Jurnal Farmasi Galenika (*Galenika Journal of Pharmacy*) (e-Journal) 2022; 8 (1): 65 – 74
ISSN: 2442-8744 (electronic); 2442-7284 (printed)
Journal Homepage: https://bestjournal.untad.ac.id/index.php/Galenika
DOI: 10.22487/j24428744.2022.v8.i1.15678

Ethanol Extract of Miana Leaf (*Coleus atropurpureus* Benth) *as* Analgetic Antiinflamation in Rats (*Rattus novergicus*)

(*Ekstrak Etanol Daun Miana (Coleus atropurpureus* Benth*) sebagai Analgetik Antiinflamasi pada Tikus (Rattus novergicus)*)

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Article Info:
Received: 1 November 2021
in revised form: 12 December 2021
Accepted: 8 March 2022
Available Online: 29 March 2022

Keywords:
Antiinflamation
Analgetic
Coleus atropurpureus Benth
Miana Leaf
Rattus novergicus

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ABSTRACT

Background: Inflammation is a normal protective response to tissue injury caused by physical trauma, chemicals, or microbiological substances. The manifestation clinic of inflammation is pain. Miana leaves (*Coleus atropurpureus* Benth.) have chemical contents such as essential oils, phenols, flavonoids, and polyphenols. Flavonoids can protect lipid membranes from damage and inhibit cyclooxygenase, which is the first pathway for the synthesis of pain mediators such as prostaglandins. **Objectives:** This study aims to determine the effectiveness of ethanol extract of Miana leaves (*Coleus atropurpureus* Benth) (EEML) as analgesic and anti-inflammatory in rats (*Rattus novergicus*). **Material and Methods:** This study used *The Pre-Posttest-Only Control Group Design*, twenty male rats were divided into 5 groups namely negative control, positive control (diclofenac sodium), treatment with EEML dose of 150, 300, and 600 mg/kg body weight (BW). Anti-inflammatory was assayed by using the rat hind paw edema method where the condition of inflammation in rats is induced by 5% egg white by sub plantar. The measurement of edema in rat feet was using a caliper every 30 minutes for 5 hours. While The measurement of analgesic testing was using the nociception test method which evaluates pain responses such as staggering gait, vocalization, and writhing. The inflammatory parameters data were analyzed with the one-way anova for the width of edema foot and kruskal walis for the thickness of edema foot. While the observing pain response data were analyzed with the Kruskal Walis test. **Results:** The ethanol extract of Miana leaves has analgesic anti-inflammatory activity especially at a dose of 600 mg/kg bw which are non-significant ($p > 0.05$) with a positive control group (sodium diclofenac). **Conclusions:** This study concluded the potency of Miana leaves as analgetic anti-inflammatory drugs.

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How to cite (APA 6th Style):
Sukmawati, Santi, I., Wati, A., Aulya, R. (2022). *Ethanol Extract of Miana Leaf (Coleus atropurpureus* Benth*) as Analgetic Antiinflamation in Rats (Rattus novergicus)*. *Jurnal Farmasi Galenika: Galenika Journal of Pharmacy (e-Journal), 8*(2), 65-74. doi:10.22487/j24428744.2022.v8.i1.15678

