016), lipid accumulation is found in hepatocytes marked with small vacuoles found in the cytoplasm. As a result, the nucleus was pushed to the edge. Lipid degeneration can occur because of excessive consumption of fat and protein consumed by white rats.

Instability between triglyceride mice and globular lipids can cause degeneration (Linton et al., 2019). These circumstances occur when lipid transportation and lipid synthesis in the liver while fat exertion reduces the amount of fat in liver cells. An organ was too long exposed to a strange substance will stimulate changes toward hepatocytes and become reversible. However, if this condition constantly occurs, structure changes, including fat degeneration, become irreversible (Fortes, 2017; Westbrook et al., 2016).

In Figure 2, damaged hepatocytes were found in all treatments with different percentages. The damaged includes picnotic specifically darker and more condensed nucleus. In P0 treatments (drink and eat inadlibitum) necrosis was also found. This condition can occur by some factors such as stressed conditions and unsuitable feed given to white rats. Medical plants that have consumed underwent a barrage of disintegration, absorption, distribution, metabolism, and excretion. The liver also does a series of those processes. The liver is one of the most vulnerable organs towards drugs and the accumulation of metabolites and foreign substances that can harm the organs. In addition, long-term consumption of an extract from plants can destroy the liver (Abou, 2016).

Given extract of nanas bongsai with three different dosage serials towards the kidney found that these give adverse effect towards kidney histology namely picnosis. However, this damage did not affect the glomerulus structure. Histology structure of white rat kidney still in normal condition bowman capsule still covering the whole bowman chamber. While in the histology of white rat liver was found minor damage, including hydropic degeneration. Lipid degeneration as well as necrosis. However, this damages still in low percentage. Based on the scoring result, both organs are still in normal condition, and the percentage is less than 25%.

CONCLUSION

The present study showed the absence of toxic effect of nanas bongsai (Ananas comosus var. microstachys L.) on kidney and liver of white rat (Rattus norvegicus), and suggest that popular consumption of nanas bongsai powder by humans will not cause toxicity effect on the kidney and liver function. From the results of this study, it is necessary to carry out further analysis in carrying out phytochemical tests of the nanas bongsai standardizing the use of this raw material to see its effect in overcoming and reducing pain during menstruation and tissue damage to vital organs. Furthermore, the results of this study are expected to be developed into phytopharmaca ingredients.

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