rr retno widyowati <rr-retno-w@ff.unair.ac.id>

Submission Confirmation

1 message

SPJ <em@editorialmanager.com> Thu, Dec 8, 2022 at 12:32 PM
Reply-To: SPJ <support@elsevier.com>
To: Retno - Widyowati <rr-retno-w@ff.unair.ac.id>

Dear Retno,

We have received your article "The Pro-Inflammatory Cytokine IL-Iβ Alteration by Deer (Rusa unicolor) Antler Extract on Osteoarthritis Rat Model" for consideration for publication in Saudi Pharmaceutical Journal.

Your manuscript will be given a reference number once an editor has been assigned.

To track the status of your paper, please do the following:

1. Go to this URL: https://www.editorialmanager.com/spj/

2. Enter these login details:
Your username is: Retno_Widyowati

If you need to retrieve password details, please go to: https://www.editorialmanager.com/spj/l.asp?i=204939&l=W61JEEOQ.

3. Click [Author Login]
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4. Click [Submissions Being Processed]

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Kind regards,

Editorial Manager
Saudi Pharmaceutical Journal

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rr retno widyowati <rr-retno-w@ff.unair.ac.id>

Please edit your submission

2 messages

SPJ <em@editorialmanager.com> Thu, Dec 8, 2022 at 5:20 PM
Reply-To: SPJ <support@elsevier.com>
To: Retno - Widyowati <rr-retno-w@ff.unair.ac.id>

Re:
Title: The Pro-Inflammatory Cytokine IL-Iβ Alteration by Deer (Rusa unicolor) Antler Extract on Osteoarthritis Rat Model

Dear Dr Widyowati,

Your submission entitled "The Pro-Inflammatory Cytokine IL-Iβ Alteration by Deer (Rusa unicolor) Antler Extract on Osteoarthritis Rat Model" has been received by Saudi Pharmaceutical Journal.

However, before we can proceed with the review process we ask you to address the following:

1-MANUSCRIPT (Without Author Information) should have the Title and Abstract.
MANUSCRIPT (Without Author Information) should not contain any author information including names, affiliation, and contact details.

Please log onto Editorial Manager as an Author:

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1. Go to the menu item "Submissions/Revisions Sent Back to Author".
2. Click "Edit Submission/Revision".
3. Click on the relevant submission step on the left-hand menu;
4. Provide or modify the item/information as requested.
5. Go to "Attach Files" and "Build PDF for my Approval".
6. View and Approve your new PDF file including the changed item(s), or if needed, Edit again.

Thank you for submitting your work to the journal, and if you have any questions, please don't hesitate to contact me.

Yours sincerely,

Editorial Office
Saudi Pharmaceutical Journal

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rr retno widyowati <rr-retno-w@ff.unair.ac.id>

Your PDF has been built and requires approval

1 message

SPJ <em@editorialmanager.com> Fri, Dec 9, 2022 at 10:07 AM
Reply-To: SPJ <support@elsevier.com>
To: Retno - Widyowati <rr-retno-w@ff.unair.ac.id>

Saudi Pharmaceutical Journal
Title: The Pro-Inflammatory Cytokine IL-Iβ Alteration by Deer (Rusa unicolor) Antler Extract on Osteoarthritis Rat Model
Authors: Retno - Widyowati; Suciati Suciati; Dewi Melani Hariyadi; HSin-I Chang; Ngurah IPG Suryawan; Nurliana Tarigan; irawati sholikhah; Chrismawan Ardianto; Ahmad Dzulfikri Nurhan; Ilham Bagus Safitaras

Dear Retno,

The PDF for your submission, "The Pro-Inflammatory Cytokine IL-Iβ Alteration by Deer (Rusa unicolor) Antler Extract on Osteoarthritis Rat Model" has now been built and is ready for your approval. Please view the submission before approving it, to be certain that it is free of any errors. If you have already approved the PDF of your submission, this e-mail can be ignored.

To approve the PDF please login to the Editorial Manager as an Author:

https://www.editorialmanager.com/spj/
Your username is: Retno_Widyowati

Then click on the folder 'Submissions Waiting for Author's Approval' to view and approve the PDF of your submission. You may need to click on 'Action Links' to expand your Action Links menu.

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Your submission will be given a reference number once an Editor has been assigned to handle it.

Thank you for your time and patience.
Kind regards,
Editorial Office
Saudi Pharmaceutical Journal

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rr retno widyowati <rr-retno-w@ff.unair.ac.id>

A manuscript number has been assigned: SPJ-D-22-00677

1 message

SPJ <em@editorialmanager.com> Sun, Dec 11, 2022 at 1:07 AM
Reply-To: SPJ <support@elsevier.com>
To: Retno - Widyowati <rr-retno-w@ff.unair.ac.id>

Ms. Ref. No.: SPJ-D-22-00677
Title: The Pro-Inflammatory Cytokine IL-Iβ Alteration by Deer (Rusa unicolor) Antler Extract on Osteoarthritis Rat Model
Saudi Pharmaceutical Journal

Dear Retno,

Your submission "The Pro-Inflammatory Cytokine IL-Iβ Alteration by Deer (Rusa unicolor) Antler Extract on Osteoarthritis Rat Model" has been assigned manuscript number SPJ-D-22-00677.

To track the status of your paper, please do the following:

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Thank you for submitting your work to Saudi Pharmaceutical Journal.

Kind regards,

Wajhul Qamar, Ph.D.
Editorial Office
Saudi Pharmaceutical Journal

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rr retno widyowati <rr-retno-w@ff.unair.ac.id>

Track the status of your submission to Saudi Pharmaceutical Journal

1 message

Track your Elsevier submission <no-reply@submissions.elsevier.com> Sun, Jan 1, 2023 at 6:25 PM
To: rr-retno-w@ff.unair.ac.id

Manuscript Number: SPJ-D-22-00677
Manuscript Title: The Pro-Inflammatory Cytokine IL-Iβ Alteration by Deer (Rusa unicolor) Antler Extract on Osteoarthritis Rat Model
Journal: Saudi Pharmaceutical Journal

Dear Retno Widyowati,

Your submitted manuscript is currently under review. You can track the status of your submission in Editorial Manager, or track the review status in more detail using Track your submission here:
https://track.authorhub.elsevier.com?uuid=fe5b3f02-4e16-402a-a5de-e424712c726d

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Your Submission

3 messages

SPJ <em@editorialmanager.com> Fri, Feb 10, 2023 at 5:25 PM
Reply-To: SPJ <support@elsevier.com>
To: Retno - Widyowati <rr-retno-w@ff.unair.ac.id>

Ms. Ref. No.: SPJ-D-22-00677
Title: The Pro-Inflammatory Cytokine IL-Iβ Alteration by Deer (Rusa unicolor) Antler Extract on Osteoarthritis Rat Model
Saudi Pharmaceutical Journal

Dear Retno,

The reviewers have commented on your above paper. I invite you to revise and resubmit your manuscript.

Please carefully address the issues raised in the comments.

If you are submitting a revised manuscript, please also:

a) outline each change made (point by point) as raised in the reviewer comments (Highlight changes in the manuscript)

AND/OR

b) provide a suitable rebuttal to each reviewer comment not addressed (Highlight changes in the manuscript)

To submit your revision, please do the following:

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4. Click [Submissions Needing Revision]

Please note that we allow 21 days for author revisions.

