Chronic Toxicity Effect of *Mimosa pudica* Leaf Extract Towards Histology Profile of Stomach and Duodenum in Mice

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**ABSTRACT**

In Indonesia, *Mimosa pudica* Linn. (*M. pudica* L.) is a wild plant (weed) from the Mimosaceae family. This plant is widely used as a traditional medicine for various types of diseases such as insomnia, acute eye inflammation, urolithiasis (urinary stones), fever, and bronchitis. This study aimed to find out the effect of *M. pudica* leaf extract on the histological damage of the stomach and duodenum of mice. This study used a Completely Randomized Design with 24 male mice, which were divided into control placebo (P₀) given CMC-Na 0.5%; and *M. pudica* leaf extract doses of 200 mg/kg body weight (P₁); 400 mg/kg body weight (P₂); and 600 mg/kg body weight (P₃). The extract was administered orally for 28 days. On day 29, the animal was dissected to collect its digestive organs. The histological preparation of the stomach and duodenum of mice was performed by using the paraffin method and Hematoxylin and Eosin staining to observe the histological damage, namely congestion, hemorrhage, epithelial cells desquamation, inflammatory cell infiltration, edema, and hyperplasia. Quantitative data were analyzed with a one-way ANOVA test and Duncan's post hoc test with the SPSS version 22. The results showed that the *M. pudica* leaf extract increased hemorrhage and hyperplasia damage at doses of 400 and 600 mg/kg body weight in mice’s duodenum.


**INTRODUCTION**

Indonesia has various kinds of herbal plants that are efficacious for healing diseases. About 10% of all plants in Indonesia are predicted to be medicinal plants. At least 80% of species of medicinal plants in Southeast Asia, whether native or introduced, can be found in Indonesia. (Cahyaningsih et al., 2021). Although many types of plants have the potential to be used as herbal medicines, most have not been tested and have not been monitored at all. The problem is the lack of knowledge about the mechanism, the potential for adverse reactions, contraindications, and interactions with other drugs used and with the food consumed. Safety continues to be a major issue in the use of herbal medicines; therefore, research and testing are needed to ensure that all herbal medicines are safe and of good quality (Ekor, 2014).

Herbal plants are now widely circulated in the market and are used as alternative medicines for disease recovery processes. One of the plants that has long been used by the people of Indonesia as a medicinal ingredient is *M. pudica* Linn, from the Fabaceae family. According to Ahmad et al. (2012), all parts of this plant have the potential to have medicinal properties. In India, the *M. pudica* plant is cultivated because of its many benefits and is very useful for the people of India, as a vegetable, spice, cosmetic oil, and medicinal plant.
Herbal or traditional medicines made from plant materials that are used by drinking directly will go through a digestive process in the digestive tract and then be absorbed by the intestines. The leaves of *M. pudica* are widely used in traditional medicine, however, research on their effects on the digestive tract and finding the effective dose for traditional medicine has not been carried out yet. Phytochemical screening of the *M. pudica* leaf extract showed the presence of bioactive components such as alkaloids, coumarins, flavonoids, glycosides, phenols, quinines, saponins, tannins, and terpenoids. An alkaloid called mimosine has been isolated from the plant (Ahmad *et al.*, 2012). People must consider consuming *M. pudica* because it contains a toxic alkaloid such as mimosine.

The sub-chronic toxicity of the *M. pudica* ethanolic extract caused changes in organ histology in the form of necrosis in liver hepatocyte cells and proximal tubule cells of the kidney, as well as atresia in ovarian follicles (Tarung, 2015). Based on this background, it is necessary to conduct research on the effect of the ethanol extract of *M. pudica* leaves on the histology of the digestive tract (stomach and duodenum) in experimental mice.

**MATERIALS AND METHODS**

**Preparation of *M. pudica* Crude Extract**

The leaves of *M. pudica* Linn were dried for about 3-6 days, then blended into a fine powder and weighed. The samples were extracted by soaking them in 70% ethanol in a closed jar so that they were not exposed to light for 3-7 days (maceration process). The macerate was filtered with Whatman filter paper and then evaporated with a rotary evaporator until it formed a crude extract.

