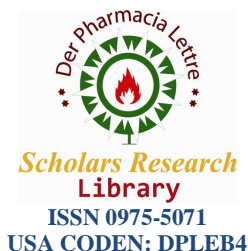




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The effect from methanol extract of jackfruit leaves (*Artocarpus heterophyllus* Lam) in rheumatoid arthritis rat induced collagen type II

Ira Asmaliani¹ and Maria Immaculata Iwo²

¹Department of Pharmacology, Pharmacy Faculty, Universitas Muslim Indonesia

²Department of Pharmacology and Pharmacy Clinic, Institute Technology of Bandung

ABSTRACT

Plants are the essential and integral part in complementary and alternative medicine. Metabolite secondary in plant can be use as alternative medicine and heal some disease. The jackfruit (*Artocarpus heterophyllus* Lam) has been used as traditional folk medicine for asthma, wound healing, ear ache, antiulcer, adsorbent, antibacterial, anti-inflammatory, anemia, dermatitis, cough, diarrhea, and fever. This research aimed to study the activity from methanol extract of jackfruit leaves (MEJL) in treating rheumatoid arthritis. The active compounds of jackfruit were extracted by maceration using methanol. This study begins with an acute anti-inflammatory test followed by anti-rheumatoid arthritis in rat model. In the acute anti-inflammatory test, Wistar rats were induced having acute inflammation by injecting intradermal of carrageenan lambda into hind limb footpad. Animal model of rheumatoid arthritis (RA) were performed in Wistar rat by injecting collagen type II bovine emulsified in CFA for immunization phase and in the IFA as booster phase. Two doses of extracts were tested i.e., 150 and 200 mg/kg body weight for MEJL. The parameters were observed i.e., arthritis score, TNF- α level and histology of joint. In the AR model test, both doses of MEJL can reduce arthritis scores significantly ($p < 0.05$) with the highest percentage of reduction i.e. 41.02% occurred after administered of MEJL at dose of 150 mg/kg body weight. MEJL at dose of 150 mg/kg body weight can reduce level of TNF- α significantly ($p < 0.05$) compared than control. Through histological examination of hind limb joints of RA rats showed an improvement of the joint cartilage indicated by the absence of pannus formation and cartilage erosion after treated with MEJL dose of 150 mg/kg body weight. It can be concluded that MEJL dose of 150 mg/kg body weight have a better activity in treating rheumatoid arthritis compare to MEJL dose of 200 mg/kg body weight.

Keywords: rheumatoid arthritis, jackfruit (*Artocarpus heterophyllus* Lam.), scoring arthritis, TNF- α , histology of joint.

INTRODUCTION

Plants have the ability to form a secondary metabolites like flavonoids, alkaloids, steroids and phenolic substances which are used to restore health and heal many diseases. This secondary metabolite has been reported for immunomodulation activity and many formulations of these plant products are available to enhance the immune system [6].

The jackfruit (*Artocarpus heterophyllus* Lam) is widely distributed in tropical region and this family Moraceae has been used as traditional medicine [8]. It is used for asthma, wound healing, ear ache, antiulcer, adsorbent, antibacterial, anti-inflammatory, anemia, dermatitis, cough, diarrhea, and fever [7].

Methanol extract of *Artocarpus heterophyllus* leaves have shown analgesic and immunomodulation effect [6]. Three phenols compounds were characterized as artocarpesin, norartocarpetin, and oxyresveratrol were evaluated by determining their inhibitory effects on the production of pro inflammatory mediators in murine macrophage cells [4].

Rheumatoid arthritis is an autoimmune disease that characterized by inflammation, the involvement of humoral and cellular immune system and synovial hyperplasia that can cause joint destruction [3]. This disease involves inflammation response and suppression immune system.

The aim of this study is to investigate its anti-rheumatoid arthritis activity on leaves of *Artocarpus heterophyllus*. Rheumatoid arthritis rat was made by induced emulsion of collagen type II in complete freund's adjuvant (CFA) or in incomplete freund's adjuvant [1]. The similarity of collagen induced arthritis (CIA) model with human RA is involvement of symmetric joints, hyperplasia synovial, infiltration of inflammatory cells, formation of pannus and production of rheumatoid factor [2].

MATERIALS AND METHODS

Animals

Twenty adult female Wistar rats (age 2-3 month, 150-200 g) were obtained from Institut Teknologi Bandung, Indonesia. The rats were housed five per cage prior to initialization of experiments and were acclimated for 1 week. They were housed in temperature room and had free access to pellets with water available ad libitum.

