Anti-inflammatory Effect of *Phyllanthus niruri* L. from Indonesia (Pre-clinical Study)

Em Sutrisna¹,*, Maryati², Sri Wahyuni², Tanti Azizah S²

**ABSTRACT**

Indonesian people often use *Phyllanthus niruri* L. (*P. niruri* L.) to treat pain. Pain is the one of symptoms of inflammation. Some people take traditional medicine to treat this pain, because some analgesic drugs have several adverse effects. The aim of this research is to explore the anti-inflammatory effect of ethyl acetate fraction of ethanolic extract of *P. niruri* L. (EAPN) from Indonesia in rats induced by carrageenan. A total of 25 rats were divided into 5 groups. Group 1 (diclofenac sodium 50 mg/kg bw), group 2 (aqueous extract), groups 3,4, and 5 were ethyl acetate fraction at dosage of 250; 500 and 1000 mg/kg bw respectively. Group 1 was treated by diclofenac sodium at dose of 50 mg/kg bw, group II was treated by aqueous 2mL/rats, groups III, IV and V were treated by EAPN at dosage of 250; 500 and 1000mg/kg bw respectively. Thirty minutes later, all rats were injected by carrageenan 0.1 mL 1% w/v sub cutaneous into back rat's left paw. Paw volumes were measured by Plethysmometer on minutes 0, 60, 90, 150, 210, 330. Area under the curve (AUC) were calculated from these results (AUC 0-30; AUC 0-90; 0-150; AUC 0-210 and AUC 0-330). The AUC of EAPN at dosage of 250; 500 and 1000mg/kg bw smaller than negative control significantly (*P*<0.05). EAPN contains alkaloids, flavonoids, terpenoids, saponins and triterpenes. EAPN has anti-inflammatory effect in rats induced by carrageenan.

**Key words:** Anti-inflammatory, *Phyllanthus niruri* L., Carrageenan.

**INTRODUCTION**

Indonesia is the largest biodiversity country in the world after Brazilia. Ironically, the development of phytopharmaca in Indonesia is very slow. Traditional Indonesian society often use traditional medicinal plants to cure the disease. One plant that is often used to treat diseases is *P. niruri* L. This plant was used to treat pain.¹ *P. niruri* L. is a species of the genus Phyllanthus. This genus consists of more than 600 species of plants.² Some other communities in the world, such as in Africa, *P. fraternus* (a species of the genus Phyllanthus) is used to treatment of diabetes, flu, colic, dyspepsia, fever, jaundice.³

Pain is one sign of inflammation. Inflammation is part of the body's immune response to various dangerous stimuli, such as bacteria, viruses, damaged cells, toxic compounds, radiation and chemical exposure.⁴ Inflammation can caused by infection and non-infection.⁵ Due to this stimulation, the body will try to eliminate it and begin the healing process.⁶ Cardinal sign of inflammation are swelling/edema, pain, fever, redness, and loss of function.⁷

At the present, the treatment of inflammation using NSAIDs (non-steroidal anti-inflammatory drugs). Research by Teslim et al, that published in 2014, found there are many side effect of this drugs, among others: high blood pressure, low blood pressure, indigestion, gastro-intestinal bleeding, ulcer, vomiting, diarrhea, abdominal pain, heart attack, kidney disorders, dizziness, comma/unconsciousness, nausea, constipation, gastric pain and liver problems.⁸ Due to many sides effects of NSAIDs, many people used medicinal traditional plant to treat pain and inflammation. Currently, there is no study which is reporting on anti-inflammatory effect of effect ethyl acetate fraction of ethanol extract *P. niruri* L.

The aim of this research is to explore the effect ethyl acetate fraction of ethanol extract *P. niruri* L as an anti-inflammatory.

**METHOD**

This research has been approved by Health Research Ethics Committee of Medical Faculty of Universitas Muhammadiyah Surakarta with number 2064/A.2/KEPK-FKUMS/III/2019.

**Plant materials**

Dry matters of simplisia was found from Gede market, Surakarta, Central of Java in March 2019. The rats wistar strain were found from Pharmacology laboratory of UMS.

Drug and chemical: Diclofenac sodium (Kimia Pharma) and Carrageeenan (Sigma Aldrich) were found in April 2019.

**Preparation of extracts**

A total of 300 g of plant samples were macerated in 4 liters of ethanol. This immersion is done for 3 days, and re-maceration is carried out. The results of maceration were then fractionated with ethyl acetate and then placed in the rotary evaporator until an ethyl acetate fraction was obtained.

Ant-inflammatory activity
A total of 25 rats were divided into 5 groups. Group 1 was treated by diclofenac sodium at dose of 50 mg/kgbw, group II was treated by aqueous 2 ml/rat, groups III, IV dan V were treated by EAPN at dosage of 250; 500 and 1000 mg/kgbw respectively. Thirty minutes later, all rats were injected by carrageenan 0.1 ml. 1% w/v sub cutaneous into back rat’s left paw. Paw volume were measured on minutes 0; 60; 90; 150; 210; 330 by Plethysmometer. Area under the curve (AUC) were calculated on 0-30; AUC 0-90; 0-150; 0-210 and AUC 0-330.

