

## Original article

# Effect of propolis on maternal toxicity

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**Keywords:**

Indonesian propolis;  
Pregnancy; Stingless bee

**ABSTRACT**

Propolis is a natural product that has been extensively used to treat several diseases. However, the safety evaluation of propolis on maternal toxicity has not been reported. This study was aimed to analyze the effect of propolis administration during pregnancy on the function of maternal liver and kidney. A total of 25 pregnant mice were equally divided into five groups ( $n \geq 5$ ): control group (Tween 80 1%); low-dose (380 mg/kg b.w) and high-dose (1400 mg/kg b.w) of ethanol extract of propolis from South Sulawesi; low-dose (380 mg/kg b.w) and high-dose (1400 mg/kg b.w) of water extract of propolis from Banten. Propolis was administered for 18 days of gestation. Maternal weight, serum ALT, AST, urea, creatinine and the histopathological changes of liver and kidney were analyzed to determine maternal liver and kidney function. The result showed that neither ethanol extract of propolis from South Sulawesi nor water extract of propolis from Banten at low and high dose decreased maternal weight gain. No significant alteration was found in the serum ALT, AST, urea and creatinine between all groups. In addition, the present study found no specific histopathological changes of liver and kidney in all groups. This study concludes that propolis administration during pregnancy is relatively safe for mothers.

**1. INTRODUCTION**

Propolis is a resinous product collected by bees from various plants and used to construct their hives and to protect the colony<sup>1,2</sup>. This substance has also an important role in bee immunity<sup>3</sup>. In the other hand, propolis has been used as a human medicinal product to treat various diseases due to its antibacterial, antiviral, antifungal, antiseptic, anti-inflammatory, and antioxidant properties<sup>4</sup>. In addition, propolis indicates to possess antidiabetic and antihyperlipidemic properties<sup>5,6</sup>. Therefore, propolis has been proposed as a new drug in the future<sup>7</sup>.

Propolis appears to be a relatively safe for consumption. Burdock<sup>8</sup> found that propolis did not show any adverse effect at a dose of 1400 mg/kg. In addition, acute and sub-chronic toxicity studies showed that propolis did not alter any physiological function in experimental animals<sup>9,10</sup>. An observational study concludes that propolis is safe for human use, yet the allergy reaction may appear<sup>11</sup>. Although the toxicity study of propolis has been extensively conducted, the information regarding the effect of propolis use during pregnancy is still limited. Indeed, several metabolism changes happen during pregnancy and might cause an alteration in the absorption, distribution, and elimination of drugs. These changes could happen due to plasma volume expansion, decreased

albumin concentration, increased glomerular filtration, changes in drug metabolizing enzymes and gastrointestinal function<sup>12</sup>.

The consumers of the natural products are mostly women<sup>13</sup>. Pregnant women use the natural products for some reasons including its health benefits and potentially no side effect<sup>14</sup>. However, several natural products, including green tea, fenugreek, asparagus, ginseng, ginkgo biloba, and ginger were reported to possess an embryo toxicity effect<sup>15-18</sup>. Moreover, the safety evaluation of natural product in pregnancy has been an important question<sup>19</sup>. Our last publication showed that propolis at daily dose (380 mg/kg) did not appear to inhibit fetal development<sup>20</sup>. However, the information regarding its effect on maternal health had not been reported. Therefore, the present paper wanted to report the effect of propolis administration during pregnancy on maternal toxicity.

## 2. MATERIALS AND METHODS

### 2.1. Preparation of propolis extracts

Propolis samples were obtained from Banten and South Sulawesi province, Indonesia. Propolis from Banten was manufactured by *Tetragonula laevicep*, while that from South Sulawesi was manufactured by *Tetragonula irioi*. These samples were extracted by water and 70% ethanol, respectively. Thus, there were two kinds of tested propolis, namely water extract of propolis from Banten (WEB) and ethanol extract of propolis from South Sulawesi (EES). Propolis from Banten was *Mangifera*-type propolis, while propolis from South Sulawesi was *Calophyllum*-type propolis. We tested these samples because both samples possessed high antiemetic activity and are probably used for nausea and vomiting treatment during pregnancy<sup>21</sup>.

Propolis was prepared using ultrasound-assisted extraction. Propolis was cut into small pieces then dissolved in the solvent (water or 70% ethanol) with ratio of 1:10. Ultrasound was applied for 4 h. Subsequently, the mixture was filtered and evaporated to obtain the dry extract. Samples were stored at -20°C until used<sup>21</sup>.