**Animal Experiment**

The experimental animals used in this study were male Swiss Webster mice (*Mus musculus* L.), 20-25 grams of body weight, aged 2.5-3 months, and physically healthy without any abnormalities. The animals were adapted for one week (acclimatization). The cages were plastic tubs, 33x25x14 cm, covered with woven wire. In each cage, husks were added to absorb urine.

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**Research Design and Animal Treatments**

This study used a Completely Randomized Design (CRD) using 24 male mice. Animals were divided into four groups: a group of placebo controls (Po) which were given CMC-Na 0.5% (Na CMC or sodium carboxymethyl cellulose is a suspending agent that is often used as stabilizer in drug suspension preparations). The Na-CMC powder is weighed by 0.5 gram and then dissolved in distilled water until it reaches 100 ml; and three groups of multilevel doses of *M. pudica* leaf extract: 200 mg/kg body weight (*P*1), 400 mg/kg body weight (*P*2), and 600 mg/kg body weight (*P*3).
weight (P2), and 600 mg/kg body weight (P3) based on doses at our preliminary study. The extract was administered orally by gavage in the form of a suspension in 0.5% CMC-Na solution at a rate of 0.5 ml/animal/day for 28 days.

The stomach and intestines of samples were collected on day 29. Animals were sacrificed by neck dislocation. The organs were then washed with a NaCl solution, dried with filter paper, and weighed. Furthermore, the organs were placed in a tube or pot and soaked with 10% Neutral Buffer Formalin, a fixation agent. Histological preparations of the stomach and intestines were carried out by paraffin method with Hematoxylin & Eosin staining. Histology preparations were photographed microscopically with a strong magnification (400x) light digital microscope (Optilab®) using the Optilab Viewer program (Micronos®) from an Optilab camera connected to a laptop. Calculation of the number of damaged cells was carried out using the Image Raster application (Micronos®). The number of damaged cells was counted in each of the 5 fields of view (left, right, top, bottom, and middle) of each slide (Setyawati et al., 2017).

**Histological Scoring**

Variable damage to the histology of the digestive tracts of male mice in this study was assessed using a scoring method based on Ratnasari’s research (2019), which included the presence of damages namely hemorrhage, edema, inflammatory cell infiltration, congestion, hyperplasia, and desquamation of epithelial cells of the stomach and duodenum. All damages were counted and then divided into four categories, namely a value of normal (-) or 0% occurrence with score 1; mild damage (+) or <20% occurrence with score 2; moderate damage (++) or 20-50% occurrence with score 3; and severe damage (+++) or >50% occurrence with score 4.

**RESULTS AND DISCUSSION**

**Stomach Histology of Male Mice Treated with M. pudica Leaf Extract**

The stomach histological normality test showed an abnormal distribution of data for the parameters of hemorrhage, edema, congestion, hyperplasia, and desquamation of epithelial cells so the Kruskall-Wall’s test was performed. The results showed a non-significant difference (P>0.05) between the control and all treatments (Table 1). The histology of the mice’s stomachs is presented in Figure 2.

In the histology of the mice’s stomach (Figure 2), damages were found in the form of hemorrhage (Figure 2.B.2), congestion (Figure 2.C and D.5), hyperplasia (Figure 2.D.6), desquamation of epithelial cells (Figure 2.B.3) and inflammatory cell infiltration (Figure 2.C.4) although these were not statistically significant (Table 1). Inflammation is an important mechanism needed by the body to defend itself from various hazards that disturb the balance and improve the structure and function of tissue disorders. The presence of inflammatory cell infiltration in the histology of the stomach may be a cell response to a toxic agent, disease, or various toxic agents. It is also a protective response to maintain structure and improve tissue function (Chen et al., 2018). In this study, inflammatory cell infiltration was found in the treatment groups as a response to mimosine substance in the *M. pudica* leaf extract.

