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Antiarthritic Activity of Pacar Air (*Impatiens balsamina* Linn.) Herb Extract in Animal Model of Rheumatoid Arthritis – An Autoimmune Disease

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Abstract: The objective of this study was to investigate the activity of *Impatiens balsamina* herb in animal model of rheumatoid arthritis. The active compounds of leaf and stem of *Impatiens balsamina* Linn. were extracted using ethanol 96% by maceration method. Anti-arthritis activity of the test extracts was determinedin rat adjuvant-induced arthritis (AIA) model. The rats were injected with Complete Freund's Adjuvant (CFA) intraplantarly to induce arthritic animal model. The anti-arthritic effect of the extracts was determined by measurement of the paw volume, joint edema, and TNF-α levels determined using ELISA technique. The rats were successfully arthritis induced using 0.25 mL of CFA. The administration of extract for 14 days started from day 8thduring chronic arthritic condition significantly ($p<0.05$) reduced the total volume of paw and joint edema while no effect onbody weight.Highest inhibitionpercentage was caused by high dose of *Impatiens balsamina*extract (500 mg/kg bw)with inhibition percentagefor paw and joint edemawere 29.6% and 24.9%, respectively. The TNF-α levels in all doses of extract groupsdecreased and significantly lower ($p<0.05$) compared to that of control. *Impatiens balsamina* extract has immunosuppressive activity and able to reduce arthritis symptoms in adjuvant induced arthritisrat model. *Impatiens balsamina* extract also has the activity to minimize symptoms of arthritis, so it is a potentially alternative drug for rheumatoid arthritis.
Keywords: *Complete Freund's Adjuvant* (CFA), *Impatiens balsamina*, rheumatoid arthritis, TNF-α.

Introduction

Rheumatoid arthritis (AR) is a systemic autoimmune disease characterized by chronic polyarthritis, progressive inflammation and synovial proliferation followed by erosion of the joints and cartilage[1]. Rheumatoid arthritis (AR) itself is one of the autoimmune diseases and arthritis that often occurs affects more than 1.3 million people in the United States with 75% of whom are women[2].

Impatiens balsamina was used in traditional Chinese medicine, Taiwan and Thailand to treat arthritis, fractures, and superficial infection[3]. Another study showed *Impatiens balsamina* herb has anti-inflammatory activity in carrageenan-induced mice[4]. *Impatiens balsamina* plants also contains high naphthoquinone. Research onnaphthoquinone shows immunosuppressant activity by nitric oxide from macrophages potentially carcinogenic with immunosuppressive effects by 98%[5].

This research will assess anti-arthritic effects of *Impatiens balsamina* Ethanolic extract in Adjuvant-Induced Arthritis (AIA) animal models, which is one of the rheumatoid arthritis animal models.

Experimental

Materials

Stems andleaves of *Impatiens balsamina* Linn., ethanol 96%, distilled water, hydrochloric acid, sulfuric acid, isopropanol, toluene, chloroform, ammonia, dragendorff reagent, Mayer reagent, magnesium powder, amyl alcohol, solution of Fe(III) chloride, gelatin, sodiumchloride 0,9%, Chinese ink Pelikan®B-17,thin-layer chromatography plates (GF_{254}), Carboxymethylcellulose sodium, Methylprednisolone (PT. Gracia Pharmindo), acetate acid, sterilized bidistilled water, *Rat TNF alpha ELISA Ready-Set-Go!*® (eBioscience®) kit, *phosphate buffer saline* (PBS), Tween 20, *Complete Freund's Adjuvant* (CFA) (Sigma®).

Instruments

Grinders, oven, analytical scale, a set of distillation, *rotary evaporator* (Buchi *Rotavapor* R-215 and Buchi *Heatingbath* B-491), UV-Vis spectrophotometer (Hewlett Packard 8452), ELISA reader (Thermo Scientific Multiskan®), *Plethysmometer* (Ugo Basile 37140),

Animal

Female Wistar rats aged 8 weeks obtained from the Animal Laboratory, School of Pharmacy,Bandung Institute of Technology (ITB)

Plants Preparation

Impatiens balsamina plant was taken from Kuala Dua village, Rasau Jaya district, Kubu Raya Regency, West Kalimantan. Determination of the plant were performed at Biology Laboratory of Mathematics and Natural sciences, University of Tanjungpura, Pontianak.

