The Effect of *Mimosa pudica* Root Extract to Histopathological Representation of *Rattus norvegicus* Liver Injected with *Naja sputatrix* Venom

Pengaruh Ekstrak Akar *Mimosa pudica* terhadap Gambaran Histopatologi Liver *Rattus norvegicus* yang Diinjeksi dengan Bisa *Naja sputatrix*

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**Abstrak**

Tujuan dari penelitian ini adalah untuk mengetahui pengaruh *Mimosa pudica* akar ekstrak memberikan representasi histopatologi *Rattus norvegicus* hati disuntikkan oleh *Naja sputatrix* racun. Tiga puluh tikus dibagi menjadi lima kelompok yang berisi enam tikus setiap. Mereka adalah dua kelompok kontrol dan tiga kelompok perlakuan, yang diberikan 250, 500, dan 1000 mg / kg BB dari *Mimosa* ekstrak pudica akar secara lisan. Selama tujuh hari pertama masing-masing kelompok diberi aquadest 0,1ml. Pada hari ke-8, pengobatan dimulai dengan menyuntikkan *Naja sputatrix* LD50 (0,13 uL / gram BW) IM di gluteus musculus dan kemudian dilanjutkan dengan pemberian *Mimosa* ekstrak pudica akar secara lisan untuk kelompok perlakuan. Pada hari yang sama, 4 jam setelah pengobatan terakhir, evaluasi histopatologi dilakukan untuk mencetak kerusakan hati berdasarkan hepatosit degenerasi dan nekrosis menggunakan HE noda dengan 400x pembesaran. Data skor dianalisis menggunakan Kruskal Wallis dan Mann-Whitney. Hasil penelitian menunjukkan 250 mg / kg BB, 500 mg / kg BB dan 1000 mg / kg BB dosis *Mimosa* ekstrak pudica akar dapat mengurangi kerusakan hati berdasarkan kemacetan hati, hepatosit degenerasi dan nekrosis di Tikus (Rattus norvegicus) yang disebabkan oleh *Naja sputatrix* racun dan memberi perbedaan yang signifikan (p <0,05) antara kelompok perlakuan. *Naja sputatrix* racun dapat menyebabkan denyut jantung dan pengurangan tingkat pernapasan saat *Mimosa pudica* dapat meningkatkan denyut jantung tikus dan tingkat pernapasan.

**Keywords**: *Mimosa pudica*, snake venom, liver damage

**Background of Research**

Venomous snakes bite and insect stings are bio-toxin case that the most frequently encountered (Mount, 1989). Not only human cases of snakes bite, it can also occur on pets or other domesticated animals. Among domesticated animals, dogs are the most frequently bitten and killed by snakes (Osweiler, 1996). Unlike the dog, snakes bite cases in cats is rarely encountered because the cat reflexes to avoid snake bites better.
Snake bite cases in Asia mostly caused by *Naja sp* (cobra) and *Bungarus sp* (Valenta, 2010). Among the species of cobra, *Naja sputatrix* is the species which is the most often encountered in Indonesia, especially in Java. *Naja sputatrix* can be encountered in little humid places, swampy areas, paddy fields, and forests in the southern part of Indonesia such as Java, Bali, Lombok, Sumbawa, Komodo and Flores (Valenta, 2010). The spread of this species in large areas in Indonesia led to the interactions that occur between these species by humans is often the case, this may increase the incidence of venomous snake bites are caused by species *Naja sputatrix*.

Giving antivenin are the main treatment that is given to victims of snake bites (Shashidharamurthy et al., 2008). The treatment is considered the most effective to venomous snake bite cases. Anti-venom serum or commonly called antivein or antivenom is a biological product that is used as a treatment for venomous snake bites (Offerman et al., 2002). The use of anti-venom serum can reduce the risk of complications and decrease the mortality rates of snake bite victims (Soh and Rutherford, 2006). However, the use of anti-venom serum can cause adverse side effects for the victim (Premawardhena et al., 1999).

The traditionally treatment of snake bites that using many herbs have been studied, including *Mimosa pudica*, incubation of *Mimosa pudica* root extract with *Naja kaouthia* venom can neutralize 2LD50 of *Naja kaouthia* venom with 100% success rate (Vejayan et al., 2007).

**Material of the Research**

This research was conducted at the Laboratory Animals Model at Veterinary Medicine of Airlangga University for the treatment of experimental animals. Making of *Mimosa pudica* root extract was done at Tropical Disease Centre. *Rattus norvegicus* liver histopathological representation was observed at the Laboratory of Pathology Veterinary, at Veterinary Medicine of Airlangga University. Implementation of this research was carried out in May 2016.