ABSTRAK

Latar belakang: Inflamasi merupakan respon protektif normal terhadap luka jaringan yang disebabkan oleh trauma fisik, zat kimia atau zat-zat mikrobiologi. Manifestasi klinik dari inflamasi adalah rasa nyeri. Daun Miana (*Coleus atropurpureus* Benth.) memiliki kandungan kimia seperti minyak atsiri, fenol, flavonoid, dan polifenol. Flavonoid adalah senyawa yang dapat melindungi membran lipid dari kerusakan dan menghambat cyclooxygenase yang merupakan jalur pertama sintesis mediator nyeri seperti prostaglandin. Tujuan: Penelitian ini bertujuan untuk menentukan efektifitas ekstrak etanol daun Miana (*Coleus atropurpureus* Benth) (EEDM) sebagai analgetik dan antiinflamasi pada tikus (*Rattus novergicus*). Bahan dan Metode: Penelitian ini menggunakan *The Pre-Postest-Only Control Group Design*, dimana 20 ekor tikus jantan dibagi dalam 5 kelompok yaitu kontrol negatif, kontrol positif diberikan natrium diklofenak, perlakuan dengan variasi dosis EEDM 150 mg/kgBB, 300 mg/kgBB dan 600 mg/kgBB. Pengujian antiinflamasi menggunakan metode *Rat Hind Paw Edema* dimana kondisi radang kaki belakang pada tikus diinduksi dengan putih telur 5% secara subplantar. Pengukuran radang pada kaki dengan menggunakan jangka sorong tiap 30 menit selama 5 jam. Sedangkan pengujian analgetik menggunakan metode uji *nociception* dimana mengevaluasi respon nyeri seperti *Staggering gait, Vocalization* dan *Writhing*. Data hasil pengukuran parameter inflamasi dianalisis menggunakan uji *One Way Anova* untuk lebar kaki udema dan *Kruskal Walis* untuk tebal kaki udema. Sedangkan data pengamatan respon nyeri dianalisis dengan uji *Kruskal Walis.* Hasil: Ekstrak etanol daun Miana memiliki aktivitas sebagai analgetik antiinflamasi terutama pada dosis 600 mg/Kg BB yang hasilnya non-signifikan ($p>0,05$) dengan kelompok kontrol positif (natrium diklofenak). Kesimpulan: Penelitian ini menyimpulkan bahwa daun miana berpotensi sebagai obat analgetik antiinflamasi.

Kata kunci : Antiinflamasi; Analgetik, *Coleus atropurpureus* Benth; Daun Miana; *Rattus novergicus*

INTRODUCTION

Pain or soreness is a sign that there is a problem with a part of the body, which is a symptom, whose function is to protect and provide a warning sign of disorders in the body such as inflammation, infection with germs, or muscle spasms. Pain arises due to mechanical or chemical stimuli, which can cause damage to tissues and release certain substances called pain mediators such as bradykinin, histamine, serotonin, and prostaglandins (Mutschler, 1991). Inflammation is a normal protective response to tissue injury caused by physical trauma, damaging chemicals, or microbiological substances. Symptoms include pain (dolor), heat (heat), redness (rubor), swelling (tumor) and changes in function (functionolaesa) (Mycek M J. Harvey RA, 2001); (Narande *et al.,* 2013). Treatment that has been used to relieve pain and suppress inflammation (anti-inflammatory analgesics) is the use of synthetic drugs. One of them is the non-steroidal anti-inflammatory drug (NSAID) class which has side effects, such as gastrointestinal disorders and cardiovascular disorders. So that people turn to look for safer and potential alternative treatments by utilizing natural ingredients (Sukmawati & Saharuddin, 2018).

The leaves of Miana (*Coleus atropurpureus* Benth) contain chemicals such as essential oils, phenols, tannins, fats, phytosterols, alkaloids, flavonoids, and polyphenols (Kusumawati *et al.,* 2014). Flavonoids are compounds that can protect membrane lipids from damage and inhibit the cyclooxygenase I enzyme, which is the first pathway for the synthesis of pain mediators such as prostaglandins (Afrianti *et al.,* 2014). Empirical use in the community of Miana leaves has antipyretic, analgesic, and anti-inflammatory properties. Also, this plant relieves coughs with phlegm. In

traditional medicine, the decoction of Miana leaves is commonly used to reduce fever. This study aimed to examine the anti-inflammatory analgesic effect of the ethanol extract of Miana leaves (*Coleus atropurpureus* Benth) in rats and to determine the dosage of the extract which was effective as an anti-inflammatory analgesic.

MATERIAL AND METHODS

Materials

The materials used in this study were Miana leaves (*Coleus atropurpureus* Benth) was obtained from Antang (Makassar, Indonesia). The plants were identified at Pharmacy Laboratory, Faculty of Pharmacy, Indonesia Moslem University (UMI). Aquadest, ethanol 96% (Bratachem. Ltd), diclofenac sodium, carboxymethylcellulose sodium (Sigma-Aldrich), chloride sodium (NaCl), and egg white.