Extraction

Crude plant powder was extracted by maceration with 96% ethanol, which previously had been redistilled. Maceration is done for 4 x 24 hours and filtered as well as replacing the solvent every 24 hours. Extract was concentrated using rotary evaporator to obtain a thick extract.

Anti-Arthritis Effect Assessment

Extract effects were conducted *in vivo* using Wistar strain female rats aged 8 weeks. Assesment of Rheumatoid arthritis animal model is divided into three phases. Test phase including induction phase, treatment phase, and evaluation of test results.

Induction Phase

Induction of arthritis was conducted using Adjuvant-Induced Arthritis (AIA) which has been modified by Woode[6]. Each test animals were injected intraplantar with 0.25 mL of Complete Freund's Adjuvant (CFA) on the footpad of the left leg of rats. CFA is a suspension that contains antigen of Mycobacterium tuberculosis (H37Ra, ATCC 25177) at a concentration of 1 mg/ mL suspension. Before it is injected, CFA suspension were homogenized by vortex. Before the injections, the rats were weighed, paw and joint thickness were also measured, as well as serum on day 0 (T_0)

Treatment Phase

The Administration of the test extract was conducted on day 8^{th}based on the clinical sign of arthritis and the inflammatory reaction that shown. Clinical signs of arthritis which is characterized by the appearance of chronic phase (in day 8^{th}) such as the increasing of paw volume and joint edema that occurs after the acute phase (in day 4^{th}- 5^{th}). After the expected clinical symptoms appear, on day 8, the animals were randomized into 4 groups, i.e. control group (1% Na CMC), methylprednisolone group (15 mg/kg bw), Ethanolic Extract of

Impatiens balsamina at dose 250 mg/kg bw (EPA I), and Ethanolic Extract of Impatiens balsaminaat dose 500 g / kg bw(EPA II). Administration of the sample is done every day for 14 days from the grouping.

Evaluation phase

The evaluation is conducted to assess outcome of therapy and the development of test conditions treated animals. Evaluation is done by observing the daily body weight, paw volume, and joint edema in each animal. Serum samples were taken on day 5 via tail vein to measure TNF-α level on day 5 (T_5) and at the end of the test, to measure TNF-α level on day 21 (T_{21}).

Observation of Paws volume and Joints Edema.

During anti-arthritis effect assessment, paws and joints thickness were observed for 14 days. Paw volume were measured every day from the first day after induction by comparing the increase or decrease percentage in paw volume from day to day as compared to day 0, i.e. before induction. Joints thickness were also measured every day compared to the joints thickness on day 0. Total volume of edema of the day 8 to day 21 of each group was calculated in arbitrary units to obtain the value of Area Under the Curve (AUC) and the inhibition percentage is calculated as**:**

$$\text{Edema inhibition (\%)} = \left[\frac{AUCcontrol - AUCtest}{AUCcontrol}\right] x\ 100$$

AUC values for each group were compared and statistically analyzed with ANOVA and significance of the data were determined.

Determination ofTNF-α Levels

Each of these animals had serum samples taken from the tail vein on day 0 (T_0), day 5 (T_5), and day 21 (T_{21}). TNF-α level was determined by ELISA Technique.

Results and Discussion

Anti-arthritis Effect Testing Results of Extract

Animal model of rheumatoid arthritis (AR) is prepared by Adjuvant-Induced Arthritis (AIA) method, using Complete Freund's Adjuvant (CFA) as the immunogen. Adjuvant Induced Arthritis is commonly used in AR experiments, namely by inducing arthritis using a single injection of CFA intradermally. Complete Freund's Adjuvant contains heat-killed *Mycobacterium tuberculosis* suspended in mineral oil. Bacteria contained in the CFA will amplify the signal to be received by T cells and activate the T cells, thereby increasing immune response that may caused chronic inflammation.

