

## The Effect of Noni Leaves Extract (*Morinda citrifolia L*) on Wound Healing Percentage of Traumatic Ulcer In Oral Mucosa of Wistar Rats (*Rattus norvegicus*) by *In Vivo*

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<http://dx.doi.org/10.13005/bpj/1286>

(Received: August 02, 2017; accepted: September 25, 2017)

### ABSTRACT

Traumatic ulcer is a lesion in oral mucosa caused by trauma. Traumatic ulcer resulting pain can disturb activities and metabolism. Noni leaves (*Morinda citrifolia L*) contain flavonoid, saponin, and tannin as anti-inflammatory, anti-bacterial, and antioxidant to accelerate healing process. This research aimed to determine the effect of *Morinda citrifolia L* leaves extract on healing percentage of traumatic ulcer in oral mucosa. Ulcer was made by put hot burnisher on lower labial mucosa of twelve *Rattus norvegicus* for a second. They were divided into three groups. First group was given *Morinda citrifolia L* leaves extract, control group were given triamcinolone acetonide 0,1% and aquades. The research was performed for 10 days by measuring ulcer diameter and applying extract. Based on Independent-Samples T Test showed *Morinda citrifolia L* leaves extract has no significant effect on healing percentage compared to control groups with  $p = 0.18$  and  $0.694$  ( $p > 0.05$ ), recovery day for treat group was eighth day and for each control groups were tenth day. *Morinda citrifolia L* leaves extract has no significant effect on wound healing percentage of traumatic ulcer compare to positive control and negative control.

**Keywords:** *Morinda citrifolia* leaves extracts; traumatic ulcer; oral mucosa.


### INTRODUCTION

The most common lesions found in the oral cavity are the ulcers.<sup>1</sup> Ulcer caused by trauma is called a traumatic ulcer.<sup>2,3</sup> Types of trauma that can cause ulcers are mechanical trauma e.g. being bitten and tooth brushing vigorously,<sup>3,4</sup> Thermal trauma e.g. eating hot food or drinks,<sup>4</sup> Chemical trauma e.g. the use of mouthwash with high alcohol content, Iatrogenic trauma e.g. an error during treatment e.g. exposed with hot sonde.<sup>4,5,6</sup>

The traumatic ulcer is followed by the symptoms of pain which are caused by the opening of the peripheral nerve endings at the base of the lamina propria.<sup>7</sup> The inflicted pain interferes the function of chewing, talking, or swallowing,<sup>8</sup> thus disrupting the function of the oral and systemic body due to disruption of the body's metabolic processes.

The treatment for traumatic ulcers is eliminating the trauma-causing factor and providing a corticosteroids for relieving the pain in very severe



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case,<sup>5,10</sup> however corticosteroid has side effects that are the depletion of the oral mucous layer and local irritation.<sup>11</sup> Based on this, an alternative medicine with little or even no side effects derived from herbs or other natural materials are needed.

Noni (*Morinda citrifolia* L) is a typical tropical plant including Indonesia and distributed in Southeast Asia, Australia, Africa, and South America.<sup>12,13</sup> *Morinda citrifolia* L leaves are used as topical medicines to heal the affected parts of the skin and reducing pain.<sup>14</sup> In some areas, the infuse leaves are consumed as an analgesic.<sup>15</sup> The active ingredients found in the leaves are saponin, alkaloids, iridoid glycosides, tannins, and terpenoids, and flavonoids.<sup>14,16</sup>

The main effects of chemical compounds in *Morinda citrifolia* L leaves are related to wound healing process e.g. saponin as antibacterial, tannin as hemostatic and astringensia, alkaloids are useful as analgesic, and iridoid glycoside compounds as anti-inflammatory, terpenoid as antibacterial and astringensia which can trigger wound healing, while flavonoids as antioxidants and anti-inflammatory.<sup>16</sup>

Nayak *et al* (2007) explained in their research results, ethanol extract from *Morinda citrifolia* L leaves are capable to initiate wound healing activity.<sup>14</sup> The results of Sabirin *et al* (2013) stated that flavonoids, terpenoids and iridoid glycosides from *Morinda citrifolia* L extract as the most active substances in assisting the healing process of excision wound in the rat's back.<sup>15</sup>

Based on the description above, the authors are interested to conduct the research on the effect of *Morinda citrifolia* L leaves extract wound healing percentage of traumatic ulcer in oral mucosa of wistar rats (*Rattus norvegicus*) by *in vivo*.

