



Anti Inflammatory Activity Test of Ethanol Extract of Fennel Leaves and Fruits (*Foeniculum Vulgare* Mill.) In Wistar Rats

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Abstract

Fennel plant has various kinds of benefits such as anti-inflammatory. Fennel plants contain alkaloid compounds, flavonoids, saponins, polyphenols, tannins, anthraquinones, and steroids. The research objective was to determine the anti-inflammatory activity of the ethanol extract from fennel leaves and fruit. The research method belongs to experimental research with edema formation on rats' feet induced by 1% carrageenan. The animals' tests were randomly divided into eight treatment groups and measured the volume of their feet. Each group was given CMC Na 1% (negative control), diclofenac sodium 4.5mg/Kg weight rats (positive control), and ethanol extract of fennel leaves and fruit at a dose of 87.5 mg/Kg rat body weight, 175 mg/ Kg rat body weight, 350 mg/Kg rat body weight. Moreover, the animals' tests were induced with carrageenan 1% while the edema volume of rat leg was measured at the hours of 0.25; 0.5; 0.75; 1; 1.25; 1.5; 1.75; 2.00 after induction of carrageenan. The results of the study were edema volume data and AUC value for each treatment. The data were analyzed using the Kolmogorov-Smirnov (normality test), Levene test (homogeneity test), and One-Way Anova (effectiveness test). The normality and homogeneity test results show that there is no significant difference ($P>0.05$). In contrast, the effectiveness test between CMC Na 1% (negative control) and Diclofenac sodium 4,5 mg / Kg weight (positive control) shows the significant result ($P<0.05$), CMC Na (negative control) and all extract treatment shows the insignificant result ($P>0.05$), Diclofenac Natrium 4,5 mg/ Kg weight (positive control) with all treatments indicates the significant result ($P<0.05$).

Keywords: Anti-Inflammatory, Fennel Leaves, Fennel Fruit, Carrageenan

INTRODUCTION

Inflammation is a complex biological response of vascular tissue to harmful stimuli such as irritants, pathogens, or damaged cells/tissues. Treatment of patients with inflammation is generally to slow or limit the tissue damage process that occurs in the inflamed area. Synthetic drugs are often used to treat inflammation or inflammation, which consists of two classes of drugs, namely non-steroidal drugs (NSAIDs) and steroids. NSAIDs are reported to have side effects such as gastric ulcers and kidney problems, so safe anti-inflammatory drugs are needed, which are thought to have fewer side effects (Apridamayanti et al., 2018).

One of the medicinal plants developed as an anti-inflammatory is fennel (*Foeniculum vulgare* Mill.). *Foeniculum vulgare* Mill. commonly known as a medicinal plant belonging to the Apiaceae (Umbelliferae) family with a distinctive aromatic smell. Research that has been conducted on fennel plants shows the presence of fatty acid compounds, phenolic components, hydrocarbons, essential oils, and several other classes of secondary metabolites from different parts. Several fennel plant extracts and isolated compounds have been evaluated for several studies that have anti-aging, anti-allergic, anti-inflammatory, antimicrobial, antiviral, and anti-infective activity (Badgujar et al., 2014).

The results of the phytochemical screening of the ethanol extract of fennel leaves showed the presence of alkaloids, flavonoids, saponins, polyphenols, tannins, anthraquinones, steroids (Abdul and Qonitah, 2020). Flavonoids are one of the chemical compounds that have been researched to have an anti-inflammatory effect because flavonoids can inhibit the enzyme cyclooxygenase or lipoxygenase (Agustina et al., 2015).

Flavonoids such as eriodictyol-7-Rutinoside, Quercetin-3-Rutinoside, and Rosmarinic Acid have been isolated from the fennel plant. Flavonoids in fennel such as isorhamnetin 3-O-rhamnoside, quercetin, and kaempferol were isolated from ethyl acetate extract, while quercetin 3-O-Rutinoside, kaempferol 3-O-Rutinoside, and quercetin 3-O-glucoside were isolated from methanol extract. Oral administration of 200 mg/kg methanol extract from fennel fruit to mice and mice showed an inhibitory effect on acute and subacute inflammation. The overall result of this study is that fennel plants have an anti-inflammatory effect through the cyclooxygenase and lipoxygenase pathways. Traditionally in South Africa, the use of infusion or fennel leaf decoction can treat arthritis (Badgujar et al., 2014).

Based on previous research, there is no data on research with the same method, namely using ethanol in fennel plants, this study is a new study to determine the anti-inflammatory activity of the ethanol extract of fennel fruit and leaves.

