eISSN (Online): 2598-0580

Bioscientia Medicina: Journal of Biomedicine & Translational Research

Journal Homepage: www.bioscmed.com

The Effect of Active Gambir (*Uncaria gambir*) Fraction on TNF-α Protein Expression and Lesion Size in White Rats Gastritis Model

Ni Wayan Lisa Suasti[1*], Lunsi Oktafitria[1], Ellya Aidia[1], Rahmad Dhani[1]

[1] Bachelor of Pharmacy Study Program, Sekolah Tinggi Ilmu Farmasi Bhakti Pertiwi, Palembang, Indonesia

ARTICLE INFO

Keywords:
Gambir
TNF-α
Antiinflamatory
Gastiritis

***Corresponding author:**
Ni Wayan Lisa Suasti

E-mail address:
dr.wayanlisa@gmail.com

All authors have reviewed and approved the final version of the manuscript.

https://doi.org/10.32539/bsm.v2i3.53

ABSTRACT

Background: Gambir (*Uncaria gambir*) is empirically used to treat abdominal pain and vomittus caused by gastritis because of its anti-inflammatory effects, especially flavonoid. This study aims to determine the effect of active gambir fraction on TNF-α protein expression and wound size in white rats gastritis model. **Methods:** The research method used experimental study design in vivo with post test with control group design. Rats were divided randomly in 11 groups and were induced to be gastritis for 1 day. Group 1 (negative control) was given aquadest of 5 mL, group 2 (positive control) was administered ranitidine 10 mg/kgBW, groups 3, 4, and 5 were given n-hexane fraction, groups 6, 7 and 8 were given a water fraction, and groups of 9, 10, and 11 were given ethyl fractions with each group receives dose of 20, 40, and 80 mg/kgBW and all groups were treated for 3 days. Rats were dissected on 5th day for examination of gastric mucosal lesion size and performed ELISA expression of TNF-α expression of gastric mucosal tissue. The results of this study were assayed by SPSS 18. **Results:** The result of the research using *Kruskal-Wallis* test showed that there were significant differences ($p < 0.05$) of the lesions size between the sample groups where control positive, ethyl fraction 20, 40, 80 mg/kgBW, and water fraction 20, 40 mg/kgBB had the gastric mucosal lesion size differed significantly with the negative control group, while the TNF-α protein expression test using *Kruskal-Wallis* showed that there was a significant difference ($p < 0.05$) TNF-α levels of all groups against the negative control. **Conclusion:** Active gambir fraction had a potention to reduce size of mucose gaster lesion and reduce expression of TNF-α protein.

1. Introduction

The World Health Organization (WHO) conducted a review of several countries in the world and got the percentage of gastritis in the world, including the UK 22%, China 31%, Japan 14.5%, Canada 35% and France 29.5%. In Indonesia, the incidence of gastritis is quite high, the prevalence is 274,396 cases from 283,452,952 people.[1] Gastritis is a condition in which the lining of the stomach, known as the mucosa, becomes irritated and inflamed. The inflammatory process can be shown by the increased expression of TNF- protein because it has a role in the inflammatory process that can increase and stimulate the adhesion of leukocyte cells. TNF- is a cytokine that is widely secreted by macrophages and has many metabolic roles such as cell proliferation, differentiation, apoptosis.[2] Gastritis which is an inflammation of the gastric mucosa (gastric) can be divided into acute and chronic gastritis. Acute gastritis is gastritis that occurs suddenly, severe, and in a short time, while chronic gastritis is gastritis that occurs in the long term. Erosive gastritis is another type of gastritis that may be acute or chronic, but the lining of the stomach is eroded either superficially or deeply, so bleeding and death can occur. Clinically, gastritis can be felt as complaints of heartburn, nausea, and vomiting that cause discomfort, even erosive gastritis can cause bloody stools, and vomiting blood

Gastritis management is drugs that reduce the amount of stomach acid in order to reduce gastritis symptoms and drugs that reduce the risk of gastritis.

can improve gastric mucosal repair, such as antacids, histamine-2 (H2) blockers, and proton pump inhibitors (PPIs). Other treatments given are antibiotics for *H. pylori infection* and discontinuation of drugs that can trigger gastritis such as NSAIDs.[3] One of the traditional gastritis management is using gambier extract. In Japan, gambier is empirically used to relieve symptoms of abdominal pain and vomiting that may be caused by gastritis.

Study of the effects of *Uncaria gambir* Roxb. on gastric ulcers and malondialdehyde levels in animals induced by ethanol showed that gambier extract at a dose of 200mg/kgBW could improve gastric ulcers.[5] In addition, studies on the effect of gambir from *Uncaria gambir* (hunter) Roxb. for pH and gastric ulcers in white male rats showed that gambier at a dose of 20 mg/kg, 40 mg/kg, 80 mg/kg orally for 2 days could heal ulcers in the gastric mucosa of male white rats with a healing percentage of 51, respectively. 93%; 55.98% and 63.90% respectively.[6]

Gambir is a natural wealth owned by Indonesia where gambier material is still available and world export demand for gambier leaves continues to increase throughout the year (2000-2004), the increase in export volume reached 87.49%,[7] so that it became the basis for researchers to make gambier as one of the candidates for the treatment of gastritis and eventually became the standard treatment.