### 2.2. Experimental animals

Mice in the present study were obtained from the Tropical Biopharmaca Research Centre, Bogor. Mice aged 8-10 weeks and weighed 25-30 g. All animals were caged under the standard condition and fed with pellet diet and water *ad libitum*. The cages were cleaned twice a week. Estrous cycle

was checked using the method of Byers *et al.*<sup>22</sup> to determine the best time for breeding.

Female mice at proestrous and estrous stage were mated with the male on a one-to-one basis in separate cages. The day 0 of pregnancy was determined by the presence of a vaginal plug. A total of 25 pregnant mice were randomly divided into five groups (n = 5), including control group (1% Tween 80), low-dose of WEB group (380 mg/kg), high-dose of WEB group (1400 mg/kg), low-dose of EES group (380 mg/kg), high-dose of EES group (1400 mg/kg). The samples were administered in a dose of 5 ml/kg. The administrations were done from 0 until 18 days of gestation. The weight of pregnant dams was measured once per two days and they were sacrificed at day 18 of gestation. All animal experiments have been approved by the Animal Care and Use Committee, IPB University (No. 64-2017 IPB).

### 2.3. Serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), urea, creatinine analysis

Blood was taken by intracardiac injection. Serum was obtained after centrifugation at 1000 rpm for 15 min and used to analyze ALT, AST, creatinine and urea concentrations using the colorimetric technique<sup>23</sup>.

### 2.4. Histopathological analysis

Liver and kidney were harvested after laparotomy to examine the histopathological changes. The organs were fixed in 10% neutral buffer formalin. After trimming, washing, dehydrating in alcohol, clearing in xylene and embedding in paraffin, the tissues were sectioned by microtome at 4-6 µm and stained with hematoxylin and eosin<sup>24</sup>. Liver and kidney histopathological scoring system used Roenigk classification and semiquantitative method, respectively<sup>25,26</sup>.

### 2.5. Statistical Analysis

Data were presented as mean ± standard deviation. The differences in maternal weight gain, organs weight, and biochemical profiles of blood were determined using ANOVA with Duncan's post-hoc multiple range test. Meanwhile, ANOVA with a post-hoc Bonferroni correction was applied to analyze the differences of histopathological score between the groups. The significant level was considered at  $p < 0.05$ .

### 3. RESULTS

No pregnant mice died during the administration. The present study showed that propolis did not appear to decrease maternal body

weight gain ( $p > 0.05$ ). Furthermore, the gravid uterine-corrected weight gain did not differ between the groups (Table 1). This implies that the difference in maternal weight gain was not associated with the fetal weight differences.

**Table 1.** Maternal weight gain.

Groups	Weight gain (g)	Gravid uterus weight (g)	Corrected weight gain (g)
Control	18.77 ± 2.04	14.99 ± 4.56	4.02 ± 1.00
Low-dose of EES	19.02 ± 0.86	15.64 ± 1.08	3.38 ± 0.97
High-dose of EES	18.99 ± 1.45	13.43 ± 2.79	6.67 ± 1.35
Low-dose of WEB	20.66 ± 2.74	16.94 ± 3.39	5.77 ± 0.54
High-dose of WEB	18.98 ± 2.67	14.86 ± 4.25	4.12 ± 1.07

Corrected weight gain = [(weight at day 18 - gravid uterus weight) - weight at day 0]

EES : ethanol extract of propolis from South Sulawesi

WEB : water extract of propolis from Banten

**Table 2.** Weight of organs.

Groups	Weight of liver (g)	Relative weight of liver (%)	Weight of kidney (g)	Relative weight of kidney (%)
Control	2.55 ± 0.38	7.45 ± 1.48	0.22 ± 0.05	0.65 ± 0.06
Low-dose of EES	2.42 ± 0.34	7.32 ± 1.05	0.20 ± 0.02	0.63 ± 0.04
High-dose of EES	2.63 ± 0.64	7.85 ± 0.89	0.20 ± 0.03	0.60 ± 0.04
Low-dose of WEB	2.28 ± 0.23	6.59 ± 0.27	0.20 ± 0.02	0.59 ± 0.04
High-dose of WEB	2.46 ± 0.37	7.82 ± 1.02	0.22 ± 0.04	0.70 ± 0.10 <sup>a</sup>

