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Diah Purwaningsari, Jusak Nugraha, Sri Puji Astuti Wahyuningsih, Suhailah Hayaza, Raden Joko Kuncoroningrat Susilo and Win Darmanto[1]

Department of Biology and Department of Medicine, Universitas Airlangga, Surabaya 60115, Indonesia.

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Abstract

The present study was carried out to elucidate effect of Polysaccharide krestin (PSK) in treating rheumatoid arthritis (RA). Wistar male rats (*Rattus norvegicus*) (60) were divided into six groups. Measurement of MMP3 expression was done by using immunohistochemical methods and foot diameter was measured using calipers. The results showed a significant difference decrease in the treatment groups in MMP3 concentration and foot diameter.

Key words: Polysaccharide Krestin (PSK), Rheumatoid Arthritis, Rat

Coriolus versicolor mushroom is often used as alternative medicine especially in Asian countries. Rheumatoid arthritis (RA) is a chronic inflammatory disease which causes persistent synovitis, systemic inflammation, and auto immune bodies (Scott *et al*., 2010; Kita *et al*., 2012). Polysaccharide krestin from *C. versicolor* has immunomodulator activity and it can act as an anticancerless agent and suppress autoimmune diseases. However, data regarding the effect of PSK as immunomodulators on RA disease is still lacking, so the present study was undertaken.

Materials and Methods

The taxonomic identification of the *Coriolus versicolor* was carried out as per Cui and Chisti, (2003). 66 Nos of Wistar (*Rattus norvegicus*) male rats aged 16 weeks weighing 200-250 g. approved by Ethical Clearance at the Animal Care and Use Committee, were used in the study (Reference Number: 541-KE). They were divided into 6 groups of 11 each, housed in controlled environment (25 ± 5°C, the humidity of 50 ± 10% and 12 light / dark cycle). Arthritis was induced by injecting 0.1 ml of Complete Freund's Adjuvant (CFA) (PCode 1001821390, Sigma-Aldrich, St. Louis, MO, USA) interdermally in tail and 0.05 ml CFA, as a booster after 2 weeks in two feets (Mahdi *et al*., 2017). The booster dose of CFA was considered as day-0. 1 week control group (K1), 2 weeks control group (K2), 3 weeks control group (K3), the PSK treatment group at the dose rate 50 mg/kg bw for 1 week (K4), 2 weeks (K5), for 3 weeks (K6), respectively.

Mice were anasthetized with ether and then were sacrificed. The ankle joint were extracted, presented in fixative solution and labeled. The joint tissue was given monoclonal antibody of MMP3 anti-rat [EP1186Y] ab52915 for immunohistochemical examination (David, 2014). The brown colour in cell nucleus and citoplasm has shown positive results. The MMP3 expression percentage was measured by: (the number of positive cells/number of total cells) x 100%. The measurements of joint swelling were taken using vernier caliper to assess arthritic effect at the middle of both feet. The data was analyzed statistically using the Static Package for the Social Science (SPSS) program which included testing the One Way ANOVA test ($\alpha = 0.05$).

Results and Discussion

The statistical tests showed that there was no significant difference in the body weight between experimental groups (Fig. 1A). The statistical test results related to MMP3 expression indicated significant differences between the treatment groups and the control groups

[1]*Corresponding author* : Email : windarmanto@fst.unair.ac.id

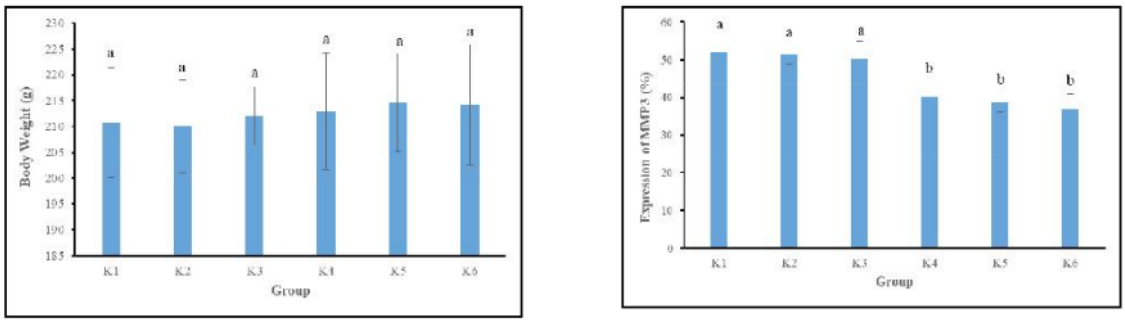

Fig 1. Body weight and expression of MMP3 from each group after treatment; A: Body weight gain; B: Expression of MMP3 changes. The different letter indicated a significant difference ($p \leq 0.05$).

(Fig. 1B and 2). The results revealted in PSK could reduce MMP3 expression.

The foot thickness in K4 and K5 were significantly different from control groups, while K6 was not. However, when rats were sacrificed, all treatments showed a significantly different foot thickness compared to the respective control groups. This result proved that the provision of PSK was able to reduce foot thickness significantly as evident in Table I.

MMP3 is a proteolytic enzyme that play an important role in joint destruction in RA through the breakdown of various extracellular components, including collagens (type III, IV, V, IX, and XI), proteoglycans and activating pro-MMP such as pro MMP7, pro MMP8 and pro MMP9 (Fadda *et al*., 2016).