Plant collection

Jackfruit leaves (*Artocarpus heterophyllus* Lam) was collected from Lembang, Bandung, West Java, Indonesia.

Preparation of extract and fraction

Dry powder of jackfruit leaves (500 g) was extracted with methanol using maceration apparatus for 3 days. The extract was concentrated by rotary evaporator.

Preparation of collagen emulsion

Type II collagen was derived from Chondrex.Ltd. The collagen emulsion prepared by mixing bovine type II collagen in complete freund's adjuvant (1: 1) using a homogenizer (immunization) and for booster, type II collagen was emulsified with incomplete freund's adjuvant (1:1).

Anti-rheumatoid arthritis effect by collagen induced arthritis.

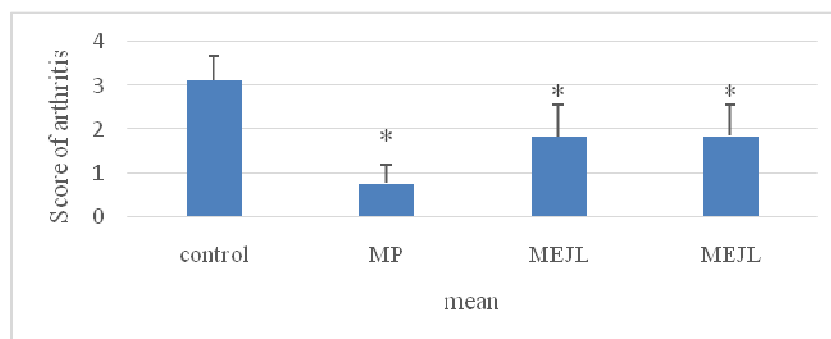
Female Wistar rats were divided into 4 groups: control group (Na.CMC 0,3%), methylprednisolone (MP) group dose of 45 mg/kg body weight, and methanol extract of jackfruit leaves (MEJL) doses 150 and 200 mg/kg body weight. An arthritis rat model was made by Collagen Induced Arthritis (CIA) model. On day 1, rats induced with collagen emulsion (immunization) at the base of tail. Furthermore, on days 7, rat was induced with emulsified collagen (booster) at the base of tail 1-2 cm below the injection site of immunization. Day 21 was the peak severity of arthritis that we can start treatment according to the experiment protocol during 25 days. Parameters were observed by measure score of arthritis, level of TNF- α and histology of joint tissues.

RESULT AND DISCUSSION

In previous study, three phenols from methanol extract of breadfruit leaves had activity to inhibit macrophage to produce mediator pro-inflammatory [4].

The activity of MEJL against rheumatoid arthritis is examined in rheumatoid arthritis rat. It was using CIA model by inducing collagen emulsion that were divided into two phase, immunization and booster phase to improve the successful of this disease.

Scoring arthritis was observed by presence of nodules and swelling in hind and forelimbs. Based on data below, MEJL group doses of 150 and 200 mg/kg body weight can reduce swelling significantly ($p < 0.05$). The mean of arthritis score each group can see in Figure I.



Note: *: ($p < 0.05$) compare to control group

Figure I. Mean of Arthritis Score

The percentage of lowering arthritis score can see in Table I.

Table I. Activity of lowering arthritis score from MEJL

Groups	Doses (mg/kgbw)	Score	Lowering of arthritis score (%)
Control	0	3,12±0,57	0%
MEJL	150	1,84±0,71*	41 %
MEJL	200	1,85±0,70*	40,7 %
Methylprednisolone	45	0,78±0,40*	75 %

Note: *: ($p < 0.05$) compare to control group

Next parameter was the levels of TNF- α . On the last day, bloods of rats were collected by intra cardiac and analyzed by ELISA method. The levels of TNF- α was measured at 450nm. We can see the level of TNF- α in table II.