2. Methods

the design of this study was anexperimental study *in vivo* with *posttest with control group design*.

Subjects

The subjects of this study were white rats (*Rattus norvegicus*) Wistar strain, 2-3 months old, weighing between 150-200 grams. The sample size was calculated using the federer's formula: (n-1) x (t-1) 15 where n is the sample size and t is the group. The result of the calculation is 3 rats in each group.

Preparation of the active fraction of gambir

Simplicia gambir is dried first by aeration, then mashed. Furthermore, maceration with 1:10 ethanol solvent, then, the maceration was evaporated with a *rotary evaporator* to obtain a thick extract. Next, the liquid-liquid fraction with n-hexane, ethyl acetate, and water.

Research procedure

Thirty-three rats were divided into 11 groups. Group 1 (negative control) consisted of 3 white rats induced gastritis and given 5 mL aquadest for 3 days. Group 2 (positive control) consisted of 3 white rats induced gastritis and given ranitidine 10 mg/kgBW for 3 days. Groups 3, 4 and 5 of 3 white rats in each group, induced gastritis, obtained n-hexane fraction ethanol extract with each group receiving doses of 20, 40, and 80 mg/kgBW for 3 days. Groups 6, 7, and 8 consisted of 3 white rats in each group, induced gastritis, obtained water fraction ethanol extract with each group receiving doses of 20, 40 and 80 mg/kgBW for 3 days. Groups 9, 10 and 11 consisted of 3 white rats in each group, induced gastritis, obtained the ethyl fraction of ethanol extract with each group receiving doses of 20, 40, and 80 mg/kgBW for 3 days.

Measurement of TNF- α protein expression

Measurement of TNF- protein expression used the TNF- ELISA and Sunlong biotech Rat ELISA methods. The ELISA procedure is based on the test procedure in the manual.

Measurement of the size lesion

The gastric mucosalmeasurement of the size of the lesion in the gastric mucosa was carried out with the help of a caliper. Each lesion found in the gastric mucosa was assessed for the length and width of the lesion to obtain the extent of each gastric mucosal lesion. Then, the total area of each lesion was added up to get the total area of the lesion.

Data Analysis

The results were tested using SPSS 18. Data were tested for bivariate and multivariate analysis. The Mann-Whitney bivariate analysis was used and the Kruskal-Wallis multivariate test was used.

3. Results

Analysis of the size of gastric mucosal lesion

The results of the Kruskal-Wallis test showed that there were significant differences in gastric mucosal lesions between the sample groups. This test was followed by the Mann-Whitney test to determine the relationship between each sample group. The results of the Mann-Whitney test showed that the positive control group, the ethyl fraction 20, 40, 80 mg/kg BW, and the water fraction 20, 40, 80 mg/kg BW had mucosal lesion areas that were significantly different from the negative control group. The 80 mg/kg BW gambier ethyl group had the most significant effect, even compared to positive control, in reducing the lesion area.

Table1. The size of the lesion mucosal gaster in rats each group

Group	size Mean (cm2)	*p value* *	Difference Mean (%)
Negative	1.34 ± 1.09	1.000	to
Positive	0.13 ± 0.12	0.049	090.29
n-hexane 20 mg / kg	1.04 ± 0.01	0.513	- to
n-hexane 40 mg / kg	0, 84 ± 0.84	0.513	22.3937.31
n-hexane 80 mg / kg	0.97 ± 1.08	0.513	- to
Ethyl 20 mg /kgBW	0.09 ± 0.00	0.046	27.6193.28
Ethyl 40 mg/kgBW	0.06 ± 0.03	0.046	- to
Ethyl 80 mg/kgBW	0.04 ± 0.06	0.049	95.5297.01
Water 20 mg/kgBW	0.27 ± 0.14	0.049	- to
Water 40 mg/kgBW	0.23 ± 0.17	0.049	79.8582 ,84
Water 80 mg/kgBW	0.13 ± 0.11	0.049	- 90,29

*Mann-whitney***,** p<0,05

Measurement of TNF-α protein expression

The results of the Kruskal-Wallis test showed that there was a significant difference between TNF- protein expression between the sample groups. This test was followed by the Mann-Whitney test to determine the relationship between each sample group. The results of the Mann-Whitney test showed that the positive control group and all test groups had TNF- protein expression that was significantly different from the negative control group. The 80 mg/kg BW ethyl group had the best significant effect, even compared to positive control, in reducing TNF- α protein expression.