Identification of alkaloids
A total of 0.5 g extract was inserted in the test tube + 2 mL ethanol 70% then stirred and 5mL Hcl 2N was added and heated it in a bath water. After getting cold, the mixture was filtered and some filtrate was added with Meyer’s reagent drops. The emergence of orange red precipitate indicated the presence of alkaloids.

Identification of flavonoids
A total of 0.5 g of extract is put in a test tube + 2 mL ethanol 70% then it was stirred and added with 1mL of chloroform and 1mL of acetic anhydrous Hcl were added. The orange to red solution indicated the presence of flavonoids.

Identification of saponin
A total of 0.5 g of extract was put in a test tube + 2 mL ethanol 70% then stir and magnesium powder 0.5 g and 3 drops of concentrated H2SO4 was added. The red color ring showed the presence of saponin.

Identification of triterpenoids
A total of 0.5 g of extract was put in a test tube + 2 mL ethanol 70% then stir and magnesium powder 0.5 g and 3 drops of concentrated H2SO4 was added. The red color ring showed the presence of steroids.

Identification of steroids
0.5 g of extract was put in a test tube + 2 mL ethanol 70% then it was stirred. 2 mL of chloroform and concentrated H2SO4 were added by dropping them slowly through the wall of the test tube. The formation of red rings showed the presence of steroids.

RESULTS
Phytochemical screening
The compound content in EAPN is scientifically validated through identification of phytochemicals. This compound is thought to be responsible for the anti-inflammatory effect. The results of the qualitative chemical analysis of EAPN are tabulated in Table 1.

Anti-inflammatory activity
Carrageenan is used to induce inflammation. The use of carrageenan refers to research conducted by Mostofa, et al., 2017. Diclofenac sodium (50 mg/kgbw) was used as a synthetic drug during the anti-inflammatory evaluation of EAPA. The results of the volumes of rat paw edema in the five groups are presented in Table 2.

From this table, it can be concluded that EAPN at dosage of 250; 500 and 1000 mg/kgbw can decrease edema volume on minutes 150-330.

DISCUSSION
The results of this research is in line with previous research. Research by Porto at al., 2013, showed that Phyllanthus niruri spray-dried standardized extract has anti-inflammatory effect on mus musculus Swiss male mice (18-30 g) by carrageenan (1% w/v, 50 μL). Methanol extract of Phyllanthus niruri decreased edema in Swiss albino rats induced by carrageenan. This extract showed a reduction of 46.80%; 55.32% and 69.14% at doses of 100; 200 and 400 mg/kg respectively. In this research, inflammation was induced by injecting 0.1 ml carrageenan (1% w/v) into the left hind paw. The aqueous extract revealed anti-inflammatory activity significantly on carrageenan (p<0.001) and chronic granuloma (p<0.001) rodent models.

Ethanolic and aqueous extract of P. fraternus whole plant possess anti-inflammatory activity in on carrageenan-induced paw edema in Sprague-Dawley rats.

Table 1: The phytochemical profile of ethyl acetate fraction of Ethanolic extract of P. niruri (EAPN).

<table>
<thead>
<tr>
<th>Active compounds</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloid</td>
<td>Positive</td>
</tr>
<tr>
<td>Flavonoid</td>
<td>Positive</td>
</tr>
<tr>
<td>Tannin</td>
<td>Positive</td>
</tr>
<tr>
<td>Saponin</td>
<td>Positive</td>
</tr>
<tr>
<td>Triterpen</td>
<td>Positive</td>
</tr>
<tr>
<td>Steroid</td>
<td>Negative</td>
</tr>
</tbody>
</table>

