INTERNATIONAL JOURNAL OF BIOSCIENCES AND BIOTECHNOLOGY · Vol. 9 No. 1 · September 2021
eISSN: 2655-9994
pISSN: 2303-3371
https://doi.org/10.24843/IJBB.2021.v09.i01.p03

IN VIVO ANTI-INFLAMMATORY ACTIVITY OF DADAP LEAVES (*Erythrina subumbrans* (Hassk.) Merr)

Ni Luh Kade Arman Anita Dewi[1]*, Ni Luh Sintya[1], Maria Malida Vernandes Sasadara[1], Erna Cahyaningsih[1], Putu Era Sandhi Kusuma Yuda[1], Puguh Santoso[1]

[1]Faculty of Pharmacy, Mahasaraswati University
Corresponding author: armannita@unmas.ac.id

ABSTRACT

Leguminosae is widely known for its anti-inflammatory properties due to the presence of several beneficial phytochemicals, including alkaloids, flavonoids, steroids, and saponins. Dadap (*Erythrina subumbrans* (Hassk.) Merr), a Leguminosae member, has empirically been used to treat inflammation. This research aimed to prove the anti-inflammatory effect of Dadap leaves scientifically. Dadap leaves were macerated in 96% ethanol to produced crude ethanol extract, followed by in vivo anti-inflammatory assay with the paw edema method. Three different doses of extract: 200, 300, and 400mg/kg BW, were administrated to Wistar rats to observed its ability in reducing carrageenan-induced inflammatory. All extract doses produced similar anti-inflammatory activity as compared to diclofenac sodium as a positive control. There is no significant difference among the three different doses. The results indicated that all treatment doses produced anti-inflammatory activity, but 200mg/kg BW administration was most efficient.

Keywords: anti-inflammatory, Dadap, carrageenan.

INTRODUCTION

Inflammation is a normal protective response to tissue injury caused by physical trauma, damaging chemicals, or microbiological substances. Inflammation can also be interpreted as an attempt by the body to activate or damage invading organisms, remove irritants and regulate tissue repair (Howland et al., 2006). The inflammatory process's occurrence will be characterized by characteristic features, including the appearance of redness, swelling in the area of inflammation, a feeling of heat, and the onset of pain (Saputri & Zahara, 2016)

Many chemical drugs can prevent inflammation, as NSAID (Non-Steroid Anti-Inflammatory) is considered the most common (Howland et al., 2006). Anti-inflammatory is the term for an agent or drug that acts against or suppresses the inflammatory process. Long-term use of anti-inflammatory drugs can cause a decrease in the function of various organs such as the kidneys, liver, digestive system organs, and heart (Gunaydin & Bilge, 2018). A safe anti-inflammatory agent with fewer side effects is needed so that natural anti-inflammatory agent is considered essential to explore.

The use of natural ingredients both as medicine and for other purposes tends to increase. Research on various nutritious plants continues to be carried out, especially for traditional medicine when modern medicine is slowly moving away from society. Traditional medicine in medicinal plants (herbal medicine) is widely used because it is more natural, original, and relatively safe. With the development of science and technology, medicine has begun to shift towards natural products because of nature's trend. This decade, it is estimated that around 80% of the world's population, or about four billion people rely on herbal medicine to support health (Ekor, 2014).

One plant with anti-inflammatory effects is Dadap (*Erythrina subumbrans* (Hassk.) Merr). Dadap is a member of Leguminosae. Marabahan (South Kalimantan) people have empirically used to treat inflammation by consuming Dadap leaves boiled water. Another popular method is applying mashed Dadap leaves to the swollen body part (Hendrawan et al., 2019).

Leguminosae is widely known for its anti-inflammatory properties due to the presence of several beneficial phytochemicals, including alkaloids, flavonoids, steroids, and saponins (Mantena & Tejaswini, 2015). Flavonoid is well-known for various activities such as anti-inflammatory, antioxidant, and antimicrobial. Flavonoids protect lipids membrane against damaging reductions. Flavonoids can also inhibit the release of inflammatory mediators such as histamine and prostaglandins (Ciumărnean et al., 2020).

This research aimed to investigate the anti-inflammatory potential of Dadap leaves. A qualitative phytochemicals assay was obtained, followed by in vivo anti-inflammatory assay. Three different extract doses were administrated to mice induced by carrageenan. The percentage of inflammation reduction in each group was observed to determine the anti-inflammatory effect.