METHODS

The type of research is experimental. The location of this research was conducted at the Laboratory of Pharmacy Pharmacology, Sahid University, Surakarta.

Materials and Tools

A set of glassware (pyrex, Germany), analytical scales (ACIS, Indonesia), plastimograph (local, Indonesian), macerator set, oven (Memmert, Germany), flannelette,

spatula, and vacuum rotary evaporator (Biobase, China). The materials used are filter paper, aqua bidestilata (Ikapharmindo, Indonesia), leaf and fennel fruit *Simplicia* (Cepogo, Boyolali, Central Java, Indonesia), 1% carrageenan (Bratchem), 96% ethanol (Merck, Germany), CMC Na 1%. (Bratachem), NaCl 0.9% (Wina, Indonesia), Diclofenac Sodium 50 mg (Chemical Planet).

Making Ethanol Extract of Fennel Leaves and Fruits

The fruit and fennel leaf powders were extracted using 96% ethanol by maceration at room temperature for 3 x 24 hours. Maceration was carried out by putting the powder into a macerator then adding 96% ethanol solvent in the ratio of fennel leaves and fennel fruit (1: 5) and stirring every 12 hours then leaving it for 24 hours in a place protected from sunlight. After 3 days the macerate is filtered and squeezed. The obtained macerate is then filtered with a Buchner funnel and evaporated using a rotary evaporator then evaporated in a water bath at 60 ° C until a thick consistency is obtained (Abdul and Qonitah, 2019).

Anti-Inflammatory Test

In this study, the test animals used were male white rats Wistar strain aged 2-3 months and bodyweight 150-250 gr. Each of the 3 test animals in each experimental group. The anti-inflammatory test procedure is that the rats are fasted 18 hours before the test, then drinking water is still given. The rats were weighed and grouped randomly. There were 24 rats randomly divided into 8 groups. The left hind leg of each mouse to be induced was marked on the ankle, then the volume was measured first by inserting the mouse's foot into the plastismograph to the boundary mark. Edema volume was calculated from the difference in the volume of rats' feet after and before 1% carrageenan induction. After obtaining the volume value of rat foot edema, the Area Under Curve (AUC) value is determined by the formula:

$$AUC_{t_n-1}^{t_n} = \frac{V_{t_n} + V_{t_n-1}}{2} (t_n - t_{n-1})$$

Information:

- $AUC_{t_n-1}^{t_n}$: Area under the inflammation percentage curve against a time of the treatment group
 V_{t_n} : udem volume (mL)
 t_n : time (hour)

The AUC value is the average area under the curve which is the relationship between the average volume of edema per unit time (Apridamayanti et al., 2018).

RESULTS AND DISCUSSION

The yield obtained is the percentage weight (w/w) between the yield and the weight of the *simplicia* powder used by weighing. 96% ethanol solvent lasts longer in storage because it contains less water content so that bacteria are less likely to grow (Abdul et al., 2020). The yield of the extract can be seen in table 1.

Table 1. The yield of Fennel Leaf and Fruit Ethanol Extract

Extract	Powder weight (kg)	Extract weight (kg)	Yield (%)
Fennel leaves	0,4629	0,0493	10,65
Fennel fruit	0,8038	0,0394	4,91

The table above obtained the highest yield in fennel leaf extract compared to fennel fruit extract. This is influenced by the plant part, the extraction solvent, and the particle size of the fennel fruit used (Abdul et al., 2020).

In this study, the test animals were divided into 8 groups, group I was given CMC Na 1% (negative control). Group II was given diclofenac sodium 4.5 mg / Kg BW (positive control), group III was given ethanol extract of fennel leaves at a dose of 87.5 mg / Kg BW, group IV was given ethanol extract of fennel leaves at a dose of 175 mg / Kg BW, group V was given ethanol extract of fennel leaves at a dose of 350 mg / Kg BW, group VI was given ethanol extract of fennel fruit at a dose of 87.5 mg / Kg BW, group VII was given ethanol extract of fennel fruit at a dose of 175 mg / Kg BW, group VIII was given extract ethanol of fennel fruit at a dose of 350 mg / Kg BW. All of the extracts were administered orally to rats, then measurements were taken with a mercury plastismograph every 15 minutes for 2 hours to assess the anti-inflammatory effect in the form of volume and percentage of inhibition of rat foot edema. Methanol extract of fennel fruit (200 mg/kg) showed significant inhibition of carrageenan-induced leg edema compared to the control group (Badgular et al., 2014). The dosage variation was obtained from ethanol extract research (Septiani, 2018). containing flavonoids, namely 87.5 mg / Kg BW, 175 mg / Kg BW, and 350 mg/kg BW of rats.

