Ethanolic extract of *Lannea coromandelica* stem bark: Histopathology and infiltration of inflammatory cells in the rat’s gastric

Achmad Ramadhan,
Hastuti Herman,
Sutrisnawati Sutrisnawati

Department of Biology Education,
Faculty of Teacher Training and Education, Tadulako University, Palu, Indonesia

**Abstract**

This study determined the efficacy of extract of the stem bark of *Lannea coromandelica* (ESBLc) on histopathology and inflammatory cell infiltration in the gastric of rats induced by mefenamic acid. We grouped 20 rats (*Rattus norvegicus*) into 5; Group 1 (positive control, mefenamic acid + sucralfate suspension), Group 2 (negative control, mefenamic acid), Group 3 (mefenamic acid + ESBLc 1575 mg/kg), Group 4 (mefenamic acid + ESBLc 3150 mg/kg), and Group 5 (mefenamic acid + ESBLc 3600 mg/kg). The dose of mefenamic acid used was 23.25 mg/kg, given orally for 7 days. Gastric histopathological observations were carried out qualitatively, and inflammatory cell infiltration was analyzed quantitatively by one-way ANOVA. The qualitative and quantitative analysis results showed that ESBLc had efficacy in restoring damaged gastric tissue of rats; statistically, 3150 mg/kg and 6300 mg/kg effectively reduced inflammatory cell infiltration. ESBLc recovered the function of gastric organs of *Rattus norvegicus L.* induced by mefenamic acid, including improved mucosa and reduced inflammatory cell infiltration in the gastric. The doses of ESBLc, which effectively reduced inflammatory cell infiltrations, were 3150 mg/kg and 6300 mg/kg BW.

**Key words:** Ethanolic extract, gastric, histopathology, inflammatory, *Lannea coromandelica*

**INTRODUCTION**

Various steroidal and non-steroidal anti-inflammatory drugs (NSAIDs) are commonly implemented in treating inflammatory diseases,[1] including mefenamic acid NSAIDs. About 7 days of intervention of 23.25 mg/day may cause gastric abnormalities in the form of acute inflammation and erosion of the gastric mucosal epithelium in rats.[2] Treatment of stomach inflammation usually uses drugs from the antacid class; H₂ antagonist drugs work by reducing the production of stomach acid. Examples of ulcer drugs included in this drug class are cimetidine, famotidine, ranitidine, sucralfate, and several other drugs.[3]

Some currently available agents used to treat ulcers have not given the desired results, and their usefulness is limited due to many side effects. Therefore, alternative control methods are needed.[4] Plant-based products seem promising in the new quest for a better ulcer treatment. Several medicinal plants reported having anti-ulcer properties were tested on experimental animals, including *Musa paradisiaca*, *Loranthus micranthus*, and *Acalypha wilkesiana*.[5] *Lannea coromandelica*

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belong to the Anacardiaceae family and includes tropical
trees, widely distributed in tropical countries, including
India and Bangladesh,[8] conventionally various parts of
this plant are used by several ethnic communities in Central
Sulawesi Province, Indonesia as a medicinal plant. The bark
is beneficial for treating bruises, wounds, gout, ophthalmic
ulcers, ulcerative stomatitis, sprains, odontalgia, dysentery,
and diarrhea.[7] In addition, the decoction of plant leaves
was found effective as cough medicine, ulcer medicine, and
appetite enhancer.[8]

*L. coromandelica* plants contain several phytochemical
compounds, including saponins and flavonoids.[9] The
ethanolic extract of the bark of Java (*L. coromandelica*)
contains secondary metabolites of flavonoids,
carbohydrates, gums, mucilages, and tannins.[10] The
chemical content of the bark of *L. coromandelica* contained
13 compounds identified as quercetin, including aralia
cerebroside, (2S,3S,4R,10E)-2-[(2′R)-2′-hydroxytetra
cosanoyl amino]-10-octadecene-1,3,4-triol, sitosterol
palmitate, sitosteryl-3β-glucopyranoside-6′-O-palmitate,
5,5′-dibuthoxy-2,2′-bifuran, protocatechuic acid, myricadiol,
isovalinil, p-hydroxybenzoic acidethyl ester, stearic acid,
palmitic acid, and trans-cinnamic acid.[11]

This study determined the efficacy of extract of the stem
bark of *L. coromandelica* (ESBLc) on histopathology and
inflammatory cell infiltration in the gastric of rats induced
by mefenamic acid.