MATERIALS AND METHODS

Research Design

This study was experimental to investigate the anti-inflammatory effect of Dadap (*Erythrina subumbrans* (Hassk.) Merr) leaves extract in male rats, with a pretest-posttest only control group design. Twenty-five experimental animals were divided randomly into five groups.

Plant material and extract preparation

Dadap (*Erythrina subumbrans* (Hassk.) Merr) was collected from Badung, Bali. Plant determination was carried out by UPT LIPI, Plant Conservation Agency of the

eISSN: 2655-9994
pISSN: 2303-3371
https://doi.org/10.24843/IJBB.2021.v09.i01.p03

Botanical Garden "Eka Karya" Bedugul Bali. Fresh and clean Dadap leaves were macerated for three days with 96% ethanol in a ratio of sample and solvent was 1:2. Maceration was done in dark conditions and constant stirring for 1 hour each day. The filtrate obtained was then remacerated with half of the ethanol volume used before for another day. The filtrates from the first and second maceration were collected, and the solvent was evaporated under vacuum at 40 °C to furnish dry ethanol extract.

Phytochemical Screening

Protocols for alkaloid, saponin, tannin, steroid, triterpenoid, and flavonoid screening assay are referred to Pandey et al. (2014) and Santoso et al. (2018) (Pandey et al., 2014; Santoso et al., 2018). Alkaloid screening was done using Mayer and Dragendorf reaction. Saponin screening was obtained using 2N HCl to form a foam, which indicates the presence of saponin. Tannin screening was done using 10% FeCl3, in which the presence of tannin is indicated by dark blue and greenish-black color. Steroid and triterpenoid screening was observed by adding 1ml chloroform, 1ml acetic acid anhydrous, and 4ml H2SO4. Brown to violet ring forming indicating presence of triterpenoid, and greenish-blue ring indicating the presence of steroid. Flavonoid screening was observed by forming yellow sedimentation and yellow solution with 10% Pb-acetic and 20% NaOH, respectively.

Animal's experiments

A total of 25 male Wistar rats (*Rattus norvegicus*) were obtained from Bikul Bali, were then placed under controlled environmental conditions: temperature of 22±2℃, 55±5% relative humidity, and a constant light/dark cycle. A standard diet with pellets and water was supplied *ad libitum*. All experiments were performed under the Animal Ethics Committee of the Faculty of Medical and Health Sciences Udayana University (No. 1381/UN14.2.2.VII.14/LT/2020). Rats were divided into five groups: the control group (Negative) was given aquadest, the positive control group (Positive) was given diclofenac sodium suspension, and treated groups received treatment of Dadap leaves extract in three different doses: 200 mg/kg Body Weight (BW) (P1), 300 mg/kg BW (P2), and 400 mg/kg BW (P3).

Rats were placed under fasting conditions for 18 hours before the experiments. The test method used was the paw edema method. Artificial edema was induced by injection of 0.1% carrageenan in 10 ml of 0.1 ml 0.9% NaCl solution, intraplantar on the rats' feet. The volume was observed with a plesthymometer before

the injection of carrageenan and treatment (positive and extract treatment) as V0 and the volume was observed 4 hours after carrageenan and treatment injection as Vt. The anti-inflammatory activity was indicated by the percentage of a decrease in the inflammatory volume at 4 hours. The difference in inflammation reduction was compared between treatment groups to determine the ratio of inflammation reduction between groups. The reduction percentage was calculated by the following formula (Singh et al., 2010)

$$Anti - inflammatory\ activity = \frac{Vt - Vo}{Vo}\ x\ 100\%$$

Statistical Data Analysis

Data of volume changes and anti-inflammatory activity were analyzed using One Way ANOVA followed by Bonferroni Post Hoc Test. All statistical analyzes used a 95% confidence level. Statistical analysis was conducted using IBM SPSS Statistics 25.

RESULTS AND DISCUSSION

Study on natural medicine is extensively and continuously be done. Natural medicine provides various proven pharmacological activities with a relatively high level of safety and low side effect. Dadap (*Erythrina subumbrans* (Hassk.) Merr) is commonly used for several pharmacology activities. Phytochemical screening revealed flavonoids, saponin, tannins, steroids, and triterpenoids in Dadap leaves extract (Table 1). This result is reinforced in previous research where the ethanol extract of Dadap leaves contains alkaloids, flavonoids, and steroids (Mantena & Tejaswini, 2015). Flavonoids are well-known phytochemicals for their anti-inflammatory activity by inhibiting cyclooxygenase and lipoxygenase (Panche et al., 2016).

