Effect of Natural Extract Leaves Waru (Hibiscus tiliaceus L) on Structure Liver of White Male Rats (Rattus novergicus Berkenhout 1769)

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Received: 7 December 2017, Accepted: 27 December 2017
Published online: 30 December 2017

Abstract: The waru plant (Hibiscus tiliaceus L) is one of the potentially medicinal plants and belongs to the Family Malvaceae. Waru Plants are trusted and used by the community to clean up the blood postpartum, fever, vomiting blood, cough and shortness of breath. Study of side effects especially the use of natural extract of medicinal plants has not been done, especially on the leaves of waru and one of the organ that are often damaged because the metabolism of chemical compounds is the liver. The purpose of this research is to study the change of histology structure of liver tissue caused by the giving of natural extract of waru leaf. This study used 9 rats divided into 3 groups namely: P0 (control), P15 (given the natural extract waru dose 100 mg/kgBB for 15 days), and P30 (given the natural extract waru dose 100 mg/kgBB for 30 days). Preparation of histological preparations using parafin method, Hemayoxylin-Eosin staining. At P0, the average damage was 2.87%, while at P15 5.58%, and there was an increase at P30 equal to 11.32%. The hearts of white rats undergo histopathological changes in the form of hydropic degeneration, fat degeneration, necrosis, vascular congestion, and sinosoid congestion. In this case the giving of natural extract of leaves waru with dose 100 mg/kgBB allegedly affect the structure of liver cells. But the change are still within normal limits due to the degree of minor damage of 25%.

Keywords: Waru; Liver; Male Rat; Histopathological of the Liver.

1. Introduction

Medicinal plants are plants that contain chemical compounds that can serve as drugs. One of the potentially medicinal plants is the waru plant (Hibiscus tiliaceus L) which is the Family of Malvaceae. Waru plants are trusted and used by the community to clean up the blood postpartum, fever, vomiting blood, cough and shortness of breath. Based on research that has been done by Sartika [1] empirically has been proven that waru plants also potentially can shed kidney stones or as an antiurolithiasis agent. Then by Kumar [2] can improve the liver tissue of rat that have diabetes.

However, the plant as a drug can also cause toxic effects due to duration of use, and safety use is not widely known. Community are more likely to use medicinal plants without knowing the effects of chemical witch contained in these plants against organs. Studies of side effects, especially the use of natural plant extracts of medicinal so far has not been done, especially on leaves waru. Based on research that has been done Al-hasawi and Al-Harbi [3] using natural extracts of leaves from Rhazya stricta for 15, 30, and 45 days caused damage to liver tissue in male rats. It is thought to be a metabolic compound of the plant that can alter the structure of cells in liver tissue.

The liver is an organ that serves as a detoxifier, detects chemical compounds that enter the body [4] Then the chemical compound is overhauled so that it will easily dissolve in water and toxicity levels
are reduced. However, the liver especially in mammals if it is damaged constantly will cause the formation of connective tissue followed by regeneration of liver cells so that the liver structure becomes irregular. The build-up of toxic substances in the hepatic parenchyma can injure the liver cells and lead to varying histopathological changes [5].

Research on liver histopathology by Fauziah et al [6] describes the picture of liver tissue damage caused by neem leaf extract causing liver cell degeneration and necrosis. Research using ethanol extract from opposite leaves resulted in parenchymal degeneration, hydropic degeneration, necrosis, fat degeneration and hemorrhage in the liver of white mice. Histopathology study of liver organ of white rats especially using natural extract of waru leaves has never been studied. So need to do further research about histopathology study on internal organs, especially liver that uses natural plant extract of waru which is believed as toxic by society.

2. Material & Methodology

2.1. Time and Place of Research

This study was conducted from April 2015 to September 2016. Preparation of plant extract and rod maintenance was done in Zoology laboratory, microscopic observation was done in Mikroteknik Laboratory of Biology Department Faculty of Mathematics and Natural Sciences of Riau University.

2.2. Tools and Materials

Tools used in this study is measuring cylinders, digital scales, filter paper, glass funnel, label paper, oven, paraffin blocks, stirrer bar, strainers, sample bottles, glass bottles, glass cups, a set of surgical instruments, glass objects, Light, digital camera canon brand, hotplate, water bath, oil bath, microtom, microtom knife, ha staining set.

