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Histological Study of White Rats (*Rattus norvegicus*) Kidney Following The Consumption of Obat Pahit from Riau Archipelago

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Abstract

Obat Pahit from Lingga Malay ethnic is a traditional medicine which has believed by the local people to maintain the body stamina (immunomodulators and antioxidants). This study aimed to investigate the effect of *Obat Pahit* Potion on kidney histological structure of white male rats (*Rattus norvegicus*). This research was an experimental study with a Randomized Complete Block Design consisted of 15 treatments and 3 replications. The experiment groups consisted of control groups (Stimuno, distilled water, and CMC-Na 1%) and three treatment groups of *Obat Pahit* Potion (Kalan, SP4, Linau) with 4 different dosages. The samples of a kidney of white rats were prepared for histological observation using paraffin method and Hematoxylin-Eosin staining. The results of this study showed that three kinds of *Obat Pahit* with four different dosages showed the expansion of glomerular cells on kidney tissue. Damage that occurs in the kidneys is still in small amounts and normal range which is less than 25%. The results of this study provide information for the society that the consumption of *Obat Pahit* Potion will not cause toxicity effect on the kidney.

How to Cite

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INTRODUCTION

Malay Lingga Empire as a motherland of Malay ethnic (Malays) has a rich of indigenous knowledge, especially regarding ethno medicine. People in Lingga Island have a unique tradition to maintain their immunity that the knowledge is inherited from generation to generation. One of the most prominent traditional medicines is *Obat Pahit* Potion which has believed by local ethnic as an antiaging agent and can be used to maintain the stamina (immunomodulators and antioxidants) (Fitmawati, Sofiyanti, Roza, Isnaini, Hazimi, et al., 2017; Fitmawati et al., 2017).

A local wisdom of Lingga Malay ethnic is consuming *Obat Pahit* Potion in a long period which is believed to affect the cell regeneration of vital organs. Based on the result of research by Fitmawati et al. (2017), *Obat Pahit* potion has a high potential as the source of natural antioxidants and as a raw material of natural immunomodulator, so that potential to be developed as herbal medicine (Fitmawati et al., 2017). Usually, people consume *Obat Pahit* without any dose provision because there is no scientific data on the safe use of *Obat Pahit* potion. This study was carried out in order to determine the toxic effect of *Obat Pahit* potion at various doses on the kidney function of the rat.

A kidney is a major organ in metabolizing toxic compounds besides liver. It receives 25-30% blood containing a lot of chemical compounds and is at high risk to be exposed to toxic compounds. Moreover, the kidney also maintains liquid balance, electrolytes and salt in the body (Anyanwu et al., 2017; Aboonabi et al., 2014; Dollah et al., 2013; Brzoska et al., 2003). Kidneys often face the problem of toxicity if the human body were extremely exposed to anti-nutritional substances (Morgado & Neves, 2012; Tomino, 2014). As a consequence, the possibility of pathological changes is immensely high (Noori et al., 2013). The kidney damage also was determined by histological examination of kidney tissue. This research was a preclinical test of *Obat Pahit* utilization. This research aimed to determine the effect of various dosages of *Obat Pahit* on kidney histology. The benefit of this study can provide information to the society about the doses of *Obat Pahit* potion that can be used and prove the effect of toxicity on kidney organ.

METHODS

Boiling process of *Obat Pahit*

One hundred g of *Obat Pahit* was added to 100 ml of water and was heated using earthen pot until boiling. The decoction was taken as much as 200 ml which was used for the stringing process. *Obat Pahit* potion was administered twice (morning and evening) for 3 days.

In Vivo assay

In this research, 45 male white rats aged three months (170-260 g body weight) were used. This research used a Randomized Complete Block Design consisted of 15 treatments with 3 replications. Control group consisted of zero control with the administration of distilled water, positive control with the administration of Stimuno (drugs that can increase the stamina) and negative control with the administration of CMC-Na 1%. The potion used consisted of three *Obat Pahit* potions namely Kalan, SP4 and Linau with four different serial dosages. The treatment was performed orally twice in a day for 11 days using a disposable syringe. Chloroform was used to kill all white rats On the 11th day. Dosage determination was based on Laurance and Bacharach (1964) by converting common dosage consume by a human (100 ml) with white rats conversion factor (0.018) and it was obtained conversion dosage of 1.8 ml/200g weight that had been set as two route dosage. The given doses were 0.9 ml/200g bw (P1), 1.8 ml/200g bw (P3) and 2.7 ml/200g bw (P2), respectively.