The methods used in this research are animal cages made from plastic, wire mesh to cover the cage, the feeding and drinking place, and liters. *Mimosa pudica* root extract with water extraction method was done by using scissors, trays, measuring cups, distilled water, a spatula (stirrer). Making the histopathological representation include: surgical scissors, sterile scalpel & forceps, object & cover glass, trays, cartons, tongs, organ pot, Bunsen fire, paper labels for preparations histopathology, section board, syringe 1cc, syringe 3cc, and light microscope.

Materials used in this study are *Mimosa pudica* root extract from Kebun Raya Purwodadi, *Rattus norvegicus* liver, *Naja sputatrix* venom from Surabaya, pellet feed, water, husk to the base enclosure, and aquadest.

**Methods of the Research**

Rats were with a number of 30 head reared in Laboratory Animals Model at Faculty of Medicine Universitas Airlangga, randomized by means of a lottery and was divided into five groups, and then adapted to the environment for one week. In the second week of experiment, animals were injected with *Naja sputatrix* venom, 5 minutes later treated with *Mimosa pudica* root extract. Heart rate and respiratory rate were observed before venom injection, 1 minute after venom injection and 4 hours after *Mimosa pudica* root extract treatment. 8 hours after *Mimosa pudica* root extract treatment, rats were euthanized by cervical dislocation. Feeding and drinking was ad libitum.

*Mimosa pudica* Root Extraction Method

*Mimosa pudica* root extraction using water extraction method (normal water extraction), as Mahanta and Mukherjee...
did. The fresh Mimosa pudica root left in the open air, dried under the sun, and pulverized to a powder. 4 grams of the powder was added into beaker glass and was added 200 ml of distilled water, then stirred for about 3 hours at room temperature. The extract was filtered using muslin cloth then concentrated at 40° C. Then it was placed in freeze dry equipment.

Liver Damage Scoring
The grading technique used in this research was a modification of Knodell (1981). Forms of lesions observed and the score of each lesions are for degeneration: 0: none, 1: degeneration change in <1/3 of the field of view, 3: degeneration change in 1/3-2/3 of the field of view, 4: degeneration change in >2/3 of the field of view; for necrosis: 0: none, 1: necrosis in <25% of the field of view, 3: necrosis in 25-50% of the field of view, 4: necrosis on >50% of the field of view, 5: necrosis in 25-50% of the field of view with bridging necrosis, 6: necrosis in >50% of the field of view with bridging necrosis, 10: multilobular necrosis; for congestion: 0: none, 1: Mild (congestion <25% of whole field), 2: Moderate (congestion 25-50% of whole field), 3: Severe (congestion >50% of whole field).

Research Result and Discussion
Histopathology examination of white rat (Rattus norvegicus) liver injected with Mimosa pudica root extract post Naja sputatrix venom injection was done microscopically by Hematoxylin Eosin (HE) staining and 400 (40x) magnification. The variables observed in this observation were hepar that undergo congestion, degeneration and necrosis.

Congestion
Analytic result of hepatic congestion observation is shown in the table 1.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>X ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative control (C-)</td>
<td>7.167 ±</td>
</tr>
<tr>
<td>Positive control (C+)</td>
<td>5.680 ±</td>
</tr>
<tr>
<td>Mimosa pudica 250mg/kg BW (T1)</td>
<td>24.500 ±</td>
</tr>
<tr>
<td>Mimosa pudica 500mg/kg BW (T2)</td>
<td>4.899 ±</td>
</tr>
<tr>
<td>Mimosa pudica 1000mg/kg BW (T3)</td>
<td>16.500 ±</td>
</tr>
<tr>
<td>*The different superscript show there is significant difference between treatment groups (p&lt;0.05).</td>
<td></td>
</tr>
</tbody>
</table>

Statistical analysis of hepatocyte congestion in Negative control (C-) shows significant difference to other treatment groups. Same result shown in Positive control (C+) which shows significant difference to other treatment groups. Mimosa pudica root extract 250 mg/kg BW (T1) shows significant difference to both Mimosa pudica root extract 500 mg/kg BW (T2) and Mimosa pudica root extract 1000 mg/kg BW (T3) treatment groups. While Mimosa pudica root extract 500 mg/kg BW (T2) shows no significant difference to Mimosa pudica root extract 1000 mg/kg BW (T3) treatment groups shown by the difference of superscript (Figure 1).