I look forward to receiving your revised manuscript.

Yours sincerely,

Aws Alshamsan, Ph.D
Editor-in-Chief
Saudi Pharmaceutical Journal

Reviewers' comments:

Reviewer #1: In the manuscript entitled "The Pro-Inflammatory Cytokine IL-Iβ Alteration by Deer (Rusa unicolor) Antler Extract on Osteoarthritis Rat Model" the authors evaluated impact of 70% ethanol extracts of deer antler in

reducing cytokine IL-1β to rat model of OA. Many previous studies have reported the effects of ethanol and aqueous extracts of deer antler inhibited cell inflammation and stimulated osteoblast differentiation in bone turnover and rheumatoid arthritis models. In this regard, the authors have attempted to make a contribution but the evidence seems insufficient and not enough convincing as just the reduction of IL-1b does not look considerable for the paper publication, the author must include the real-time changes in osseous tissues by histopathological changes noted before and after treatment. Further, there are serious issues in the method section that must be addressed:

* In method section, The reference for MIA at a dose of 4 mg that was dissolved in saline (50 µl) must be given.

* After MIA injection, for how long the paw edema was measured? and why authors did not monitor the baseline values as they are critical to make the comparison. How the paw volume was measured? plz make it part of the methodology.

* In the first line of animal grouping section, please correct @5 and write n=5. Further, this section must be rewritten with a detailed elaboration of how and through which route the animals of all groups were given the respective treatments. and either once or twice daily? this paragraph must be revised as required information is missing and an experimental layout must be incorporated to show the animal grouping and their treatments as well as the edema and hyperalgesia monitoring time points with blood withdrawal day for ELISA, to make the method section more reader-friendly.

* The weight is a parameter that affects the animal's movement in this type of model so, rats should have been monitored for their weight variation during the entire course of study.

* The animals section must be separately written from material section and should include the housing conditions and how many rats per cage (write cage dimensions as well) were kept as overcrowding must bring discomfort to the rats with edema and pain.

* Authors should write the unit i.e. seconds immediately after writing the Latency response in the hyperalgesia experiment section. Further, in the same section, The author mentioned that "they were eliminated from the warm plate straight away after 70 responses occurred"? the line seems confusing, what do they mean by 70 responses? Plz, elaborate. Moreover, if authors eliminated any animal from any group, plz write in the manuscript in results as the number of animals is already just 5 per group.

* How the ELISA was performed? Plz, incorporate the details if kits were used.

* In the statistical analysis section, plz write the name of the post hoc test used.

* In the results section, the units of diameter and latency are missing in table 1 and 2.

* What did the author conclude from outcomes, was the tested therapy effective as an anti-inflammatory or by providing analgesic effects? Or by both? Moreover, the conclusion section must incorporate the limitations of the study.

Reviewer #2: I have read the manuscript titled " The Pro-Inflammatory Cytokine IL-Iβ Alteration by Deer (Rusa unicolor) Antler Extract on Osteoarthritis Rat Model" (SPJ-D-22-00677).
Authors have tried to elaborate the effectiveness of Deer Antler extract in managing osteoarthritis in rat model. Though the results look exciting, but the article is very poorly written and needs a lot of improvement.
Firstly, authors need to highlight the novelty of their work as few articles on this subject are already published in many journals. Secondly, to convince the efficacy of this method authors needs to identify and share the information about the chemical constituents present in this extract.
Besides that here are few minor concerns regarding the manuscript
1. The language of the manuscript requires major improvement as there are many grammatical errors and

many sentences need to be rephrased.
2. Abstract of the article needs major revision and p values mentioned in it should be checked and corrected.
3. Previous studies on this subject should be mentioned in the introduction section and should also be discussed in the discussion section.
4. Methodology should be elaborated regarding the extract formation particularly about the source of antler powder.
5. Few of the results should be elaborated through graphs for easy understanding of readers.

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rr retno widyowati <rr-retno-w@ff.unair.ac.id> Fri, Feb 10, 2023 at 6:56 PM
To: irawaty sholikhah <irawatysholikhah55@gmail.com>

Kita diskusikan senin ya untuk njawab ini

Bismilah banyak yg hrs dikoreksi … dan semoga hasilnya bagus bisa accepted .. Aamiin

Dikirim dari iPhone saya

Awal pesan yang diteruskan:

> **Dari:** SPJ <em@editorialmanager.com>
> **Tanggal:** 10 Februari 2023 17.25.48 WIB
> **Kepada:** Retno - Widyowati <rr-retno-w@ff.unair.ac.id>
> **Subjek: Your Submission**
> **Balas-Ke:** SPJ <support@elsevier.com>
>
> Ms. Ref. No.: SPJ-D-22-00677
> [Quoted text hidden]

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irawaty sholikhah <irawatysholikhah55@gmail.com> Fri, Feb 10, 2023 at 7:09 PM
To: rr retno widyowati <rr-retno-w@ff.unair.ac.id>

Baik bu, ngih bismillah responya positif,,🙏🙏
[Quoted text hidden]

Reviewer #1: In the manuscript entitled "The Pro-Inflammatory Cytokine IL-Iβ Alteration by Deer (Rusa unicolor) Antler Extract on Osteoarthritis Rat Model" the authors evaluated impact of 70% ethanol extracts of deer antler in reducing cytokine IL-1β to rat model of OA. Many previous studies have reported the effects of ethanol and aqueous extracts of deer antler inhibited cell inflammation and stimulated osteoblast differentiation in bone turnover and rheumatoid arthritis models. In this regard, the authors have attempted to make a contribution but the evidence seems insufficient and not enough convincing as just the reduction of IL-1b does not look considerable for the paper publication, the author must include the real-time changes in osseous tissues by histopathological changes noted before and after treatment. Further, there are serious issues in the method section that must be addressed:

Author Response : Thank you for the suggestions and valuable comments. This study did not include bone tissue changes by histopathological changes. But in our opinion, there are three test parameters that we observe, such as physical parameters, namely the measurement of the joint diameter of rats before and after treatment; functional parameters, namely measurement resistance (hyperalgesia) to responses in the thermal stimulation of the hot plate method application in the rats before and after treatment; and biochemical parameters, namely the measurement of proinflammatory IL-IB cytokine levels in the rat's blood serum before and after treatment, is enough to prove this study.

1. In method section, The reference for MIA at a dose of 4 mg that was dissolved in saline (50 µl) must be given.

   Author Response : Thank you for the suggestion, we have add the reference for MIA dose in method section
2. After MIA injection, for how long the paw edema was measured? and why authors did not monitor the baseline values as they are critical to make the comparison. How the paw volume was measured? plz make it part of the methodology.

   Author Rensponse : Thank tou for the valuable comments.

   Swelling measurements in rat joints were carried out for seven weeks (weeks 0, 1, 2, 3, 4, 5, 6, 7) after intra-articular MIA injection (diameter measurement data can be seen in Table 1 and Table 4). For the baseline value used in measuring joint swelling, the authors compared the value of joint diameter in rats injected with MIA intraarticularly (negative

group) with rats not injected with MIA intraarticularly (healthy group). Swelling volume in rat joints was measured using a calibrated screw micrometer. Measurements were made on the part of the joint that experienced swelling due to the MIA injection. This measurement is done to determine the swelling that occurs during the developmental stages of osteoarthritis with an interval of several days.