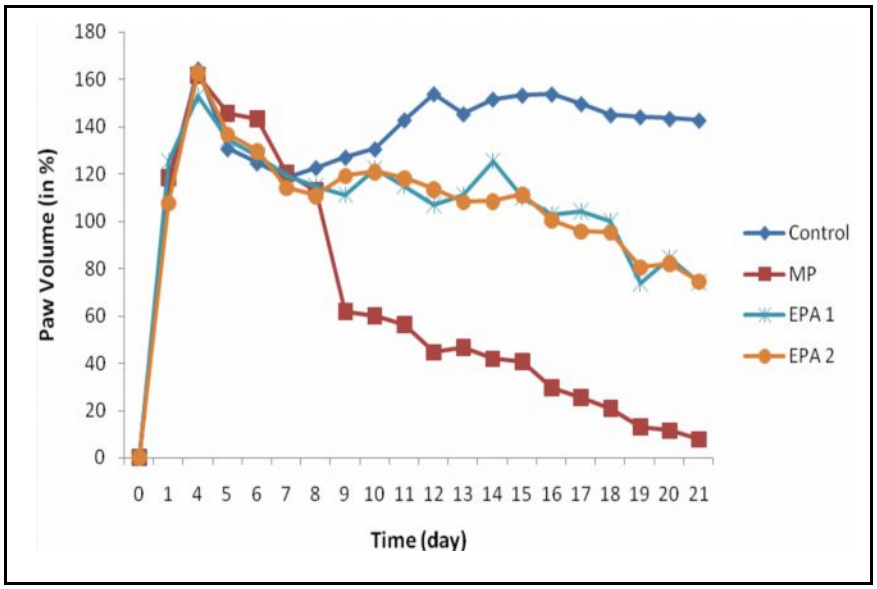
AIA animal model is marked by the emergence of two (2) phases, namely the acute phase and chronic phase. Induction of arthritis is successful, if animal models continues to experience chronic phase after acute phase has passed. Assessment for the category of animals who experience chronic phase is characterized by an increase in swelling or edema volume on the paw and joints that injected, after a decline in the volume of edema in the acute phase.

Based on research by Weichman[7]rheumatoid arthritis animal model, acute phase will occur between day 4^{th} to day 6^{th} and followed by the onset of the chronic phase between days 9^{th}-12^{th} after CFA induced.

In this study, administration of the test substance wasstarted on day 8^{th} after CFA induction, which isthe arthritishas started that is characterized by the onset of the chronic phase and the test substance administered daily for 14 days, with a total of 21 days' observation after CFA induction.

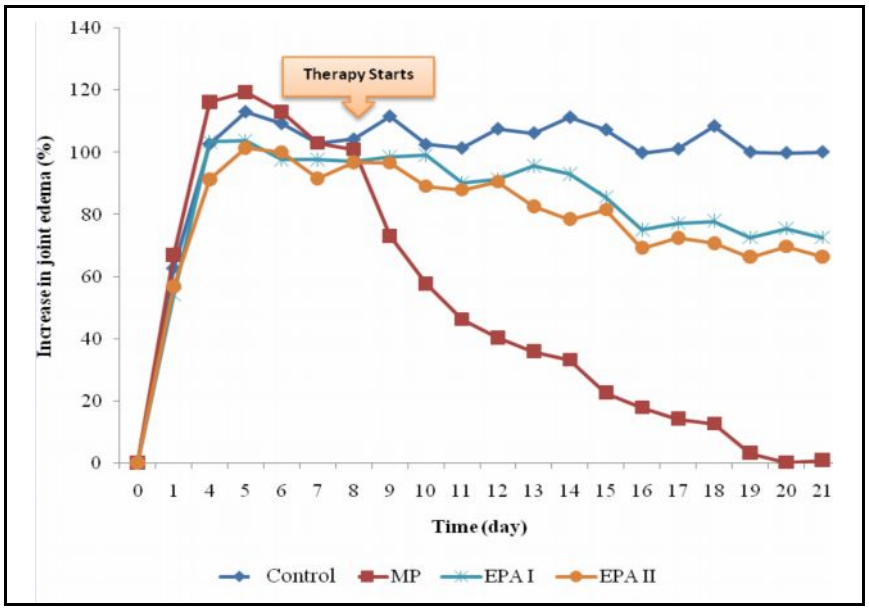
Control group shows an increase in the paw volume at day 4^{th} and increased joints edema at day 5^{th}, which gradually decrease. The increasein paw volume and joints thicknessis started again between day 8^{th} and day 9^{th}. This suggests that the acute inflammatory phase occurs between the day 4^{th} and 5^{th}, followed by a chronic phase that occurs between the day 8^{th} and 9^{th}. In the control group, an increase in the volume of swollen

paw were highly significant ($p < 0.05$) on day 8th to day 21th, so it can be concluded that induction with 0.25 ml of CFA managed to cause arthritis until the end of observation. Percent increase in swollen legs and thick joints from day 0 to day 21 can be seen in Figure 1 and Figure 2.



