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Research Article

Anti-Inflammatory Effects of Ripe Areca Nut Ethyl Acetate Fraction in Wistar Rats with Knee Joints Osteoarthritis

Efek Anti-Inflamasi Fraksi Etil Asetat Biji Pinang terhadap Tikus Wistar Model Osteoarthritis

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ABSTRACT

Osteoarthritis (OA) is the most common form of arthritis. Meloxicam are one of NSAIDs commonly used that have many side effects. Areca nut (Areca catechu L), which contains flavonoids, can provide anti-inflammatory effects. This study aimed to determine the potential anti-inflammatory effect of ethyl acetate fraction from ripe areca nut in OA rat model. Twenty five of adults, male Rattus novergicus strain Wistar were induced to OA with Monosodium Iodoacetate (MIA), then divided into five groups that each group were given ethyl acetate fraction of ripe areca nut doses of 15mg/kgBW, 30mg/kgBW, and 60mg/kgBW, meloxicam 1.35mg/kgBW and aquadest as negative control for 14 days. Diameter of the knee joint is measured by digital metric kaliper to assess joint edema. The Histopathological examination with Haematoxyllin-Eosin stain to assess degree of inflammation on synovial. Among the areca groups, dose 30mg/kgBW had knee joint diameter reduced better and lower synovitis score. In conclusion, ethyl acetate fraction of the ripe areca nut group has anti inflammation effect in OA model but not dose-dependent.

Keywords: *Areca nut, ethyl acetate fraction, histopathology, knee joints, osteoarthritis*

ABSTRAK

Osteoarthritis (OA) adalah bentuk artritis yang paling umum. Meloxicam adalah salah satu *Non-Steroidal Anti-Inflammatory Drugs* (NSAID) yang umum digunakan yang memiliki banyak efek samping. Pinang kuning (*Areca Catechu L*) yang mengandung flavonoid dapat memberikan efek anti inflamasi. Penelitian ini bertujuan untuk mengetahui potensi efek anti-inflamasi fraksi etil asetat biji pinang matang pada model tikus OA. Dua puluh lima tikus dewasa, *Rattus Novergicus Strain Wistar* diinduksi OA dengan *Monosodium Iodoacetate* (MIA), kemudian dibagi menjadi lima kelompok yang masing-masing kelompok diberi fraksi etil asetat pinang kuning dosis 15mg/kgBB, 30mg/kgBB, dan 60mg/kgBB, meloxicam 1,35mg/kgBB dan aquadest sebagai kontrol negatif selama 14 hari. Diameter sendi lutut diukur dengan kaliper metrik digital untuk menilai edema sendi. Pemeriksaan histopatologi dengan pewarnaan Haematoxyllin-Eosin untuk menilai derajat inflamasi pada sinovial. Di antara kelompok pinang, dosis 30mg/kgBB memiliki pengurangan diameter sendi lutut yang paling baik dan skor sinovitis yang paling rendah. Kesimpulannya, fraksi etil asetat kelompok pinang kuning memiliki efek anti inflamasi pada model OA tetapi tidak tergantung dosis.

Kata Kunci: Biji pinang kuning, fraksi etil asetat, histopatologi, *osteoarthritis*, sendi lutut

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INTRODUCTION

Osteoarthritis (OA) is the most common form of arthritis, affecting one in three people older than 65 years old and occurring more in women than men. The prevalence of OA is increasing because of the increased risk from physical inactivity, obesity, and joint injury. Joint pain associated with OA leads to functional limitations, sleep deprivation, fatigue, depressed mood, and interdependence (1).

According to WHO data, 40% of patients with OA in the elderly worldwide complain of limited joint motion. As many as 5% of Osteoarthritis patients in Indonesia are aged over 40 years, 30% are aged 40-60 years, and 65% are older than 61 years; all of these represent a high number. Damage to these synovial joints increased along with age. One to two million older adults in Indonesia are estimated to suffer from disabilities due to OA. Considering these situations, there are many challenges to the impact of OA due to the increasing aging population (2).

Joint changes during aging can contribute to the development of OA. Increased manifestations of the senescence-associated secretory phenotype (SASP), for example, lead to increased production of matrix metalloproteinases (MMPs) in joints. In OA, synovial fluid contained several inflammatory mediators, including plasma proteins (C-reactive protein, proposed as a marker of OA progression and progression), prostaglandins (PGE2), leukotrienes (LKB4), cytokines (TNF, IL1β, IL6, IL15, IL17, IL18, IL21), growth factors (TGFβ, FGFs, VEGF, NGF), nitric oxide, and complementary components (3).

Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) are drugs recommended in national and international guidelines for pain management in OA with severe and musculoskeletal pain and those unresponsive to paracetamol (acetaminophen) therapy. Meloxicam is one of anti-inflammatory drug that is generally used to reduce symptoms, but with some intolerable side effects, especially by the elderly for long-term use. In this case, alternative medicines that have the same effect as NSAIDs are needed (4).

In Indonesia, one of the herbal plants that contains anti-inflammatory agent is from the Areceae family, namely the areca nut (*Areca catechu L*). Ripe areca nut has several chemical compounds, including flavonoids, terpenoids, tannins, quinones, and alkaloids. One of the flavonoids in areca nut that has an anti-inflammatory effect by inhibiting the processes of cyclooxygenase-1, cyclooxygenase-2, and 5-lipoxygenase is proanthocyanidin. This substance can also inhibit the pathway of arachidonic acid metabolism, a molecular radical activity or the formation of histamine and prostaglandins. In this case, proanthocyanidins have an effect like NSAIDs (Non-Steroid Anti-Inflammatory Drugs) (5). This study aimed to determine the anti-inflammatory effect of fractions ethyl acetate of areca seed extract in osteoarthritis model.

METHOD

Experimental Design

All procedures during the experiment were approved by the Research Ethics Committee of Universitas Jambi through ethical clearance number 1560/UN21.8/PT.01.04-2021.

Induction of the Osteoarthritis Model

Before treatment, all 25 Wistar rats were given an intra-articular injection of monosodium iodoacetate in the knee joint based on previous methods (6). OA was induced through a single intra-articular injection of MIA. MIA were prepared as follows 5 mg MIA was dissolved with 10 mL of sterile saline, and a 0.4mL of solution were injected in the knee joint as single dose. Under anesthesia with ketamine and xylazine, both knees were shaved and disinfected. Then, an incision was made at the center of the knee to expose the patellar ligament. Each rat was positioned on its back, and the leg was flexed 90° at the knee joint. The patellar ligament was palpated below the patella, and MIA was injected into the medial side of the ligament of both knees using a 29-gauge, 0.5-inch needle carefully (7).

Treatment

Male 16-week-old Wistar rats, with 200-250 gram bodyweight were housed in a controlled temperature and humidity room at the Animal Experimental Laboratory of Health Faculty, Universitas Jambi, and were provided standard laboratory food and water (ad libitum). A light-dark cycle was set to 12:12 hours, and all procedures were conducted in the light phase. Twenty five of rats were divided equally into five groups (each group with 5 rats). Group 1 were given meloxicam 1.35mg/kgBW, group 2 were given aquadest, groups 3, 4, and 5 were given ethyl acetate fraction of ripe areca nut dose of 15mg/kgBW, 30mg/kgBW, and 60mg/kgBW, respectively. The treatment were given once daily using oral feeder for 14 days after 7 days post OA induction (day 8^{th} until day 21^{th}).

Extraction and Fractination

The ripe areca nut was extracted using the maceration method. The dried powdered ripe areca nut were sifted and weighed as much as 300 grams. Extraction with maceration method is conducted by adding 3000 ml of 96% ethanol solvent with a ratio of 1: 10 for three days (7-9).The extract was homogenized every 3 hours by tilting the bottle at 180° and shaking it up and down for 3 minutes. After three days, the macerate was filtered using a paper filter and a funnel into an Erlenmeyer bottle and concentrated using a rotary evaporator at a speed of 200 rpm and a maximum temperature of 50°C. It yielded, a thick ethanolic extract of 75 ml of areca nut (9-12). The thick ethanol extract of ripe areca nut was weighed and dissolved with distilled water in a ratio of 1:10, which had been heated to 60°C, and stirred until homogeneous.Then, it was mixed with ethyl acetate into a separating funnel with a ratio of 1:10 and homogenized by closing and then tilting 180°, shaking up and down ten times, then opened the faucet so that the gas was wasted. This step was repeated until the gas disappeared. It was then allowed to stand until there were two separate layers (±30 minutes). After the beaker was placed under the separatory funnel, then the bottom cover of the separatory funnel was opened slowly to drain the residue. The ethyl-acetate layer collected was concentrated with a rotary evaporator to obtain the ethylacetate fraction (13).

Measuring Knee Joint Diameter

Measurements were made using a digital metric calliper with millimeter units to measure knee joint diameter.

Measurements were made from the posterior side of the rat's knee and the lateral side of the rat then the diameters were added up and divided by two.

