Perbandingan Efek Anti-inflamasi antara Propolis dan Celecoxib terhadap Tikus dengan Sinovitis Lutut

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ABSTRAK

Pendahuluan. Sinovitis merupakan proses awal peradangan sendi, ditandai dengan meningkatnya jumlah makrofag pada sinovium yang berperan penting terhadap kerusakan kartilago dan tulang melalui pembentukan fibroblas. Pemberian anti-inflamasi non-steroid (AINS) pada nyeri sendi sering dilakukan, namun efek samping pemberian menimbulkan permasalahan tersendiri di bidang kesehatan. Propolis suatu bahan alami banyak dikonsumsi sebagai penghilang nyeri sendi lutut, mengandung bioflavonoid dan *Caffeic Acid Polyphenol Ester*. Beberapa penelitian membuktikan efek propolis sebagai anti-inflamasi, namun mekanisme kerjanya dalam menekan jumlah makrofag dibandingkan dengan anti inflamasi lain belum pernah diteliti. Dengan demikian, penelitian ini bertujuan membandingkan efek propolis terhadap celecoxib sebagai anti-inflamasi dalam sinovitis lutut tikus.

Bahan dan cara kerja. Pada penelitian eksperimental ini digunakan tikus jantan galur Wistar yang dibagi menjadi tiga grup. Masing-masing grup diberikan peptidoglikan saja, peptidoglikan dan propolis per oral, serta peptidoglikan dan celecoxib per oral. Skor dari jumlah makrofag dan sinovitis sendi lutut tikus diamati dengan imunohistokimia CD-68 dan pewarnaan hematoksilin-eosin pada hari ke-1, ke-3 dan ke-14. Perbedaan skor masing-masing grup dianalisis dengan ANOVA.

Hasil. Peningkatan jumlah makrofag dan sinovitis untuk semua grup penelitian terjadi pada hari ke-3, selanjutnya menurun pada hari ke-14. Terdapat perbedaan bermakna penghambatan jumlah makrofag antara grup 1 dengan kedua grup lainnya pada hari ke-3 ($p<0.05$). Hal tersebut membuktikan bahwa terdapat peran anti-inflamasi. Pada hari ke-3 dan ke-14, jumlah makrofag grup 2 lebih sedikit dibandingkan dengan grup 3 dengan perbedaan hingga 5 kali lipat ($p<0.05$).

Kesimpulan. Propolis menghambat jumlah makrofag 4-5 kali lebih kuat dibandingkan dengan celecoxib pada sinovitis sendi lutut.

Kata kunci: propolis, celecoxib, makrofag, sinovitis

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Comparison between Propolis and Celecoxib as Anti-inflammatory Agent in Rat with Knee Synovitis

ABSTRACT

Introduction. Synovitis is an inflammation occurs in a joint marked by an increase in macrophage numbers in synovium resulting in cartilago and bone destruction by production of fibroblasts. Administration of non-steroid anti inflammation drug (NSAID) in management of arthritis and synovitis has its own complications, including gastrointestinal and bleeding disorder. Propolis, a natural bee product, is recognized as one of traditional pain killers at knee joint pain containing flavonoid and caffeic acid phenolic esters (CAPE). Several studies show its anti-inflammation effect, but its effect compared to other NSAID is still unknown. Therefore the aim of the study is to compare Propolis and celecoxib anti-inflammation effect in rat with knee joint synovitis.

Materials and Methods. In this experimental study, Wistar strain rats were used. They were divided into three groups. Each group were given peptidoglycan only, peptidoglycan followed by Propolis, and peptidoglycan followed by celecoxib. Scoring based on number of macrophages and synovitis degree were evaluated by immunohistochemistry CD 68 and HE staining. It was evaluated in day 1, 3, and 14. Those scores were collected and analyzed using ANOVA.

