Article # 255/19 for revision and referee comments attached.

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Sun, Aug 18, 2019, 7:21 PM

to me

Sir / Madam,

Revise the paper according to the referee's comments and corrections marked on the manuscript. Resubmit the revised article as per IVJ format for further action.

Sincerely

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email : ivj83@yahoo.com
Web : www.ivj.org.in

2 Attachments • Scanned by Gmail

W

diah purwaningsari <diah.purwaningsari@gmail.com>

to Ind

Tue, Aug 20, 2019, 3:30 PM

Dear Editorial Team,

Hereby attached is the revised manuscript with ID 225/19 that we have already changed based on the reviewers comments and journal guidelines. We also attached the comments given by the reviewer in pdf file.

Looking forward to hearing from you, Thank you

Best regards

Diah Purwaningsari

•••

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THE INDIAN VETERINARY JOURNAL

(The Official Organ of the Indian Veterinary Association)

Dr. S. SUKUMAR
MANAGING EDITOR

No.11, Chamiers Road, Nandanam
Chennai – 600 035, India.

ARTICLE NO: 255/19

Date : 14.8.19

Author is requested to note :

- Revise the paper according to the referee's comments and corrections marked on the manuscript.
- Return the original manuscript and the referee's comments sent herewith.
- Resubmit the revised article as per IVJ format – one hard copy and one soft (CD) for each article separetely.

EDITOR's COMMENTS

1) Title of the article to be revised as shown.
2) Only the full address of the place where the work was carried out, need be furnished below the name of author.
3) Abstract should not exceed 100 words.
4) Introduction should be presented without the sub title and Contained to 5 lines and Material & Methods to 10 lines giving only the important steps of procedure.
5) Result & Discussion to be abridged.
6) For HP Slides Stain used & magnification may be mentioned.
7) A short summary of 3-4 lines to be included.
8) References should be in IVJ format. Name of journal cited should be in approved abbreviated form in Italic
9) All the suggestions & queries of the referee may be addressed and following the editors Comment, revised article ~~and a copy may be~~ submitted as full research article of 5-6 pages inclusive of figures after going thro' the enclosed IVJ guidelines, for further review.

To

Dr Win Darmanto

Managing Editor

RETURN THIS PAPER WITH YOUR REPLY WITHIN 90 DAYS

13/8/19

RR 255/19

To
The Managing Editor,
The Indian Veterinary Journal,
No.11, Chamiers Road, Nandanam,
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Sub: Review Report of IVJ article (No. 255/19)

Respected Sir,

In reference to your letter Dt. 10.07.19 (received on 18.07.19), I've meticulously reviewed the **article entitled "Effect of Polysaccharide Krestin……..Rat Joint Foot" by Purwaningsari *et al.*** to improve its quality & suitability for publication in The Indian Veterinary Journal. **The general comments for authors are as follows.**

a) The corrections/modifications are marked in the text material of the manuscript by ball point pen.
b) Revise "**Abstract**" with **Key words** as suggested.
c) Revise the introductory part as marked.
d) Revise **Materials and Methods** section as suggested**. Give details on the arthritis in experimental rats and mention the method of immunohistochemical examination with reference.**
e) Revisions/modifications in **Results and Discussion** part are to be done as suggested/marked in the text..
f) **Original / colour micrographs of good quality are required for better authentication of the related results.**
g) **Include "Summary" part.**
h) References & other sections must be written as per IVJ format / Guidelines to Authors.
i) This article may be accepted for publication in IVJ only after complying with these suggestions/ modifications (including providing the original micrographs).

Thank you for having faith in me for such an interesting, noble assignment in the greater interest of the veterinary profession. I'll be happier to do the same in future.