Relative weight of organ: [weight of organ (g)/(maternal weight (g) - gravid uterus weight (g))] x 100%

<sup>a</sup>: the difference between the intervention groups was significant but not with the control group

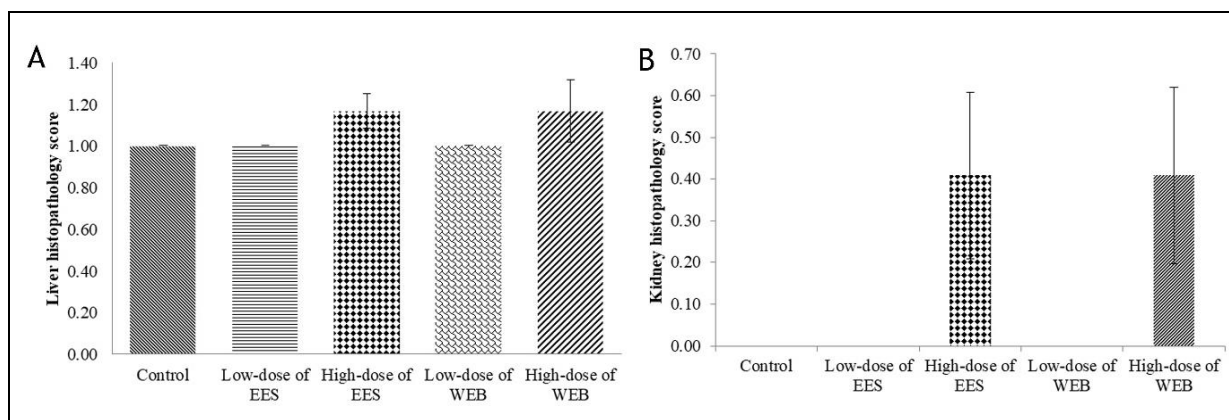
EES : ethanol extract of propolis from South Sulawesi

WEB : water extract of propolis from Banten

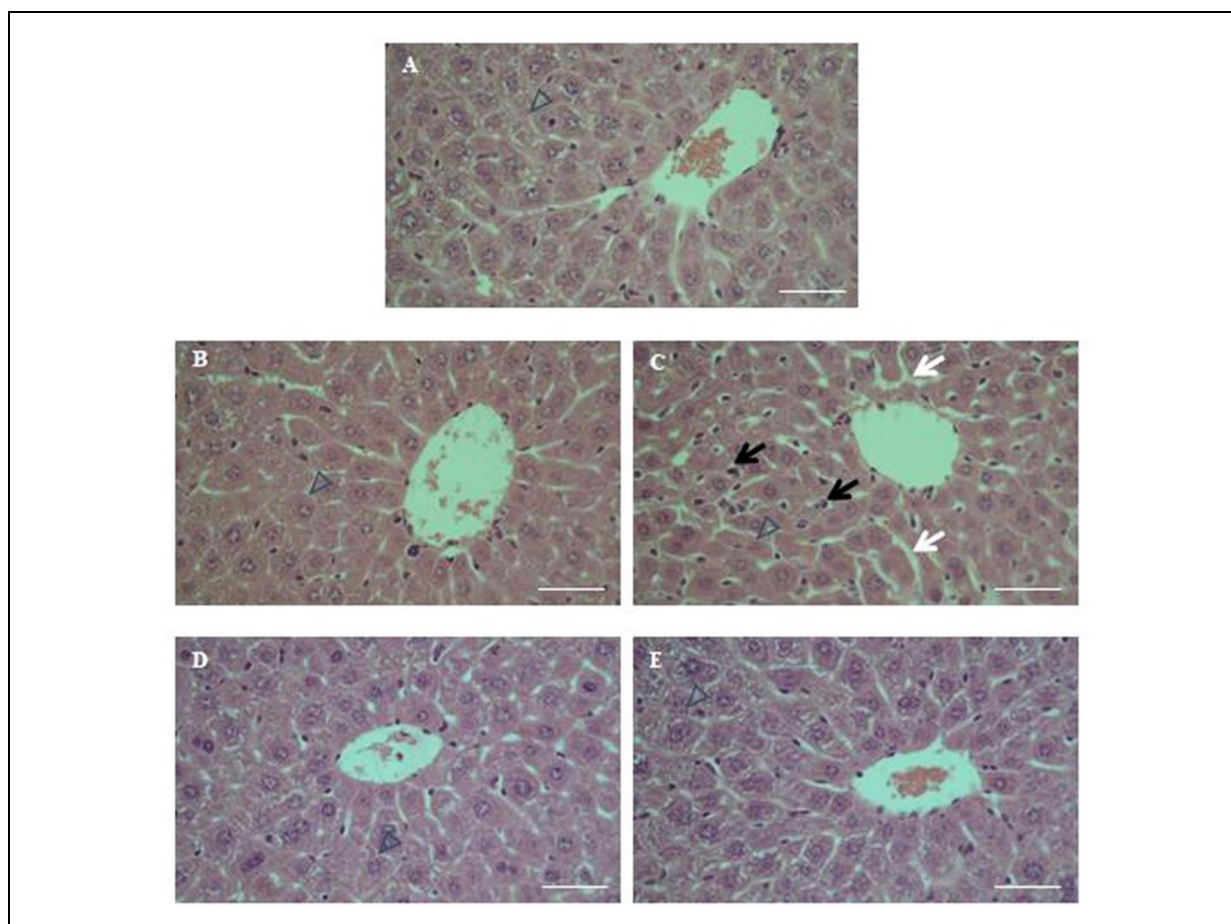
There were no remarkable differences in the organ weight and serum parameters. No significant differences were observed either in maternal weight-corrected (relative weight) or uncorrected liver and kidney weight between the control and intervention groups ( $p > 0.05$ ). However, the significant difference in the