Materials used during the study were male white rats, waru leaf, feed, aquades, BNF (Buffered Neutral Formalin) 10%, 70% alcohol, and stratified alcoho (30%, 40%, 50%, 60%, 70%, 80%, 90%, absolute I, II, and III), xylol (I, II, and III), entail and hematoxylin and eosin staining solutions.

2.3. Procedures

Make a Simplisia Leaf

Plant leaves are cleaned and dried to obtain powder with sieved.

Creating Natural Leaf Extracts

Waru leaf powder 25 gr is added with aquades as much as 250 ml in stir well until the powder of leaves waru really mixed with aquades. The leaf extract is filtered and let stand for 24 hours.

In Vivo Test

Nine male white rats aged 3 months weighing about 200 gr - 300 gr divided into 3 groups of treatment:
- Normal control group (P0): rats fed and clean drinking water for 30 days.
- Treatment group (P15): rat fed with natural extract of waru leaves with dose of 100 mg / BB for 30 days.
- Treatment group (P15): rat fed with natural extract of waru leaves with dose of 100 mg / BB for 15 days.

Sampling of Liver Samples of Aseptic White Rats

Microscopic Observation

Preparation of histology of the liver are:
- The liver organ is cut using a scalpel blade with a thickness of 0.3 to 0.5 mm.
- Fixation by inserting sample into 10% BNF solution for 24 hours, then sample soaked with 70% alcohol.
- Dehydration is done by inserting the sample into the alcohol of stratum ranging from 70%, 80% (9 hours), 90% (9 hours), absolute I 96% (1hour), absolute II 96% (2 hours), absolute III 96% (1 hour), Xylol I (1 hour), xylol II (1 hour), xylol III (1 hour).
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- Paraffin infiltration (Xylol-Paraffin) is carried out by inserting the sample into paraffin I, paraffin II, and paraffin III respectively for 30 minutes.
- Embedding is done by way of samples inserted into the mold block and left to harden.
- Paraffin blocks are cut using microtome with thickness ranging from 3-4 μm, soaked in hotplate with temperature 400. Then paraffin ribbon is taken using glass object and dried in oven.
- The nitrogenation is carried out by means of the sample incorporated into xylol I, xylol II, xylol III, absolute 96%, 90%, 80%, 70%, 60%, 50%, 40%, 30% alcohols each solution for 2 minutes.
- Sample coloring using Hematoxylin-Eosin (HE). The sample was put into aquades for 2 minutes and soaked in Hematoxylin solution for 7 minutes. After the Hematoxylin stain, the sample was rinsed with running water about 10 minutes. Then, the samples were inserted into 30%, 40%, 50%, 60%, 70%, respectively 2% dyeing liquor, then the sample was immersed in Eosin solution for 5 minutes. After Eosin staining, the samples were included in alcohols of 70%, 80%, 90%, absolute alcohol, xylol I, xylol II, xylol III each 2 times dye.
- Mounting is the process of closing the sample with a glass cover glued to the entelan.

2.4. Data Analysis

The histopathological data of mouse liver in microscopic was analyzed descriptively. Microscopic data of the liver obtained by calculation 5 field of view, each field of view is calculated percentage of cell number that is damaged to total cell number. After that the data is tested using One Way ANOVA test and if there is real difference is done further test of DMRT using SPSS 17.0 program. Calculation of damage percentage using mouse liver evaluation and scoring formulas on 5 field based on [7]:

\[
\text{Percentage of cell damage} = \frac{\text{The number of cell damage}}{\text{Total cell count}} \times 100\%
\]

3. Results and Discussion

Microscopic observation on the 15th and 30th day after the rat determination and taken the organ of his heart, obtained a total percentage of hepatocyte cell damage in white male rats (Table 1).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Repeat</th>
<th>Average</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 (%)</td>
<td>2 (%)</td>
</tr>
<tr>
<td>P0</td>
<td>2.89</td>
<td>2.86</td>
</tr>
<tr>
<td>P15</td>
<td>5.43</td>
<td>5.42</td>
</tr>
<tr>
<td>P30</td>
<td>11.26</td>
<td>9.89</td>
</tr>
</tbody>
</table>

Remarks: P0: rats fed and clean drinking water for 30 days; P15: rats bundled with natural extract of waru leaves dose 100 mg/kgBB 15 days; P30: rat blighted natural extract of leaves waru leaves dose 100 mg / kgBB 30 days.