## MATERIALS AND METHOD

This research is experimental laboratory with *Post-Test Only Design* method conducted in three places, Pharmacology Laboratory of Veterinary Faculty of Syiah Kuala University to extract *Morinda citrifolia* L leaves, phytochemical test at Chemical Research Laboratory Faculty of Mathematics and Science, and Experimental Laboratory Animal

Faculty of Veterinary University of Syiah Kuala University to make traumatic ulcer and measure the width of *Rattus norvegicus*. The study was conducted in January 2017.

The sample of this study was *Rattus norvegicus* which was male, aged 2-3 months, weight of 120-250 gram, and healthy. The samples amount were twelve *Rattus norvegicus*. Twelve rats were then divided into 3 groups, the treatment group of 4 rats were applied *Morinda citrifolia* L leaves extract, the positive control group of 4 rats were applied *triamcinolone acetonide* 0.1%, and the negative control group of 4 rats were applied aquadest.

### **Morinda citrifolia** L. Leaves extraction procedure

1 kg of fresh *Morinda citrifolia* L washed leaves which were neither too old nor young, then dried for 7-10 days in a room temperature. Afterwards the leaves were mashed by using a blender. Then, the *Morinda citrifolia* L leaves which had become powder form, was extracted using maceration technique. *Morinda citrifolia* L leaves powder was put into a tightly closed erlenmeyer flask with addition of ethanol solvent 96% until the powder submerged for 3 days.<sup>19</sup>

After the maceration completed, the powder was filtered with a *buchner* funnel that had been previously coated with filter paper and accommodated into the eruvier flask to obtain filtrate and residue. Then the filtrate was evaporated using a *rotary evaporator* at a temperature of 40°C to obtain a viscous extract.<sup>14</sup> The results of liquid extraction were stored at temperature of 0-4 °C.<sup>20</sup> According to Kitao and Sekine (1994) flavonoids were unstable against the influence of light (cit Yati, 2007),<sup>21</sup> therefore a dark storage container is required to avoid the effect of light on the extraction results. Phytochemical test was performed for *Morinda citrifolia* L leaves extract results to determine the content of it's active compounds.

### **Animal Experiment Procedure**

The animal was placed in a cage for 7 days with husks covering the cage floor at 24°C, in a dark cycle of 12 hours, in a light cycle less than 12 hours with food and water given.<sup>22</sup> The rats were anesthetized by intramuscular injection on the left

hind leg in the *musculus tricep* with a combined dose of 33 mg / kg of Ketamine Hydrochloride and 13 mg / kg of Xylazine Hydrochloride (2%), that was 0.08 mL Ketamine Hydrochloride and 0.16 ml of Xylazine Hydrochloride (2%).<sup>8</sup> Anesthesia effects had worked characterized by the absence of movement, no pain response (clamped with tweezers in the ear), no sound, and no reflexes (palpebral, pupils, and pedals).<sup>23</sup>

The traumatic ulcer was made by providing thermal trauma using a 3 mm diameter *burnisher*. The *burnisher* was heated by using a spirit light for 60 seconds and was touched on the labial mucosa below the rat for one second and as deep as one-third *burnisher*.<sup>24</sup>

On the first day, the positive control, the negative control, and the treatment group were given thermal trauma. The second day the measurement performed, the ulcer was measured using a periodontal prob vertically, horizontally and two diagonal sides to get the average diameter.