3. In the first line of animal grouping section, please correct @5 and write n=5. Further, this section must be rewritten with a detailed elaboration of how and through which route the animals of all groups were given the respective treatments. and either once or twice daily? this paragraph must be revised as required information is missing and an experimental layout must be incorporated to show the animal grouping and their treatments as well as the edema and hyperalgesia monitoring time points with blood withdrawal day for ELISA, to make the method section more reader-friendly.
   Author Response: Thanks for the valuable suggestions and comments. We have added some information suggested by reviewers and we have also revised the use of sentences in the method section to make it easier for readers.

   This research was conducted using 25 rats which were divided into five groups (n = 5 rats). The groups were divided into (1) healthy group (S, was given food and water *ad libitum* but not induced by MIA), (2) negative group (N, rats injected 4 mg MIA dissolved in 50 µl saline and treated 0,5% Carboxy Methyl Cellulose (CMC), (3) positive group (P, rats injected with MIA 4 mg dissolved in 50 µl saline and treated with glucosamine sulfate (250 mg/kg BW) (Lipa Pharmaceuticals Ltd, NSW, 2566, Australia), (4) low dose group (L, rats injected with 4 mg MIA dissolved in 50 µl saline and treated deer antler extract 250 mg/kg BW), and (5) the high dose group (H, rats injected with 4 mg MIA dissolved in 50 µl saline and treated deer antler extract 500 mg/kg BW). Intraarticular MIA injection (4 mg MIA dissolved in 50 µl saline) (Sigma-Aldrich, Darmstadt, Germany) was performed in all groups except the healthy group to obtain the OA rat model. After 3 weeks MIA induction, all rats were blood drawn to see IL-1β levels were measured (pretest), followed by administration of deer antler extract orally once a day and carried out every day for 28 days based on the group. Furthermore, measurements of swelling in the rat joint diameter and hyperalgesia tests were measured with a calibrated micrometer screw and hot plate for seven weeks (at weeks 0, 1, 2, 3, 4,

5, 6, and 7). The latency time is measured with a stopwatch. At week 7, blood was taken to measure IL-1β levels in blood serum (as a posttest).

4. The weight is a parameter that affects the animal's movement in this type of model so, rats should have been monitored for their weight variation during the entire course of study.

   Author Response: Thank you for the suggestion. During the study, we monitored the weight of the experimental animals used by providing food in the same amount and type every day. We also controlled it by weighing the rats every two days to see their progress.

5. The animals section must be separately written from material section and should include the housing conditions and how many rats per cage (write cage dimensions as well) were kept as overcrowding must bring discomfort to the rats with edema and pain.

   Author Response: Thank you for your suggestion. We have change according to what the reviewer suggested.

   Twenty-five male Wistar rats (Rattus norvegicus) purchased from the Faculty of Pharmacy, Airlangga University, Surabaya, Indonesia, were in good health. Rats aged 3-4 months (200-300 g) were acclimatized for seven days at the Animal Laboratory of the Faculty of Pharmacy, Airlangga University. Rats were maintained in a room at a temperature of 22±3°C, with a relative humidity of 30-70% and 12 hours of light and 12 hours of darkness. Rats were kept in cages with dimensions of 45cm(L) x 30cm(D) x 20cm(H), with each cage consisting of one rats. The cage is waterproof, robust, easy to clean, and free from noise. This research has followed the Guidelines for the Care and Use of Laboratory Animals issued by the National Institutes of Health which was revised in 1985, and has been approved by the ethical committee of Universitas Airlangga. Faculty of Veterinary Medicine, Airlangga University (No.2.KE.176.09.2019).

6. Authors should write the unit i.e. seconds immediately after writing the Latency response in the hyperalgesia experiment section. Further, in the same section, The author mentioned that "they were eliminated from the warm plate straight away after 70 responses occurred"? the line seems confusing, what do they mean by 70 responses? Plz, elaborate. Moreover, if authors eliminated any animal from any group, plz write in the manuscript in results as the number of animals is already just 5 per group.

Author Response: Thank you for your valuable comments and suggestions. We've changed the sentences to make it easier to understand. we apologize because in this section we have errors in writing.

7. How the ELISA was performed? Plz, incorporate the details if kits were used.

   Author Response: Thank you for your valuable comment. ELISA was performed using a commercial mouse IL-1β ELISA kit (Bioenzy, Germany). Rat blood was taken as much as 1-2 mL, then centrifuged at 3000 rpm for 10 minutes to get some of the serum. secondly to 96 well plates each was added standard solution (50 µl), blood serum (40 µl), and 1L-1β antibody (10 µl). Then, mixed with streptavidin-HRP (50 µl) and incubated at 37°C for 60 minutes. Then each well was washed 5 times with buffer (0.35 ml) and left for 30 seconds. After that, substrates A and B (50 µl), and stop solution (50 µl) were added to each well for 10 minutes, and the reactions were measured with an ELISA reader (EZ 2000) at a wavelength of 450 nm. This reaction occurs when there is a color change from blue to yellow. These instructions are in accordance with the instructions from a commercial mouse IL-1β ELISA kit (Bioenzy, Germany).

8. In the statistical analysis section, plz write the name of the post hoc test used.

   Author Response: Thank you for valuable suggestion. We have add name of post hoc test in statictical analysis section.

9. In the results section, the units of diameter and latency are missing in table 1 and 2.

   Author Response: Thank you for valuable comment. We have added the missing diameter and latency units in the results section specifically in tables 1 and 2.

10. What did the author conclude from outcomes, was the tested therapy effective as an anti-inflammatory or by providing analgesic effects? Or by both? Moreover, the conclusion section must incorporate the limitations of the study.

    Author Response: Thank you for your valuable comment and suggestion. Based on the results obtained in the current study, it was shown that 70% ethanol extract from deer antler could reduce levels of the pro-inflammatory cytokine IL-1β, reduce joint swelling, and increase latency time by MIA injection in animal models. These findings indicate that deer antler extract 250 mg/kg BW and 500 mg/kg BW can be a potential treatment for OA as well as glucosamine sulfate.

Reviewer #2: I have read the manuscript titled " The Pro-Inflammatory Cytokine IL-Iβ Alteration by Deer (Rusa unicolor) Antler Extract on Osteoarthritis Rat Model" (SPJ-D-22-00677). Authors have tried to elaborate the effectiveness of Deer Antler extract in managing osteoarthritis in rat model. Though the results look exciting, but the article is very poorly written and needs a lot of improvement.

Firstly, authors need to highlight the novelty of their work as few articles on this subject are already published in many journals. Secondly, to convince the efficacy of this method authors needs to identify and share the information about the chemical constituents present in this extract.

Author Response:

Irawati 23/2/23 23.19
Comment [1]: Mohon maaf bu, untuk bagian ini saya bingung untuk menjawabnya

Besides that here are few minor concerns regarding the manuscript

1. The language of the manuscript requires major improvement as there are many grammatical errors and many sentences need to be rephrased.