Methods

Animal Handling

Animals used in this study was obtained from Surabaya City. About 20 male Wistar rats with the age of ± 2 months and a bodyweight of 100–200 g are kept in individual cages which are covered with gauze. Adaptations were carried out for 2 weeks before use in the study. This study obtained ethical clearance from KEPK UMI and RSIS YW-UMI Makassar with the letter number: 041/A.1/KEPK-UMI/VI/2020

In vivo analgetic anti-inflammatory test

Rats were divided into 5 treatment groups by random allocation. Each group consisted of 4 rats. The anti-inflammatory test was carried out using the *Rat Hind Paw Edema* method. The first stage, on day 0 all rats' feet width and thickness were measured with calipers as initial data. Furthermore, all rats were induced with 5% egg white as much as 0.5 ml subplantarly (under the skin of the rats' feet). Then left for 5 hours until inflammation of the rats' feet occurs. Furthermore, the width and thickness of the rats' feet were measured using a caliper as induction data. Next the preparations were tested as follows: Group I: The rats were given 1% Na CMC orally, group II: The rats were given diclofenac sodium suspension 5,136 mg/kg bw, Group III: The rats were given ethanol extract of Miana leaf (EEML) 150 mg/kg bw, Group IV: The rats were given EEML 300 mg/kg bw, and Group V: The rats were given EEML 600 mg/kg bw. The entire administration was carried out by oral. Measurement of the feet of male rats (*Rattus novergicus*) was carried out every 30 minutes to 300 minutes. After that, the thickness and width of the feet were recorded for each treatment. Then, the percentage of anti-inflammatory power (% AIP) was determined and analyzed.

In vivo nociception test method

The rats that have been induced by 5% egg white on the hind paw, the behavior of the rats were observed every 30 minutes to 300 minutes when inflammation occurred and got assessed in the form of scoring. The scoring of the pain response observations is shown in Table 1 below :

Table 1. Scoring of pain response observation

Types of pain response parameters	Score
If there are all signs of pain response (*Staggering gait, Vocalization, Writhing*)	3
If there are 2 signs of pain response (*Staggering gait, Vocalization, Writhing*)	2
If there is 1 sign of pain response (*Staggering gait, Vocalization, Writhing*)	1
If there is no sign of pain response	0

RESULT AND DISCUSSION

The results of the research on the effectiveness test of the ethanol extract of Miana leaves (*Coleus atropurpureus* Benth) as an anti-inflammatory analgesic in male rats (*Rattus novergicus*) can be seen in the table and figure below.

Changes in inflammation or edema of rat feet that were observed every 30 minutes for 5 hours in each treatment group based on measurements of the width and thickness of foot edema can be seen in Figures 1 and 2 below :

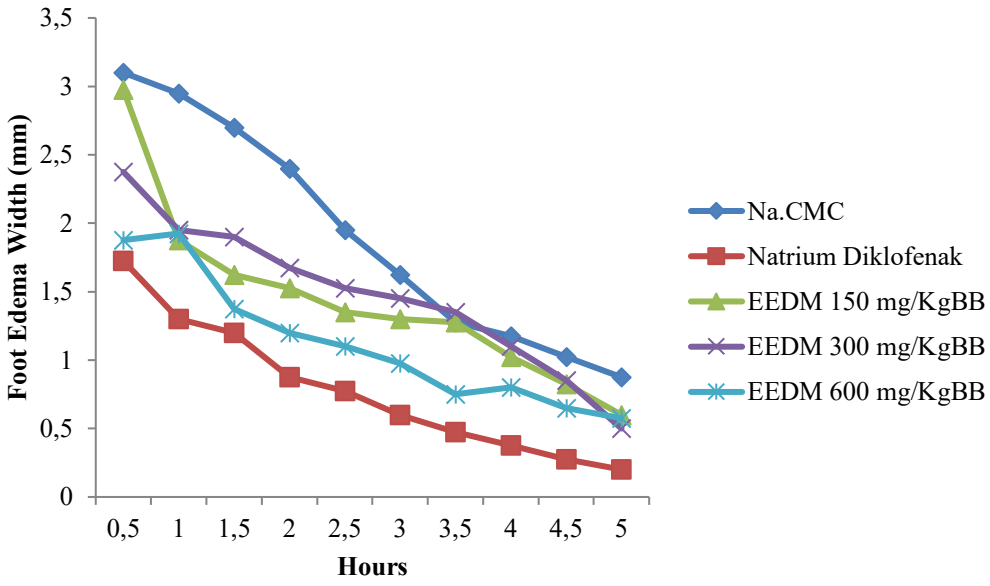

Figure 1. Diagram of changes in rat foot inflammation based on the width of foot edema for 5 hours

