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Effectiveness of neem (*Azadirachta indica a.juss*) bark extract as a gastroprotektor

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ABSTRACT

Background: Gastric ulcer is a digestive problem that has a high prevalence rate, peptic ulcer can cause complications such as bleeding in the gastrointestinal tract, perforation, recurrence, cancer and even death. The neem stem bark extract has a compound that can be a gastroprotective activity so that it can reduce the number of gastric ulcer and gastric acid levels. Gastroprotective effects of neem bark extract in combination have been widely studied can reduce gastric ulcer, but no one has examined the extract of neem bark as a single gastroprotector.
Objective: This study aims to determine the dose of extract that can prove the extract of neem stem bark can inhibit the ulcer index by macroscopic observation and microscopic images of in rats
Methods: This research is a purely experimental study carried out in July – September 2019. The neem stem bark extract was given to Wistar male white rats with various dosages for seven days orally, after 24 hours of fasting, induced gastric ulcer with ibuprofen 400 mg/kg. Mice were dissected to activate the ulcer index after 8 hours of induction of gastric ulcer.
Result: The ulcus index of negative group (ibuprofen), positive group (ranidin), and extracts of mimba rods 250, 500, 1000, and 2000 CMG/kgBB in a row were 7.13, 16.76, 7.41, 2.32, and 2.00 (p= 0.034). Microscopic observations suggest a much better picture of histology after this extract.
Conclusion: The extract of a mimba bark has gastroprotective activity at 2,000 mg/kgBB.

Latar Belakang: *Tukak lambung merupakan salah satu gangguan pencernaan yang memiliki angka prevalensi yang tinggi. Tukak lambung dapat menyebabkan komplikasi seperti perdarahan pada saluran cerna, perforasi, kekambuhan, kanker bahkan kematian. Ekstrak kulit batang mimba memiliki senyawa yang dapat menjadi aktivitas gastroprotektif sehingga dapat menurukan jumlah tukak lambung dan kadar asam lambung. Efek gastroprotektif ekstrak kulit batang mimba secara kombinasi sudah banyak diteliti dapat menurunkan tukak lambung namun belum ada yang meniliti ekstrak kulit batang mimba secara tunggal sebagai gastroprotektor.*
Tujuan Penelitian: *Penelitian ini bertujuan untuk mengetahui dosis ekstrak yang dapat membuktikan bahwa ekstrak kulit batang mimba dapat menghambat indeks tukak secara pengamatan makroskopis dan didukung oleh gambaran mikroskopis pada tikus putih jantan wistar yang diinduksi ibuprofen.*
Metode: *Penelitian ini yaitu penelitian eksperimental murni yang dilaksanakan pada bulan Juli – September tahun 2019. Ekstrak kulit batang mimba diberikan pada tikus putih jantan wistar dengan beberapa variasi*

dosis selama 7 hari secara oral. Setelah dipuasakan 24 jam tikus diinduksi tukak lambung dengan ibuprofen 400 mg/kg. Tikus dibedah untuk diamati indeks tukak setelah 8 jam induksi tukak lambung.
Hasil: *Indeks tukak kelompok negatif (Ibuprofen), kelompok positif (Ranitidin), dan Ekstrak kulit batang mimba dosis 250, 500, 1000, dan 2000 mg/kgBB berturut-turut adalah 7.13; 16.76; 7.41; 2.32, dan 2;00 (p=0,034). Pengamatan mikroskopis menunjukkan gambaran histologi lebih baik setelah diberikan ekstrak ini.*
Kesimpulan: *Berdasarkan penelitian ini dapat disimpulkan bahwa ekstrak kulit batang mimba dosis 2000 mg/kgBB memiliki aktivitas gastroprotektif.*

INTRODUCTION

Health development is currently facing two significant problems; the communicable (infectious) and non-communicable diseases. The latter condition is increasing in morbidity and mortality, which are mostly caused by unhealthy lifestyles such as lack of physical activity, unhealthy eating patterns, and mental-emotional disorders (stress). Those unhealthy lifestyles may induce a spectrum of digestive disorders that resulted from an imbalance of defensive and aggressive factors (endogenous and exogenous). This disorder may cause complications such as bleeding in the gastrointestinal tract, perforation, recurrence, cancer and even death. However, many patients often ignored these conditions that lead to chronic and more severe form of gastrointestinal problems.