   Author Response: Thank you for the reviews and suggestions that make our article improve. We have read in more detail and rewritten som parts that are not quite right, especially on the grammar in the text so that it is easy to understand.
2. Abstract of the article needs major revision and p values mentioned in it should be checked and corrected.

   Author Response: Thank you for the reviews and suggestions given. We have rewritten abstract and corrected the abstract as suggested.
3. Previous studies on this subject should be mentioned in the introduction section and should also be discussed in the discussion section.

   Author Response: Thank you for the reviews and suggestions provided. we have added previous research in the introduction section and we have also discussed it in the discussion section as suggested. We have mark changes and additions with a highlight
4. Methodology should be elaborated regarding the extract formation particularly about the source of antler powder.

Author Response: Thank you for the reviews and suggestions given. We have added about extract formation particularly of deer antler in Method section.

Deer antler powder of *Rusa unicolor* was received from UPTD of East Kalimantan, Indonesia. The 500 g of deer antler powder was extracted with 70% ethanol (2.0 L x 3) by using maceration method at room temperature for 24 hour. After being filtered with Whatman paper no. 41, the filtrate was obtained. Then, the filtrate was evaporated by a BUCHI rotary evaporator at 40°C and 40 rpm to create a thick extract with a constant weight and resulting deer antler 70% ethanol extract was 16.8 g.

5. Few of the results should be elaborated through graphs for easy understanding of readers.

   Author Response: Thank you for the reviews and suggestions provided. We've transformed the resulting data into a graph for easier understanding for the reader.

Saudi Pharmaceutical Journal

The Pro-Inflammatory Cytokine IL-Iβ Alteration by Deer (Rusa unicolor) Antler Extract on Osteoarthritis Rat Model

--Manuscript Draft--

Manuscript Number:	SPJ-D-22-00677R1
Article Type:	Original Article
Keywords:	Osteoarthritis, Deer antler, MIA, IL-Iβ
Corresponding Author:	Retno - Widyowati, ph.D Airlangga University Faculty of Pharmacy Surabaya, East Java INDONESIA
First Author:	Retno - Widyowati, ph.D
Order of Authors:	Retno - Widyowati, ph.D
	Suciati Suciati, Ph.D
	Dewi Melani Hariyadi, Professor
	HSin-I Chang, Ph.D
	Ngurah IPG Suryawan, Master
	Nurliana Tarigan, Bachelors
	irawati sholikhah, Bachelors
	Chrismawan Ardianto, Ph.D
	Ahmad Dzulfikri Nurhan, Master
	Ilham Bagus Safitaras, Master
Abstract:	Osteoarthritis is a disease associated with the degradation of articular cartilage, inflammation of the intra-articular area, and subchondral bone replacement. Traditionally, deer antler extract in East Kalimantan, Indonesia, is used to treat many disorders, including as an anti-inflammatory. In this study, we used an in vivo model to investigate 70% ethanol extract from deer antlers that affected the development and progression of OA. The OA model in rats was produced by intra-articular injection of monosodium iodoacetate (MIA). The development of OA was observed three weeks. Subsequently, the treatment of deer antler extract at doses 250 and 500 mg/kg BW orally in OA model rats for four weeks was performed to assess their effect of reducing IL-1ß levels, joint diameter, and hyperalgesia. Successful induction was demonstrated by significant differences ($p<0.05$) compare to healthy group in decreased latency time, increased joint diameter and IL-1ß levels. Treatment with deer antler extracts significantly ($p<0.05$) reduced IL-1ß levels, joint swelling, and increased response hyperalgesia. So deer antler 70% ethanol extract has therapeutic effects on inflammation through reduced IL-1ß in rat model of experimental MIA-induced osteoarthritis. This study suggests that deer antler extract may have a potential novel agent for treating OA.
Suggested Reviewers:	Ram Kumar Sahu, Doctor Ass. Professor, Assam University ramsahu79@gmail.com He is an expert in the field of drug discovery
	Katsuyoshi Matsunami, Doctor Professor, Hiroshima University matunami@hiroshima-u.ac.jp
	Chao Che-Yi, ph.D Professor, Asia University cychao@asia.edu.tw She is an expert and conducts research in the field of activity testing in experimental animals

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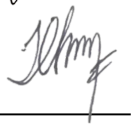
10.	Ilham B Sagitaras		December 5, 2022

Editor in Chief: Professor Aws Alshamsan Date:-

Cover Letter

Dear Mr. / Ms.

Aws Alshamsan

Editors-in-Chief

Saudi Pharmaceutical Journal

Enclosed is a manuscript to be considered for publication in Saudi Pharmaceutical Journal. Below are our responses to your submission requirements.

1. Title of the manuscript:

The Pro-Inflammatory Cytokine IL-Iβ Alteration by Deer (*Rusa unicolor*) Antler Extract on Osteoarthritis Rat Model

2. Why the manuscript is important in its field and why the manuscript should be published in Saudi Pharmaceutical Journal?

The prevalence of osteoarthritis is increasing every year. The use of synthetic drugs causes high side effects and is expensive. So many patients are disobedient in using them. Therefore, this research is very important to be carried out to produce potential new drugs derived from natural ingredients that are safe and inexpensive. Deer antlers are empirically used to strengthen bones. This research is important to produce the potential of deer antler as anti-osteoarthritis. The Saudi journal was chosen because it fits the results of the research conducted. In addition, this journal has a good reputation in distributing research results

3. Statements.

This paper is our original work. We confirm that this manuscript has not been published in part or whole elsewhere in any language, and it has not been submitted to any other journal for reviews.

We certify that all authors named deserve authorship, and that all authors have agreed to be so listed and have read and approved the manuscript and its submission to Saudi Pharmaceutical Journal.

4. Whether the authors have published or submitted any related papers from the same study.

None

5. Financial arrangements or relationships that may pose conflict of interest.

There is no financial relationship that may give rise to a conflict of interest.

6. The authors have no conflicts of interest to declare

Please address all correspondence concerning this manuscript to me at rr-retno-w@ff.unair.ac.id

Thank you for consideration of this manuscript.

Sincerely

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Title Page

Title : The Pro-Inflammatory Cytokine IL-Iβ Alteration by Deer (*Rusa unicolor*) Antler Extract on Osteoarthritis Rat Model

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Abstract

Osteoarthritis is a disease associated with articular cartilage degradation, intra-articular area inflammation, and subchondral bone replacement. Cytokine IL-1β has a prominent function in the inflammations process that passes in the joints. The 70% ethanol extracts of deer antler (250 and 500 mg/kg BW) and glucosamine sulfate (250 kg/BW) were evaluated for four weeks in reducing cytokine IL-1β to rat model OA-induced Monosodium iodoacetate. Measurements of joint diameter in rat's knee and hyperalgesia were performed on weeks 0, 1, 2, 3, 4, 5, 6, and 7. The presence of a significant difference in the stimulation thermal latency ($p=0.00$) and the resulting increase in swelling of joint diameter ($p=0.00$) are evidence that MIA has successfully induced the rat modeling of OA. A significant decrease in cytokine IL-Iβ levels was shown on week 3 after MIA injection ($p=0.00$). Both concentrations of deer extracts significantly reduced knee joint diameter ($p=0.00$), latency thermal stimulation ($p=0.00$), and cytokine IL-1β levels ($p=0.00$). Based on the results, it can be concluded that the 70% ethanol extract of deer antler is a potential medicine for OA therapy.