Table 1.Extract phytochemical screening test results Ddadap leaves

Phytochemicals	Reagent Test	Results	Description
Alkaloids	Mayer	No precipitation	-
	Dragendorf	No precipitation	-
Flavonoids	10% lead acetate, Mg, Klorhidrat and Amil alcohol	precipitate formed saffron	+
Saponin	Aquadest and 2N HCl	formed a stable foam within 30 seconds	+
Tannins	$FeCl_{3A}$	greenish black color is formed	+
Steroids and triterpenoids	Chloroform and Libermann Burchard	A green and blue solution is formed (steroids)	+

(-) No presence of phytochemicals ; (+) presence of secondary metabolites

eISSN: 2655-9994
pISSN: 2303-3371
https://doi.org/10.24843/IJBB.2021.v09.i01.p03

The anti-inflammatory assay of Dadap leaves extract was carried out using the paw edema method by observing the test substance's ability to prevent swelling due to carrageenan induction. Inflammation volume changes and anti-inflammatory activity for each group are shown in Figure 1. All extract treatments reduced volume in 4 hours of treatment compared to volume without carrageenan and treatment injection, except for the negative group, which showed volume increase. Statistically, there were differences in volume reduction in positive and extract treatment. The negative control showed no anti-inflammatory effect, and mainly it produced increasing instead of decreasing volume. Treatment of 400 mg/kg BW of extract produced the highest activity (Figure 1) compared to other groups suggesting dose-dependence. Statistically, extract treatment in all doses produced no different activity as compared to the positive control. Nevertheless, among three dosses showed no significant different as compare each other.

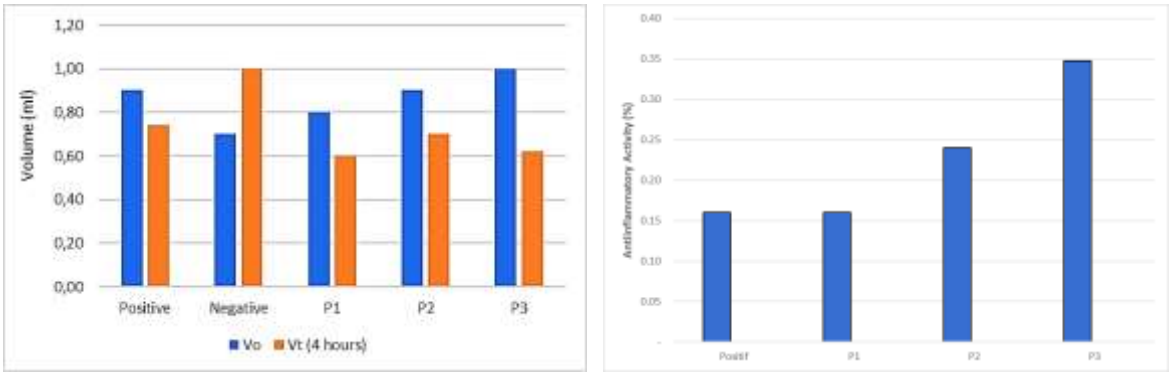

Figure 1. Volume changes (left) and anti-inflammatory activity (right) for each group. The highest volume reduction was produced by the treatment with P3 (400mg/kgBW). The negative control showed inflation in Vt as compared to Vo, indicating no anti-inflammatory produced.

Carrageenan induces cell injury by triggering the inflammatory mediator's release that initiates the inflammation process and produces edema. Inflammatory mediators, especially prostaglandin E1 and E2, produced in this condition, decrease the vascular permeability and last for 6 hours and gradually decrease within 24 hours (Santoso et al., 2018).