Results. Increasing number of macrophages and synovitis degree for all groups occur on day 3 and continuously decreasing until day 14. There is a significant difference in number of macrophages between grup 1 and the other two groups on day 3 ($p<0.05$). It shows that there is an anti-inflammation effect of both propolis and celecoxib. On day 3 and 14, the number of macrophages in grup 2 were five times lower than grup 3. ($p<0.05$)

Conclusions. Propolis anti-inflammation effect shows 4-5 folds stronger than celecoxib in knee joint synovitis.

Keywords: celecoxib, macrophage, propolis, synovitis

Introduction

Arthritis is a term used to describe more than hundred different joint disorders; meanwhile synovitis is an inflammatory process in synovial membrane that follows arthritis.[1] Synovitis still remains as a worldwide health problem regarding complications due to dysfunction of the affected joint as result of progressivity and inadequate treatment. Annual survey in United States in 2008 stated that there are approximately 50 million people diagnosed as arthritis,[2] 744,000 people treated, 9,367 die and 19 million people living with restricted joint mobility.[1]

The principle management of synovitis either conservative or operative is relieving pain, joint resting and immobilization, debridement and surgical synovectomy, besides the causative therapy. Symptomatic therapy to relieve pain using non-steroidal anti-inflammatory drugs (NSAID) has led to health problems due to their side effects, such as gastrointestinal system discomfort and bleeding disorders. The long term duration of using NSAID, even with newest class of selective inhibitor of cyclooxygenase-2 (COX-2) can cause its own complications.

Some of herbal have been proposed as alternative in treatment of joint pain. They provide efficacy and reduce NSAID side effect. One of those substances is Propolis which is derived from bee hive and its saliva. It has been widely consumed as food supplement and is efficacious to relieve joint pain empirically.[7] Its active compound, caffeic acid phenolic esters (CAPE) and flavonoids, has anti-free radical and anti-inflammatory effect.[7] The dose 100 mg/kgBW/day of Propolis for 7 days orally has proven inhibit swelling, granuloma and exudate in rat leg significantly.[10]

There have been many scientific publications about the role of Propolis as a free radical scavenger and anti-inflammatory properties, but its effectiveness in synovitis com-

pared with NSAIDs have not been investigated. The aim of this study is to compare the antiinflammatory effect between Propolis and celecoxib in rat with knee joint synovitis.

Materials and methods

This experimental study was conducted using Propolis from the PT MNI®-Melia Nature Indonesia and GMP standardized, Celecoxib from Pfizer®, and Peptidoglycan 1 mg sterile powder from Sigma-Aldrich® 77 140.

Subjects were Wistar strain rat derived from PT Biofarma. Inclusion criterias were male, weighing 200-250 gr, and healthy. Exclusion criterias were less active appearance and separating themselves when adaptation to the environment around the cage. Rat were divided into three groups after 2 weeks adapatation. Group 1 was negative control group given 100 ug single dose of Peptidoglycan. Group 2 was treatment group given 100 ug Peptidoglycan intra-articularly and single dose Propolis 100 mg/kgBW/day orally. Group 3 was a positive control group given 100 ug Peptidoglycan with Celecoxib 50 mg/kgBW/day orally.

All groups were sacrificed on day 1, 3 and 14. Knee joints of each group were preserved in paraffin block for histopathology and immunohistochemistry evaluation. Histopathology changes of synovitis were evaluated by Hematoksillin Eosin staining using Krenn's scale. Macrophages activity was assessed by numbers of macrophages seen through immune-expression CD-68 using by immunohistochemistry staining. The scoring were analyzed by ANOVA.