For the percentage of healing using the formula:

$$L_{(\%) } = \frac{(d_1)^2 - (d_2)^2}{(d_1)^2} \times 100\%$$

Group 1 was applied with *triamcinolone acetonide* 0.1%, group 2 was applied with aquadest 2 times a day in the morning and afternoon, and group 3 was applied with *Morinda citrifolia L* leaves extract twice a day every morning and evening. Each

**Table 1: *Morinda citrifolia L* Leaves Extract Phytochemical Test Results**

No.	Phytochemical Test	Result
1.	Alkaloid : Mayer	+
	Wagner	-
	Dragendorff	+
2.	Steroid	+
3.	Terpenoid	-
4.	Saponin	+
5.	Flavonoid	+
6.	Tanin	+

Explanation: + = found, - = not found.

materials were applied by using *micro applicator* topically as much as 2 mL. The *Morinda citrifolia L* leaves extract provision and ulcer diameter measurement were performed for 10 days.

## RESULTS

The results of phytochemical test of *Morinda citrifolia L* leaves can be seen in Table 1.

Based on Table 2, The mean percentage of traumatic ulcer healing in the positive and negative control group in D8 was 79.11% and 95.73%, while in the *Morinda citrifolia L* leaves extract group had reached 100% (Table 2.).

The result of data normality test using SPSS showed that the data percentage of treatment and positive control group was normally distributed with *p* value = 0,397 (*p* > 0,05), also the data percentage of treatment and negative control group were normally distributed with *p* value = 0,365 (*p* > 0, 05), then followed by unpaired t test.

From the homogeneity test results, in the treatment group with positive control *p* value = 0.087 was obtained and with negative control *p* value = 0.712 which signified the same data variant. The result of unpaired t test of treatment group

**Table 2: Healing Mean Percentage in *Morinda citrifolia L* Leaf Extract Treatment, Positive Control, and Negative Control Group (%)**

	Day	R1	R2	R3
Healing Mean Percentage (%)	D1	28,13	-8,96	2,3
	D2	45,73	-29,29	30,91
	D3	68,98	-28,49	48,59
	D4	83,12	12,04	77,02
	D5	93,98	67,95	64,49
	D6	95,23	67,95	88,78
	D7	98,89	79,24	86,65
	D8	100	79,11	95,73
	D9	100	95,59	97,11
	D10	100	100	100

Explanation: R1 = Healing Mean Percentage of Treatment Group, R2 = Healing Mean Percentage of Positive Control group, R3 = Healing Mean Percentage of Treatment Negative Control Group

**Table 3: Unpaired T Test and Homogeneity Test of Traumatic Ulcer Healing Percentage between *Morinda citrifolia L* Leaves Extract Treatment and Triamcinolone acetonide 0,1% as Positive Control and Aquadest as Negative Control Group**

	Group	Levene Homogeneity Test <i>p</i>	Unpaired T Test <i>p</i>
Healing Percentage	Positive Control Treatment	0,000	0,000
	Negative Control Treatment	0,124	0,087

with positive control showed  $p$  value = 0,138 which indicated no significant effect. The result of unpaired t test between treatment and negative control group with  $p$  value = 0,694 ( $p > 0,05$ ) showed no significant effect. These results can be seen in Table 3.

### DISCUSSION

The *Morinda Citrifolia L* leaf is a herbal plant that has been used as a topical medicine to heal the wound of the skin and reduce pain.<sup>14</sup> Sabirin *et. al* (2013) stated that the *Morinda Citrifolia L* leaf has benefit to heal an incision wound.<sup>15</sup> Nayak *et al* (2007) explained in their research that ethanol extract from the leaf of *Morinda Citrifolia L* was able to initiate wound healing activity.<sup>14</sup>

*Morinda Citrifolia L* leaves extract were applied one day after the making of ulcer every morning and evening. The frequency of the extract provision refers to the selection of traumatic ulcer therapy according to the Field (2003) which recommended the use of topical corticosteroid for 2-3 times.<sup>26</sup> Besides, the good usage principle of topical medicine is by setting aside the medicine for 3-5 minutes in order to maximum attachment to occur on oral mucosa of rat.<sup>27</sup>