**MATERIALS AND METHODS**

The bark of *L. coromandelica* was obtained from the Sigi
Regency, Central Sulawesi Province, Indonesia, and
subsequently identified by a laboratory assistant at the
Biology Education Laboratory, Tadulako University, for
3 months. A total of 500 g of dried simplicia stem bark of
*L. coromandelica* was put into a maceration container, and
96% ethanol was added until the bark was submerged.
Next, stirred and placed for 3 × 24 h and filtered to get
the filtrate. Finally, the filtrate was concentrated with a
rotary evaporator and weighed to calculate the yield.
Phytochemical tests on the bark extract of *L. coromandelica*
included alkaloids, saponins, polyphenols, and flavonoids.

**Effects of ethanolic extract of *Lannea coromandelica*
stem bark on rats**

The white male rats of the Wistar strain, weighing 200–250 g,
aged 8–10 weeks, and as many as 20 individuals were
used in this study. During adaptation (7 days), they were
treated and given *ad libitum* feed. The induction agent
used to irritate the stomach was mefenamic acid 500 mg,
a type of NSAID. The dose given to rats was 23.25 mg/
day. These doses cause abnormalities in the stomach as
acute inflammation and erosion of the gastric mucosal
epithelium.[12] Mefenamic acid was administered orally for
7 days. Furthermore, the rats were orally given the ethanolic
Experimental animal care and research protocols center on
values and guidelines approved by the Laboratory Animal
Care and Use Guidelines. We grouped the animals into 5,
according to the dose [Table 1]. The selection of doses given
to experimental animals was based on traditional use in the
community as an ulcer reliever and has been converted to
the use of experimental animals. Histological slide method
of gastric organs based on the Theory and Practice of
Histological Techniques guidebook.[13]

**Data analysis**

The data from the observation of gastric histopathology
were analyzed qualitatively, and the changes in gastric
histology (inflammatory cell infiltration) were tested for
normality with the Shapiro–Wilk test. The difference
was identified using one-way ANOVA test with a 95%
confidence level, followed by Duncan’s multiple range
test at α=0.05.

**RESULTS**

**Phytochemicals of ethanolic extract of *Lannea coromandelica* stem bark**

The results of the phytochemical test showed that ESBLc
was positive in alkaloids, polyphenols, saponins, and
flavonoids [Table 2].

**Histopathology of white rat’s gastric**

According to the gastric histology [Table 3 and Figures 1, 2],
there was desquamation of the gastric mucosal epithelium,
mucosal erosions, and inflammatory cell infiltration with
severe damage in Group 2 (negative control). Furthermore,

### Table 1: Inflammatory cell infiltration in the stomach of experimental animals after being given extract of the stem bark of *Lannea coromandelica*

| Groups  | Treatment                                           | Inflammatory cell infiltration (mean±SD) |
|---------|-----------------------------------------------------|------------------------------------------|
| Group 1 | 23.25 mg/kg mefenamic acid + 1 ml sucralfate suspension (positive control) | 12.00±0.816                              |
| Group 2 | 23.25 mg/kg mefenamic acid without ESBLc (negative control) | 19.00±1.826                              |
| Group 3 | Mefenamic acid dosage of 23.25 mg/kg bodyweight+ESBLc-1575 mg/kg | 14.25±1.258b                             |
| Group 4 | Mefenamic acid dosage of 23.25 mg/kg bodyweight+ESBLc-3150 mg/kg | 13.25±1.258a,b                          |
| Group 5 | Mefenamic acid dosage of 23.25 mg/kg bodyweight+ESBLc-6300 mg/kg | 12.75±0.957a,b                          |

*a, b* Statistically significant differences (P<0.05). Each value is mean±SD (n=4). SD: Standard deviation, ESBLc: Extract of the stem bark of *Lannea coromandelica*.
Groups 3 and 4 had mucosal desquamation and erosion at a moderate level, but the inflammatory cells decreased. Moreover, Groups 1 and 5 experienced cell mucosal desquamation and erosion, with inflammatory cell infiltration in the mild category, which improved [Figure 1].

**White rat’s gastric inflammatory cell infiltration**

ESBLc had significant activity against white rats’ gastric recovery induced mefenamic acid (*P* < 0.05). The decrease in inflammatory cell infiltration after ESBLc was given as the dose increased. The administration of 3150 and 6300 mg/Kg ESBLc effectively suppressed the amount of inflammatory cell infiltration [Table 1].