### Table 1. The average number of bleedings, edema, congestion, hyperplasia, and desquamation of epithelial cells in the stomach of male mice treated with *M. pudica* leaf extract

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Hemorrhage</th>
<th>Edema</th>
<th>Congestion</th>
<th>Hyperplasia</th>
<th>Desquamation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ranking</td>
<td>P0</td>
<td>4.50 (ns)</td>
<td>9.00 (ns)</td>
<td>4.50 (ns)</td>
<td>7.60 (ns)</td>
</tr>
<tr>
<td>Means</td>
<td>P1</td>
<td>9.10 (ns)</td>
<td>11.10 (ns)</td>
<td>10.00 (ns)</td>
<td>9.50 (ns)</td>
</tr>
<tr>
<td></td>
<td>P2</td>
<td>12.80 (ns)</td>
<td>12.90 (ns)</td>
<td>14.60 (ns)</td>
<td>15.40 (ns)</td>
</tr>
<tr>
<td></td>
<td>P3</td>
<td>15.60 (ns)</td>
<td>9.00 (ns)</td>
<td>12.90 (ns)</td>
<td>9.50 (ns)</td>
</tr>
<tr>
<td>Chi-Square</td>
<td></td>
<td>10.6</td>
<td>3.93</td>
<td>9.10</td>
<td>9.04</td>
</tr>
<tr>
<td>P</td>
<td></td>
<td>0.14</td>
<td>2.69</td>
<td>0.28</td>
<td>1.51</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>P0</td>
<td>1.0 ± 0.000</td>
<td>1.0 ± 0.000</td>
<td>1.0 ± 0.000</td>
<td>1.0 ± 0.894</td>
</tr>
<tr>
<td></td>
<td>P1</td>
<td>1.4 ± 0.985</td>
<td>1.0 ± 1.788</td>
<td>1.3 ± 3.577</td>
<td>1.2 ± 1.814</td>
</tr>
<tr>
<td></td>
<td>P2</td>
<td>1.6 ± 2.607</td>
<td>1.1 ± 1.788</td>
<td>1.6 ± 3.033</td>
<td>1.6 ± 4.147</td>
</tr>
<tr>
<td></td>
<td>P3</td>
<td>1.8 ± 1.673</td>
<td>1.1 ± 0.000</td>
<td>1.5 ± 3.847</td>
<td>1.2 ± 3.898</td>
</tr>
</tbody>
</table>

Note: The control, given a solution of 0.5% CMC-Na (P0), treatment doses of 200 mg/kg BW (P1), 400 mg/kg BW (P2), and 600 mg/kg BW (P3); (ns) = non-significant
The normality test for the average number of inflammatory cell infiltrates in the histology of the stomach of mice showed no normal data distribution, therefore, the one-way ANOVA test was performed. Statistic tests showed no significant difference (P>0.05) between the control and the treatments with *M. pudica* leaf extract for 28 days (Table 2).

Histology of the stomach treated with *M. pudica* leaf extract at doses 200, 400, and 600 mg/kg BW in this study showed some damages, but they were not significantly different compared to the control (P0). There was no significant difference between the control and the treatment of *M. pudica* leaf extract on damage in the form of hemorrhage, edema, congestion, hyperplasia, desquamation of epithelial cells (Table 1) and inflammatory cell infiltration (Table 2) in the stomach of male mice. This suggests that *M. pudica* leaf extract is still classified as safe for the stomach. The stomach organ has a thicker lining than the rest of the digestive tracts. The stomach also has defensive factors, including bicarbonate, prostaglandins, and blood flow. The thick layer of gastric mucus is a defense against autodigestion, providing protection against mechanical trauma and chemical agents (Hehi et al., 2013).

**Duodenal Histology of Male Mice Treated with *M. pudica* Leaf Extract**

The small intestine functions as a place for food distribution and absorption of nutrients into the blood vessels and lymph vessels. The small intestine is divided into three parts, namely the duodenum, jejunum, and ileum. In general, the structures of the small intestine are the mucous membrane, lamina propria, submucosa, lymphatic tissue, and serous and muscular layers. Epithelial cells cover the entire free surface of the mucous membrane and are simple cylindrical epithelium (Pearce, 2016).

Duodenum histology normality test showed abnormal data distribution for parameters of edema, inflammatory cell infiltration, congestion, and desquamation of epithelial cells so the Kruskall-Walli’s test was performed. Histology of the duodenum in this study showed insignificantly different (P>0.05) damages (Table 3) such as congestion (Fig. 3.B.1), desquamation of epithelial cells (Fig. 3.D.4), edema, and inflammatory cell infiltration.