Research with the RA model showed that the activity of D-glucan isolated from *Pleurotus ostreatus* has significant reduction in rat's hind paw volume and arthritic score, which is dramatically increased due to the arthritis process (Bauerova *et al*., 2009). Although, the study has not examined MMP3 levels, in a study conducted by Chou *et al*. (2011), the levels of MMP3 with hyaluronan showed that the material was able to reduce damage in animal models and reduce the number of positive cells to significant MMP3 levels. Polysaccharide from *Pleurotus pulmonarius* similar to PSK contains glycan active protein which has also been shown to reduce the thickness of rat hind feet induced inflamation by formalin and carrageenan. The administration of polysaccharide is able to reduce the thickness of rat feet on the 5th, 10th and 15th days of treatment with an inhibition percentage of 83.3%, exceeding the inhibiting capacity of diclofenac which is only 33.3% (Adebayo *et al*., 2012).

The analysis of foot thickness data after 1week treatment revealed that after the PSK administration, a different test was performed on all treatment groups that all groups shown significant differences when compared to the thickness of the feet during induction. This explains that the provision of PSK for 1 week has not been strong enough to reduce foot thickness in AA model animals, but administration for 2 weeks and 3 weeks have been able to provide a significant decrease in foot thickness.

Table I. Comparison of foot thickness measurement in each group

Group	Foot Thickness (mm)			
	Before Induction	After Induction	After Treatment at 1 week	Before Sacrificed
K1	5.073±0.179^{a}	10.473±0.398^{a}	10.245±0.314^{a}	10.245±0.314^{a}
K2	5.145±0.242^{a}	10.436±0.338^{a}	10.155±0.225^{a}	10±0.257^{ab}
K3	5.027±0.241^{a}	10.423±0.633^{a}	10.018±0.438^{ab}	9.791±0.365^{b}
K4	4.909±0.138^{a}	10.427±0.766^{a}	9.718±0.366^{b}	9.718±0.366^{b}
K5	5.036±0.169^{a}	10.509±0.931^{a}	9.545±0.745^{b}	8.755±0.671^{c}
K6	4.936±0.191^{a}	10.582±0.994^{a}	9.636±0.947^{ab}	7.2±0.642^{d}

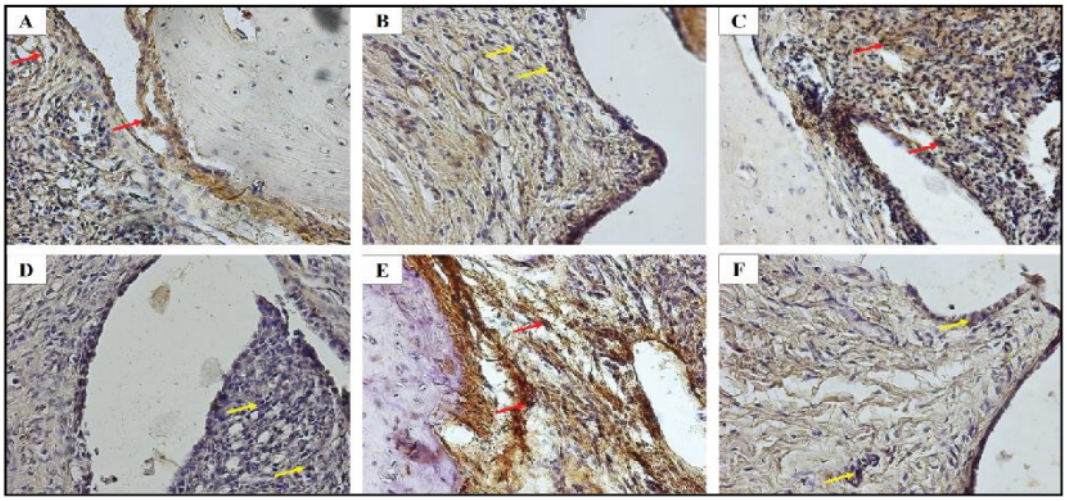

Fig 2. MMP3 expression in rat foot joints of AA model. A: control group at 1 week; B: group giving PSK 50 mg/kg BB at 1 week; C: control group at 2 weeks; D: group giving PSK 50 mg/kg BB at 2 weeks; E: control group at 3 weeks; F: group giving PSK 50 mg/kg BB at 3 weeks. Positive cells (red arrows) are colored brown on the nucleus, cytoplasm, or both. Negative cells (yellow arrows) are colored blue on the nucleus and cytoplasm (200x magnification).

This study also revealed that there was no significant difference in all control groups. Among the three treatment groups, K6 group showed significant reduction in foot thickness, so it can be concluded that the administration of PSK at a dose of 50 mg/kg BW for 3 weeks in RA induced rats has shown best results on reducing foot thickness.

Summary

In conclusion, this present study show the scientific evidence of the effectiveness of the PSK as anti-arthritic in the rat adjuvant arthritis model, possibly by reducing the MMP3 levels and foot diameter, which is mediated by activation of the glycan from PSK. The positive effect of PSK in rats with rheumatoid arthritis may be beneficial for future therapy in arthritic patient

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