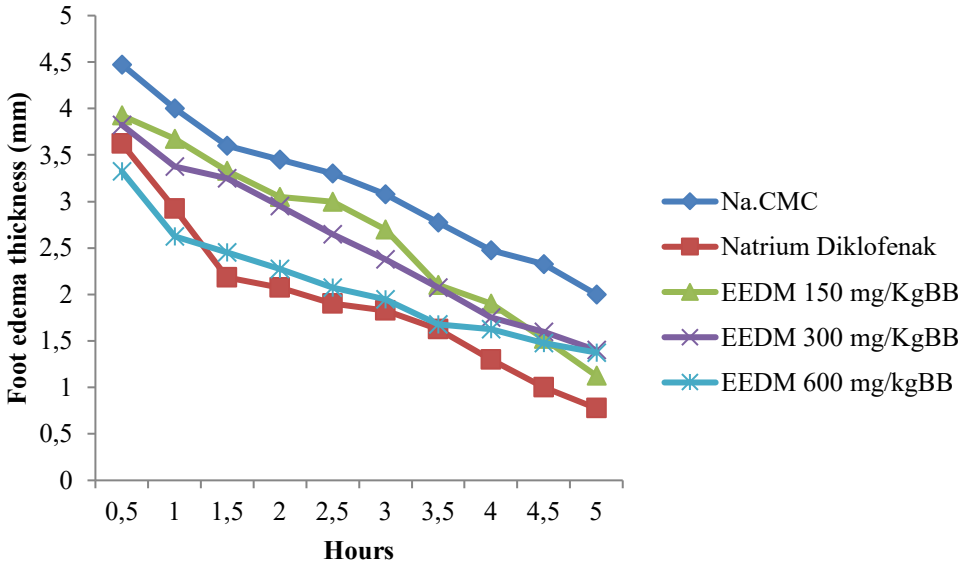

Figure 2. Diagram of changes in rat foot inflammation based on the thickness of foot edema for 5 hours

The results of measurements of the width and thickness of edema in rats' feet that have been induced by 5% egg white solution in Figures 1 and 2 show that there is a decrease in inflammation every 30 minutes for 5 hours of observation. In the diagram, it can be seen that the ethanol extract of Miana leaves at a dose of 600 mg / kgBW was not significantly different from the positive control group using the Diclofenac sodium comparison. This is also supported by the results of the calculation of the percentage of anti-inflammatory power (% AIP) in table 2 and 3 below

Table 2. Result of determination of percentage of anti-inflammatory power of ethanol extract of miana leaves in rats based on udema foot width parameters.

Test Groups	Average AUC Score (mm.minute)	% AIP
Negative Control (Na CMC 1%)	9,30	-
Positive Control (Natrium Diclofenac) [c]	3,83	58,81
EEML 150 mg/KgBW [a;d]	7,02	24,51
EEML 300 mg/KgBW [a;d]	7,19	22,68
EEML 600 mg/KgBW [b;c]	5,44	41,50

EEML= Ethanol Extract of Miana Leaves; AIP = Antiinflamation Power; AUC = Area Under Curve
[a] $P < 0.05$ with positive control (*LSD test*) ; [b] $P > 0.05$ with positive control (*LSD test*)
[c] $P < 0.05$ with negative control (*LSD test*); [d] $P > 0.05$ with negative control (*LSD test*)

Table 3. Data on the determination of percentage of anti-inflammatory power of ethanol extract of miana leaves in rats based on measurement of udema foot thickness parameters

Test Groups	Average AUC Score (mm.minute)	% AIP
Negative Control (Na CMC 1%)	15,24	-
Positive Control (Natrium Diklofenak) [c]	9,39	38,38
EEML 150 mg/KgBW [a;d]	12,85	15,68
EEML 300 mg/KgBW [a;d]	12,24	19,68
EEML 600 mg/KgBW [b;c]	10,06	33,98