In the study, the anti-inflammatory effect of the Dadap (*Erythrina subumbrans* (Hassk.) Merr) leaves extract was observed. Phytochemical screening of Dadap leaves extract showed the presence of flavonoids, Flavonoids can modulate anti-inflammatory

activity through a broad spectrum of mechanisms involving various key points in the regulation of the inflammatory process. These activities are related to the mechanism of flavonoids against various simultaneous activities on different molecular targets. Several pathways that can mediate the antiphlogistic action of flavonoids include antioxidant and pro-oxidant effects, inhibition of the expression of genes that play a role in the inflammatory process, interactions with signaling pathways, and interactions with pro-inflammatory proteins, including inhibition of specific enzymes (Hanáková et al., 2017). Flavonoid is also reported for its activities in inhibiting the cyclooxygenase and lipoxygenase enzymes, producing in inhibition of eicosanoids (prostaglandins and leukotrienes) as well (Panche et al., 2016). Modulating pro-inflammatory enzymes is considered the essential mechanism in anti-inflammatory activity (Hanáková et al., 2017). Eicosanoid release is considered the starting point for a general inflammatory response (Dennis & Norris, 2015). Dadap leaves extract also contains steroid compounds known to inhibit the phospholipase A2 enzyme, which is responsible for releasing arachidonic acid. Arachidonic acid is then metabolized by cyclooxygenase and lipoxygenase enzymes. Inhibition of arachidonic acid is then followed by inhibition of cyclooxygenase and lipoxygenase enzyme production (B. Wang et al., 2021; Z. J. Wang et al., 2010). Dadap leaves extract also contains astringent tannins that affect the cytoplasmic membrane's permeability, causing shrinkage of the cell membrane. Saponins also play an essential role as an anti-inflammatory by inhibiting exudate formation and vascular permeability increase from reducing inflammation (Dermiati et al., 2018). Saponins are detected to be present in Dadap leaves extract as well. Saponins are reported to be able to inhibit the production of lipopolysaccharide (LPS)-induced nitric oxide (NO) and prostaglandin E2 (PGE2). This inhibitory activity occurs through suppression of inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2) mRNA and the expression of proteins that play a role in inflammation. Saponins are also reported to be able to inhibit the production of pro-inflammatory cytokines, such as TNF- and IL-1β, through suppression of transcription processes through attenuation of NF-kB translocation from the cytoplasm to the nucleus, which is accompanied by blocking of the phosphoinositide 3-kinase (PI3K)/Akt signaling pathway and mitogen-activated protein kinase (MAPK) (Jang et al., 2013).

eISSN: 2655-9994
pISSN: 2303-3371
https://doi.org/10.24843/IJBB.2021.v09.i01.p03

Based on the results of these studies, Dadap (*Erythrina subumbrans* (Hassk.) Merr) leaves extract produced potential oral anti-inflammatory activity. This plant is potential to be developed in herbal anti-inflammatory preparations. Further research is still needed, primarily to assess its toxicity level and product formulation.

CONCLUSIONS

Dadap (*Erythrina subumbrans* (Hassk.) Merr) leaves extract contains secondary metabolites, including flavonoids, steroids, triterpenoids, saponins, and tannins. In vivo anti-inflammatory assay showed the potential of Dadap leaves extracts in reduce carrageenan-induced inflammatory. Administration of 200mg/kg BW of Dadap leaves extract produced a similar effect to diclofenac sodium, while administration dose of 300 and 400mg/kg BW produced higher anti-inflammatory activity than diclofenac sodium, with administration dose of 400mg/kg BW considered the most active.

ACKNOWLEDGEMENTS

The authors are grateful to the authorities of the Faculty of Pharmacy, Universitas Mahasaraswati Denpasar for approving this study.

REFERENCES

Ciumărnean, L., Milaciu, M. V., Runcan, O., Vesa, S. C., Răchisan, A. L., Negrean, V., Perné, M.-G., Donca, V. I., Alexescu, T.-G., Para, I., & Dogaru, G. (2020). *molecules The E ff ects of Flavonoids in Cardiovascular Diseases*. 1–18.

Dennis, E. A., & Norris, P. C. (2015). Eicosanoid Storm in Infection and Inflammation. *Nature Reviews Immunology*, *11*(8), 511–523. https://doi.org/10.1038/nri3859.Eicosanoid

Dermiati, Kamal, A., Tibe, F., & Anggi, V. (2018). Uji Antiinflamasi Ekstrak Etanol Kulit Barang Ceremai (Phyllanthus acidus L . Skell) Terhadap Edema Kaki Tikus. *Farmakologika Jurnal Farmasi*, *XV*(1), 1–8.