Histological Analysis

Rat terminated in days 22 by ketamine overdose anesthetic. Knee Joint were fixed in 10% neutral-buffered formalin for 3 days and decalcified using decalcifier solution (Osteosoft, Merck,101728). Decalfication process follow the manual instruction. After softened, tissue were washed with 10% neutral-buffered formalin, dehydrated and embedded in paraffin wax. The 4 um sectioned slides were stained with Haematoxylin-Eosin (HE). The score of synovitis were assessed by pathologist blindly with semiquantitative methode as follows 0.) normal synovium, 1.) synovial lining 4-5 cell thick, increased cellularity with some inflammatory cells as mild inflammation, 2.) synovial lining 6-7 cell thick, dense cellularity with inflammatory cells but no lymphoid aggregates as moderate inflammation, 3.) synovial lining more than 7 cell thick, dense cellularity with inflammatory cells, may contain lymphoid aggregates as severe inflammation (14).

Statistical Analysis

The software used for statistical tests is IBM SPSS Statistics 25. The differential test used was the Kolmogorov-Smirnov for distribution of data, followed by One-Way ANOVA and Post-Hoc Test (Duncan), with significant value p<0,05.

RESULTS

Monosodium Iodoacetate Injection for Osteoarthritis Model

The average knee joint diameter of Wistar rats (Figure 1) on day 7 was higher than the average knee joint diameter of rats on day 0 (D0). It indicated a successful Monosodium Iodoacetate (MIA) injection with an increase in rat knee joint diameter compared to that before the injection of Monosodium Iodoacetate (MIA).

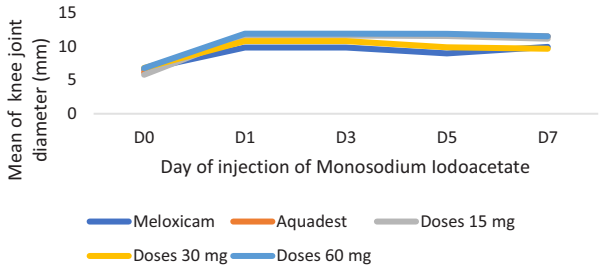

Figure 1. The mean knee joint diameter after the injection of Monosodium Iodoacetate (MIA)

Knee Joint Diameter

After 14 days treatment, on days 21 there was a significant different of diameter of joint knee among the groups (p=0,000). The smallest diameter of joint knee was meloxicam group, followed with group areca nut extract dose 30mg/kgBW, dose 15mg/kgBW and dose 60mg/kgBW. Groups that given aquadest as negative control group had the highest diameter of joint knee on days 21 (Figure 2).

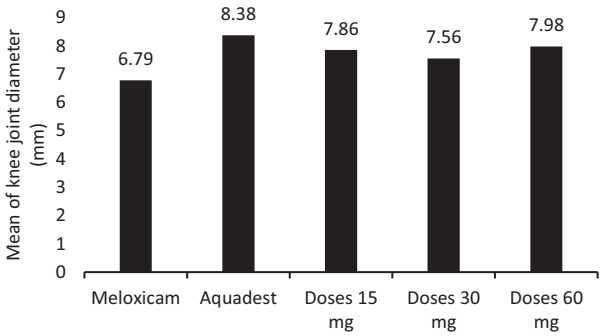

Figure 2. Mean of knee joint diameter after 14 days treatment (day 21)

There were a significant difference of synovitis score after 14 days treatment among the groups (p=0.01). Among groups given areca extract, dose of 30mg/kgBW had the lowest synovitis score. However, meloxicam as a positive control had better synovitis score than areca extract dose 30mg/kgBW (Figure 3).

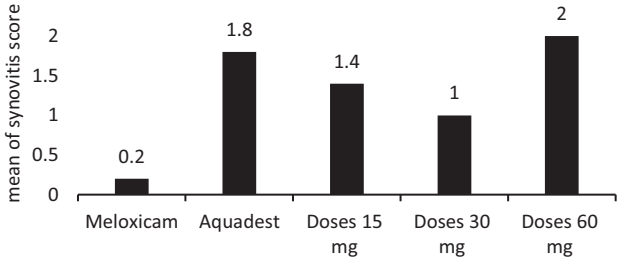

Figure 3. Mean of synovitis score after 14 days treatment

As seen in Figure 4, (A) Meloxicam Group and (D) Dose 30mg/kgBW show the degree of inflammation normal to mild or grade 0-1, marked with an arrow there is a smooth cartilaginous surface appearance, with matrix and chondrocytes positioned properly and well aligned in the zone. There was no enlargement/distortion of the chondrocytes and no changes in chondrocyte proliferation. (B) Negative Control with aquadest, (C) Dose 15mg/kgBW and (E) Dose 60mg/kgBW indicate a moderate or grade of inflammation, marked with an arrow there is focal discontinuities of the superficial zone of cartilage. In this grade, abrasion causes loss of a small portion of the superficial matrix parallel to the surface. The exfoliated fragments in the image above appear as a matrix of ''flakes'' or ''fibrils'' in the synovial fluid.