Analysis of Normality and Homogeneity

Analysis of normality in this study used the Kolmogorov-Smirnov test with a significance of 0.074. Value ($P > 0.05$) so that it can be seen that the data is normally distributed, while the homogeneity analysis in this study uses the Levene test with a significance of 0.168. Value ($P > 0.05$) so it can be said that the data used is homogeneous. The data is homogeneous and normally distributed so that it is continued with the One-Way Anova statistical test to determine the significance of each sample treatment.

Anti-Inflammatory Activity Test of Fennel Leaf Ethanol Extract

The anti-inflammatory activity test was carried out using the test animal foot edema method, using a foot volume measuring device on the test animal, namely a plastimograph filled with mercury.

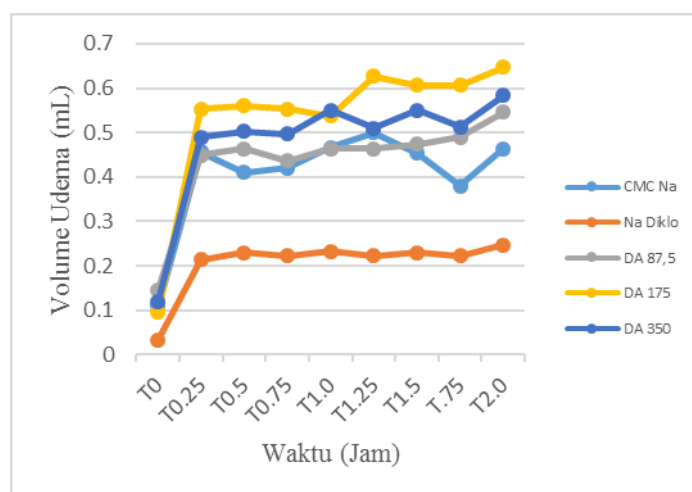


Figure 1. Graph of Average Udema Volume of Fennel Leaf Ethanol Extract, (Diclo Na): Diclofenac Sodium, (DA 87.5): Fennel leaves 87.5 mg/KgBW, (DA 175): Fennel leaves 175 mg/KgBB and (DA 350): Fennel leaves 350 mg / KgBB.

The figure shows that the negative control group and the ethanol extract group of fennel leaves have almost the same volume of edema. This means that the ethanol extract of fennel leaves did not show anti-inflammatory activity at a dose variation of 87.5; 175 and 350 mg/kgBw. Traditionally in South Africa, the use of infusion or fennel leaf decoction can treat arthritis. One of the compounds contained in fennel leaves is flavonoids such as eriodictyol-7-Routoside, Quercetin-3-Rutinoside, and Rosmarinic Acid (Badgujar et al., 2014). Flavonoids are thought to have anti-inflammatory activity (Abdul et al., 2020). However, in this study the volume of rat foot edema induced by carrageenan in the ethanol extract treatment group of fennel leaves did not show a decrease, this is likely the mechanism of anti-inflammatory action of the ethanol extract of fennel leaves is different from diclofenac sodium.

Table 2. Average AUC and SD mean of fennel leaf ethanol extract

Treatment Group	N	AUC Data Average \pm SD	Anov <i>a</i>
Control negative (-)	3	0.1104 \pm 0.0252	
Control positive (+)	3	0.0569 \pm 0.0045	
Ethanol extract of fennel leaves 87.5 mg / Kg BW	3	0.1174 \pm 0.0156	0,000
Ethanol extract of fennel leaves 175 mg / Kg BW	3	0.1461 \pm 0.0073	*
Ethanol extract of fennel leaves 350 mg / Kg BW	3	0.1307 \pm 0.0158	

The results of the AUC value statistical analysis showed that the negative control group was not significantly different from the dose treatment group. Diclofenac sodium as a positive control had an anti-inflammatory effect on carrageenan-induced male white rats Wistar strain while the ethanol extract of fennel leaves and fruit did not show any anti-inflammatory effect. There was a significant difference ($P < 0.05$) between 4.5 mg / Kg BW of Diclofenac sodium (positive control) and fennel leaf ethanol extract at all doses, but it was not potential because the AUC value of fennel leaf ethanol extract was higher than Diclofenac sodium or close to its value. of CMC Na 1% (negative control). The results of the study of anti-inflammatory activity in the ethanol extract of fennel leaves showed that

diclofenac sodium 4.5 mg / Kg BW (positive control) gave a good therapeutic effect in the form of edema inhibition. The ethanol extract of fennel leaves may have a small anti-inflammatory effect or may have an anti-inflammatory effect with a different pathway, namely the lipoxygenase pathway because there is no decrease in inflammation after carrageenan induction for 2 hours.