Observation of the structure of rat liver showed changes in hepatocyte cells in all treatment groups. Changes that occur based on the calculated damage hepatocyte cell has a total percentage of damage Different. This is in accordance with the opinion of Lu [8] that the liver is often the target organ because most foreign compounds enter the body through the gastrointestinal system and after being absorbed are brought by the portal vein to the liver. In the P0 group fed and drinking water, hepatocyte cell damage was found in 3 repetitions, respectively 2.89, 2.86, and 2.86 (Table 1). In line with research conducted by Bhara [9], that groups given only feed and drinking water which are not oxidant substances can also cause damage to mouse hepatocyte cells. Another possibility of hepatocyte cell damage is found in group P0 due to the aging process and cell death that is physiologically experienced by all normal cells. According to Iber and Latham [10], every cell in the body will always experience the aging that ends death on the cell, so that later will be replaced with new cells through the regeneration process. Meanwhile, in the treatment group given the natural extract of leaf waru dose 100 mg / kgBB found hepatocyte cell damage in 3 repetitions respectively, P15 i.e. 5.43, 5.42, 5.90 and P30 ie 11.26, 9.89, 12.80 (Table 1). According to Robins [11] damage to hepatocyte cells can be caused by foreign compounds that enter the liver, the dose used, and the length of treatment day.
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Medicinal plants entering the body will experience a series of processes that are absorption, distribution, metabolism and excretion including the natural extract of waru leaves. The liver is the main metabolic organ that is susceptible to drugs or accumulation of metabolite compounds, so that often damaged. Medicinal plants entering the body will experience the process of metabolism in the liver and followed by changes in chemical structures catalyzed by enzymes produced by hepatocyte cell microsomes or known as biotransformation process. The medicinal plant will be converted into a less active or inactive metabolite. The process of metabolism in the liver is not only the process of detoxification or elimination of the compounds that occur, sometimes will experience the process of drug transformation into intermediate compounds that are reactive and toxic to the liver structure [12]. In addition, damage can also occur due to several factors, namely stress and immune factors in rats. In Table 1 shows the mean value of percentage of hepatocyte cell damage in all treatment groups increased. At P0 the average of low hepatocyte cell damage is 2.87. According to research conducted Larasati [13] animal liver in the untreated control group there will not be very severe damage. This is because the liver is not exposed to phytochemical compound contained in plants. While on P15 and P30 given the natural extract of leaves waru dose 100 mg/kgBB, the average damage of hepatocyte cells increased ie P15 5.58 and P30 11.32 (Table 1). This is possible because the liver is exposed by phytochemical compounds contained in the plant and the duration of natural extract of leaves of waru in rats. Natural extract of waru leaf contains phytochemical compound in the form of alkaloids, flavonoids, tannins and phenol.

The results of data analysis of mean hepatocyte cell damage in the treatment group were tested for normality using Kolmogrov Sminov One-Sample Test showed that the data was normally distributed with \( p = 0.43 \) \((p > 0.05)\). Homogeneity test of variance showed homogenous data with \( p = 0.093 \) \((p > 0.05)\). Based on One Way ANOVA test result on hepatocyte cell damage in all treatment groups obtained Sig 0.000 value, where this value is smaller than \( \alpha = 0.05 \) so that H1 is accepted and Ho is rejected, which means the data between treatment group there are significant difference and Where Ho is the data between treatment groups there is no significant difference. The differences between treatment groups showed that P15 and P30 treated with natural extract of leaves of waru at doses of 100 mg/kgBB for 15 days and 30 days had a significant effect on hepatocyte cell damage rate. Based on research that has been done Ryan et al [14] using Rosella ethanol extract for 7 and 34 days showed an increase in damage to hepatocyte cells. And another study by Wicaksono [15] using Kaliandra leaf extract for 7 to 21 days also showed an increase in hepatocyte cell damage. In this case, the provision of leaf extract using a long period of time can give a real effect on the increase of hepatocyte cell damage. According Lengkong [16], the use of a plant extract for a long time will not fix the liver cells, but it gives the opposite effect (Negative). Further analysis was done by DMRT (Duncan Multiple Range Test) with significance level \( \alpha = 0.05 \) to know the difference of mean of hepatocyte cell damage between treatment group. The results of statistical calculations with the DMRT test were obtained (Table 2).