corrected kidney weight (relative weight) was observed at high dose of WEB group in comparison to the other intervention groups (Table 2). This study also found that serum ALT, AST, urea, and creatinine were statistically not significant between the groups (Table 3).

Propolis administration generally did not



**Figure 1.** Liver and kidney histopathological score of pregnant mice after propolis administration for 18 days of gestation. EES: ethanol extract of propolis from South Sulawesi; WEB: water extract of propolis from Banten. Liver histopathological scoring system used scale from 1 to 4 (1 = mild or none; 2 = moderate or severe; 3a = mild fibrosis, portal fibrotic septa, extension into the lobuli and portal tract enlargement; 3b = moderate or severe fibrosis; 4 = cirrhosis, regenerating noduli and bridging of the portal tracts), while kidney scoring system used scale from 0 to 5 (0 = normal; 1 = < 10% injury, minimal; 2 = 10 -25%, mild; 3 = 26-50%, moderate; 4 = 51-75%, severe; 5 = >75%, very severe). ANOVA with a post-hoc Bonferroni correction did not find any differences in either liver or kidney histopathological score between the groups ( $p > 0.05$ ).



**Figure 2.** Maternal liver section after administration of propolis for 18 days of gestation. A: control; B: low-dose of ethanol extract of propolis from South Sulawesi; C: high-dose of ethanol extract of propolis from South Sulawesi; D: low-dose of water extract of propolis from Banten; E: high-dose of water extract of propolis from Banten; bar = 50  $\mu$ m; magnification = 40x. Mild cell degeneration (blue triangle) was found in all groups. Sinusoidal dilatation (white arrow) and Kupffer cells (black arrow) infiltration were found in high-dose of ethanol extract of propolis from South Sulawesi group.

appear to alter liver and kidney tissue architecture. The histopathology score of both tissues showed no significant difference (Figure 1). However, sinusoidal dilation and Kupffer cells infiltration were found in the high-dose of EES group. In addition, mild cell degeneration tissue was found in all groups (Figure 2). Indeed, no specific histopathological change was also found in kidney tissue. Nevertheless, we found the expansion of Bowman's space, mild protein accumulation on glomerulus, and hyperplasia of mesangial cells either in high-dose of EES or high-dose of WES group (Figure 3).

#### 4. DISCUSSION

Body weight is one of the main indicators of maternal toxicity<sup>27</sup>. No inhibition of maternal weight gain probably indicated that propolis did not cause maternal toxicity. The findings were supported by previous toxicological studies reported that propolis did

not alter weight gain<sup>9,10</sup>. Maternal weight gain is a gross parameter to examine the fetal development or overall health status of pregnant mother. However, the correlation between maternal and developmental toxicity is not always linear. Therefore, maternal toxicity could not be used as the single indicator for developmental toxicity and should be handled case by case basis<sup>27</sup>.