EEML = Ethanol Extract of Miana Leaves; AIP = Antiinflamation Power; AUC = Area Under Curve
[a] P<0.05 with positive control (*Mann-Whitney*); [b] P > 0.05 with positive control (*Mann Whitney*);
[c] P < 0.05 with negative control (*Mann-Whitney*); [d] P > 0.05 with negative control (*Mann-Whitney*)

Statistical analysis using the one-way anova test followed by LSD test on the measurement parameters for foot edema width and Kruskal Walis followed by Mann-Whitney on measurement parameters for foot edema thickness. The results of the analysis are shown in table 2 and table 3. In the two tables, it is shown that the positive control group using Diclofenac Sodium as a comparison showed a significant difference with the negative control group. This indicates that the drug has anti-inflammatory activity. The EEML group at a dose of 150 and 300 mg/kg bw showed a significant difference from the positive control group, but when compared with the negative control group the results were not significant (nonsignificant). Thus it can be concluded that the two EEML doses, namely 150 and 300 mg / kg bw do not provide good anti-inflammatory power.

Testing the effectiveness of EEML at a dose of 600 mg/kg bw, the statistical results concluded that there was a significant difference with the negative control group and the difference was not significant or the same as the positive control group using the diclofenac sodium comparison. Thus it can be concluded that 600 mg/kg bw of EEML provides good anti-inflammatory power

Diclofenac sodium is a non-selective NSAID (non-steroidal anti-inflammatory drug). Acetic acid group, and derivatives of acid phenylacetate. This drug has anti-inflammatory, analgesic, and antipyretics. Diclofenac sodium has cyclooxygenase enzyme (COX) inhibiting activity through inhibition of prostaglandin formation which is a mediator of pain, so that can be used to treat all kinds of pain, migraine and gout (Hutahuruk *et al.,* 2014).

Diclofenac sodium is a strong anti-inflammatory phenylacetate derivative with relatively mild side effects compared to other types of drugs (Meltyza, E., Anita, IR, 2014). This drug works by inhibiting the cyclooxygenase enzyme so that the conversion of arachidonic acid to PGE2 is impaired. The cyclooxygenase and PGE2 enzymes are associated with the second phase, namely the inflammatory

phase. Therefore, a significant effect of diclofenac sodium occurs in the second phase (inflammatory phase) (Sari, et el., 2018).

In the first phase, it is also called the neurogenic phase because the pain in this phase is caused by direct stimulation of sensory nerve fibers due to compound induction (Sari, et el.,2018). In this study, the neurogenic phase occurred after 5% egg white induction in Hind Paw rats. Pain sensation occurs when inflammation begins where there is an irritation reaction to the leg tissue. This results in pain through behaviors such as withdrawal, licking, immobility, and vocalization. Observation of this behavior is termed a nociception test, which evaluates the ability of animals, usually rodents, to detect harmful stimuli such as feelings of pain, which are caused by stimulation of nociceptors (Barrot, 2012).

In this study, there are three types of behavior observed when the experimental animal experiences a sensation/response to pain, including (1) Staggering gait or abnormal gait or walking abnormally indicating muscle relaxation or ataxia; (2) Vocalization, where rat with loud voices showing a painful stimulus; (3) Writhing indicates tissue irritation or stimulation of sensory receptors.

Based on the test results of the Ethanol Extract of Miana Leaf (EEML) as an analgesic based on the nociception test shown in table 4 with the Kruskal Walis statistical analysis followed by the Mann-Whitney test, it was concluded that the pain response caused in the negative control group was significantly different from all treatment groups either control positive or EEML dosage. The positive control group using Diclofenac Sodium as a comparison showed a significant difference with the negative control group. This indicates that the drug has analgesic activity. Meanwhile, EEML at a dose of 600 mg/kg bw showed a pain response that was not significantly different from the positive control group using the Diclofenac sodium comparison. The two EEML doses, namely 150 and 300 mg/ kg bw showed significant differences with the positive control group. Thus, it can be concluded that the ethanol extract of Miana leaf (EEML) has an analgesic effect on rats with edema in the hind paw and the best dose of EEML as an analgesic is 600 mg/kg bw.