Preparations and Staining of Paraffin Sections

Sacrificing the animal was according to approved euthanasia technique. Tissues were excised and fixed in 10% formalin at room temperatures in no longer than 24 hours. Then the tissues were rinsed with running tap water for an hour. The next following step was dehydration using graded alcohol (ethanol) 30%, 50%, 70%, 90% for 45 minutes of each concentration. Then, the tissues were cleared using xylol I and II for 45 minutes and followed with the infiltration process using the paraffin. The next process was deparaffinization using xylol I and II, graded alcohol 96%, 80% and 70% for two minutes of each. The staining process was using Hematoxylin-Eosin (HE). Slides were put into Hematoxylin for 8 minutes and were rinsed using water three times and then put into eosin for 4 minutes. The last step was put into graded alcohol 70%, 80%, 95% respectively for two minutes and xylol I and II for 3 minutes of each.

Statistical Analysis

Kidney histology was observed with macroscopic and microscopic observation and

was analyzed descriptively. Data were analyzed using ANOVA (analysis of variance) followed by Duncan Multiple Range Test (DMRT). If values were less than 0.05, it was considered as significant (Mattjik & Sumertajaya, 2002). Scoring parameters of tubules and glomerulus were based on Baldatina (2008) (Table 1).

RESULTS AND DISCUSSION

Observation of Macroscopic Structure

Macroscopic observation performed including color and shape (Figure 1). The observation of color and shape in treatment showed that the shape of the kidney resembles a bean and the color is brownish red. In the upper side, the shape looks convex while the underside is concave.

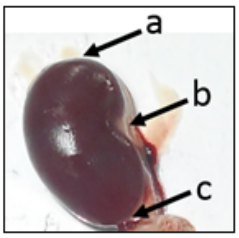

Figure 1. Macroscopic structure of white rats kidney; a. Anterior, b. Renal hilum, c. Posterior.

There are three kinds of *Obat Pahit* potion (Kalan, SP4, Linau) applied with four different doses showing that the kidney is in a normal condition which characterized by the shape of a bean, brownish red. According to Snell (2006), the characters indicate that the kidneys are in good condition.

Observation of Microscopic Structure

Based on microscopic observation, it was seen that there were several damages in preparations of the kidney. There is a different percentage of the damaged glomerulus in control (zero, positive and negative control) and three serial dosages. Percentage and scoring of damaged glomeruli is presented in Table 2.

In this research, the observation towards glomerulus was by counting abnormal glomerulus. The highest damaged glomerulus is in the negative control, approximately 5.06±0.63. Based on the data, the results of zero control should be 0% (Table 2). However, there are several factors affecting the kidney such as the earlier condition of the kidney before treatment and the physiology of the kidney (Aboonabi et al., 2014). In addition, stress condition experienced by white rats can increase cytosol enzyme that is able to affect the kidney and heart (Sanchez et al., 2002).

Based on the ANOVA test, there is an expansion of glomerulus obtained with P-value about 0.000. P value that is $P<0.05$ means that there is a significant percentage of histology damaged in every treatment. Based on the DMRT test, there is a significant difference between Kalan potion at D1 until D4 and at SP4 at D1 and D2 with the positive control (stimuno) and negative control (CMC-Na 1%). There is an insignificant difference between SP4 potion at D3 and D4 as well as Linau potion at D1 until D4. The Kalan potion used in different doses causes a different percentage of cell damage on glomerulus. Kalan potion in dose 2 is the safest from the other doses. While S4 and Linau potion in dose 3 are the safest from the other doses. Based on scoring the glomerulus cell of white rats show the rates of damage cell at positive control, zero control, negative control and all treatments at 0-25%. The rates of damage glomerulus underwent in all groups (control and treatment) is zero which means that the damage rates of each control group are still in normal condition.

Table 1. Scoring parameters of tubules and glomerulus

Score Range (%)	Change of HP (Histophatologic)
0 < 25	Expansion of bowman capsules, necrosis, tubules expansion, fatty tubules, necrosis and congestion
25-50	Expansion of bowman capsules, necrosis, tubules expansion, fatty tubules, necrosis and congestion
50-75	Expansion of bowman capsules, necrosis, tubules expansion, fatty tubules, necrosis and congestion
> 75	Expansion of bowman capsules, necrosis, tubules expansion, fatty tubules, necrosis and congestion

Note: Scoring number 0: Normal, 1: Light , 2: Medium , 3: Heavy; Source: Baldatina (2008) Percentage of damage cell (%) = Damage cell x 100% All cell