Anti-Inflammatory Activity Test of Fennel Ethanol Extract

This study showed the lowest volume of diclofenac sodium edema among the negative control group and the carrageenan-induced treatment group. Carrageenan is known to induce an acute inflammatory response in three phases. The main phase is mediated by histamine and 5-hydroxytryptamine (Serotonin), the second phase is mediated by bradykinin, and the last phase is prostaglandin induced. The inflammatory response of carrageenan through these 3 phases can be seen from the increase in the size of the edema growing rapidly and persisting at a maximum volume of about 5 hours around induction (Septiani, 2018). In the positive control group that was given diclofenac sodium at a dose of 4.5 mg/200 g BW the volume of the rats' feet increased at 0.25 hours then the volume was stable until the 2nd hour. This shows that diclofenac sodium has a good therapeutic effect in the form of edema inhibition that occurred at hour 1. The decrease in volume of edema in the positive control group was greater than that of the ethanol extract of fennel fruit at a dose of 87.5; 175; 350 mg/kg BW, meaning that the inhibition potential of diclofenac sodium is greater than the ethanol extract of fennel fruit. This is because diclofenac sodium works by stabilizing the lysosomal membrane, inhibiting the release and activity of inflammatory mediators (histamine, serotonin, prostaglandins), inhibiting cell migration to the site of inflammation, and suppressing pain (Amalia, 2016).

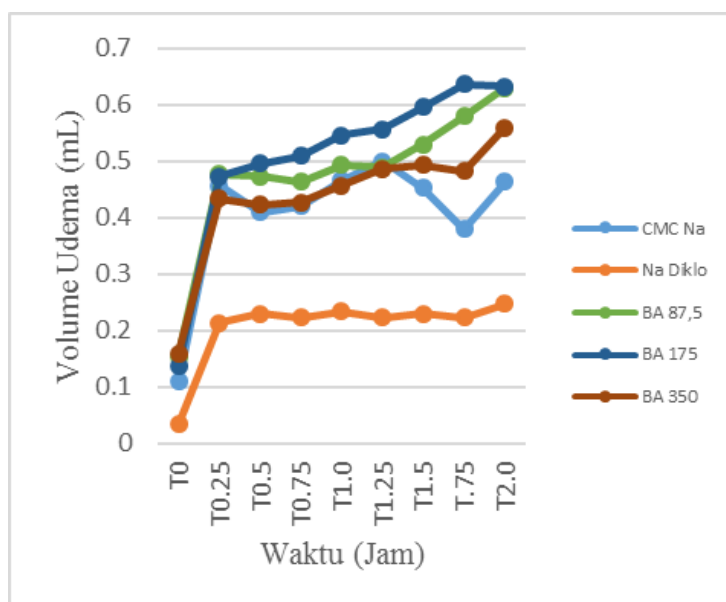


Figure 2. Graph of Average Udema Volume of Fennel Fruit Ethanol Extract, (Diclo Na): Diclofenac Sodium, (BA 87.5): Fennel Fruit 87.5 mg / KgBW, (BA 175): Fennel Fruit 175 mg / KgBB and (BA 350): Fennel Fruit 350 mg / KgBB.

Diclofenac sodium is an NSAID drug that works to inhibit the cyclooxygenase enzyme which plays a role in the metabolism of arachidonic acid into prostaglandins, prostaglandin mediators formed 3 hours after carrageenin induction. The absorption of diclofenac sodium is rapid and complete, diclofenac sodium is 99% bound to plasma protein, experiences the First-pass effect of 40-50%, and has a half-life of 1-2 hours, an onset of 30 minutes, and a duration of 8 hours (Septiani, 2018). The volume of rat edema after being induced by carrageenan in the ethanol extract treatment group of fennel fruit was almost the same as the negative control group. This means that the ethanol extract of fennel fruit did not show any anti-inflammatory effect at the dose variation of 87.5; 175; 350 mg/kg BW. This may be because the time to observe the volume of edema was not long.