Table II. TNF- α level from MEJL groups

Groups	Dose (mg/kg body weight)	TNF- α level (ng/mL)
Control	0	1,22 ± 0,007
MEJL	150	0,93 ± 0,216*
MEJL	200	1,14 ± 0,102
Methylprednisolone	45	0,96 ± 0,064*

Note: *: ($p < 0.05$) compare to control group

TNF- α is an important cytokine in the inflammatory response. TNF- α will produced by macrophages and synovial fibroblasts. Macrophages will release some pro-inflammatory cytokines including TNF- α , IL-1, IL-6 and VEGF. VEGF will cause angiogenesis or blood vessel proliferation which facilitates the influx of active cells. Influx of inflammatory cells will lead to the formation of *pannus* and will allow access to the cartilage. Activation of macrophages and synovial fibroblasts will also activate cells in *pannus* and then release MMPs (matrix metalloproteinase) who can damage the cartilage. TNF- α also activates chondrocytes in cartilage matrix and increase the secretion of inflammatory cells and MMPs then causing cartilage damage. This damage is irreversible and causes a narrowing of the joint until to bone erosion. Based on data, the levels of TNF- α from MEJL dose of 150 mg/kg body weight had than the control group and were significantly ($p < 0.05$). This shows that MEJL dose of 150 mg/kg body weight have mechanism by lowering the levels of TNF- α in rheumatoid arthritis rat.

Left hind limb was isolated to histopathology parameter. Below is an overview of the hind limb joints each treatment group.

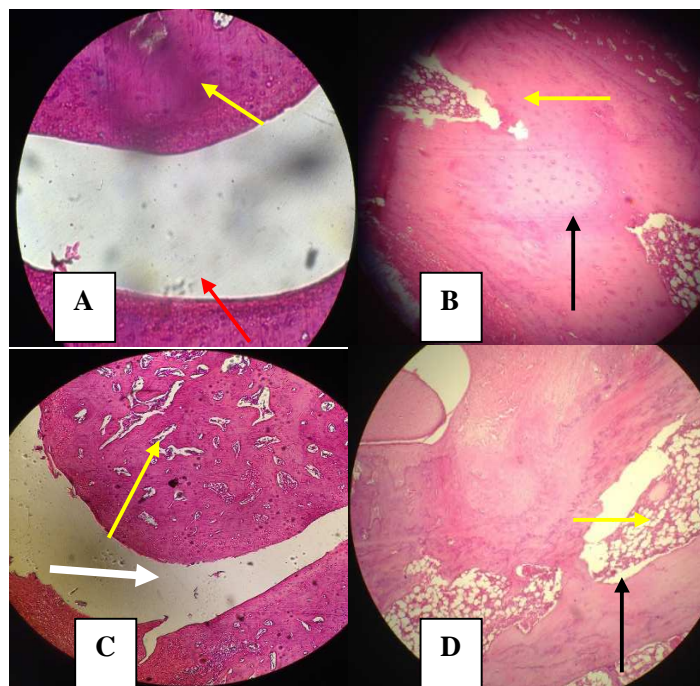


Figure 2. Sectional hind limb joints in rat after given (A) methyprednisolone, (B) Control group, (C) MEJL dose of 150 mg/kgbw, and (D) MEJL dose of 200 mg/kgbw

Description: Yellow arrow showed the infiltration of inflammatory cells. White arrow showed hypertrophy. Black arrow showed pannus formation (D) and even the destruction of cartilage (B). Red arrow shows the normal cartilage.

In rheumatoid arthritis, an autoimmune reaction occurs in the synovial tissue that can induce inflammatory cells. Normally, the synovial tissue does not contain immune cells. However, in the state of RA, there is infiltration of inflammatory cells that will trigger a chronic inflammation and formed oedema. Oedema also characterized by dilatation of joint cavity that filled with fluid. This situation was seen in all groups except the MP group. MP group also contain an inflammatory cells but in small quantities and did not showed oedema. The existence of inflammatory cells was also seen in the group MEJL dose of 150 mg/kg body weight. Chronic inflammation causes hypertrophy and proliferation of the synovial membrane which will form *pannus*. Hypertrophic synovium membrane was seen in MEJL group dose of 150 mg/kg body weight but did not formed a *pannus*. The existence of *pannus* formation can be seen in the group MEJL dose of 200 mg/kg body weight. *Pannus* formation is a sign of chronic phase of rheumatoid arthritis which release of platelet-derived growth factor, prostaglandins and substances which eventually will lead to destruction of cartilage and bone erosion [5]. There is no erosion of cartilage in two doses of MEJL. This showed that the MEJL dose of 150 mg/kg body weight has activity in treating rheumatoid arthritis better than dose of 200 mg/kg body weight which can see in the absence of pannus formation.

CONCLUSION

Extract methanol of jackfruit leaves (MEJL) dose of 150 mg/kg body weight has activity in treating rheumatoid arthritis better than dose 200 mg/kg body weight.

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