Gastric ulcer is a disease of the digestive tract with high prevalence rate. Based on the WHO 2011, the incidence of gastric ulcers in the world, around 1.8-2.1 million of the population every year. In the United States, peptic ulcer disease affects about 4.5 million people every year, with 20% due to Helicobacter pylori. Hospitalization occurs in around 180,000 patients, and 5,000 people die every year.[1] In Indonesia, the incidence of peptic ulcers has been reportedly high in several capital cities. In Medan (North Sumatra), the rate is reaching 91.6%, Jakarta 50%, Denpasar 46 %, Palembang 35.5%, Bandung 32.5%, Aceh 31.7%, Surabaya 31.2% and Pontianak 31.2%.[2]

The use of natural ingredients for gastric ulcers has been introduced empirically in many regions. The Neem plant is known for its anti-inflammatory and antimicrobial activity. Besides, the Neem Bark water extract (Azadirachta indica A. Juss) is potentially having a gastroprotective effect. It shows a strong anti-secretory and anti-ulcer properties in vivo studies (both in human and animals) from its phenolic compounds.[3,4] Anti-secretory activity occurs through inhibition of H+/K+-ATPase, whereas anti-ulcer property occurs from the inhibition of peptide acid dilution, inhibition of gastric mucus depletion, prevention of damage to the oxidative mucous layer caused by decreased levels of glutathione and decrease the lipid peroxide as the primary cause of gastric lesions.[5]

However, there is still little clinical evidence on the dosage of this ingredient to successfully preventing ulcer in vivo. Therefore, this study aims to determine the extract dosage, which can prove the efficacy of the neem stem bark extract in inhibiting the ulcer index by macroscopic observation and microscopic examination.

METHODS

Material and method

This research is a pure experimental study, with the subject of research is the Wistar male white rat (Rattus norvegicus) that required 2-3 months with a body weight of 150-200 grams and acclimatized for 7 days. An Ethics Approval (Ethical Permit) was obtained from the Diponegoro University Research Ethics Committee with No.402/EA/KEPK-FKM/2019 dated September 4, 2019.

Wistar male white rats were divided randomly into six groups as follow; group I was the negative control, group II was the positive control with the administration of ranitidine 400 mg/kg, with the rest were administration with neem bark extract 250 mg/kg, 500 mg/kg, 1000 mg/kg, and 2000 mg/kg for group III, IV, V, and VI respectively.

All groups were given for seven days and fasted for 24 hours. On the 8th day, all groups were induced ibuprofen 400 mg/kg at 8-hour

surgical intervals based on preliminary tests that had been carried out. Doses of ibuprofen 400-800 mg/day can be absorbed quickly by the intestine and provide a rapid analgesic effect.[6] Ibuprofen is a non-steroidal anti-inflammatory drug (NSAID) that is difficult to dissolve in the water. Therefore a suspension is carried out in 1% CMC solution. Induction of gastric ulcer was given at 24 hours after the 8th day of Neem bark extract administration. Following 8 hours of induction of peptic ulcers, the animals' gaster were surgically removed and processed for macros and microscopically observations.

Macroscopic Observation

Mice were dissected, and their stomach organs removed after being fasted for 24 hours. Gastric organs are opened along the minor curvature, washed with 0.9% sodium chloride and then stretched to facilitate measurement of the length of the lesion. According to Bancroft & Cook, 1984, ulcer index was calculated based on the area of ulcer severity and number of ulcers. The severity of ulcers, assessed by the length of the lesion, can be done using callipers and then scored. The formula determines the ulcer index[7]:

$$Ulcer\ index = UN + US + 0.1\ (\%1)$$

UN = Average of number of ulcer per animal
US = Average of severity score
% one = Percentage of an animal with ulcer

The ability of test material to reduce or prevent ulcers was assessed by the protection ratio formula[8]:

$$\%\ inhibition = \frac{Control\ group - intervention\ group\ x\ 100\%}{Control\ group}$$

Microscopic Observation

Histopathological preparations were made according to the standard method of making histopathological preparations in the Anatomical Pathology laboratory of the Faculty of Veterinary Medicine, Gadjah Mada University. Stomach soaked in 0.9% physiological NaCl solution for 15 minutes then gastric tissue was fixed in a buffered neutral formalin (BNF) solution of 10% for 24 hours then dehydrated in a stratified ethanol solution after clearing three times using xylol and then infiltrated tissue back into liquid paraffin three times. If the tissue has been infiltrated, embed it into liquid paraffin and cool at room temperature to form a paraffin block. Cutting is done using a microtome in a horizontal direction with a thickness of 3µ - 4µ. The next process is staining with hematoxylin eosin (HE) to see the histological changes in the stomach.

Histopathological examination of the stomach with HE staining was performed on five microscope fields with 100x magnification. Variable histopathological changes in the stomach were observed only based on the picture that can be seen from the results of rat gastric photographs. The occurrence of inflammation is characterized by cell death in the mucosal part, the presence of inflammation that occurs in the sub-region edematous mucosa, which is characterized by stretching the muscular tissue.