Table 2. TNF- α Protein expression in white rats

Group	Protein Expression TNF- α	p value	Difference in mean (%)
Negative	66.85 ± 4.42	1,000	0
Positive	16.01 ± 1.24	0.049	- 79.05
MeanHexane 20 mg/kgBW	31.19 ± 2.68	0.049	- 53.35
Hexane	32.73 ± 2.30	0.049	- 51.03
40 mg/kgBW Hexane	± 4.56	0.049	- 63.21
80 mg/kgBW	24.5930.58 ± 1.00	0.049	- 54.25
Ethyl 20 mg/kgBW	20.67 ± 1.60	0.049	- 69.08
Ethyl 40 mg /kgBW	12.52 ± 1.11	0.049	- 81.27
Ethyl 80 mg/kgBW	32.33 ± 2.88	0.049	- 51.63
Water 20 mg/kgBW	29.50 ± 2.06	0.049	- 55.87
Water 40 mg/kgBW	25.40 ± 3.11	0.049	- 62, 02
Water 80 mg/kgBW			

Mann-whitney, p<0.05

4. Discussion

Gambier ethyl fraction showed an effect dose dependent in reducing lesion size, even better than positive control. The dose of ethyl fraction of 80 mg/kgBW had the best effect in reducing the area of the lesion, followed by the ethyl fraction of doses of 40 and 20 mg/kgBW. The ethyl gambir fraction of 80 mg/kgBW also showed the best effect in reducing TNF- protein expression in the gastric mucosa of white rats even when compared to the positive control group. This may be related to the effect of the ethyl fraction which has an anti-inflammatory effect that inhibits inflammation, whereas ranitidine is only able to reduce gastric acid secretion by selectively inhibiting H2 receptors so that ranitidine only inhibits further inflammatory processes.

The ethyl fraction attracts a large number of flavonoids because flavonoids are semipolar, although the water fraction can also attract small amounts of flavonoids. Catechin belongs to the class of flavonoids that have the potential to be anti-inflammatory and are present in gambier herbs. This content gave good results in reducing the expression of TNF- α protein in the gastric mucosa and succeeded in reducing the lesion area. The larger the dose of ethyl acetate fraction, the more flavonoids that can be withdrawn so that the dose of ethyl acetate fraction shows a form dose dependent in reducing TNF- α protein expression and the size of gastric mucosal lesions. In addition to flavonoids, the anti-inflammatory effect is also shown by saponins which can be attracted by the water fraction so that the water fraction also shows a good effect in reducing TNF- α expression and gastric lesions. The n-hexane fraction also has a good effect in reducing TNF- α expression which may be due to the steroid content in it, but the n-hexane fraction does not have the ability to reduce the lesion area as obtained from other fractions. This may be due to the steroid content which has been the subject of debate in the formation of ulcers due drug-induced to reduced gastric protective function, namely reduced mucus production, angiogenesis, and gastric epithelial repair.[9,10]

5. Conclusion

The active fraction of gambir has the potential to reduce the size of gastric mucosal lesions and reduce the expression of TNF- protein.

6. Acknowledgments

We thank dr. Rachmat Hidayat, M.Sc. and Maisha Pusrita, ST. in the process of implementing this research laboratory.

7. References

1. Zaqyyah, H. Relationship of Knowledge about the Causes of Gastritis with Gastritis Prevention Behavior. Banjarmasin. 2017; 1(1).
2. Siregar, A., Halim, S., Ricky RS. Serum TNF-α. IL-8, VEGF Levels in Helicobacter pylori Infection and Their Association with Degree of Gastritis. Faculty of Medicine, University of North Sumatra. 2015; 47(2).
3. US Department of Health and Human Services. Gastritis. National Institute of Diabetes and Digestive and Kidney Disease. 2015.
4. Kalaiselvi, P., Rajashree, K., Priya, LB, and Padma, VV. Cytoprotective Effect of Epigallocatechin-3-gallate Against Deoxynivalenol-induced Toxicity Through Anti-oxidative and Anti-inflammatory Mechanisms in HT-29 c. Food and Chemical Toxicology. 2013; 56: 10–118.
5. Irramah M, Julizar, Lili Irawati. Effect of Uncaria gambir roxb on gastric ulcers and malondialdehyde levels of experimental animals induced by ethanol. Padang: Andalas Medical Magazine. 2017; 40(1): 1-10.
6. Suhatri, Zet R. Debhi MI Effect of Giving Gambir from Uncaria gambir(Hunter) roxb on pH and gastric ulcers in male white rats. Padang: Journal of Pharmacy Higea. 2015; 7(1).
7. Dhalim Azmi. Problems of Gambir (Uncaria gambir L.) in West Sumatra and Alternative Solutions. Bogor. 2006; 5(1): 46-59.
8. Azis, N. The Role of H-2 Receptor Antagonists in the Treatment of Peptic Ulcers. Sari

Pediatrics. 2002; 3(4): 222-226.
9. Guslandi, M., Tittobello, A. Steroid ulcers: a myth revisited. BMJ. 655–656
10. Carpani de Kaski, M., Rentsch, R., Levi, S., Hodgson, HJ. Corticosteroids reduce regenerative repair of epithelium in experimental gastric ulcers. gut. PubMed. 1995; 37: 613–616.