ANTI-ULCER ACTIVITY OF AFRICAN LEAVES (*VERNONIA AMYGDALINA* DEL.) ETHANOL EXTRACT ON MALE RAT

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Received: 10 November 2017, Revised and Accepted: 14 December 2017

ABSTRACT

Objectives: This study aims to evaluate the anti-ulcer activity of African leaves (*Vernonia amygdalina* Del.) ethanol extract on male rat.

Methods: African leaf powder was extracted used ethanol 96% by percolation, then made into a suspension preparation used 0.5% Na-CMC with 3 dose variations (100, 200 and 400 mg/kg). Further tested its effectiveness in healing peptic ulcer in gastric-induced rats using acetosal dose 800 mg/kg with oral administration every day until surgery at 3, 8, and 14. Observations include macroscopic (number and index of ulcer) and microscopic (histopathological test).

Results: A significant healing of ulcer was observed. The extract 100, 200, and 400 mg/kg group showed significant (p<0.05) reduction in number and index of ulcer as compared to the negative control. The most effective dose is 200 mg/kg because it has the greatest ability to reduce the number and index of ulcers and has the fastest recovery day.

Conclusion: Results of this study indicated that African leaves (*V. amygdalina* Del.) ethanol extract has potential anti-ulcer activity.

Keywords: African leaves, Peptic ulcer, Acetosal, *Vernonia amygdalina* Del.

INTRODUCTION

Multiple factors and harmful substances continuously exposed to the gastric mucosa that may trigger the onset of peptic ulcers, this being the most prevalent gastrointestinal disease worldwide [1]. About 14.5 million people worldwide are estimated affected by peptic ulcers with 4.08 million deaths per year [2].

Peptic ulcers are wound on the mucosal layer (epithelial layer) of the gastric and mucosal irritation with diameter 5 mm or more in depth down to submucosa. The pathogenesis of peptic ulcers occurs when there is an imbalance between aggressive and defensive factors [3]. Among the defensive factors, we can include mucus and bicarbonate production, cellular regeneration, and adequate blood flow, while the main aggressive factors comprise gastric acid, pepsin secretion, and reactive oxygen species [4].

Herbal medicine is one of the other ways of treatment outside the medical science, and traditional medical needs to be fostered, developed, and supervised to be accountable for its benefits and safety [5]. Herbal medicine in Indonesia is a nation’s cultural heritage, so it needs to be explored, researched, and developed to be used more widely by the community. Tropical Indonesia has the second largest biodiversity in the world after Brazil has about 25,000–30,000 plant species that makeup 80% of all plant species in the world and 90% of all Asian plants [6].

*Vernonia amygdalina* Del. or African leaf is bushes originating from the African and other parts of Africa, especially Nigeria, Cameroon, and Zimbabwe. These plant can be found in the yard, along rivers and lakes, and in the forests [7]. These plants contain flavonoids, tannins, saponins, and terpenoids [8]. Tannin has an antimicrobial effect that can help defend against *Helicobacter pylori*. In addition, tannins can also precipitate microprotein at the site of the ulcer to form a thin protective layer that prevents the attack of irritant factors of proteolytic enzymes [9]. Saponin activates the protective factor of mucous membranes [10]. Flavonoids improve mucosal blood circulation and increase prostaglandins, but the most important is its role as an antioxidant that will counteract free radicals that play a role in the pathogenesis of peptic ulcers [11].

Based on the use of African leaves in the community and the pharmacological effects produced by compounds contained in African leaves, this is the underlying importance of the authors to conduct research with the title "Testing the effectiveness of African leaf (*V. amygdalina* Del.) ethanol extract for peptic ulcer healing on male rats."

MATERIALS AND METHODS

Materials

Drugs and chemicals used in this study were Aquades, ethanol 96%, acetalos 100 mg (generic), formalin, Na, CMC, Na2HPO4, NaH2PO4, NaOH 0.01 N, methyl red indicator, phenolphthalein indicator, and sucrate (Propapsa®).

Preparation of extracts

*V. amygdalina* Del. was collected from Meunasah Teungoh village, Nurussalam subdistrict, East Aceh District, Nanggroe Aceh Darussalam Province, Indonesia. The leaves were washed and dried at 30°C–35°C, then grind until dried powder was obtained. The dried powder was percolated using ethanol 96%, then the obtained percolate was evaporated and freeze-dried.

Animals

Animals used were healthy male rats weighing 200 g (± 10%) in suitable cages, given appropriate food and drink, and acclimatized for 2 weeks before being treated. Before rat treatment is fasted for 24 h with the aim of obtaining a relatively clean gastric from food.

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The rats are sacrificed by dislocation. The rats were dissected for
Before treatment rats are fasted for 24 h without food, but still beverages.

Macroscopic observation
untreated group still showed a gastric ulcer (1.33 ulcers) until day 14.