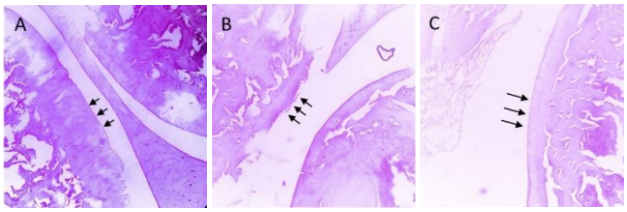

Figure 4. Histopathological assessment of regions in the sagittal section at the center of the patellofemoral joint

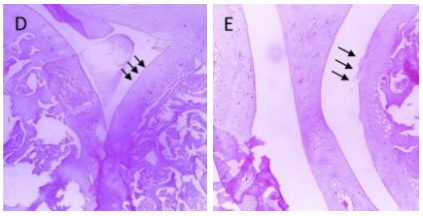

Figure 4. Histopathological assessment of regions in the sagittal section at the center of the patellofemoral joint

DISCUSSION

The anti-inflammatory effect of the ethyl acetate fraction of ripe areca nut in OA model were assessed by the decrease of diameter of the joint swelling and histopathological appearance in the knee joint. Monosodium iodoacetate is known to induce osteoarthritis in animal models. After the injection, an inflammatory reaction in the joints was triggered, which causes destruction of the proteoglycan matrix, subchondral sclerosis, subchondral cysts, osteophyte formation, and impaired joint function, similar to osteoarthritis in humans (15).

Edema is a sign of inflammation that can be observed by measuring the diameter of the joint. A decrease in joint diameter may indicate a decrease in edema and inflammation. The data showed there were significant differences between the mean diameter of the joint knee. Among the areca nut treatment groups, the ethyl acetate fraction at a dose of 30mg/kgBW showed greater effect in reducing knee joint edema than other doses. However, meloxicam had greater effect on reducing knee joint edema than the extract. Histopathology of the synovitis score had similar result with the edema of knee joint. The histopathological description of the synovial layer dose of 30mg/kgBW was normal to mild inflammation, while dose of 15mg/kgBW and 60mg/kgBW showed mild to moderate inflammatory. Dose of meloxicam that we used were animal dose from conversion of the highest dose as maximum tolerated dose in human, 15mg per daily. In clinical setting, meloxicam is usually given in gradual dose started from lower dose 0.75 mg daily (16).

The flavonoids contained in areca nut have anti-inflammatory properties with different mechanisms, consisting of inhibition of regulatory enzymes and transcription of elements that have important functions in the management of mediators involved in inflammation. Flavonoids are powerful antioxidants that can scavenge free radicals and reduce their formation. Therefore, flavonoids have profound effects on many immune cells and immune mechanisms that may be important in the inflammatory process (17). In addition, flavonoids contain proanthocyanidin chemicals, which have anti-inflammatory effects by inhibiting the action of cyclooxygenase-1, cyclooxygenase-2, and 5-lipoxygenase. Proanthocyanidins also work to inhibit arachidonic acid metabolic pathway, prostaglandin formation, histamine release, or molecular radical scavenging activity (18). The non dose dependent manner of our ethyl acetate fraction of areca nut extract need to be further investigated. We suggest that different types of flavonoid in areca nut could affect its anti-inflammatory effect.

In conclusion, ethyl acetate fraction of areca nut extract had anti inflammatory effect on OA rat models in non dependent manner. Further studies are needed to determine the mechanism action of flavonoid in areca nut on the OA model and the effect on the long duration of treatment.