Table 4. Test results of ethanol extract of Miana leaves has activity as analgesic based on the nociception test

Group Test	Average Pain Response Score at the minute-										Number of Pain Response
	30	60	90	120	150	180	210	240	270	300	
Negative Control (Na CMC 1%)	3	3	3	3	2	2	2	2	2	1	23
Positive Control (Natrium Diclofenac) [c]	3	3	2	2	1	1	1	1	1	1	16
EEML 150 mg/KgBW [a;d]	3	3	2	2	2	2	2	2	1	1	20
EEML 300 mg/KgBW [a;d]	3	3	2	2	2	2	2	1	1	1	19
EEML 600 mg/KgBW [b;c]	3	3	2	2	2	2	1	1	1	1	18

Pain response includes *Staggering gait, Vocalization, Writhing*. Score 3 = If there are all signs of pain response; Score 2 = If there are 2 signs of pain response; Score 1 = If there is 1 sign of pain response;
[a] $P<0.05$ with the positive control (*Mann-Whitney*); [b] $P > 0.05$ with the positive control (*Mann Whitney*);
[c] $P < 0.05$ with negative control (*Mann-Whitney test*); [d] $P > 0.05$ with negative control (*Mann-Whitney*)

The effectiveness of the ethanol extract of Miana leave as an anti-inflammatory is due to the chemical content of flavonoids. The flavonoids consist of quercetin, kaempferol, and myrisetin. Quercetin is generally the largest component in a plant (Koirewoa *et al.,* 2012). According to (Sativa *et al.,* 2014) types of flavonoids are known to play a role in the anti-inflammatory activity, one of which is quercetin. Based on the results of the research that has been done by Sukmawati (2019), it can be concluded that the ethanol extract of miana leaves (*Coleus atropurpureus* Benth) contains quercetin. The results of the quantitative analysis obtained that the quercetin content in ethanolic extract of miana leaves (*Coleus atropurpureus* Benth) was 3,122 mg/g or 0,312% (Sukmawati *et al.,* 2019).

This compound has an anti-inflammatory mechanism by inhibiting the cyclooxygenase enzyme so that it does not form inflammatory mediators. Another mechanism of flavonoids in two ways, which are by inhibiting capillary permeability and inhibiting arachidonic metabolism where these flavonoids play an important role in maintaining permeability and increasing capillary blood vessel resistance. Therefore, flavonoids are used in pathological conditions such as damage to blood vessels due to inflammation causing an increase in capillary permeability so that blood will come out of the tissue capillaries (Sukmawati *et al.,* 2018). Flavonoids are analgesic and have the effect of inhibiting the metabolism of arachidonic acid, namely inhibiting cyclooxygenase and lipooksigenase (Andriyono, 2019).

Besides, the essential oil namely citral has the potential as an anti-inflammatory analgesic. Citral compounds can inhibit the formation of edema by inhibiting the release of histamine and / serotonin in the first phase. In the next phase, citral inhibits the cyclooxygenase enzyme which plays a role in the formation of prostaglandins and leukotrienes (Saputri & Zahara, 2016). Phenolic compounds, namely

polyphenols, catechins, epicatechins, procyanidins, and other phenolic compounds that can inhibit pain-inducing pro-inflammatory mediators, such as PGE2, TNF-α, IL-6, and IL-1β so that the significant effect of these extracts does not occur in the first phase (neurogenic phase) (Sari, *et al.,*2018).

CONCLUSION

Based on the results of this study, it can be concluded that the ethanol extract of Miana leaves (*Coleus atropurpureus* Benth) has a pharmacological effect as an anti-inflammatory analgesic. The ethanol extract of Miana leaves (*Coleus atropurpureus* Benth) at a dose of 600 mg/kgBW is the most effective as an anti-inflammatory analgesic

ACKNOWLEDGEMENT

We would like to express our gratitude to all parties involved in this research activity, which are LP2S Universitas Muslim Indonesia as a sponsor of activity funds, the Faculty of Pharmacy as the provider of activity facilities, the Head of the Pharmacy Laboratory, and laboratory assistant as a supervisor in technical activities.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest

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