Gastric ulcers were present on the day 8 in the ALEE 100 group and ALEE 200
100
8
Positive control group, ALEE 200 and 400 mg. The decrease in
index of ulcers occurred starting on the 3rd day with the greatest
decrease starting from the positive control group (0.95), ALEE 200 mg
(1.47), ALEE 100 mg (1.54), ALEE 400 mg (1.64), and the group without
1.54
1.64
(1.50). The decline in the index value of peptic ulcers showed
that giving of ALEE was able to reduce the severity of gastric ulcers that
occurred in rat stomach. The graph of the decrease in the peptic ulcer
index can be seen in Fig. 1.

The decrease in gastric ulcer index can be seen in Fig. 1, where on the
8th day, the positive control group, ALEE 200 mg. and ALEE 400 mg, the
value of the gastric index has 0. The ALEE group of 100 mg and
without treatment on 8th day still had an index of ulcers 0.22 and 1.48.

Based on statistical results using one-way ANOVA method, there was
a significant difference (p<0.05) to data of amount and index of peptic
ulcer on days 3, 8, and 14. This shows that ALEE suspension preparation
has a healing effect of peptic ulcer. Then proceed with post hoc Tukey
honestly significant difference test to see the real difference of each rat
1.48
Tukey
treatment. The results of the post hoc Tukey test show that the three
ALEE suspension preparations show significantly different results than
the negative controls.

Microscopic observations were performed by histopathologic tests on
rat stomach tissue. The result histopathologic test of rat's gastric with
10×10 magnification can be seen in Fig. 2. Histopathological test results
in gastric mucosa of rats administered by sucralfate and suspension of
ALEE showed that mucosal cell cohesion was good and there was no
erosion of epithelial cells on the day 14.

DISCUSSION
Orally, administration of acetalosal doses 800 mg/kg for 2 days may
form peptic ulcers, and this can be visually observed on the surgical
stomach and evidenced by the erosion of gastric mucosal cells seen in
the histology induced rat. Acetalosal can cause ulcers by damaging the
gastric mucosal defenses and systemic inhibition of gastric mucosal
protectors through inhibition of cyclooxygenase activity [13]. The
use of nonsteroidal anti-inflammatory drugs (NSAIDs) over long
periods induces gastric ulcer development through the suppression
of prostaglandin synthesis [14]. Prostaglandins have an integral role

Macroscopic observation is also done by calculating the index of peptic ulcer in each group. The result of calculation of the mean peptic ulcer
index is shown in Table 2.

Index ulcers in the 0 day gastric ulcer control group were 2.00,
considered the initial condition of peptic ulcers. The decrease in
the index of ulcers occurred starting on the 3rd day with the greatest
decrease starting from the positive control group (0.95), ALEE 200 mg
(1.47), ALEE 100 mg (1.54), ALEE 400 mg (1.64), and the group without
(1.64)
(1.54)
(1.47)

The gastric ulcer index at day 14 became 0 in the positive control group,
ALEE 100, 200, and 400 mg, while the untreated group had not shown
a cure with a gastric index of 0.42.

Preparation of Na-CMC suspension 0.5%
A total of 0.5 g Na-CMC is sown in a mortar containing±10 ml of hot
distilled water, silenced for 15 min, then crushed until a transparent
mass is obtained, then crushed until homogenous, diluted with
distilled water, homogenized, and fed into a 100 ml tin flask, sufficient
volume with distilled water to the mark line.

Preparation of African leaf ethanol extract (ALEE) suspension
Each extract was prepared with Na-CMC 0.5% with different doses,
100, 200, and 400 mg/kg. Each dose was weighed and mixed with Na-
CMC 0.5% to homogeneous to 10 ml volume.

Preparation of acetalosal suspension
Weigh the acetalosal tablet equivalent to 800 mg, insert in the mortar,
added 0.5 cm Na-CMC to the homogeneous, and then, the volume is
10 ml.

Induction of peptic ulcer
Rat was given an acetalosal dose of 800 mg/kg for 2 days, 2 h after
induction rats divided into 6 groups. Group I of 3 induced rats will
be completely dissected and considered to be the initial condition of
gastric ulcers. Groups II, III, IV, V, and VI each consist of 3 subgroups
(days 3, 8, and 14).
• Group I: Control peptic ulcer
• Group II: No treatment (negative control)
• Group III: Treated with sucralfate 360 mg/kg (positive control)
• Group IV: Treated with 100 mg/kg of ALEE orally
• Group V: Treated with 200 mg/kg of ALEE orally
• Group VI: Treated with 400 mg/kg of ALEE orally.

All the rats in each group were treated daily, and then, the animals were
sacrificed on the 3rd, 8th, and 14th days.

Macroscopic observation
Before treatment rats are fasted for 24 h without food, but still beverages.
The rats are sacrificed by dislocation. The rats were dissected for
his stomach and then opened and observed the occurrence of ulcers in
the rat's stomach.

Macroscopic observations included observations of the number of
peptic ulcers and the index of ulcers. Observation of peptic ulcers is
done by visually observing the number of ulcers formed in the gastric.
The calculated gastric ulcer is then measured in length and width using
a sliding term which then the data will be used for the calculation of the
peptic ulcer index by the following formula [12]:

\[
\text{Total area of ulcer} = \frac{\text{Total area of gastric mucosa}}{100}
\]

Microscopic observation
The histological observation of gastric mucosal is performed using a
light microscope with 10×10 and 10×40 magnification.