With warm regards,

Effect of Polysaccharide Krestin on MMP3 Expression and Foot Diameter in Rheumatoid Arthritis Disease from Rat Joint Feet

Diah Purwaningsari[1)], Jusak Nugraha[2)], Sri Puji Astuti Wahyuningsih[3)], Suhailah Hayaza[3)], Raden Joko Kuncoroningrat Susilo[3)], Win Darmanto[3)*]

[1)] Faculty of Medicine, Universitas of Hang Tuah, Surabaya, 60111, Indonesia.
[2)] Faculty of Medicine, Universitas Airlangga, Surabaya, 60115, Indonesia.
[3)] Faculty of Science and Technology, Universitas Airlangga, Surabaya, 60115, Indonesia.

*windarmanto@fst.unair.ac.id

Abstract Polysaccharide krestin (PSK) from *Coriolus versicolor* is known to cure rheumatoid arthritis (RA) disease. This study used animal experiments, Wistar male rats (*Rattus norvegicus*). The grouping of the study was divided into six groups. Measurement of MMP3 expression using immunohistochemical methods and measurement of foot diameter using calipers. The results showed a significant difference in the treatment group in the form of a decrease in MMP3 concentration. On foot diameter after treatment 1 week and before sacrifice showed significance in the treatment group. In conclusion, PSK has the ability to treat RA by reducing MMP3 expression and foot diameter.

Key words: *Coriolus versicolor*, Joint Feet, Polysaccharide Krestin, Rheumatoid Arthritis, Rat

Introduction

The mushroom is often used as alternative medicine by the community. *Coriolus versicolor* mushroom is one of the fungi that is often used for this treatment, especially in Asian countries. This fungus also belongs to the Polyporaceae family (Maehara et al., 2012; Sun et al., 2012; Meng et al., 2016). Polysaccharide krestin from *C. versicolor* also has activity as an immunomodulator so that it can act as an anticancer and suppress autoimmune diseases. However, further research is needed to explain the molecular mechanism of the immunomodulating effects of metabolites from fungi (Lull et al., 2005; Lee et al., 2010; Saroj et al., 2012). Rheumatoid arthritis (RA) is a chronic inflammatory disease that can cause persistent synovitis, systemic inflammation, and autoantibodies (Scott et al., 2010; Kita et al., 2012).

Research conducted by Zheng (2008) shows that there is a role for TGF-β as an anti-inflammatory cytokine in inducing the formation of FoxP3 Treg in mice and humans and can suppress effector T cells in autoimmune diseases. In addition, TGF-β also has antiproliferative activity on CD4+ cells due to its ability to inhibit IL-2 production and rearrangement in inhibiting the cell cycle. Moreover, data regarding the effect of PSK as immunomodulators on RA disease is still lacking, so further research needs to be done.

Materials and Methods

The taxonomic identification of the *Coriolus versicolor* was carried out by Dr. Ni'matuzahroh from the Department of Biology, Faculty of Science and Technology, Airlangga University, Surabaya, Indonesia. The chemicals used in this study were from Sigma-Aldrich (USA) and Merck (Germany). *Coriolus versicolor* extraction based on Cui &

Chisti (2003), which was as much as 200 g of *C. versicolor* powder added with distilled water 3 L and boiled at 80 - 98°C for 2-3 hours. Furthermore, the dissolved part would be filtered with Whatman paper so that the extract solution was obtained. The freeze drying process was carried out to obtain an extract of *C. versicolor*. The extract was then precipitated with 90% ammonium sulfate. In this process, a crude PSK was produced and dissolved in a saline solution. Then the solution was dialyzed for 24 hours using dialysis tubing. In this process, there was a separation of unused small molecules in the solution. After that, the spectrophotometer was examined to determine the concentration of the material in the solution.