We conducted the further examination throughout organ investigation. Liver and kidney weight are the two most common female organs used in the toxicity studies. The organ weight changes may indicate the pathological implication<sup>28</sup>. The previous study also found no alteration in those two organs after propolis administration<sup>29</sup>. In contrast, Mohammadzadeh *et al.*<sup>9</sup> found an increase in relative weight of the liver after sub-chronic administration of propolis. However, they speculated that the vehicle solution (30% ethanol) was the reason for those changes.



**Table 3.** Serum ALT, AST, urea, and creatinine concentration.

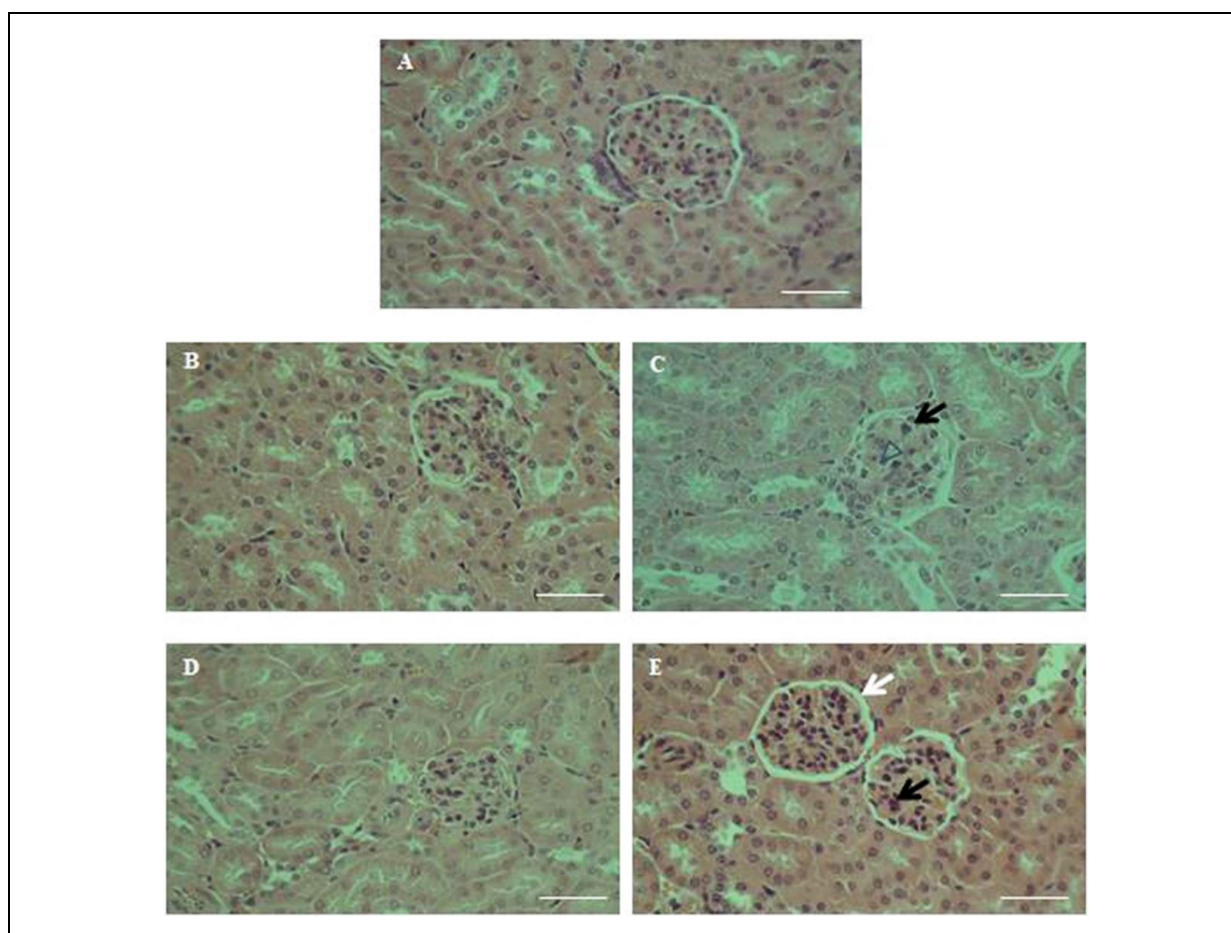
Groups	ALT (U/L)	AST (U/L)	Urea (mg/dl)	Creatinine (mg/dl)
Control	157.65 ± 32.77	86.70 ± 32.71	0.27 ± 0.10	32.52 ± 7.47
Low-dose of EES	145.78 ± 60.89	54.36 ± 19.96	0.31 ± 0.14	28.56 ± 6.59
High-dose of EES	134.74 ± 90.73	89.90 ± 39.89	0.24 ± 0.13	32.00 ± 6.41
Low-dose of WEB	139.48 ± 46.78	54.08 ± 25.84	0.45 ± 0.18	30.78 ± 4.54
High-dose of WEB	142.36 ± 22.12	73.05 ± 27.29	0.45 ± 0.31	31.88 ± 7.83