Results

On day-1, histopathology finding using hematoxilin eosin staining shows mild synovitis in 1-2 layers of synovial lining with normal cellularity and absence of multinucleate cells. The infiltration of inflammatory cells are found at perivascular areas and the mean score range between 2 to 3 (Figure 1). The mean Krenn's score for each research groups are 2.6 for group 1, 2.2 for group 2 and 3. There is no significant difference among the three study groups ($p < 0.05$). On day 3, there is an increase of synovitis severity degree compared to day 1. The range of Krenn's score at day 3 are between 3.6 to 5. Histopathology findings are synovitis variation in 4-5 layers of synovial lining, increased cellularity, macrophage and aggregation inflammatory cells. There is significant difference ($p<0.05$) between Group 1 and Group 2-3. (Figure 2) On day 14, histopathology finding of synovitis is improved. Mean score range between 1.2 to 2.2 (Figure 1). There is no difference between Group 1 and Group 3. Histopathology changes showed 2-3 layers of synovial lining with hypo cellularity but in some preparations are still found without aggregation multinucleated macrophages inflammatory cells. There is statistically significant differences ($p <0.05$) between Group 2 (1.2), group 1 (2.2) and group 3 (2.2).

Among the three days of observation, data on day-3 was chosen to evaluate the difference between the three groups of studies statistically by using ANOVA in PASW/SPSS 18. There were significant differences between Group 2 among the two other groups (1 and 3) ($p <0.05$). When compared between the two anti-inflammatory substances, Propolis is more superior to Celecoxib to inhibit synovitis process as 6% (0.6 / 3.6 x 100%).

Immunohistochemistry CD-68 staining in all three groups can be seen in Figure 3. The ratio between the numbers of macrophage cells that labelled by CD-68 as compared with other inflammatory cells was calculated in percentages and incorporated into the score. The highest number of macrophages that labelled CD-68 is found in group 1 observed on day 3. Whereas the lowest number of macrophage cells is found in group 2 observed on day 14. On day-3, there is difference in number of macrophage cells between group 2 with group 1 and 3. Group 2 average score is 1.75 with SD 0.500 while the Group 1 is 3 with SD 0.816.

Discussions

Administering a single dose Peptidoglycan 100 mg intra-articularly in knee joint to all groups, leads to synovitis changes as early as 2 hours after injection and clearly visible through the light microscope on day 1 treatment. The result is consistent with the previous research.[11] It also causes activation and proliferation of macrophage from the resident cell (synoviocytes type A)[12], and polymorphonuclear (PMN) cell as inflammatory responses.[13]

The effect of oral anti-inflammatory substances to the number of macrophages in synovitis still can not be identi-

Table 1. Krenn's score to evaluate histopathological finding in synovitis[11]

Scale	Synovial Lining	Resident cell/Macrophage	Inflammatory cell
0	1-2 layers	Normocellular	No infiltration
1	2-3 layers	Mild hypercellular	Mild increased at perivascular
2	4-5 layers	Moderate hypercellular, multinucleated	Moderate increased, aggregation and follicle formation
3	≥ 5 layers, ulcer (+), Multinucleated	Severe hypercellular, with pannus and granuloma formation	Wide aggregation with thickening of infiltrate

0-1= normal, 2-3 = mild synovitis, 4-6 = moderate synovitis and 7-9 = severe synovitis.

fied clearly because the optimal dose of these substances has not been revealed in this study. In HE staining infiltration of inflammatory cells is found migrating to perivascular area. However it is difficult to identify and distinguish whether the proliferation of macrophages from activation migrating inflammatory cell or activation of resident cell. The CD-68 immunohistochemistry staining is able to differentiate origin of macrophage whether inflammatory cells or resident cell.[14]

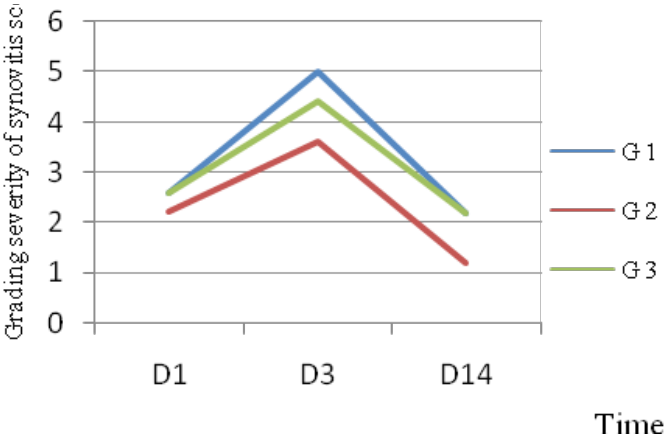

Figure 1. Grading severity score of synovitis (Krenn's Score). G1= Peptidoglycan Group, G2= Peptidoglycan and Propolis Group, G3 = Peptidoglycan and Celecoxib Group; D1=Day 1, D3=Day 3, D14=Day 14