### Table 2. DMRT Test Results of hepatocyte cell damage in the treatment group

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Average</th>
</tr>
</thead>
<tbody>
<tr>
<td>P0</td>
<td>2.8700a</td>
</tr>
<tr>
<td>P15</td>
<td>5.5833b</td>
</tr>
<tr>
<td>P30</td>
<td>11.3167c</td>
</tr>
</tbody>
</table>

Remarks: P0: rats fed and clean drinking water for 30 days; P15: rats bundled with natural extract of waru leaves dose 100 mg / kgBB (15 days); P30: rat blighted natural extract of leaves waru dose 100 mg / kgBB (30 days)

Based on Table 2 can be seen on P0 given feed and drinking water and P15 and P30 given natural extract leaf waru dose 100 mg/kgBB give effect to hepatocyte cell damage. But in P30 hepatocyte cell damage was found to be greater than that of P0 and P15 in which 30 days of natural extracts showed more influence on hepatocyte cell damage. However, the damage found can still be tolerated because at P30 the damage found is still below 25% or still categorized as normal damage.

The liver of white rats undergoes histopathologic changes in hepatocyte cells with damage found in the form of necrosis, hydropic degeneration and fat degeneration. It can be seen the number of damaged hepatocyte cells in Table 3.
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Table 3. The number of damaged hepatocyte cells

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dh(%)</th>
<th>Dl(%)</th>
<th>NK(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P0</td>
<td>7.02</td>
<td>0.41</td>
<td>1.18</td>
</tr>
<tr>
<td>P15</td>
<td>11.79</td>
<td>1.25</td>
<td>3.71</td>
</tr>
<tr>
<td>P30</td>
<td>22.62</td>
<td>2.11</td>
<td>9.22</td>
</tr>
</tbody>
</table>

Remarks: Dh: hydropic degeneration; Dl: fat degeneration; Nk: necrosis

![Image of histology](image)

**Figure 1.** P0, b: P15, and c: P30. Histology of the liver of white rats (P30) ie vs. central vein, kp: vascular congestion, dh: hydropic degeneration, dl: fat degeneration, n: necrosis, ks: synosoid congestion (40x16).

From Table 3 seen from the number of hepatocyte cells that suffered damage in the form of hydropic degeneration, fat degeneration and necrosis increased in all groups. In P15 also found vascular congestion (Figure 1b) and P30 vascular congestion and sinusoid congestion (Figure 1c). The hydropic degeneration found in all treatment groups in both P0, P15 and P30 shows the presence of vacuoles in the cell cytoplasm and the cells are swollen and the cell nucleus is pale (Figure 1a, 1b, and 1c). According to Robbins et al. [11], hydropic degeneration occurs due to the disruption of the active transport resulting in the cell unable to pump the Na + ions out so that the concentration of Na + ions inside the cell rises. It affects the osmosis process that causes the water influx into the cell causing the cell to become swollen like a vacuole and enlarged nucleus, also clearly visible granules in the nucleus. This degeneration is a temporary change (reversible) and includes mild damage because it can heal and hepatocyte cells will return to normal. In P15 and P30 cells of hydropic degeneration can be due to the high concentration and the duration of giving the natural extract of waru leaves in rats. According Fahmi et al. [17] giving extract concentration given high and given in a long time will affect the condition of
cell structures haptosis. According to Astuti et al. [18], if the intensity of exposure of a substance to an organ is too long it will cause changes in hepatocyte cell structure, the change is generally reversible. If damage occurs continuously can have an impact on other structures in the form of fat that is irreversible degeneration.