Table 2. Percentage and scoring of damaged glomeruli

Potion		Damage Percentation (%)	Damage Scoring
Control	Distilled water (K0)	1.690±0.28^{ab}	0
	Stimuno (K+)	1.133±0.15^{a}	0
	CMC Na 1% (K-)	5.060±0.63^{d}	0
Kalan	D1 (0.9 ml)	2.420±0.55^{bc}	0
	D2 (1.8 ml)	2.393±0.58^{bc}	0
	D3 (2.7 ml)	2.823±0.39^{bc}	0
	D4 (3.6 ml)	2.987±0.50^{c}	0
SP4	D1 (0.9 ml)	2.600±1.13^{bc}	0
	D2 (1.8 ml)	2.417±0.22^{bc}	0
	D3 (2.7 ml)	2.040±0.55^{abc}	0
	D4 (3.6 ml)	2.143±0.69^{abc}	0
Linau	D1 (0.9 ml)	2.147±0.69^{abc}	0
	D2 (1.8 ml)	2.157±0.30^{abc}	0
	D3 (2.7 ml)	2.037±0.28^{abc}	0
	D4 (3.6 ml)	2.153±0.76^{abc}	0

Note: Numbers that are followed by the same alphabet are not significanly different ($p<0.05$). Scoring results value 0: normal, 1: light, 2: medium, 3: heavy. D1: dosage 1, D2: dosage 2, D3: dosage 3, and D: dosage 4.

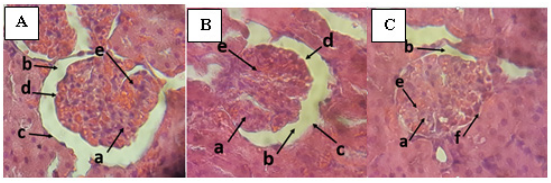

Figure 2. Histology Structure of *Rattus norvegicus*. Control group. *Hematoxylin-Eosin* staining. Magnification of 640x. A: Control 0, B: positive control, C: Negative control. a: Glomelurus, b: Bowman capsules, c: Pars parietal epithelium, d: Pars visceralis epithelium, e: picnosis core

The positive and zero control shows a few damage cells found in glomerulus namely pycnosis core in small number. Based on the histology observation, positive and zero control were in normal condition with a round nucleus. In addition, the pars visceral epithelium and pars parietal epithelium seems clear and the Bowman capsules covering glomerulus Figure 2.A; B). Whereas in the negative control, it was found many damage cells that the nucleus is having pycnosis with the expansion of glomerulus. This is indicated by the narrowing of Bowman capsule so that the pars visceral epithelium and pars parietal epithelium, so they cannot be distinguished anymore (Figure 2.C).

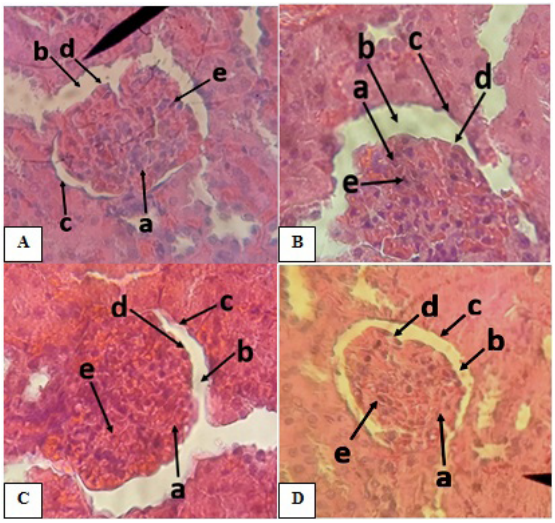

Figure 3. Histology Structure of *Rattus norvegicus*. *Hematoxylin-Eosin*. Staining Magnification 640x. A: Dosage 1, B: Dosage 2, C: Dosage 3, D: Dosage 4.. a: Glomelurus, b: Bowman capsules, c: Pars parietal epithelium, d: Pars visceralis epithelium, e: picnosis core

Figure 3, 4, and 5 show the histology of kidney with Kalan, SP4 and Linau potion treatment. Given potion with three different dosage serial towards the kidney found that these give adverse effect towards kidney histology namely pycnosis. However, this damage did not affect the glomerulus structure. Histology structure of white rat kidney is still in normal condition. Bow-

man capsule is still covering the whole Bowman chamber. While in the histology of white rat liver, it is found small damage including hydropic degeneration, Lipid degeneration as well as necrosis. However, this damages still in low percentage. Based on scoring result, both of these organs are still in normal condition and the percentage less than 25%.