Table 3. Average AUC and SD mean values of ethanol extract of fennel fruit

Treatment Group	N	AUC Data Average \pm SD	Anova
Control negative (-)	3	0.1104 \pm 0.0252	
Control positive (+)	3	0.0569 \pm 0.0045	
Ethanol extract of fennel fruits 87.5 mg / Kg BW	3	0.1280 \pm 0.0037	0,000*
Ethanol extract of fennel fruits 175 mg / Kg BW	3	0.1392 \pm 0.0089	
Ethanol extract of fennel fruits 350 mg / Kg BW	3	0.1167 \pm 0.0147	

Based on the results of the average AUC value, it showed that CMC Na 1% (negative control) and Diclofenac Na 4.5 mg / Kg BW (positive control) had a significant difference ($P < 0.05$), while for CMC Na 1% (control negative) with all treatments of fennel fruit ethanol extract there was no significant difference ($P > 0.05$) and treatment of Diclofenac sodium 4.5 mg / Kg BW (positive control) with all fennel fruit ethanol extract treatments there were significant differences ($P < 0, 05$). There is a significant difference between 4.5 mg / Kg BW Diclofenac sodium and fennel fruit ethanol extract but it is not potential as an anti-inflammatory. If a drug can reduce edema induced with carrageenan, it means that the drug has an anti-inflammatory effect. The degree of effectiveness of anti-inflammatory drugs depends on the amount of reduction in edema by the drug (Amalia, 2016).

Research (Badgujar et al., 2014) states that the flavonoid content in fennel has an anti-inflammatory effect. In fennel plant compounds, maybe the anti-inflammatory compounds are not as strong as their immunomodulatory effects so that inhibition of COX here does not function but the inhibitory mechanism is based on immunomodulators. Flavonoids such as quercetin, rutin, isoquercetin are reported to have immunomodulatory activity (He and Huang, 2011).

The methanol extract of fennel seeds contains rosmarinic acid as the main phenolic compound by 14.9% and quercetin (17.1%) and apigenin (12.5%) as the main flavonoids (Badgujar et al., 2014). This shows that the levels of phenolic and flavonoid compounds in the methanol extract of fennel seeds are greater than the phenolic content of the ethanol extract of fennel seeds (0.1777%) and the levels of flavonoids in the ethanol extract of fennel seeds are 0.0538% (Abdul and Qonitah, 2020). In this study, the flavonoid compounds may be slightly interested so that the ethanol extract of fennel leaves and fruit did not show anti-inflammatory activity.

Research from (Maghfiroh, 2019) stated that the fennel seed extract (*Foeniculum vulgare* Mill.) was able to influence the total number of leukocytes and the number of lymphocytes of Balb C mice exposed to *Salmonella typhi*. This allows it to negate the anti-inflammatory effect of the fennel leaf and fruit extracts. In the 2-hour study, the ethanol extract of fennel leaves and fruit might increase the immunomodulatory effect so that inflammation also increases because there is an increase in the volume of edema and maybe a small number of flavonoid compounds are attracted. According to (Badgujar et al., 2014) quercetin, rutin, and isoquercitrin are reported to have immunomodulatory activity. This is why the ethanol extract of fennel leaves and fruit did not show any anti-inflammatory activity in rats induced by carrageenan.

The inflammatory reaction to a foreign object begins with the introduction of the foreign object by the immune system called the innate immune system. The main cell type found in the initial inflammatory reaction is a large number of neutrophils in the inflamed tissue area. These neutrophil cells were briefly replaced by monocyte cells which rapidly differentiated with macrophages. So macrophages and neutrophils are known as inflammatory cells. In the next stage, the infection will involve lymphocytes which play a role in immune reactions. Macrophage activation results in the production of cytokines including interleukins (Farida, 2003).

Macrophages are the main cells that infiltrate tissues, remove cells and cell waste, promote angiogenesis, and produce cytokines and growth factors that suppress further inflammation and initiate healing by stimulating the division of epithelial cells, activating fibroblasts, and stimulate extracellular matrix and collagen synthesis. Macrophage activity can be significantly increased with the help of inflammatory cytokines produced by cells of the immune system, obtained by the T lymphocyte sub-group (Purwanto and Astrawinata, 2018). The value of edema volume in all treatment groups was higher than the value of some edema of CMC Na, possibly because of the compound that had a higher immunomodulatory effect than the anti-inflammatory compound.

CONCLUSION

The ethanol extract of fennel leaves at a dose of 87.5; 175; 350 mg / Kg BW and ethanol extract of fennel fruit at a dose of 87.5; 175; 350 mg / Kg BW at observation for 2 hours showed no anti-inflammatory activity compared to Diclofenac sodium.

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