In P0, P15 and P30 hepatocyte-derived lipid cells are visible clear-colored fats (Figure 1a, 1b, and 1c). According to Anggraini [19] the accumulation of fat in hepatocyte cells, usually characterized by the presence of small vacuoles in the cytoplasm. These vacuoles are enlarged to push the cell nucleus to the edge. The presence of fatty degeneration found in the P0 treatment group, according to Dannuri [20] can be attributed to the excess consumption of fat and protein in these rats. In the group of P15 and P30 hepatocyte cells with increased degeneration of fat were thought to be caused by alkaloid components contained in the natural extract of waru leaves at doses of 100 mg / kgBW, thus inhibiting the action of enzymes involved in intracellular lipid metabolism. This is in line with research conducted by El Tahir & Ashour [21], that the alkaloid components contained in a plant can cause hepatocyte cells to degenerate fat due to the length of treatment day and the high concentration given. If continuous damage occurs it can have an impact on surrounding structures of necrosis (Figure 1a, 1b, and 1c). In P0, P15 and P30 are found pycnonic nuclei where the core looks dark as a dense cloud covering all parts of the cell nucleus. According to Robbins & Kumar [22], a cell that at its core looks very dark and compact and also covers almost all parts of the nucleus of this cell is a picnotic cell. The presence of necrosis occurring in P0 which should provide a normal histological picture can be caused by feed and beverages given as well as stress factors in the mice. While the necrosis that occurs in P15 and P30 is likely due to the duration of the extract of waru leaves. According to Juhryyah [23], if exposure of a substance in the cell lasts long enough, it will reach a point until the cell can no longer compensate and can no longer continue metabolism. The reversible change will become irreversible, ie the occurrence of the death of a cell. Necrosis appears to be widespread over the length of treatment days given to mice seen in P15 and P30 (Table 3).

In P15 and P30 there is a vascular congestion in which the central venous lumen enclosed by a bright red mass represents the blood that blocks the vessels (Figure 1b and 1c). Congestion is the excess volume of blood in a part of the blood vessels. This can happen because of the large amount of blood that enters through the arteries or too little blood to the veins. Seen microscopically congestion can be characterized by the occurrence of dilatation in the walls of arteries or capillaries due to high blood volume in the section [24]. The central veins are a venule composed of a layer of endothelial cells and external tunica. External tunica is a valve that serves to prevent blood from returning to the sinusoid. Exposure of metabolite compounds can damage the mechanism of closure by the valve, resulting in blood containment in the central venous [25]. In addition, the damage found in the central vein is caused by too much blood that is accommodated so that damage that occurs in the central vein is clearly visible. At P30 not only vessels congestion but also congestion sinusoid. On the observation is seen a bright red mass which is the red blood cells that fill the liver sinusoid (Figure 1c). The sinusoid congestion found in this study was thought to be caused by the presence of alkaloid compounds contained in the natural extracts of waru leaves and in line with Atere &Ajao [26] studies, reported that if the alkaloid compounds contained in a plant are given with high concentrations it can cause sinusoid congestion In liver histology. According to Greep [27], some liver syndoses serve as a place of blood flow to the central vein, and others will be inactive and serve as a place to hold blood. Exposure of a compound can cause an increase in blood vessels in blood vessels, so much blood yeng accommodated in these vessels. Another possibility of damage that occurs in the sinusoid is also caused due to degeneration fat that formed a fat vacuole that will lead to empty space on the sinusoid and result in a wide sinusoid. Another possible cause is due to the insistence on the sinusoid wall due to the dam in the vein in the form of blood caused by an incoming compound. In general, the dams begin from the central vein to the center of the lobule [28][29].

4. Conclusion

The liver of white rats undergoes histopathologic changes in hepatocyte cells with damage in the form of hydropic degeneration, fat degeneration, necrosis, vascular congestion, and sinusoid congestion. The average hepatocyte cell damage in all treatments was increasing where in P0 it was found that the
average damage was 2.87%, while in P15 5.58%, and an increase in P30 was 11.32%. In this case the giving of natural extract of leaf of waru with dose 100mg / kgBB allegedly give influence on mouse liver cell hepatocytes changes. But the changes that occur can still be tolerated, due to changes based on the degree of damage is calculated small than 25% (Normal).

References

[12] Larasati, N. D., “Protective Effects of Honey Against Hepar Damage of White Rats (Rattus norvegicus) Adult Male Sprague Dawley-induced Ethanol Denture”, Faculty of Medicine, University of Lampung, 2011.
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