Keywords: Osteoarthritis, Deer antler, MIA, IL-Iβ

List of abbreviations : OA = Osteoarthritis

MIA = Monosodium Iodoacetate

G3PDH = Glyceraldehyde-3-Phosphate Dehydrogenase

IL-Iβ = Interleukin- 1 beta

PEG = Polyethylene Glycol

ELISA = Enzyme-Linked Immunosorbent Assay

COX-2 = Cyclooxygenase-2

TNF-α = Tumor Necrosis Factor Alpha

MMPs = Matrix Metalloproteinases

iNOS = Inducible Nitric Oxide Synthase

IL-6 = Interlekuin-6

Acknowledgments

We would like to thank the president and chair of the Institute for Research and Community Service, Universitas Airlangga, and the dean of the Faculty of Pharmacy, Universitas Airlangga for funding this research through the Mandatory Research Program. We also thank to the chairman of the UPTD East Kalimantan and the president of National Chiayi University Taiwan for the research collaboration that has been established.

Funding

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Footnotes **:** None

Declarations of interest **:** None

Type of article **:** Original Research Papers

Reviewer #1: In the manuscript entitled "The Pro-Inflammatory Cytokine IL-Iβ Alteration by Deer (Rusa unicolor) Antler Extract on Osteoarthritis Rat Model" the authors evaluated impact of 70% ethanol extracts of deer antler in reducing cytokine IL-1β to rat model of OA. Many previous studies have reported the effects of ethanol and aqueous extracts of deer antler inhibited cell inflammation and stimulated osteoblast differentiation in bone turnover and rheumatoid arthritis models. In this regard, the authors have attempted to make a contribution but the evidence seems insufficient and not enough convincing as just the reduction of IL-1b does not look considerable for the paper publication, the author must include the real-time changes in osseous tissues by histopathological changes noted before and after treatment. Further, there are serious issues in the method section that must be addressed:

Author Response: Thank you for the suggestions and valuable comments. This study did not determine bone tissue changes by histopathology. But in our opinion, there are three test parameters that we observe, such as physical parameters, namely the measurement of the joint diameter of rats before and after treatment; functional parameters, namely measurement resistance (hyperalgesia) to responses in the thermal stimulation of the hot plate method application in the rats before and after treatment; and biochemical parameters, namely the measurement of pro-inflammatory IL-Iβ cytokine levels in the rat's blood serum before and after treatment, is enough to prove this study.

1. In method section, The reference for MIA at a dose of 4 mg that was dissolved in saline (50 µl) must be given.

   Author Response: Thank you for the suggestion, we have add the reference for MIA dose in method section
2. After MIA injection, for how long the paw edema was measured? and why authors did not monitor the baseline values as they are critical to make the comparison. How the paw volume was measured? plz make it part of the methodology.

   Author Rensponse : Thank tou for the valuable comments.

   Swelling measurements in rat joints were carried out for seven weeks (weeks 0, 1, 2, 3, 4, 5, 6, 7) after intra-articular MIA injection (diameter measurement data can be seen in Table 1 and Table 4). For the baseline value used in measuring joint swelling, the authors

compared the value of joint diameter in rats injected with MIA intraarticularly (negative group) with rats not injected with MIA intraarticularly (healthy group). Swelling volume in rat joints was measured using a calibrated screw micrometer. Measurements were made on the part of the joint that experienced swelling due to the MIA injection. This measurement is done to determine the swelling that occurs during the developmental stages of osteoarthritis with an interval of several days.

3. In the first line of animal grouping section, please correct @5 and write n=5. Further, this section must be rewritten with a detailed elaboration of how and through which route the animals of all groups were given the respective treatments. and either once or twice daily? this paragraph must be revised as required information is missing and an experimental layout must be incorporated to show the animal grouping and their treatments as well as the edema and hyperalgesia monitoring time points with blood withdrawal day for ELISA, to make the method section more reader-friendly.
   Author Response: Thanks for the valuable suggestions and comments. We have added some information suggested by reviewers and we have also revised the use of sentences in the method section to make it easier for readers.

   This research was conducted using 25 rats divided into five groups (n = 5 rats). They were (1) the healthy group (S, was given food and water *ad libitum* but not induced by MIA), (2) the negative group (N, rats injected with 4 mg MIA dissolved in 50 µl saline and treated 0,5% Carboxy Methyl Cellulose (CMC), (3) the positive group (P, rats injected with MIA 4 mg dissolved in 50 µl saline and treated with glucosamine sulfate (250 mg/kg BW) (Lipa Pharmaceuticals Ltd, NSW, 2566, Australia), (4) low dose group (L, rats injected with 4 mg MIA dissolved in 50 µl saline and treated deer antler extract 250 mg/kg BW), and (5) the high dose group (H, rats injected with 4 mg MIA dissolved in 50 µl saline and treated deer antler extract 500 mg/kg BW). Intra-articular MIA injection (4 mg MIA dissolved in 50 µl saline) (Sigma-Aldrich, Darmstadt, Germany) was performed in all groups except the healthy group to obtain the OA rat model. After 3 weeks of MIA induction, all rats were blood drawn to measure IL-1β levels (pre-test), followed by administration of deer antler extract orally once a day and carried out every day for 28 days based on the group. Furthermore, swelling in the rat joint diameter and hyperalgesia were measured with a calibrated micrometer screw and hot plate for seven

weeks (at weeks 0, 1, 2, 3, 4, 5, 6, and 7). The latency time in hyperalgesia test was measured with a stopwatch. At week 7, blood was taken to measure IL-1β levels in blood serum (as a post-test).

4. The weight is a parameter that affects the animal's movement in this type of model so, rats should have been monitored for their weight variation during the entire course of study.

   Author Response: Thank you for the suggestion. During the study, we monitored the weight of the experimental animals used by providing food in the same amount and type every day. We also controlled it by weighing the rats every two days to see their progress.

5. The animals section must be separately written from material section and should include the housing conditions and how many rats per cage (write cage dimensions as well) were kept as overcrowding must bring discomfort to the rats with edema and pain.

   Author Response: Thank you for your suggestion. We have changed according to what the reviewer suggested.

   Twenty-five male Wistar rats (*Rattus norvegicus*) purchased from the Faculty of Pharmacy, Airlangga University, Surabaya, Indonesia, were in good health. Rats aged 3-4 months (200-300 g) were acclimatized for seven days at the Animal Laboratory of the Faculty of Pharmacy, Airlangga University. Rats were maintained in a room at a temperature of 22±3°C, with a relative humidity of 30-70% and 12 hours of light and 12 hours of darkness. Rats were kept in cages with dimensions of 45cm(L) x 30cm(D) x 20cm(H), with each cage consisting of one rat. The cage is waterproof, robust, easy to clean, and free from noise. This research has followed the Guidelines for the Care and Use of Laboratory Animals issued by the National Institutes of Health was revised in 1985 and has been approved by the ethical committee of Faculty of Veterinary Medicine, Universitas Airlangga (No.2.KE.176.09.2019).