Data analysis

For statistical analysis, this study used SPSS 21 with univariate and bivariate analysis using a Kruskal-Wallis test for macroscopic observation data in the form of an ulcer index and protection ratio. Microscopic observation is only a picture of ulcer damage.

RESULTS

Plant Determination Results

The plant material in this study was the Neem bark (*Azadirachta indica A.Juss*) obtained from the plantation area of Pasuruan Regency, East Java. This plant was determined in the Ecology and Biosystematic Laboratory of MIPA UNDIP to find out the truth and description of morphology.

From the results of the determination of the neem bark plant, it is known that this plant comes from the *Azadirachta* family with the species name *Azadirachta indica* A. Juss. The code of determination of the neem bark (*Azadirachta indica* A.Juss) is as follows:

1b, 2b, 3b, 4b, 12b, 13b, 14b, 17b, 18b, 19b, 20b, 21b, 22b, 23b, 24b, 25b, 26b, 26b, 27a, 28b, 29a, ... family 136: Meliaceae ... 1b , 3b, 4b, 10b,

13b, 15a, ……. Genus 9. Azadirachta ... Species. *Azadirachta indica* A.Juss. (Mimba, Nimba)

Macroscopic Examination Results

Macroscopic observations were made by counting the number of ulcers, ulcer index and the curative ratio of each treatment group. Macroscopic observations obtained the average number of ulcers and severity of ulcers in each group listed in Table 1

Table 1. Average score of ulcer number and ulcer severity

Groups	Mean score ±SD		% Ulcers	Ulcer In-dex
	Ulcer Number (UN)	Severity Score (mm)		
Negative control (Ibu-profen)	4,66 ± 0,577	3,03 ± 0,161	100	17,69
Positive control (Ranitidine)	2,00 ± 0,866*	1,80 ± 0,669*	33,3	7,13
Treatment (EKBM 250 mg/kg)	4,00 ± 1,000	2,76 ± 0,105	100	16,76
Treatment (EKBM 500 mg/kg)	2,33 ± 1,44	1,75 ± 0,433*	33,3	7,41
Treatment (EKBM 1000 mg/kg)	1,16 ± 0,288*	1,16 ± 0,288*	0	2,32
Treatment (EKBM 2000 mg/kg)	1,00 ± 0,00**	1,00 ± 0,00**	0	2,00

Note: * sig <0.05 there are significant differences with negative controls, ** there are significant differences with negative and positive controls.

Table 1 shows the average score of ulcer number and ulcer severity. The treatment group, with the administration of neem bark extract 2000 mg/kg, has a smaller ulcer index of 2.00 compared to the positive control group (ranitidine) of 7.13. Statistical results showed a dose of 2000 mg/kg had a significant difference in the number and severity of ulcers between a negative and positive control group with a p-value of 0.034 and 0.037, respectively. However, there was no difference with the positive control group indicating the equality of effects between the comparison group and the test group.

Table 2. The protection ratio

Group	Negative control Index	Ulcer Index	Protection ratio (%)
Positive control	17,69	7,13	59,69
Treatment (EKBM 250 mg/kg)	17,69	16,76	5,25
Treatment (EKBM 500 mg/kg)	17,69	7,41	58,1
Treatment (EKBM 1000 mg/kg)	17,69	2,32	86,88
Treatment (EKBM 2000 mg/kg)	17,69	2,00	88,7

Table 2 shows the protection ratio. The treatment group with the administration of neem bark extract 2000 mg/kg has a statistically significant higher protection value that can prevent ulcers compared to the positive control group (ranitidine) with a value of 88.7 % and 56.69% (p = 0.034).

Microscopic observation

Microscopic observations aimed to see the effect of the neem bark (Azadirachta indica A.Juss) on the repair of gastric damage induced by ibuprofen. The microscopic descriptions are in line with macroscopic observations which showed that 250 mg/kg and 500 mg/kg resulted

in necrosis and oedema without inflammatory cells, whereas doses of 1000 mg/kg and 2000 mg/kg showed necrosis with mild oedema. Figure 2 depicts the gastric histology of the negative control rat.