![Figure 2. Histological observation of the stomach of male mice treated with *M.pudica* leaf extract (Hematoxylin and Eosin staining; microscope magnification 400x)](image)

Note: capital letter A = control group (0.5% CMC-Na solution); B = P1 (dose 200 mg/kg BW); C = P2 (dose 400 mg/kg BW); and D = P3 (dose 600 mg/kg BW); number 1= normal cells, 2= hemorrhage, 3= desquamation of epithelial cells, 4= inflammatory cell infiltration, 5= congestion, 6= hyperplasia.
Table 2. The average number of inflammatory cell infiltration in the stomach of male mice treated with *M. pudica* leaf extract

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Type of Damage</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Inflammatory cell infiltration</td>
</tr>
<tr>
<td>P0</td>
<td>3.2 ± 4.919 (ns)</td>
</tr>
<tr>
<td>P1</td>
<td>1.5 ± 1.788 (ns)</td>
</tr>
<tr>
<td>P2</td>
<td>1.6 ± 3.847 (ns)</td>
</tr>
<tr>
<td>P3</td>
<td>1.7 ± 4.381 (ns)</td>
</tr>
</tbody>
</table>

Note: The control was given a solution of 0.5% CMC-Na (P0), treatment doses of 200 mg/kg BW (P1), 400 mg/kg BW (P2), and 600 mg/kg BW (P3); (ns) = non-significant.

The normality test for hemorrhage and hyperplasia of the mice's duodenum showed a normal data distribution, so the One-way ANOVA test was performed. Statistical test results showed a significant difference (P<0.05) between control with P1, P2, and P3 treatments for hemorrhage (Fig. 3.C and D.3). In the damage of hyperplasia (Fig. 3.B,C, and D.2), there was no significant difference (P>0.05) between control and P1 treatment. However, there was a significant difference between the control with P2 and P3 treatments after being treated with the leaf extract for 28 days (Table 4).

Congestion is an increase in the volume of blood cells in tissues and parts of the body that are experiencing pathological processes. Congestion is microscopically indicated by the presence of blood cells contained in the blood vessels, and their location fills the lumen in the blood vessels (Fig. 3.B.1). This can be caused by epithelial cells that are released due to desquamation, which are dead cells (necrosis) and become foreign objects in the body.

According to Sudiono (2014), damaged cells will release chemical compounds so that inflammatory cells are sent through the circulatory system to areas where there is damage to these cells, histologically known as inflammatory cell infiltration. This process will cause inflammation in the body. In inflammatory processes, the delivery of inflammatory cells increases blood flow which is accompanied by dilatation of blood vessels. This can trigger congestion. Edema occurs due to congestion, increased capillary permeability, and osmotic pressure of blood and fluids (Assiam et al., 2014). In this study, this kind of damage occurred insignificantly between all treatments.

Oral administration of *M. pudica* leaf extract at doses of 200, 400, and 600 mg/kg BW, caused an increase in the amount of hemorrhagic damage (Table 4). Hemorrhage is the discharge of blood from the blood vessels (bleeding or the abnormal flow of blood), histopathologically characterized by the presence of red blood cells outside the blood vessels or in the tissue (Berata et al., 2015). Hemorrhage is due to trauma (physical tissue damage), viral infections, and toxic substances that cause the wall to leak (Putra et al., 2012). It is an advanced stage of congestion that occurs because the sinusoids lose their ability to hold blood, causing the blood vessels to stretch and eventually rupture (Sudiono, 2014). Hemorrhage was found in duodenum histology in this study, presumably due to the presence of saponin compounds contained in the *M. pudica* leaf extract. This was supported by phytochemical tests, which showed the presence of saponin compounds in all parts of the *M. pudica* plant (Ranjan et al., 2013). In line with the research that saponin extracted from watermelon plant caused tissue hemorrhage (Diwan et al., 2017).

Hyperplastic damage in male mice’s duodenum histology showed a significant difference (P<0.05) between the controls and the P2 and P3 treatments. This shows that the doses of 400 and 600 mg/kg BW caused damage in the form of hyperplasia. The higher the dose, the more hyperplasia was found (Table 4 and Figure 3.B, C, and D.2). Hyperplasia occurs only in tissues that are capable of cell division, and occurs in various tissues under various conditions (Hammer and McPhee, 2019). Epithelial cell hyperplasia occurring in the duodenum can be caused by stimulation by toxic compounds causing a compensatory response from the small intestine mucosa to protect the wall by multiplying epithelial cells. It is characterized by an increase cell number in the form of tissue regeneration. In line with research conducted by Ratnasari, (2019) which proved that the administration of *Leucaena leucocephala* leaf flour containing tannins showed an increase in the number of cells (hyperplasia) in the mucosal part of the intestines of treated rats.
Figure 3. Histological view of the duodenal tract of male mice treated with *M. pudica* leaf extract (Hematoxylin and Eosin staining; microscope magnification 400x)