In congestion, blood accumulates in dilated capillaries and venules. Congestion is often accompanied by edema (Reginald, 1978). Hepatic congestion is mostly seen in positive control (C+), Mimosa pudica root extract 250 mg/kg BW (T1) and Mimosa pudica root extract 500 mg/kg BW (T2) groups.
and slightly in *Mimosa pudica* root extract 250 mg/kg BW (T3) group.

Degeneration
Analytic result of hepatocyte degeneration observation is shown in the table 2.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>X ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative control (C-)</td>
<td>4.500 ± 2.7928</td>
</tr>
<tr>
<td>Positive control (C+)</td>
<td>27.250 ± 1.9170</td>
</tr>
<tr>
<td><em>Mimosa pudica</em> 250mg/kg BW (T1)</td>
<td>20.917 ± 2.2454</td>
</tr>
<tr>
<td><em>Mimosa pudica</em> 500mg/kg BW (T2)</td>
<td>13.500 ± 6.8848</td>
</tr>
<tr>
<td><em>Mimosa pudica</em> 1000mg/kg BW (T3)</td>
<td>11.333 ± 1.8074</td>
</tr>
</tbody>
</table>

*The different superscript show there is significant difference between treatment groups (p<0.05).

Form of degeneration shown in the liver slides were mostly hydropic degeneration. The description of hydropic degeneration is explained by King and Alroy (1997) in their books with increased intracellular water causing the cytoplasm and organelles to appear swollen and vaculated while nucleus is usually not displaced to the periphery of the cell. This form of degeneration is mostly seen in positive control (C+), *Mimosa pudica* root extract 250 mg/kg BW (T1) and *Mimosa pudica* root extract 500 mg/kg BW (T2) group. (Figure 2).

The form of hydropic degeneration is mostly seen in positive control (C+) group, which was given *Naja sputatrix* LD$_{50}$ (0.13 μL/gram BW) during experiments and in several area in *Mimosa pudica* root extract 250 mg/kg BW (T1) and *Mimosa pudica* root extract 250 mg/kg BW (T2) groups.

Necrosis
Analytic result of hepatocyte necrosis observation is shown in the Table 3.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>X ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative control (C-)</td>
<td>7.917 ± 5.1227</td>
</tr>
<tr>
<td>Positive control (C+)</td>
<td>27.250 ± 2.2305</td>
</tr>
<tr>
<td><em>Mimosa pudica</em> 250mg/kg BW (T1)</td>
<td>18.833 ± 3.6148</td>
</tr>
<tr>
<td><em>Mimosa pudica</em> 500mg/kg BW (T2)</td>
<td>13.083 ± 5.4810</td>
</tr>
<tr>
<td><em>Mimosa pudica</em> 1000mg/kg BW (T3)</td>
<td>10.417 ± 8.2001</td>
</tr>
</tbody>
</table>

*The different superscript show there is significant difference between treatment groups (p<0.05).
Statistical analysis of hepatocyte necrosis in Negative control (C-) shows significant difference to (C+), *Mimosa pudica* root extract 250 mg/kg BW (T1) and *Mimosa pudica* root extract 500 mg/kg BW (T2), but shows no significant difference to *Mimosa pudica* root extract 1000 mg/kg BW (T3). Positive control (C+) shows significant difference to other treatment groups. While *Mimosa pudica* root extract 250 mg/kg BW (T1), *Mimosa pudica* root extract 500 mg/kg BW (T2), and *Mimosa pudica* root extract 1000 mg/kg BW (T3) show significant difference towards each other treatment groups shown in the superscript (Figure 2).

Necrotic cell death or necrosis is morphologically characterized by a gain in cell volume, swelling of organelles, plasma membrane rupture and subsequent loss of intracellular contents (Kroemer, 2009). Necrotic cells show increased eosinophilia (i.e., pink staining from the eosin dye—the E in the hematoxylin and eosin [H&E] stain). Compared with viable cells, the cell may have a more glassy, homogeneous appearance, mostly because of the loss of glycogen particles. When enzymes have digested cytoplasmic organelles, the cytoplasm becomes vacuolated and appears “moth-eaten”. Nuclear changes in necrosis assume one of three patterns, all due to breakdown of DNA and chromatin. Hepatocyte necrosis is mostly seen in positive control (C+), *Mimosa pudica* root extract 250 mg/kg BW (T1) and *Mimosa pudica* root extract 500 mg/kg BW (T2) groups and slightly in *Mimosa pudica* root extract 250 mg/kg BW (T3) group.