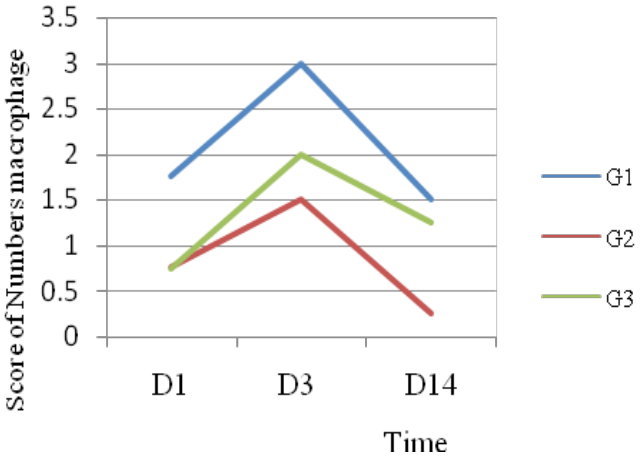

Figure 2. Score of macrophages number in IHC Staining. G1= Peptidoglycan Group, G2= Peptidoglycan and Propolis Group, G3 = Peptidoglycan and Celecoxib Group;, D1=Day 1, D3=Day 3, D14=Day 14.

On day 1, the arthrogenic effect of peptidoglycan remains strong to cause damage to the joint.[15] It causes PMN cells migration from blood vessels to the inflammation site as result of cellular inflammation response.[16] At that stage, the body's defense efforts made by PMN cells resulting in activation and proliferation of PMN cells.[15] The role of Propolis at day-1 as anti inflammation effect has not been seen clearly, because the optimal level of Propolis has not been reached. At this level, the concentration of active compound Propolis should be bigger than optimal dose.[17] The pharmacokinetics and pharmacodynamics of Propolis has not been studied so that the data regarding the bioavailability of CAPE and flavonoids in blood is unrevelaed. There is no significant difference among three groups. However, this result is consistent with the anti-inflammatory effect of Propolis against paw edema rats in which these effects occur after 48 hours[18] whereas the optimal dose of propolis was reached.

The scoring of degree synovitis on day 3 shows moderate synovitis. The cellular response of PMN cells will increase to stimulates activation and proliferation of macrophage (resident cell).[14,15] It occurs due to increased production of cytokines and pro-inflammatory chemical mediators such as IL-1β, IL-12, IL-2, IL-4, 8 and Nitric oxide (NO)[15] and will stimulate the production of TGF-β18 released by inflammatory cells and macrophages that migrate from the blood vessels[16] hence causing a chain reaction of cell damage.[14] The effect of Peptidoglycan inducing inflammation is reduced or diminshed so that the cellular response that occurs due to aggregation of PMN and macrophages that have developed.[11, 17] The role of Propolis as anti inflammation is clearly visible on day 3. Propolis reduces proliferation of synovial macrophages by inhibiting of NO, LTA and COX and terminating free radical reaction.[15] The inhibitory effect of macrophages by Propolis is better compared with Celecoxib administration. The difference is assumed that Propo-