Note: capital letter A = Control group (0.5% CMC-Na solution); B = P1 (dose 200 mg/kg BW); C = P2 (dose 400 mg/kg BW); and D = P3 (dose 600 mg/kg BW); number 1 = congestion, 2 = hyperplasia, 3 = hemorrhage, and 4 = desquamation of epithelial cells.

Table 3. The average number of edemas, infiltration, congestion, and desquamation of epithelial cells in the duodenum of male mice treated with *M. pudica* leaf extract.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Type of Damage</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Edema</td>
</tr>
<tr>
<td>Ranking</td>
<td>P0</td>
</tr>
<tr>
<td>Means</td>
<td>P1</td>
</tr>
<tr>
<td></td>
<td>P2</td>
</tr>
<tr>
<td></td>
<td>P3</td>
</tr>
<tr>
<td>Chi-Square</td>
<td></td>
</tr>
</tbody>
</table>

Note: The control, given a solution of 0.5% CMC-Na (P0), treatment doses 200 mg/kg BW (P1); 400 mg/kg BW (P2); 600 mg/kg BW (P3); (ns) = non-significant.
Epithelial desquamation is the detachment of the epithelial layer from the mucosal tissue. Desquamation of the epithelium occurs in the gastric mucosa after administration of *M. pudica* leaf extract, which is thought to be caused by tannins contained in the extract which can damage the mucosa of the stomach and small intestines. The results of the phytochemical test showed that the leaves of *M. pudica* contain 10% tannin (Joseph et al., 2019). This is in line with a study conducted by Wuragil (2007) who reported that tannins in *Acacia villosa* leaves could damage the gastric and small intestine mucosa. Presumably due to the action of hydrolyzed tannins which can cause erosion of the mucous lining of the digestive tract. Tannins changed the intestinal mucosa which was characterized by villi damage resulting in the formation of abnormal villi structures.

It was found that some of the damage that occurs in the small intestine is caused by exposure to toxic substances such as mimosine contained in *M. pudica* extract (Ahmad et al., 2012). As a drug that enters the body through digestion will be absorbed by the body, one of the absorption processes is carried out by the duodenum organ. According to Mensah et al., (2019), some chemical substances in medicinal herbal plants can be toxic. They can be aggressive factors and cause adverse reactions if the dose is inappropriate or high. They will cause negative effects in the form of toxicity that can damage the sub-structural components of an organism such as the cell (cytotoxicity), tissues and organs (organotoxicity), or the whole organism.

Safety continues to be a major issue in the use of herbal therapies. People who wish to use *M. pudica* must ensure that the dose used is safe, as studied in this study so that it does not cause histological damage to the digestive tract, which can interfere with the digestive system.

### Table 4. Hemorrhage and hyperplasia in the mice duodenal intestines.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Hemorrhage</th>
<th>Hyperplasia</th>
</tr>
</thead>
<tbody>
<tr>
<td>P0</td>
<td>1.0 ± 1.095 a</td>
<td>1.4±3.741 (ns)</td>
</tr>
<tr>
<td>P1</td>
<td>2.0 ± 4.604 b</td>
<td>1.9±8.786 (ns)</td>
</tr>
<tr>
<td>P2</td>
<td>2.1 ± 3.346 b</td>
<td>2.5±8.786 b (*)</td>
</tr>
<tr>
<td>P3</td>
<td>1.9 ± 6.294 b</td>
<td>2.8±4.472 b (*)</td>
</tr>
</tbody>
</table>

Note: The control was given a solution of 0.5% CMC-Na (P0), treatment doses of 200 mg/kg BW (P1), 400 mg/kg BW (P2), and 600 mg/kg BW (P3); (ns) = non-significant, (*) P<=0.05

### CONCLUSION

In this study, chronic exposure to *M. pudica* leaf extract at doses of 400 and 600 mg/kg body weight, caused damages in the form of hemorrhage and hyperplasia in the duodenal organ of male mice.

### REFERENCES