RESULT
The observed data on the average number of ulcers for each group are
shown in Table 1. Conditions at the beginning of ulcers (Day 0) indicate
the formation of 7 peptic ulcers. The decrease in the number of peptic
ulcers can be seen on the 3rd day which occurs in all treatment groups.
The smallest ulcers on the 3rd day started from the positive control
group and ALEE 200 mg (3.33 ulcers), ALEE 400 mg (3.66 ulcers), ALEE
100 mg (4 ulcers), and without treatment (4.66 ulcers).

Positive control group, ALEE 200 and 400 mg showed healing on the
8th day, and visual observation showed no more ulcers in rat stomach.
Gastric ulcers were presented on the day 8 in the ALEE 100 mg (0.66 ulcers)
and without treatment (2.33 ulcers). Healing of peptic ulcers on day 14
was shown in the positive control group, ALEE 100, 200 and 400 mg. The
untreated group still showed a gastric ulcer (1.33 ulcers) until day 14.

Fig. 1: Effect of the African leaf ethanol extract on the peptic ulcer
index
in stomach protection through stimulation of mucus and bicarbonate secretion and promotion of blood flow and proliferation of epithelial cells. NSAIDs are the second most common cause of gastric ulcers, particularly in seniors [15].

The healing effect of the positive control group is caused by the ability of sucralfate at the acidic atmosphere to form a viscous paste selectively binding at the base of the ulcer and becomes a barrier that protects the ulcer against the diffusion of acids, pepsin, and bile salts (local protection). Sucralfate also has cytoprotection properties by increasing the production of prostaglandins as well as stimulating the secretion of mucus and bicarbonate [16].

The healing effects of gastric ulcers that occur in the ALEE group of 100, 200, and 400 mg are caused by flavonoid, saponin, and tannin contents that play a role in the healing of peptic ulcers [17]. Sayed et al. reported that flavonoids and tannins in Vitis vinifera leaves have antioxidant activity which play a role in the healing of peptic ulcers [18]. Flavonoids and tannins are among the active compounds in plants that can provide protection against gastric ulcers by acting as a protective (protective) gastric factor [19]. Flavonoids are natural phenolic compounds with a low molecular weight that has a wide range of biological effects, including gastric anti-stomach activity [20].

Tanin is known to have styptic properties and is the ability to react with proteins in the tissues of the gastric mucosa. Its ability is useful for coating the outer layer of mucosa that makes it less permeable and more resistant to ulcers or irritation. The healing effects of peptic ulcers from tannins are to form microprotein deposits at the site of ulceration to form a protective layer that makes them more resistant to

### Table 1: Effect of the ALEE on number of ulcers

<table>
<thead>
<tr>
<th>Day</th>
<th>Control ulcer</th>
<th>Negative control</th>
<th>Positive control</th>
<th>ALEE 100 mg</th>
<th>ALEE 200 mg</th>
<th>ALEE 400 mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>7.00±1.00</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>-</td>
<td>4.66±0.57</td>
<td>3.33±0.57</td>
<td>4.00±2.00</td>
<td>3.33±1.52</td>
<td>3.66±1.15</td>
</tr>
<tr>
<td>8</td>
<td>-</td>
<td>2.33±0.57</td>
<td>0</td>
<td>0.66±0.57</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>14</td>
<td>-</td>
<td>1.33±1.15</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

All the values are express as mean±SD. SD: Standard deviation, ALEE: African leaf ethanol extract.

### Table 2: Effect of the ALEE on index of ulcers

<table>
<thead>
<tr>
<th>Day</th>
<th>Control ulcer</th>
<th>Negative control</th>
<th>Positive control</th>
<th>ALEE 100 mg</th>
<th>ALEE 200 mg</th>
<th>ALEE 400 mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>2.00±0.22</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>-</td>
<td>1.77±0.04</td>
<td>0.95±0.13</td>
<td>1.53±0.44</td>
<td>1.41±0.52</td>
<td>1.48±0.21</td>
</tr>
<tr>
<td>8</td>
<td>-</td>
<td>1.48±0.08</td>
<td>0</td>
<td>0.21±0.21</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>14</td>
<td>-</td>
<td>0.42±0.38</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

All the values are express as mean±SD. SD: Standard deviation, ALEE: African leaf ethanol extract.
CONCLUSION

Based on the results of this study, it can be concluded that ALEE has a healing effect of gastric ulcers because it can reduce the number and index of peptic ulcers and can restore gastric mucosal cell cavity damaged by peptic ulcer.

The results can be more accurately assessed by either involving a larger sample size or using a higher concentration of the extract.

ACKNOWLEDGMENTS

The authors gratefully acknowledge to all the parties involved in this research, especially to all pharmacology laboratory staff of the pharmacy faculty of Universitas Sumatera Utara.

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