Ekor, M. (2014). The growing use of herbal medicines: Issues relating to adverse reactions and challenges in monitoring safety. *Frontiers in Neurology*, *4 JAN*(January), 1–10. https://doi.org/10.3389/fphar.2013.00177

Gunaydin, C., & Bilge, S. S. (2018). Effects of nonsteroidal anti-inflammatory drugs at the molecular level. *Eurasian Journal of Medicine*, *50*(2), 116–121. https://doi.org/10.5152/eurasianjmed.2018.0010

Hanáková, Z., Hošek, J., Kutil, Z., Temml, V., Landa, P., Vaněk, T., Schuster, D., Dall'Acqua, S., Cvačka, J., Polanský, O., & Šmejkal, K. (2017). Anti-inflammatory Activity of Natural Geranylated Flavonoids: Cyclooxygenase and Lipoxygenase Inhibitory Properties and Proteomic Analysis. *Journal of Natural Products*, *80*(4), 999–1006. https://doi.org/10.1021/acs.jnatprod.6b01011

Hendrawan, I. W. D. W., Merdana, I. M., & Sudira, I. W. (2019). Pemberian Infusa

Daun Dadap (Erythrina Subumbrans) Terhadap Profil Eritrosit Ayam Broiler yang Mengalami Stres Transportasi. *Indonesia Medicus Veterinus*, *8*(2), 205–215. https://doi.org/10.19087/imv.2019.8.4.464

Howland, R. D., Mycek, M. J., Harvey, R. A., & Champe, P. C. (2006). *Pharmacology (Lippincott's Illustrated Reviews Series)* (Third Edit). Lippincott Williams & Wilkins.

Jang, K. J., Ki Kim, H., Han, M. H., Oh, Y. N., Yoon, H. M., Chung, Y. H., Kim, G. Y., Hwang, H. J., Kim, B. W., & Choi, Y. H. (2013). CytoAnti-inflammatory effects of saponins derived from the roots of Platycodon grandiflorus in lipopolysaccharide-stimulated BV2 microglial cells. *International Journal of Molecular Medicine*, *31*(6), 1357–1366. https://doi.org/10.3892/ijmm.2013.1330

Mantena, V. R. K. R., & Tejaswini, G. (2015). Anti inflammatory activity of Erythrina Variegata. *International Journal of Pharmacy and Pharmaceutical Sciences*, *7*(4), 386–388.

Panche, A. N., Diwan, A. D., & Chandra, S. R. (2016). Flavonoids: An overview. *Journal of Nutritional Science*, *5*. https://doi.org/10.1017/jns.2016.41

Pandey, A., Tripathi, S., & Pandey, C. A. (2014). Concept of standardization, extraction and pre phytochemical screening strategies for herbal drug. *Journal of Pharmacognosy and Phytochemistry JPP*, *115*(25), 115–119.

Santoso, P., Sari, N. i W. B., Yuda, P. E. S. K., & Wardani, I. G. A. A. K. (2018). Phytochemical Screening And Anti-Inflammatory Activity of Dewandaru Fruit (Eugenia uniflora L.) Extract in male White Wistar Rats (Rattus norvegicus) Using paw Edema Method Induced by Carrageenan. *Medicamento*, *4*(2), 100–106.

Saputri, F. C., & Zahara, R. (2016). Uji Aktivitas Anti-Inflamasi Minyak Atsiri Daun Kemangi (Ocimum americanum L.) pada Tikus Putih Jantan yang Diinduksi Karagenan. *Pharmaceutical Sciences and Research*, *3*(3), 107–119. https://doi.org/10.7454/psr.v3i3.3619

Singh, M., Kumar, V., Singh, I., Gauttam, V., & Kalia, A. N. (2010). Anti-inflammatory activity of aqueous extract of Mirabilis jalapa Linn. leaves. *Pharmacognosy Research*, *2*(6), 364–367. https://doi.org/10.4103/0974-8490.75456

Wang, B., Wu, L., Chen, J., Dong, L., Chen, C., Wen, Z., Hu, J., Fleming, I., & Wang, D. W. (2021). Metabolism pathways of arachidonic acids: mechanisms and potential therapeutic targets. *Signal Transduction and Targeted Therapy*, *6*(1). https://doi.org/10.1038/s41392-020-00443-w

Wang, Z. J., Wilkie, D. J., & Molokie, R. (2010). Neurobiological mechanisms of pain in sickle cell disease. *Hematology*, *2010*(1), 403–408. https://doi.org/10.1182/asheducation-2010.1.403