This study used animal experiments, Wistar (*Rattus norvegicus*) male rats aged 16 weeks with a weight of 200-250 g. All treatment procedures have been tested through Ethical Clearance at the Animal Care and Use Committee, Faculty of Veterinary Medicine, Universitas Airlangga, Surabaya, Indonesia (Approval Reference Number: 541-KE). This study was divided into 6 groups (n = 11), all of them were in a controlled environment (25 ± 5°C, the humidity of 50 ± 10% and 12 light / dark cycle). The study used six groups divided into the arthritis control group for 1 week (K1), the arthritis control group for 2 weeks (K2), the arthritis control group for 3 weeks (K3), the PSK treatment group 50 mg/kg bw for 1 week (K4), the PSK treatment group dose 50 mg/kg bw for 2 weeks (K5), the PSK treatment group dose 50 mg/kg bw for 3 weeks (K6).

Mice were anesthetized with ether then surgically performed in the ankle joint of the experimental animal, and immediately put into a fixative solution and labeled, then immunohistochemical examination was performed. The measurements of joint swelling were performed using a caliper tool to assess arthritis activity. Measurements were made in the middle of both feet in a serial manner. The data obtained from this study include the expression of rat body weight, MMP3, the thickness of the foot diameter. The data generated were then 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 results of statistical tests showed that there was no significant difference in the measurement of body weight between experimental groups (Fig. 1A). The statistical test results related to MMP3 expression indicate that there were significant differences between the treatment groups and the control group (Fig. 1B and 2). This shows that giving PSK could reduce MMP3 expression.

The results of statistical tests on induction foot thickness showed that there was no significant difference between the treatment groups and the control group. However, an examination of 1 week foot thickness showed significant differences in K4 and K5 while K6 did not show significant differences with the control group. The results of measurement of foot thickness before sacrifice showed that the treatment group had a significant difference with the control group so that the provision of PSK was able to reduce foot thickness significantly can be seen in Table 1.

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 others such as pro MMP7, pro MMP8 and pro MMP9 (Fadda et al., 2016).

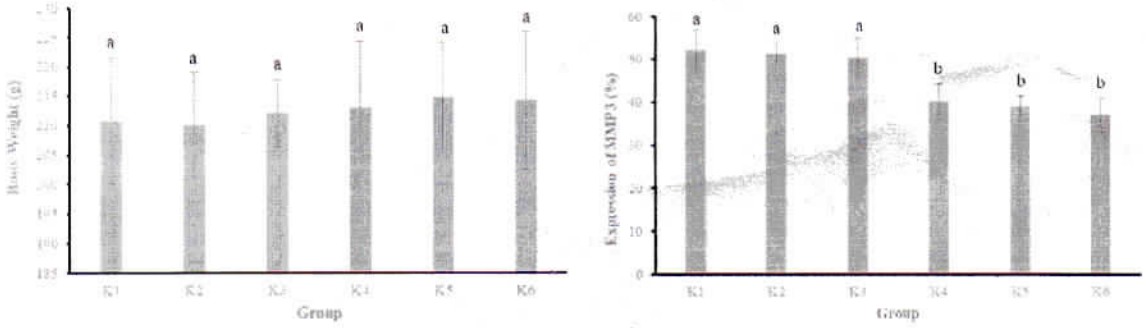

Figure 1. Body weight and expression of MMP3 from each group after treatment; A: Body weight gain; B: Expression of MMP3 changes. The different letter indicated a significant difference (p = 0.05).

Table 1. 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]

Research with the RA model showed that the activity of D-glucan isolated from *Pleurotus ostreatus* has a significant reduction in the clinical parameters of changes in hind limbs, which is dramatically increased due to the arthritis process, and the increase occurred five times higher than in normal controls (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 obtained results that the material is able to reduce damage in animal models and reduce the number of positive cells to MMP3 levels significant) Polysaccharide from *Pleurotus pulmonarius* similar to PSK contains glycan active protein which has also been shown to reduce the volume of rat hind feet induced by formalin and carrageenan to cause an inflammatory condition. The administration of polysaccharide is able to reduce the volume 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). Research with PSK materials using RA model has not been found in previous studies.

The results of the analysis of foot thickness data after 1 week treatment, it was found that in the first week after the PSK administration a different test was performed on all treatment groups and it was found that all groups experienced 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.