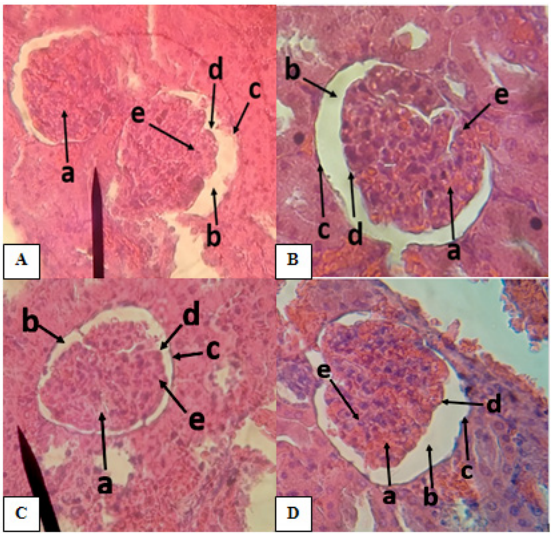

Figure 4. Histology structure of *Rattus norvegicus* kidney. SP4. Hematoxylin-Eosin Staining. Magnification of 640x. A: Dosage 1, B: Dosage 2, C: Dosage 3, D: Dosage 4. a: Glomelurus, b: Bowman capsules, c: Pars parietal epithelium, d: Pars visceralis epithelium, e: picnosis core

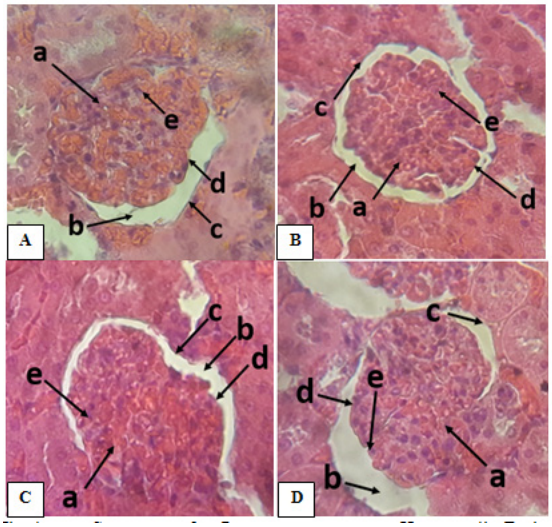

Figure 5. Histology Stucture of *Rattus norvegicus*. Hematoxylin-Eosin Staining. Magnification 640x. A: Dosage 1, B: Dosage 2, C: Dosage3, D: Dosage 4. a: Glomelurus, b: Bowman capsules, c: Pars parietal epithelium, d: Pars visceralis epithelium, e: picnosis core

The damaged cell found in slides is necrosis. This condition shows that the nucleus is denser and darker. However, this damage did not affect the glomerulus structure. In addition, Bowman capsule covering the glomerulus and borders between pars visceral epithelium with pars parietal epithelium can be seen clearly. Normal glomerulus marked by whole Bowman capsule covering it and shaped like a bowl (Monfared, 2013). Normal glomerulus has a polyhedral shape, rounded nucleus, clear cytoplasm and the epithelium can be seen clearly (Septiana & Kurniati, 2009).

Some forms of damage commonly occur in organs include nephrotoxicity, neurotoxicity, hepatotoxicity, immunotoxicity, and cardiotoxicity (Dian, 2010). Toxic substances that cause damage to the kidneys show microscopic images of degenerative symptoms that can cause another damage of necrosis (Susilo, 2014). Necrosis is a cell death with loss nucleus characteristics, increased eosinophils, the cells that more gloss (glossy) than normal cells and can be triggered by external factors (Kumar et al., 2010). Whereas the damage of hydropic degeneration is characterized by swelling and appearing cells compared to normal cells (Suhita et al. 2013).

Based on the research by Assiam et al. (2014), construction that occurs in the Bowman capsule is caused by glomerular enlargement which is characterized by increased glomerular volume. The damage found in every treatment still in a small percentage and in normal limits. This small percentage did not affect the shape of the glomerulus. The damage of Glomerulus will interfere with the production and will filtrate control function. Glomerulus has a function as a blood filter that consisted of blood capillary. The filtration and enhancement of capillary permeability cause plasma protein leakage and red blood cell, this leakage cause destruction in glomerulus filtration membrane and expansion as well as edema in Bowman capsule. Constriction in Bowman capsule occurs can cause expansion 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, glomerulus is vulnerable to be exposed to low circulation toxin compared to the other tissue (Tomino, 2014).

Given extract of *Obat Pahit* potion with three different dosage serial towards the kidney found that these give adverse effect towards kidney histology namely pycnosis. However, this damage did not affect the glomerulus structure. Histology structure of white rat kidney is still in normal condition bowman capsule still covering

the whole Bowman chamber. The results of this study showed that the absence of toxic effect of *Obat Pahit* Lingga Malay Ethnic on histology structure of kidney white rat (*Rattus norvegicus*), and suggest that consumption of *Obat Pahit* extract by human will not cause toxicity effect on the kidney function.

CONCLUSIONS

As a conclusion, the results of the present study showed the absence of toxic effect of *Obat Pahit* potion (Kalan, SP4, Linau) with 4 different dosages, on kidney of white rat (Rattus norvegicus), and suggest that popular consumption of *Obat Pahit* potion by human will not cause toxicity effect on the kidney function.

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