Table 2: The AUC of edema volume on minutes 0-60; AUC 0-90; AUC 0-150; AUC 0-210 and AUC 0-330.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Diclofenac sodium at dose of 50 mg/kgbw</th>
<th>Aquous 2 mL/rats</th>
<th>EAPN at dose 250 mg/kgbw</th>
<th>EAPN at dose 500 mg/kgbw</th>
<th>EAPN at dose 1000 mg/kgbw</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUC (ml ± SD)</td>
<td>1.53 ± 0.63</td>
<td>1.59 ± 0.71</td>
<td>1.59 ± 0.79</td>
<td>0.84 ± 0.44</td>
<td>1.46 ± 0.94</td>
</tr>
<tr>
<td>AUC 0-60</td>
<td>3.00 ± 1.01</td>
<td>3.00 ± 1.01</td>
<td>3.00 ± 1.41</td>
<td>2.25 ± 0.74</td>
<td>2.92 ± 1.43</td>
</tr>
<tr>
<td>AUC 0-90</td>
<td>7.14 ± 0.71</td>
<td>7.11 ± 0.68</td>
<td>4.74 ± 1.14*</td>
<td>4.08 ± 0.47*</td>
<td>4.65 ± 0.81*</td>
</tr>
<tr>
<td>AUC 0-150</td>
<td>8.04 ± 1.49*</td>
<td>9.93 ± 0.97</td>
<td>5.76 ± 1.47*</td>
<td>4.56 ± 1.04*</td>
<td>5.66 ± 1.13*</td>
</tr>
<tr>
<td>AUC 0-210</td>
<td>10.92 ± 2.73</td>
<td>14.01 ± 2.12</td>
<td>7.20 ± 1.73*</td>
<td>5.04 ± 0.69*</td>
<td>6.84 ± 1.68*</td>
</tr>
<tr>
<td>AUC 0-330</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Note: *: Significantly different from negative control on Anova (p<0.05)
This extract contains some phytocchemical, among others: alkaloids, flavonoid, tannin, saponin and triterpen. Mechanism of anti-inflammatory effect of this extract is not clear. Some alkaloids have been reported to have antihypertensive, analgesic, antidepressant, muscle relaxant, antimicrobial, anti-inflammatory, anti-tumor, diuretic, symp thermo-mimetic and antiviral activities.13,14 Flavonoid allegedly has anti-inflammatory effect by several mechanism, among others: inhibit enzymes xanthine oxidase, aldose reductase, cyclooxygenase, phosphodiesterase, Ca(2+)-ATP-ase and lipoxygenase etc.15 The mechanism of flavonoids is thought to inhibit NF-kappaB and activate protein-1 (AP-1) and erythroid 2-related factor 2 (Nrf2) factors.16 One form of tannin is Corilagin (β-1-O-galloyl-3,6-(R)-hexahydropinoyl-d-glucose). Coralgin is a tannin isolated from several plants, including Phyllanthus niruri and has the effect of inhibiting the release of cytokines such as TNF-α, IL-1β and IL-6 and has the effect of inhibiting the release of cytokines such as TNF-α, IL-1β and IL-6 and the production of nitric oxide, both of which are inflammatory mediators.17,18 Research by Bogi et al, found that saponin extracted from Zizyphus lotus (L.) Lam inhibited paw edema dan production of nitrite.19 Research by Patricia et al.5,15 found natural triterpenes α,β-amyrin isolated from Protium paniculatum Oil-Resins inhibited the expression of COX-2 and also inhibited the formation of paw or ear edema in rats and mice.20

CONCLUSION

Ethyl asetat fraction of ethanolic extract of P. niruri has anti-inflammatory effect on rat’s paw induced by carrageenan.

ACKNOWLEDGMENT

We thanks to Ministry of Research, Technology and Higher Education of the Republic of Indonesia for funding our research.

REFERENCES

Sutrisna, et al.: Anti-inflammatory Effect of Phyllanthus niruri L. from Indonesia (Pre-clinical Study)

ABOUT AUTHORS

Name: EM sutrisna: Correspondence author
Affiliation: Medical faculty of Universitas Muhammadiyah Surakarta
Expertise: Pharmacology
Article:
1. The potential antidiabetic and anti-inflammatory activity of Zingiber zerumbet ethanolic extracts and Channastriata powder on albino Wistar mice
2. Hypoglycemic effect of 70\% ethanolic extract of tinospora crispa L. (Bratawali) stem from Indonesia in wistar rat induced by alloxan
3. Hypolipidemic of ethanolic extract of Salam bark (Syzygium polyanthum (Wight) Walp.) from Indonesia (Preclinical study)
4. Antidiabetic activity of ethanolic extract of Eugenia polyanthawight leaf from indonesia in diabetic rat wistar strain induced by alloxan
5. Hypolipidemic effect of Tamarindus indica L fruit on Triton X-100-induced hyperlipidemia in Wistar rats

Name: Maryati: second author
Affiliation: Pharmacy faculty of Universitas Muhammadiyah Surakarta
Expertise: Molecular biology
Article:
1. The enzyme activities of Caf1 and Ccr4 are both required for deadenylation by the human Ccr4–Not nuclease module
2. A fluorescence-based assay suitable for quantitative analysis of deadenylase enzyme activity
3. Discovery, synthesis and biochemical profiling of purine-2, 6-dione derivatives as inhibitors of the human poly (A)-selective ribonuclease Caf1

Name: Tanti Azizah Sujono, Msi
Affiliation: Pharmacy faculty of Universitas Muhammadiyah Surakarta
Expertise: Pharmacology
Article:
1. Antidiabetic activity of durian (Durio zibethinus Murr.) and rambutan (Nephelium lappaceum L.) fruit peels in alloxan diabetic rats
2. Antidiabetic activity of durian (Durio zibethinus Murr.) and rambutan (Nephelium lappaceum L.) fruit peels in alloxan diabetic rats
3. Antidiabetic and antihypercholesterolemia activities of rambutan (Nephelium lappaceum L.) and durian (Durio zibethinus Murr.) fruit peel extracts

Name: Sri Wahyuni, Apt: third author
Affiliation: Pharmacy faculty of Universitas Muhammadiyah Surakarta
Expertise: Pharmacology