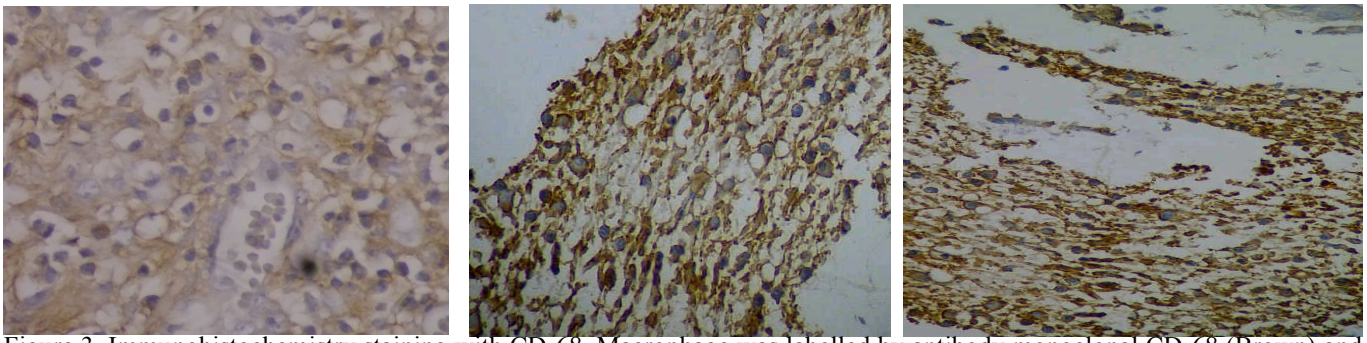

Figure 3. Immunohistochemistry staining with CD-68. Macrophage was labelled by antibody monoclonal CD-68 (Brown) and inflammatory cell (Blue). A. Group 1 at D-3, B. Group 2 at D-3 and C. Group 3 at D-3. Magnification 100x.

lis suppresses both cellular responses[7,9] and non-cellular inflammation, whereas Celecoxib only suppress non-cellular processes through selective inhibition of COX-2.

On day 14, the histopathology findings of all groups show improvement of the inflammatory response. It occurs due to induction of Peptidoglycan to produce cytokines and anti-inflammatory mediators is diminshed.[11] It also occurs due to healing process of synovitis, in which inflammation and infiltration of cells are minimal so that the production of cytokines and chemokine in inflammation cascade diminshed. Propolis action as macrophages immunomodulator[9] in the healing process can be seen from the milder degree of synovitis than the other two groups. Although there is inhibition of macrophage activation and proliferation, Propolis stimulates the release cytokines and anti-inflammatory chemical mediators such as IL-8 and TGF beta. Meanwhile, inhibition of COX-2 by Celecoxib,[6] leads to decreased level of prostaglandin E, results in decreasing number of macrophages in group 3. Its ingition effect is lower compared to group 1 and 2 but the inhibitory effect is still stronger than group 3.

Synovitis has been described that an increase whether both inflammatory cells that migrate through the blood vessels or macrophage resident cells are settled. Macrophage can stimulate and activate fibroblasts responsible for the release of cartilage collagen-degrading enzymes which stimulate osteoclasts and bone resorbtion and activate the immune system by stimulation of IL-1 and TNF-α.[6,14,15] Influence of fibroblast activation does not occur because of diminshed effect of Peptidoglycan as inflammation-induced.

Based on the observation histopathologically at day-3, it is assumed that Propolis is also able to inhibit proliferation of inflammatory cells in synovitis, which is proven by HE and immunohistochemistry staining of CD-68. According to the literature, the content of CAPE and Flavonoids of Propolis has shown to inhibit macrophage inflammatory processes in the synovial, it can also inhibit the activity and proliferation of inflammatory cells and a layer of the synovial in vitroly.[14,17] These results suggest that the inhibitory effect of Propolis on cell inflammatory cells and inflammatory processes also occur in vivo situation. But the exact mechanism of response inhibition of Propolis to the non-cellular inflammation response of cytokines and chemical mediators both pro-inflammatory and anti-inflammatory is not fully understood. Further research to eluccidate the role of Propolis against non-cellular responses such as pro-inflammatory cytokines and anti-inflammatory and pharmacodynamics in synovitis should be studied.

Conclusions

Propolis has an anti-inflammatory effect stronger than Celecoxib with variation between 4-5 times higher in reducing the number of CD-68 macrophages in rat with knee joint synovitis. Further research should be studied in order to reveal anti-inflammatory effect of Propolis against non-cellular responses and its side effects in long term use.

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