Description : MP = Methylprednisolone
EPA I = Impatiens balsamina extract dose I(250 mg/kg bw)
EPA II = Impatiens balsamina extract dose II (500mg/kg bw)

Figure 1. Percent increase in leg swelling curves of AIA rats after administration of the test extract



Description : MP = Methylprednisolone
EPA I = Impatiens balsamina extract dose I(250 mg/kg bw)
EPA II = Impatiens balsamina extract dose II (500mg/kg bw)

Figure 2. Curves percent increase in thick joints of AIA rats after administration of the test extract

Ethanolic Extract of *Impatiens balsamina* dose 250 mg/kg bw (EPA I), and Ethanolic Extract of Impatiens balsamina dose 500 mg/kg bw (EPA II) was administered from day 8th to 21thsignificantly reduces paw volume ($p<0.05$) compared to control group. Meanwhile, comparison group given Methylprednisolone (MP) shows a decrease in paw volumethat was significantly different ($p<0.05$) than the control group and the group extracts. Inhibition percentage of paw volume can be seen in Table 1.

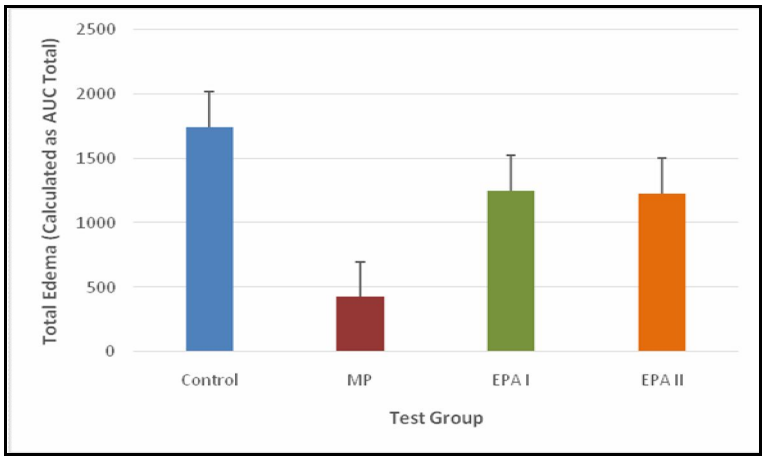


Figure 3.Total Edema in Paw of AIA Rats during the administration of Extract

Table 1. AIA rats paw edema inhibition after extract administration

Test groups	Dose (mg/kg bw)	Inhibition of paw edema (%)
Control	-	0
MP	15	75.7
EPA I	250	28.5
EPA II	500	29.6

Description: MP = Methylprednisolone
EPA = *Impatiens balsamina* herb extract

Similar as the value of total edema in paw, total of joint edema from day 8thto day 21th can be obtained from the calculation of Area Under the Curve (AUC). Increase in joint edemaand percent inhibition of increase in joint edema of each treatment group can be calculated. Percent inhibition is shown on table 2.

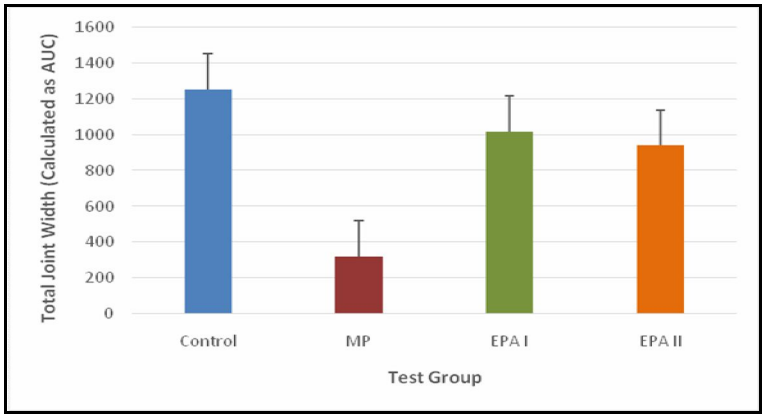


Figure 4. Total of joint edemaof AIA ratsafter administration of test extracts

Table 2. Inhibition ofJointedemaafter administration of the test extract

Test groups	Dose (mg/kg bw)	Inhibition of increase in thickness Joints(%)
Control	-	0
MP	15	74.37
EPA I	250	18.73
EPA II	500	24.92