6. Authors should write the unit i.e. seconds immediately after writing the Latency response in the hyperalgesia experiment section. Further, in the same section, The author mentioned that "they were eliminated from the warm plate straight away after 70 responses occurred"? the line seems confusing, what do they mean by 70 responses? Plz, elaborate. Moreover, if authors eliminated any animal from any group, plz write in the manuscript in results as the number of animals is already just 5 per group.

Author Response: Thank you for your valuable comments and suggestions. We've changed the sentences to make it easier to understand. We apologize because in this section we have errors in writing.

7. How the ELISA was performed? Plz, incorporate the details if kits were used.

   Author Response: Thank you for your valuable comment. ELISA was performed using a commercial mouse IL-1β ELISA kit (Bioenzy, Germany). Rat blood was taken as much as 1-2 mL, then centrifuged at 3000 rpm for 10 minutes to get some of the serum. The 96 well plates each were added standard solution (50 µl), blood serum (40 µl), and 1L-1β antibody (10 µl). Then, mixed with streptavidin-HRP (50 µl) and incubated at 37°C for 60 minutes. Then each well was washed 5 times with buffer (0.35 ml) and left for 30 seconds. After that, substrates A and B (50 µl), and stop solution (50 µl) were added to each well for 10 minutes, and the reactions were measured with an ELISA reader (EZ 2000) at a wavelength of 450 nm. This reaction occurs when there is a color change from blue to yellow. These instructions are in accordance with the instructions from a commercial mouse IL-1β ELISA kit (Bioenzy, Germany).

8. In the statistical analysis section, plz write the name of the post hoc test used.

   Author Response: Thank you for your valuable suggestion. We have added the name of post hoc test in the statistical analysis section.

9. In the results section, the units of diameter and latency are missing in table 1 and 2.

   Author Response: Thank you for your valuable comment. We have added the missing diameter and latency units in the results section specifically in tables 1 and 2.

10. What did the author conclude from outcomes, was the tested therapy effective as an anti-inflammatory or by providing analgesic effects? Or by both? Moreover, the conclusion section must incorporate the limitations of the study.

    Author Response: Thank you for your valuable comment and suggestion. Based on the results obtained in the current study, it was shown that 70% ethanol extract from deer antlers could reduce levels of the pro-inflammatory cytokine IL-1β, reduce joint swelling, and increase latency time by MIA injection in animal models. These findings indicate that deer antler extracts at 250and 500 mg/kg BW could be a potential treatment for OA as well as glucosamine sulfate.

Reviewer #2: I have read the manuscript titled " The Pro-Inflammatory Cytokine IL-Iβ Alteration by Deer (Rusa unicolor) Antler Extract on Osteoarthritis Rat Model" (SPJ-D-22-00677). Authors have tried to elaborate the effectiveness of Deer Antler extract in managing osteoarthritis in rat model. Though the results look exciting, but the article is very poorly written and needs a lot of improvement.

Firstly, authors need to highlight the novelty of their work as few articles on this subject are already published in many journals. Secondly, to convince the efficacy of this method authors needs to identify and share the information about the chemical constituents present in this extract.

Author Response: Thank you for your comments and suggestions. Much research has been done on deer antler extract related to bone disease, but no research has been carried out regarding the exploration of the potential of this extract to increase the cytokine IL-1β. By doing this research, the mechanism of the extract in bone disease can be known. The deer antler extract contains many ingredients such as protein, lipid, ash, calcium, collagen, chondroitin sulfate, and glucosamine which play a role in supporting this mechanism of the extract.

Besides that here are few minor concerns regarding the manuscript

1. The language of the manuscript requires major improvement as there are many grammatical errors and many sentences need to be rephrased.

   Author Response: Thank you for the reviews and suggestions that make our article improve. We have read in more detail and rewritten, especially on so that it is easy to understand.
2. Abstract of the article needs major revision and p values mentioned in it should be checked and corrected.

   Author Response: Thank you for the reviews and suggestions given. We have rewritten the abstract and corrected it as suggested.
3. Previous studies on this subject should be mentioned in the introduction section and should also be discussed in the discussion section.

Author Response: Thank you for the reviews and suggestions provided. we have added previous research in the introduction section and we have also discussed it in the discussion section as suggested. We have marked changes and additions with a highlight

4. Methodology should be elaborated regarding the extract formation particularly about the source of antler powder.

   Author Response: Thank you for the reviews and suggestions given. We have added about extract formation particularly of deer antlers in Method section.

   Deer antler powder of *Rusa unicolor* was received from UPTD of East Kalimantan, Indonesia. The 500 g of deer antler powder was extracted with 70% ethanol (2.0 L x 3) by maceration method at room temperature for 24 hour. Then it filtered with Whatman paper no. 41 to obtain the filtrate. The filtrate was evaporated by a BUCHI rotary evaporator at 40°C and 40 rpm to create a thick extract with a constant weight and resulting in 16.8 g of 70% ethanol extract of deer antler.

5. Few of the results should be elaborated through graphs for easy understanding of readers.

   Author Response: Thank you for the reviews and suggestions provided. We've transformed the resulting data into a graph for easier understanding for the reader.

The Pro-Inflammatory Cytokine IL-Iβ Alteration by Deer (*Rusa unicolor*) Antler Extract on Osteoarthritis Rat Model

Abstract

Osteoarthritis is a disease associated with the degradation of articular cartilage, inflammation of the intra-articular area, and subchondral bone replacement. Traditionally, deer antler extract in East Kalimantan, Indonesia, is used to treat many disorders, including as an anti-inflammatory. In this study, we used an in vivo model to investigate 70% ethanol extract from deer antlers that affected the development and progression of OA. The OA model in rats was produced by intra-articular injection of monosodium iodoacetate (MIA). The development of OA was observed three weeks. Subsequently, the treatment of deer antler extract at doses 250 and 500 mg/kg BW orally in OA model rats for four weeks was performed to assess their effect of reducing IL-1ß levels, joint diameter, and hyperalgesia. Successful induction was demonstrated by significant differences (p<0.05) compare to healthy group in decreased latency time, increased joint diameter and IL-1ß levels. Treatment with deer antler extracts significantly (p<0.05) reduced IL-1ß levels, joint swelling, and increased response hyperalgesia. So deer antler 70% ethanol extract has therapeutic effects on inflammation through reduced IL-1ß in rat model of experimental MIA-induced osteoarthritis. This study suggests that deer antler extract may have a potential novel agent for treating OA.

Keywords: Osteoarthritis, Deer antler, MIA, IL-Iβ

1. Introduction

Osteoarthritis (OA) is a universal type of arthritis and is known as a degenerative joint disorder (Parker & Parker, 2003). OA is a disease with unknown pathophysiology that includes degeneration of the articular cartilage, inflammation in the intra-articular range, and replacement of the subchondral bone (Kean et al., 2004). OA is a complex disease whose etiology and pathology are not completely understood. Modeling of OA prompted by way of monosodium iodoacetate (MIA) is extensively utilized in animal models to determine the success rate of OA in experimental animals (Bendele, 2001).

Intra-articular injection of MIA triggers articular cartilage injury through inhibition of glyceraldehyde-3-phosphate dehydrogenase (G3PDH) activity in chondrocytes, leading to impaired cell demise and glycolysis. These chondrocytes have relations in histopathology with humans and OA. MIA-induced animal models also show a connection between pain and structural changes in the joints (Bar-Yehuda et al., 2009; Grossin et al., 2006).