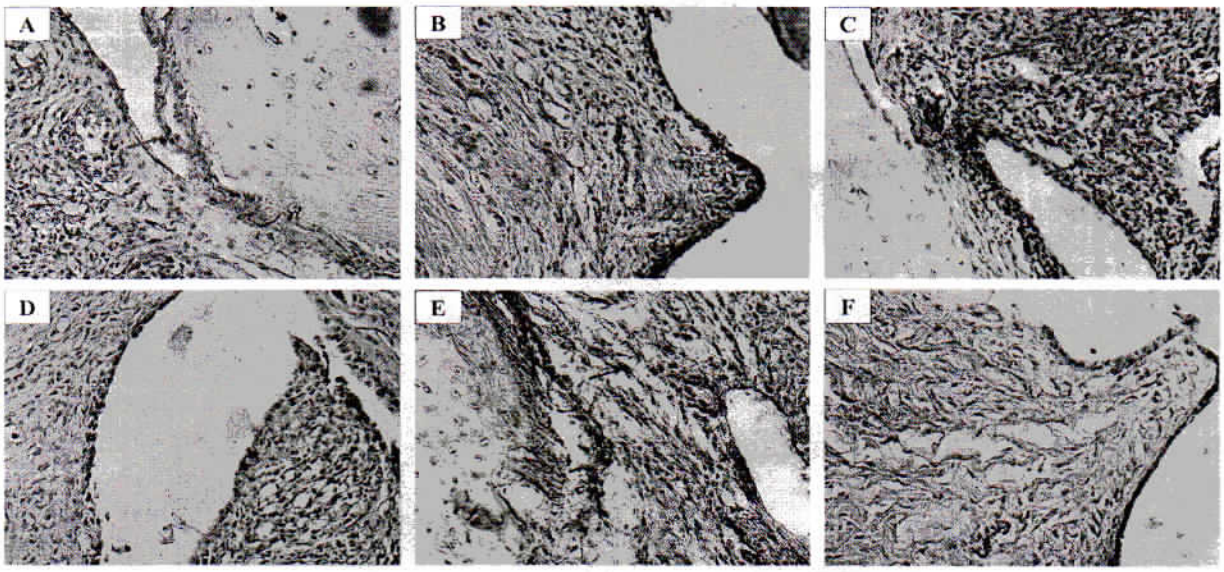

Figure 2. MMP3 expression in rat foot joints of AA model. A: control group at 1 week; B: group giving PSK 50 mg / kg BB at 1 week; C: control group at 2 weeks; D: group giving PSK 50 mg / kg BB at 2 weeks; E: control group at 3 weeks; F: group giving PSK 50 mg / kg BB at 3 weeks. Positive cells (red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

Another thing that can be concluded in this study is that the three control groups not show a significant difference, meaning that controls are sacrificed at the end of 1 week of treatment with those sacrificed at 2 weeks of treatment and 3 weeks of treatment not differ significantly. After conducting different tests on the three treatment groups, K6 group showed decrease significantly 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 obtained the best results on thick variables feet compared to giving for 2 weeks and 1 week.

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NB:

- Follow Journal Guidelines for writing different sections.
- Make necessary corrections as suggested.
- Include "Summary" part.

Ind Vet Journal <ivj83@yahoo.com>
to me

Sep 6, 2019, 1:49 PM

Dear **Dr. Diah Purwaningsari,**

We wish to inform that the under mentioned article has been accepted for publication **(255/19)**

"Effect of Polysaccharide Krestin on MMP3 Expression and Foot Diameter in Rheumatoid Arthritis in Rat."

Please remit a sum of **USD 220** towards the following charges drawn in favour of the "Editor, Indian Veterinary Journal "and payable at Chennai.

The money may be transferred into our Bank **A/c # 30281291710 Code : 09581** of **State Bank of India, Nandanam Branch, Chennai-600035**, **India**. The money should be transferred infavour of The Editor, Indian Veterinary Journal, Chennai. Under intimation to the Editor, IVJ.

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