MP = Methylprednisolone
EPA = *Impatiens balsamina* herb extract

Test extracts used from day 8th to day 21th, reduces joint thickness significantly ($p<0.05$) compared to the control group. Ethanolic Extract of *Impatiens balsamina*at dose 500 mg/kg bwhas inhibitory effect or the effect of decreasing thickness joints better, compared toEthanolic Extract of Impatiens balsamina dose 250 mg/kg bw. However, the decline is best demonstrated by a comparison group of animals which were given methylprednisolone with inhibition values of 74.37% that was significantly different ($p<0.05$) than the control group and the group of test extracts.

In addition to the presence of immune cells that plays a role in the immune response, there is also the role of protein molecules as a signal conductor called cytokines. Cytokines are released by cells in response to a stimulus and will induce an immune response through its binding to specific receptors located on the membrane of immune cells[8].

Cells which play a role in the pathogenesis of rheumatoid arthritis is T and B Lymphocytes. B lymphocytes produces autoantibody against collagen type II. Autoreactive antibodies will form a complex with self-antigens in the joints, causing type III hypersensitivity reaction which ends in local inflammation[9]. In a state of inflammation, several proinflammatory cytokines such as TNF-α, IL-Iβ and IL-6 are secreted by the cellular immune system which will stimulate the migration of macrophages and neutrophils to areas of inflammation and cartilage, resulting in worsening joint damage. Thus, we need to test the activity of the extract in suppressing the secretion of TNF-α in animal models of arthritis, to know how does this extract perform.

Table 3.Levels of TNF-α for testing the effect of test extract on AIA rats

Test groups	Dose (mg/kg bw)	TNF-α level (pg/mL)		
		H_0	H_5	H_{21}
Control	0	-	15.444 ± 2.694	18.333 ± 1.764
MP	15	-	12.556 ± 1.388	-**
EPA I	250	-	15.667 ± 1.333	11.444 ± 1.018**
EPA II	500	-	14.111 ± 2.037	10.333 ± 0.667**

Description: ** = Significantly different to controls ($p<0.01$)
- = Not Detected
H_0 = Day 0 or before induction
H_5 = Day 5 after induction
H_{21} = Day 21 after induction

From Table 3 above, CFA induction and the effect of test substances on levels of TNF-α in the blood can be seen. On day 0, i.e. before induced, TNF-α levels in the blood does not go undetected. However, 5 days after induction, where there is an acute inflammatory phase, each test group yields TNF-α levels which were insignificant with the control group ($p<0.05$). On day 21 after induction or after 14 days of administration of the test substance, the levels of TNF-α each group shows a decrease, except for the control group which tend to increase. In both groups of animals given Ethanolic Extract of *Impatiens balsamina*, their TNF-α levels were significantly different than the control group. Decreased levels of TNF-α is best shown in groups of animals treated with methylprednisolone as a comparator drug, i.e. the levels of TNF-α were not detected as TNF-α levels in normal animals before induced. The levels may be too small as it cannot be detected by ELISA reader.

Rheumatoid arthritis is an autoimmune disease that is mediated by the activity of phagocytic cells, T cells and B cells and is characterized by increased proinflammatory cytokine TNF-α, IL-Iβ and IL-6[10]. In AIA animal model, an increase in proinflammatory cytokines TNF-α is known to cause rheumatoid arthritis. According to Janeway[8], Tumor Necrosis Factor-α (TNF-α) is a proinflammatory cytokine produced by macrophages, mast cells and NK cells. Because of the ability of the extract in suppressing the production of TNF-α and non-specific immune response, the suspected mechanism of action of the extracts of the cells is to suppress the activity of macrophages and other innate immune cells.

Conclusion

Ethanolic Extract of Impatiens balsamina with doses of 250 and 500 mg/kg bw have immunosuppressive activity. The extract also had lower activity in Adjuvant Induced Arthritis (AIA) animal model, best percent inhibition is shown by Ethanolic Extract of Impatiens balsamina dose 500 mg/kg bw. Ethanolic Extract of Impatiens balsamina also has the potential to be developed as an alternative medicine for the treatment of rheumatoid arthritis.

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