**DISCUSSION**

In this study, the histopathological examination of rats’ gastric tissue showed that ESBLc could repair gastric tissue that experienced gastric epithelial cell desquamation, mucosal erosion, and gastric cell infiltration induced by mefenamic acid. The results showed that there were differences in gastric histopathology of Wistar rats in the negative control group (Group 2) who were not given ESBLc with the group given ESBLc (G3, G4, and G5) and with group 1 (positive control). Normal microscopic images with the gastric wall structure, such as normal mucosa, submucosa, muscular, and serous layers, were found in Group 1 and Group 5. Microscopic images of the Wistar rat’s gastric in the treatment group given mefenamic acid for 7 days showed infiltration of inflammatory cells of the edema gastric mucosal stroma [Figure 1b]. Mucous irritation occurs due to increased exfoliation of mucosal epithelial cells due to drugs that irritate, thereby reducing mucus secretion, a protective barrier against acidic substances, such as gastric acid. Mefenamic acid can irritate the gastric mucosa by inhibiting the biosynthesis of prostaglandins through the cyclooxygenase enzyme.[14] The mechanism of action of mefenamic acid, like other NSAIDs, is not fully understood, but involves inhibition of cyclooxygenases (COX-1 and COX-2). Mefenamic acid inhibits both isoforms of the cyclooxygenase enzyme (COX-1 and COX-2). This prevents the formation of prostaglandins, a key in protecting the integrity of the gastric mucosa by increasing local blood flow and promoting the synthesis and secretion of mucus.
and bicarbonate.\(^{[15]}\) Decreased synthesis of prostaglandins causes a decrease in mucus and bicarbonate secretion, which results in damage to the gastric mucosa. In addition, prostaglandins are vasodilators that affect widening blood vessel walls to increase blood flow to tissues. Inhibiting prostaglandins can reduce the flow of blood circulation, one of which is to the stomach. Gastric tissue can experience ischemia, which can cause the mucosa to erode.\(^{[16]}\)

NSAIDs’ anti-inflammatory and analgesic effects are based on the inhibition of cyclooxygenase, thereby inhibiting prostaglandin synthesis.\(^{[17]}\) It may decrease HCO3-secretion, weaken mucosal protection, and stop the inhibition of acid secretion. It also damages the mucosa locally through nonionic diffusion into the mucosal cells; hence, the inhibitory effect on platelet aggregation will increase the risk of bleeding. However, further research is still needed to determine the levels of prostaglandins in the blood of rats given ESBLc.

The progression of gastric tissue repair in Group 4 and Group 5 showed that ESBLc could repair gastric tissue. The improvement was due to the content of ESBLc, which is rich in phytonutrients. Based on qualitative phytochemical tests, the ethanolic ESBLc revealed alkaloids, polyphenols, saponins, and flavonoids. Several other studies have reported that these compounds positively affect their antioxidant properties.\(^{[18]}\) This phytochemical agent has yielded positive results when tested for its anti-ulcer and gastroprotective properties. The presence of significant phytoconstituents in the ESBLc makes it a potential candidate for future investigations.

Based on the one-way ANOVA test, the infiltration of inflammatory cells in the stomach of rats given ESBLc induced by mefenamic acid showed a significant difference (\(P < 0.05\)) among the five treatments above. The results showed that there was a significant difference (\(P < 0.05\)) between the positive control treatment (Group 1) and the negative control (Group 2) and Group 3, while the comparison between the positive control (Group 1) and Group 4 and Group 5 was not significantly different (\(P < 0.05\)). Thus, ESBLc at a dose of 3150 mg/kg (Group 4) and a dose of 6300 mg/kg BW (Group 5) effectively reduced the amount of inflammatory cell infiltration in the stomach of white rats. With reduced cell injury, the infiltration of inflammatory cells of lymphocytes and Polymorphonuclear leukocytes (PMNs) in the gastric mucosa is reduced.\(^{[19,20]}\) Based on the research results, the plant understudy can be a source of new anti-ulcer. However, further research should be carried out to isolate and identify the bioactive compounds and determine their specific activities.

**CONCLUSION**

ESBLc recovered the function of gastric organs of white rats (Rattus norvegicus L.) Wistar strain induced by mefenamic acid includes improved mucosa and reduced inflammatory cell infiltration in the gastric. The doses of ESBLc, which effectively reduced inflammatory cell infiltrations, were 3150 mg/kg and 6300 mg/kg BW.

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**Ethical approvals**
Prior approval to conduct animal experiments was obtained from the Ethics Committee of Tadulako University (No. 09692A/UN28.1.31/PT/2020).

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**Conflicts of interest**
There are no conflicts of interest.

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