Previously, it was believed that the damaged articular cartilage of the joints could not be healed. Therefore, the therapy was only to relieve symptoms with analgesic, anti-inflammatory, and lubricant drugs. However, in recent years several researchers have suggested that some supplements may be

able to slow down the progressive degradation of articular cartilage damage and even repair this damage. It can be seen from the decrease in levels of IL-Iβ as an inflammatory agent in bone (Maldonado & Nam, 2013). Some of the recommended supplements are chondroitin sulfate, glucosamine, and collagen hydrolysate (Bello & Oesser, 2006).

Deer antler is one of the animals with high medicinal value. It is a successful folk remedy for strengthening tendons and bones. According to the researchers, there is an imbalance between cartilage erosion and regeneration in patients with osteoarthritis caused by a deficiency of glycosaminoglycan. This substance has an important role in the structural integrity of cartilage. Through the use of cellulose acetate electrophoresis, enzymatic digestion, and chromatography techniques, glycosaminoglycan is isolated from the four different regions of deer antler, including the tip, upper, middle, and base (Sunwoo et al., 1998).

Previous studies have shown that LPS-stimulated RAW 264.7 macrophages can be inhibited (40% and 80%) by 70% ethanol and an aqueous extract of deer antlers from East Kalimantan (Widyowati et al., 2020). This extract also can successfully reduce the pro-inflammatory impact of TNF-stimulated MH7A RA-FLS cells. In *in vivo* studies triggered by zymosan, the injection of deer antler extract treatment significantly reduced clinical arthritis scores and protected synovial and cartilage damage caused by cytokine-mediated immune cells (Cheng et al., 2022). The primary components of deer antler extract are protein, lipid, ash, calcium, collagen, chondroitin sulfate, and glucosamine (Kawtikwar et al., 2010). Hyaluronic acid, keratan sulfate, and dermatan sulfate are also present, but in fewer amounts (Sui et al., 2014). Then, some research indicates that deer antlers can reduce or completely remove osteoarthritis symptoms.

Cytokine IL-1 is one of the important factors for pain response and is the focus of current research. Pronociceptive mediator nerve growth factor (NGF) is critical to pain processing mechanisms and can be regulated more effectively by IL-1. Prostaglandins, IL-6, substance P, and matrix metalloproteinase-9 (MMP-9) are released and activated due to the IL-1 signaling cascade (Moilanen et al., 2015). Therefore, this study was conducted on the efficacy test related to osteoarthritis in rats induced by MIA and treated with deer antler extract. It aimed to analyze changes in the pro-inflammatory cytokine IL-1β in an osteoarthritis rat model in the use of deer antler extract as a therapy for OA.

2. Materials and methods

2.1. Materials used

The solvent extracts were a combination of ethanol p.a (Merck) and aquadest. Other materials were 0.9% physiological salts solution, carboxy methyl cellulose (CMC), monosodium iodoacetate (MIA) (Sigma-Aldrich, Darmstadt, Germany), 10% ketamine (Agrovet, Nicaragua), IL-Iβ ELISA kit (Bioenzy, Germany), glucosamine sulfate (Lipa Pharmaceuticals Ltd, NSW, 2566, Australia), and

deer antler powder of *Rusa unicolor* was collected in the middle of August 2020 in UPTD (Technical Implementation Service Unit) of East Kalimantan, Indonesia, and voucher specimens were deposited at UPTD East Kalimantan, Indonesia.

2.2. Extraction of *Rusa unicolor* Antlers

Deer antler powder of *Rusa unicolor* was received from UPTD of East Kalimantan, Indonesia. The 500 g of deer antler powder was extracted with 70% ethanol (2.0 L x 3) by maceration method at room temperature for 24 hours. Then it filtered with Whatman paper no. 41 and evaporated by a BUCHI rotary evaporator at 40°C and 40 rpm to create a thick extract with a constant weight of 16.8 g (Hariyadi et al., 2019; Widyowati et al., 2021, 2020).

2.3. Ethical Considerations

Twenty-five male Wistar rats (*Rattus norvegicus*) purchased from the Faculty of Pharmacy, Airlangga University, Surabaya, Indonesia, were in good health. Rats aged 3-4 months (200-300 g) were acclimatized for seven days at the Animal Laboratory of the Faculty of Pharmacy, Airlangga University. Rats were maintained in a room at a temperature of 22±3°C, with a relative humidity of 30-70% and 12 hours of light and 12 hours of darkness. Rats were kept in cages with dimensions of 45cm(L) x 30cm(D) x 20cm(H), with each cage consisting of one rat. The cage is waterproof, robust, easy to clean, and free from noise. This research has followed the Guidelines for the Care and Use of Laboratory Animals issued by the National Institutes of Health was revised in 1985 and has been approved by the ethical committee of Faculty of Veterinary Medicine, Universitas Airlangga. (No.2.KE.176.09.2019).

2.4. Osteoarthritis Model

Rats in healthy conditions were injected with MIA at a dose of 4 mg that was dissolved in saline (50 µl) by a 27-G needle intra-articularly (Nagy et al., 2017). It was done below anesthesia with the usage of 10% ketamine (Agrovet, Nicaragua). Then, the swelling condition and joint damage in rats were observed daily (Khotib et al., 2020).

2.5. Animals Grouping

This research was conducted using 25 rats divided into five groups (n = 5 rats). They were (1) the healthy group (S, was given food and water *ad libitum* but not induced by MIA), (2) the negative group (N, rats injected 4 mg MIA dissolved in 50 µl saline and treated 0,5% Carboxy Methyl Cellulose (CMC), (3) the positive group (P, rats injected with MIA 4 mg dissolved in 50 µl saline and treated with glucosamine sulfate (250 mg/kg BW) (Lipa Pharmaceuticals Ltd, NSW, 2566, Australia), (4) low dose group (L, rats injected with 4 mg MIA dissolved in 50 µl saline and treated deer antler

extract 250 mg/kg BW), and (5) the high dose group (H, rats injected with 4 mg MIA dissolved in 50 µl saline and treated deer antler extract 500 mg/kg BW). Intra-articular MIA injection (4 mg MIA dissolved in 50 µl saline) (Sigma-Aldrich, Darmstadt, Germany) was performed in all groups except the healthy group to obtain the OA rat model. After 3 weeks MIA induction, all rats were blood drawn to see IL-1β levels were measured (pre-test), followed by administration of deer antler extract orally once a day and carried out every day for 28 days based on the group. Furthermore, swelling in the rat joint diameter and hyperalgesia were measured with a calibrated micrometer screw and hot plate for seven weeks (at weeks 0, 1, 2, 3, 4, 5, 6, and 7). The latency time in hyperalgesia was measured with a stopwatch. At week 7, blood was taken to measure IL-1β levels in blood serum (as a post-test). Physically, there was an expansion in the diameter of the rat's ipsilateral joint where the injection had been done, and functionally it reduced the latency time (hyperalgesia) to thermal stimulation using the hot plate method (Khotib et al., 2020).