REFERENCES

1. Hawker GA. *Osteoarthritis is a Serious Disease*. Clinical and Experimental Rheumatology. 2019; 37(5): 3–6.
2. Adhiputra IKAI. *Osteoartritis*. [Responsi Kasus]. Universitas Udayana, Bali. 2017.
3. Mora JC, Przkora R, and Cruz-Almeida Y. *Knee Osteoarthritis: Pathophysiology and Current Treatment Modalities*. Journal of Pain Research. 2018; 11: 2189–2196.
4. Cooper C, Chapurlat R, Al-Daghri N, *et al*. *Safety Of Oral Non - Selective Non - Steroidal Anti - Inflammatory Drugs in Osteoarthritis: What Does The Literature Say?* Drugs & Aging. 2019; 36(S1): 15–24.
5. Yuda DA. *Efek Anti Inflamasi Ekstrak Biji Pinang Kuning (Areca Catechu L) Pada Sendi Lutut Tikus Putih (Rattus Novergicus) Model Osteoarthritis*. [Skripsi]. Universitas Jambi, Jambi. 2019.
6. Takahashi I, Matsuzaki T, Kuroki H, and Hoso M. *Induction of Osteoarthritis by Injecting Monosodium Iodoacetate Into the Patellofemoral Joint of an Experimental Rat Model.* PLoS One. 2018; 13(4): 1-15.
7. Prayoga DGE, Nocianitri KA, and Puspawati NN. *Identifikasi Senyawa Fitokimia dan Aktivitas Antioksidan Ekstrak Kasar Daun Pepe*. Jurnal Ilmu dan Teknologi Pangan. 2019; 8(2): 111–121.
8. Ritna A, Anam S, and Khumaidi A. *Identifikasi Senyawa Flavonoid pada Fraksi Etil Asetat Benalu Batu (Begonia Sp.) Asal Kabupaten Morowali Utara*. Jurnal Farmasi Galenika (Galenika Journal of Pharmacy). 2016; 2(2): 83–89.
9. Ramadhani MA, Hati AK, Lukitasari NF, and Jusman AH. *Skrining Fitokimia dan Penetapan Kadar Flavonoid Total Serta Fenolik Total Ekstrak Daun Insulin (Tithonia Diversifolia) dengan Maserasi Menggunakan Pelarut Etanol 96 %.* Indonesian Journal of Pharmacy and Nature Product. 2020; 3(1): 8–18.
10. Cahyanto HA. *Aktivitas Antioksidan Ekstrak Etanol Biji Pinang (Areca Catechu, L).* Majalah BIAM. 2018; 14(2): 70-73.
11. Lindawati NY and Ma'ruf SH. *Penetapan Kadar Total Flavonoid Ekstrak Etanol Kacang Merah (Phaseolus Vulgaris L.) dengan Metode Kompleks Kolorimetri secara Spektrofotometri Visibel*. Jurnal Ilmiah Manuntung. 2020; 6(1): 83–91.
12. Asmorowati H and Lindawati NY. *Penetapan Kadar*

Flavonoid Total Alpukat (Persea americana Mill.) dengan Metode Spektrofotometri UV-Vis. Jurnal Ilmiah Farmasi. 2019; 15(2): 51–63.

13. Harnis ZE. *Fraksinasi Dan Karakterisasi Serta Uji Antiinflamasi Ekstrak Etanol Daun Sambung Rambat (Mikania Cordata (Burm.F) B.L Rob) Terhadap Tikus Putih Jantan.* [Thesis]. Universitas Sumatera Utara, Medan. 2019.
14. Wyatt LA, Moreton BJ, Mapp PI, *et al*. *Histopathological Subgroups in Knee Osteoarthritis.* Osteoarthritis And Cartilage. 2017; 25(1): 14–22.
15. Farry F, Ismiarto YD, Chaidir MR, and Ismono D. *Histopathological Effects of Omega-3 in Reducing Cartilage Destruction Progression in Mice with Knee Joint Osteoarthritis.* Majalah Kedokteran Bandung. 2020; 52(2): 107–112.
16. Pinandita T, Ismono D, Ismiarto YD, and Chaidir MR. *Efek Pemberian Meloxicam yang Diberikan selama Fase Inflamasi terhadap Proses Penyembuhan Tulang Tikus Paska Open Reduction Internal Fixation K-Wire Dinilai secara Radiologis.* Jurnal Sistem Kesehatan. 2018; 3(3): 135–141.
17. Maleki SJ, Crespo JF, and Cabanillas B. *Anti-Inflammatory Effects of Flavonoids*. Food Chemistry. 2019; 299: 1-11.
18. Lestari R, Djohari M, and Hasti S. *Identifikasi dan Uji Aktivitas Daya Hambat Ekstrak Etanol Biji Pinang (Areca catechu L.) terhadap Isolat Bakteri Gusi.* Jurnal Penelitian Farmasi Indonesia. 2019; 7(2): 61-69.