Based on the results of the healing application on the leaves extract group was faster compared with the other groups due to the flavonoid content in the extract. Flavonoid can stimulate the synthesis of *growth factors* that affects the proliferation of fibroblast, fibroblast has

an important role to establish new tissue structure so it can accelerate the healing of ulcer.<sup>24</sup> This is in accordance with the Sabirin (2013) research which stated the ulcer healing influenced by flavonoid content in *Morinda citrifolia L* leaves extract.<sup>15</sup> Besides flavonoid, *Morinda citrifolia L* leaves extract on this research contained saponin and tannin which also had a role in traumatic ulcers healing.<sup>14,16</sup> Saponin have an antibacterial effect that can prevent recurrent blister because the bacteria can extend proinflammatory cycle,<sup>15</sup> and tannin as hemostatic and also good astringensia in accelerarating healing.<sup>16</sup>

Traumatic ulcer healing on the positive control group which was given *triamcinolone acetonide* 0.1% was expected to recover faster if compared with the negative control group which was given aquadest only, however in fact the healing on the negative control group just as fast as the positive control group. The results of research done by Jannah (2016) showed similar thing, namely there was only 2 rats that had been recovered on the tenth day for positive and negative control group,<sup>28</sup> however, it was different in previous research done by Rahmina (2013) which stated that 8 rats on the positive control group was recovered on the seventh day and faster than aquadest group which was only 2 rats were recovered on the seventh day.<sup>29</sup>

The inhibition of traumatic ulcer healing on positive control group was allegedly caused by the use of corticosteroids which is still controversy because it can inhibit the healing.<sup>30</sup> Corticosteroid is

an anti-inflammatory medicine that work by inhibiting the mediators of inflammatory so that there will be no sign of inflammation, however the inflammatory phase inhibition causes the healing process become incomplete so it can also cause ulcer become longer to recover.<sup>31</sup> Steroid medicine which was used in this research was topical corticosteroid in ointment form, namely *triamcinolone acetonide* 0.1%. The work of this medicine is to change *lipocortin* regulation which is part of a protein that inhibits *phospholipase A2* and prostaglandins involved in the inflammatory phase.<sup>1</sup> The use of corticosteroids as anti-inflammatory is palliative therapy, that is only the symptoms are inhibited however the cause of the disease is still exist,<sup>31</sup> whereas the traumatic ulcer can be healed when the cause is omitted.<sup>3</sup>

In this research it was expected that the ulcer width reduction occurred every day, however 3 rats on the positive and negative control group experiencing width constant and width enlargement of ulcer. The enlargement or constant width of ulcer can be happened because of some factors e.g. the effect of tweezer trauma when pullout rat's labial mucosa was not consistent, medicine attachment which was less maximum because of the rats'

behavior at the time of medicine application which sometimes difficult to be controlled. Besides those, the mouth cleanliness which could not be controlled also could cause infection easy to occur because it was supported by the rat's cage dirty environment.

Based on the research results, the width reduction of ulcer traumatic on each groups were different, where on the group of *Morinda Citrifolia L* leaves extract, healing percentage of traumatic ulcer healing, all rats on *Morinda citrifolia L* leaves extract group had reached 100% healed on the 8<sup>th</sup> day, while the control groups reached 100% on the 10<sup>th</sup> day. Based on the results of the unpaired t test, the *Morinda citrifolia L* leaves extract did not affect significantly to healing percentage of traumatic ulcer compared with positive control with  $p = 0,138$  and negative control group with  $p = 0,694$  ( $p > 0.05$ ).

## CONCLUSION

Based on the results of research, it can be concluded that the *Morinda citrifolia L* leaves extract did not affect significantly to the traumatic ulcer width and healing percentage compared with positive and negative control.

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