EES : ethanol extract of propolis from South Sulawesi

WEB : water extract of propolis from Banten

Liver and kidney function can be observed by measuring the organ-specific parameters. For instance, ALT, AST, ureum and creatinine are good indicators to assess liver and kidney functions, respectively<sup>30,31</sup>. Moreover, an increase in the concentration would be the indication of organ dysfunction. The present study showed that propolis did not appear to compromise the liver and kidney function. This means the metabolism of pregnant dams seemed to work well even with propolis administration. This confirmed our maternal weight gain data. The

previous studies also found no significant changes in serum ALT, AST, urea, and creatinine concentrations after sub-chronic administration of propolis<sup>9,10</sup>. In contrast, one study showed that the serum AST and potassium ions were raised after administration of methanol extract of propolis<sup>32</sup>. It was probably due to the extraction method that only produced the concentrated extract. Thus, the methanol residue might cause the alteration. Methanol has been known since 1879 to possess toxic effect, including acidosis and brain damage<sup>33</sup>.



**Figure 3.** Maternal kidney section after administration of propolis for 18 days of gestation. A: control; B: low-dose of ethanol extract of propolis from South Sulawesi; C: high-dose of ethanol extract of propolis from South Sulawesi; D: low-dose of water extract of propolis from Banten; E: high-dose of water extract of propolis from Banten, bar = 50  $\mu$ m; magnification = 40x. Mesangial cell hyperplasia (black arrow) and protein accumulation in glomerulus (blue triangle) were found in high-dose of ethanol extract of propolis from South Sulawesi group, while mesangial cell hyperplasia (black arrow) and Bowman's space expansion (white arrow) were found in high-dose of water extract of propolis from Banten group.

There was an interesting result with regard to serum ALT and AST concentration. In spite of not statistically different, propolis administrations tend to reduce those concentrations. It is commonly known that laboratory routine procedures may cause animal stress and lead to liver injury<sup>34</sup>. The hepatoprotective activity of propolis might be the reason for our findings. Previous study found that propolis ameliorated CCl<sub>4</sub>-induced hepatotoxicity<sup>35</sup>. The anti-inflammatory, antioxidant, antihyperlipidemic and antihypertrophic activities of propolis were probably responsible to this property. Furthermore, propolis also found to possess renal protective effect<sup>36</sup>. However, we could not show the tendency through our serum parameter data.

Histopathologically, propolis administration during pregnancy did not cause any specific changes in the liver and kidney tissue. Although we found some non-specific changes, maternal metabolism changes during pregnancy might contribute. Mild cell degeneration and infiltration are common even in the control group<sup>37</sup>. Leukocytes, monocytes and lymphocytes increase during pregnancy as a response to the maternal oxidative stress<sup>38</sup>. In addition, an increase in fluid volume, systemic vasodilatation, and fluid retention response to pregnancy might cause hydronephrosis, proteinuria and glucoseuria<sup>12,39,40</sup>.

## 5. CONCLUSIONS

Propolis administration during pregnancy appears not to cause maternal toxicity. Serum ALT, AST, urea and creatinine did not differ between the groups. The histopathological examinations did not show any specific changes.

## 6. ACKNOWLEDGMENTS

We wish to thank to the Ministry of Research, Technology and Higher Education of the Republic of Indonesia for funding this research.

### Conflict of interest

Authors declare no conflict of interest.

### Funding

The Ministry of Research, Technology and Higher Education of the Republic of Indonesia under the scheme Master Program of Education Leading to Doctoral Degree for Excellent Graduates (PMDSU).

### Ethics approval

All animal experiments have been approved by the

Animal Care and Use Committee, IPB University (No. 64-2017 IPB)

### Article info:

Received April 16, 2020

Received in revised form July 3, 2020

Accepted July 27, 2020

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