Histopathological observation and examination of hepatic congestion, degeneration and necrosis in rat’s liver (*Rattus norvegicus*) is shown in figure 1.

![Comparison of hepatocyte necrosis among treatment groups. Positive control (C+), Negative control (C-), Mimosa pudica root extract 250 mg/kg BW (T1), Mimosa pudica root extract 500 mg/kg BW (T2), Mimosa pudica root extract 1000 mg/kg BW (T3). Showing: (1) Congestion around the central vein (2) Congestion around the sinusoid.](image-url)
Rattus norvegicus Heart Rate

Based on the observation of Rattus norvegicus heart rate it shows that Naja sputatrix venom contains cardiotoxin which caused cardiac disorders (Goswami, 2014), that’s why there are significant different heart rate changes in every treatment. Cardiotoxin can cause bradycardia.

From the observation C+ shows that Naja sputatrix venom can cause the most bradycardia than another treatment. The rat heart rate weaken after Naja sputatrix venom injection.

From (T1) Mimosa pudica root extract 250 mg/kg BW, (T2) Mimosa pudica root extract 500 mg/kg BW and (T3) Mimosa pudica root extract 1000 mg/kg BW show that Mimosa pudica can increase the rat heart rate.

Rattus norvegicus Respiratory Rate

Based on the observation of Rattus norvegicus respiratory rate it shows that Naja sputatrix venom contains hemotoxin which caused hypoxia and red blood cell damage (Goswami, 2014), that’s why there are significant different respiratory rate changes in every treatment.

From the observation C+ shows that Naja sputatrix venom can cause the most respiratory rate reduction than another treatment. The rat respiratory rate weaken after Naja sputatrix venom injection because of hemotoxin contained.

From (T1) Mimosa pudica root extract 250 mg/kg BW, (T2) Mimosa pudica root extract 500 mg/kg BW and (T3) Mimosa pudica root extract 1000 mg/kg BW show that Mimosa pudica can increase the rat respiratory rate.

Conclusion

In this research, 250 mg/kg BW, 500 mg/kg BW and 1000 mg/kg BW dosage of Mimosa pudica root extract can reduce liver damage based on hepatocyte degeneration and necrosis in Rat (Rattus norvegicus) caused by Naja sputatrix venom. Naja sputatrix venom can cause bradycardia and respiratory rate reduction while Mimosa pudica can increase the rat heart rate and respiratory rate.
References
Morad, T.S., M.A. Ahmad and N. Mutar. 2014. Histological and ultrastructural studies on the liver of mice after treatment with single dose of LD_{50} Naja naja snake venom. 6: 2.
Kualitas Spermatozoa Domba Merino pada Sisi Anoda Hasil Pemisahan dengan Teknik ESS (Electric Separating Sperm)

Spermatozoa’s Quality of Merino Sheep in the Anode Side By the ESS (Electric Separating Sperm) Separation Technique

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Abstract

This study is one of new applied science in artificial insemination in sheep in which sperm cells produced by males can be separated in chromosom X and Y of sex. In this study used the ESS (Electric Separating Sperm) technique as separation methods. Researchers used a new tool to separate sperm cells by using an electric tool that electrified 1.5 volts, which compared to the level of effectivenes with different time is 0 minutes, 3 minutes, 7 minutes, and 10 minutes. Data analysis using LSD forwaded anova with (Turkey) 5% by using 5 replicates per treatment. P0 in this entrol (0 min), P1 (3 minutes), P2 (7 minutes), P3 (10 minutes). Data results showed that motility of P0 52.40 ± 1.661 a, group of P1 51.40 ± 2.337 ab, group of P2 50.40 ± 1.778 a, and group of P3 48.00 ± 1.000 a. Cell viability of P0 64.40 ± 2.315 a, group of P1 60.20 ± 2.653 ab, group of P2 56.00 ± 2.302 ab, and group of P3 54.20 ± 2.746 b, and abnormalities of P0 4.60 ± 1.435 a, group of P1 7.00 ± 1.304 ab, group of P2 11.00 ± 2.345 ab, and group of P3 11.40 ± 1.030 b. The results showed that the quality of spermatozoa in microscopic oservation cells test of motility in all treatment groups showed no significant differentes, whereas the viability and abnormality observations indicate that the results between the P1 and P2 control do not have significant differences but significantly different P3.

Keywords : Separating Spermatozoa, ESS (Electric Separating Sperm), Semen quality

Pendahuluan

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