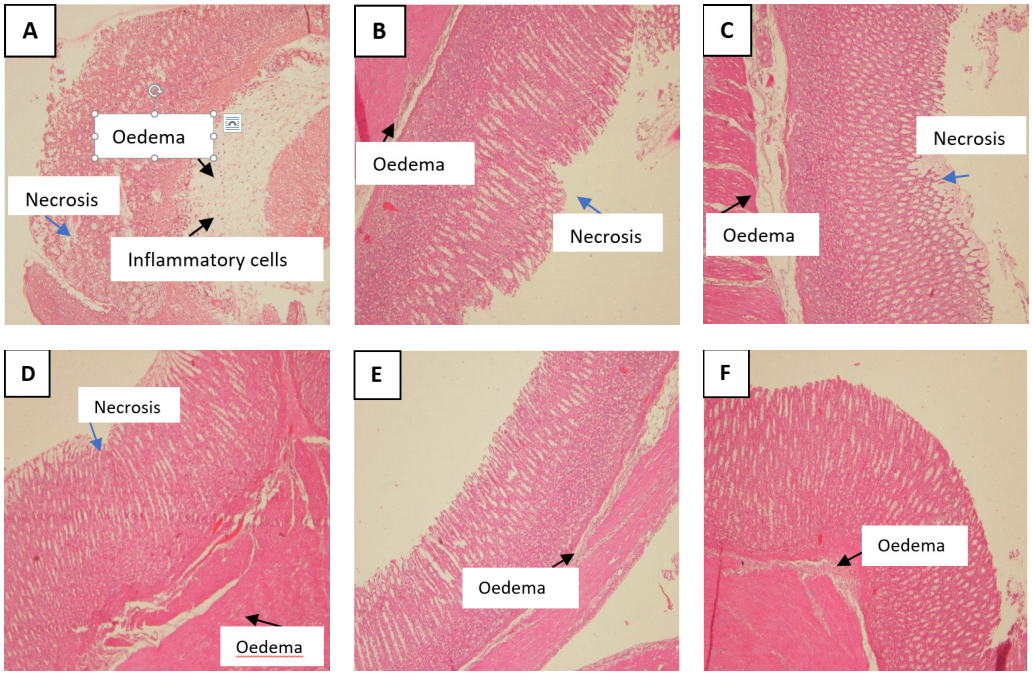

Figure 2. Microscopic photos of rat histology with hematoxylin and eosin (HE) staining, 100x Magnification
A. Negative control (ibuprofen),
B. Positive control (ranitidine),
C. EKBM 250 mg/kg (K3),
D. EKBM 500 mg/kg (K4),
E. EKBM 1000 mg/kg (K5),
F. EKBM 2000 mg/kg (K6).

DISCUSSION

Gastric ulcer is a damage or loss of mucosal tissue, submucosa, until the muscle layer of the food digestive tract area due to various reason including the drug use.10 This study used ibuprofen to induce gastric ulcer. Ibuprofen can inhibit cyclooxygenase in prostaglandin biosynthesis by disrupting the conversion of arachidonic acid to prostaglandin that resulted in irritation of the stomach.[6,13] This research suggests that the administration of Ibuprofen 400 mg/kg can cause mucosal necrosis, inflammation cells, and extensive oedema.

Neem bark extract showed the gastro-protective properties indicated by the ulcer index and protection ratio calculation.[14,15] Overall, all doses administered can reduce the risk of gastric ulcers with a lower ulcer index score when compared with negative controls, although Neem bark extract 2000 mg/kg showed the most effective doses in preventing ulcers. Previous studies by Bandyophadhyay et al. suggested that administration of 0.6 gr/kg Neem bark extract for 15 days can significantly inhibit acid production with the equivalent function of ranitidine and omeprazole without significant side effects.

The microscopic observations are following macroscopic examination. In the drug control group (Ranitidine) 300 mg/kg and treatment groups EKBM 250 and 500 mg/kg for seven days, there were no inflammatory cells, but mild mucosal necrosis and formation of oedema were present. Meanwhile, doses of 1000 and 2000 mg/kg of Neem stem bark extract showed no

necrosis with only minimal formation of oedema. This shows that administration of higher doses of neem bark extract may act as gastro-protector agent, showed by the low value of gastric damage in both, negative (Ibuprofen) and positive (Ranitidine) control groups (p=0.034).

The gastroprotective effect of neem stem bark extract (Azadirachta indica A.Juss) resulted from the compounds that act as antioxidants, anti-inflammatory, and anti-ulcer. Mimba plants are wild plants that are often found on the roadside, dry areas, and open forest.[11] This natural ingredients potentially provide many health benefits due to their active compounds such as azadirachtin, nimbolinin, nimbin, nimbidin, nimbidol, sodium nimbinate, gedunin, salaninin, and quercetin.[12] Besides; its phenolic glycoside compounds can inhibit the acid secretion by inhibiting H + K + ATPase and block oxidative damage from the gastric mucosa by blocking lipid peroxidation and scavenging from endogenous hydroxyl radicals (OH).

CONCLUSION

The treatment group with neem bark extract of 500 mg/kg provides a similar healing rate with the positive group (ranitidine 300 mg/kg). Additionally, a dose of 2000 mg/kg provides a significantly higher cure rate compared to ranitidine by observing the macroscopic and microscopic ulcer indexes.

CONFLICT OF INTEREST

None declare.

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