2.6. Hyperalgesia Experiment

Hyperalgesia experiments were carried out in each group at weeks 0, 1, 2, 3, 4, 5, 6, and 7 using the hot plate method (Ugo Basile Hot/Cold Plate 35100, Gemonio, Italy). The hot plate method is a well-established technique that relies on rats' visual cues to communicate their thermal pain and uses uncontrolled rats. The rats were placed one by one on a hot plate at 55±0.5°C for the hyperalgesia test. Heat exposure was carried out until a nociceptive response in rats occurred. The response latency and the time documented using video to observe the nociceptive response has occurred. Nociceptive responses were shown as rats licking their hind legs, rubbing their front legs, or jumping. The rats were taken off the hot plate immediately after the response was observed. Latency response is evaluated manually with a stopwatch (second).

2.7. Joint Swelling Measurement

The measurement of joint swelling was carried out on the right knee of rats after injection of MIA intra-articular in negative, positive, and treatment groups. Measurements had taken on weeks 0, 1, 2, 3, 4, 5, 6, and 7. The diameter of the rat's joint was measured using a screw micrometer that was calibrated in mm (millimeter) to determine swelling that occurs during the development stage of osteoarthritis with a time interval of several days.

2.8. ELISA Analysis of Pro-inflammatory Cytokine IL-1β Levels

The rat blood was taken 1-2 mL in the tail at weeks 3 and 7 and centrifuged at 3000 rpm for 10 minutes to get some of the serum. Proinflammatory cytokine IL-IB levels were measured using a commercial rat IL-1β ELISA kit (Bioenzy, Germany), following the manufacturer's instructions. Then, it analyzed the results using the ELISA reader instrument (EZ-2000).

2.9. Statistical Analysis

The results were performed as means ± standard deviation of 5 rats in each group. All statistical tests were performed using SPSS 23 one-way ANOVA and two-way ANOVA with 95% confidence intervals (p-value <0.05). Then, followed by LSD Post Hoc tests to establish significant differences between groups.

3. Results

3.1. Osteoarthritis Rat Models induced by Monosodium Iodoacetate (MIA)

Osteoarthritis progress in rat models OA induced by intra-articular MIA was observed for 3 weeks and evaluated by way of several parameters, namely, measurement of the joint diameter of rats (physical parameters), observation of the resistance of rat's movement to temperature stimulus by the hot plate method (functional parameters), and measurement of the pro-inflammatory cytokine IL-1β levels (biochemical parameters). The physical parameter utilized to characterize the accomplishment of an osteoarthritis model is estimating the measurement diameter of the joint of rats induced by MIA using a micrometer screw. The results showed that the negative group has a larger joint diameter than the healthy group that was not induced by MIA (p<0.05) are shown in Figure 1. It indicates that the MIA induction was successful to make the OA model in rats.

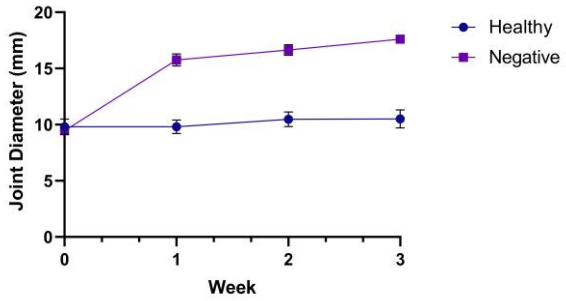

Figure 1. Rat's joint diameter during OA model development. Data are present as Mean ± SD based on n=5 for all groups.

Determination behavior of osteoarthritis can be evaluated from functional parameters or behavior changes in rats through the pain level in an inflammatory state by measuring resistance (hyperalgesia) to responses in the thermal stimulation of the hot plate method application (see Figure 2.). It showed that the negative group has hyperalgesia time in thermal stimulation faster than the healthy group with a significant difference of p<0.05.

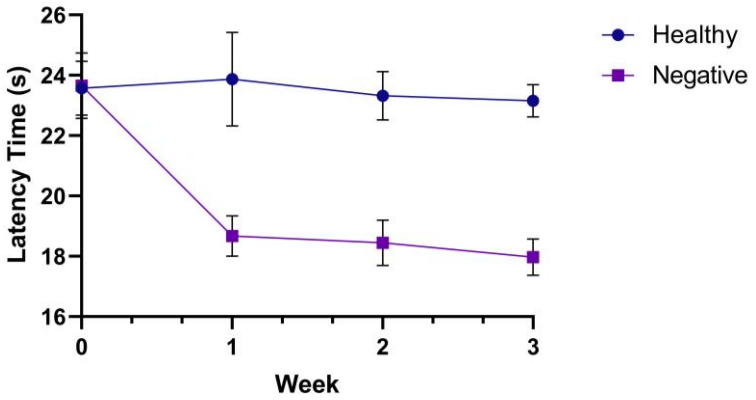

Figure 2. Hyperalgesia in rats during OA model development. Data are present as Mean ± SD based on n=5 for all groups.

On week 3 after MIA injection, rats' blood was taken to measure the levels of pro-inflammatory cytokine IL-1β using ELISA before being treated with deer antler extracts. The result showed a significant difference between the healthy and negative control group (p<0.05) in the pro-inflammatory cytokine IL-1β levels in the rats' blood serum and negative group has 4 times higher cytokine Il-1β levels compared to the healthy group (Figure 3).

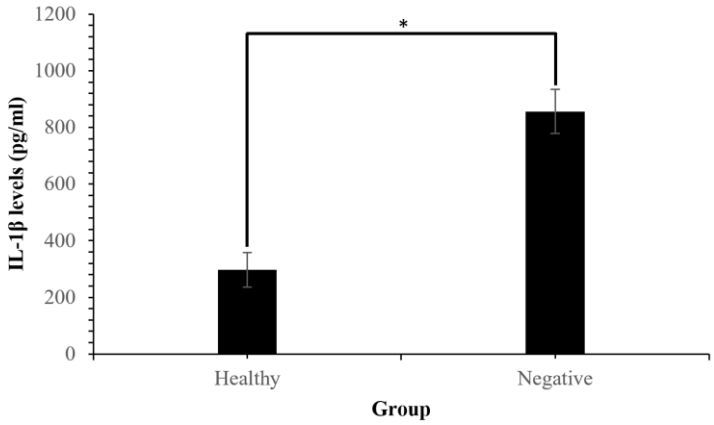

Figure 3. The pro-inflammatory cytokine IL-Iβ levels in rats' blood serum. The IL-Iβ levels were increased after MIA injection as compared to the healthy group (p<0.05). Data are present as Mean ± SD based on n=5 for all groups.

3.2. Effect of Deer Antler Extract Administration on Rats with OA

After the osteoarthritis condition, every rat in the extract group (L & H) was administered 250 mg/kg BW (L) and 500 mg/kg BW (H) of deer antler extract, then for the positive group was administered glucosamine sulfate (250 mg/kg BW) orally. Measurements of rat's right joint diameter and rat resistance time to thermal stimulation were carried out at 4, 5, 6, and 7 weeks, and the week-7 determined pro-inflammatory cytokine IL-1β levels. Based on the analysis result during therapy, the

negative, the extract (L & H), and the positive groups had significant differences ($p<0.05$) in the joint diameter parameter of rats starting from weeks 4 to 7 as shown in Figure 4. It indicated that deer antler extract and glucosamine sulfate reduce joint inflammation in rats injected with MIA.

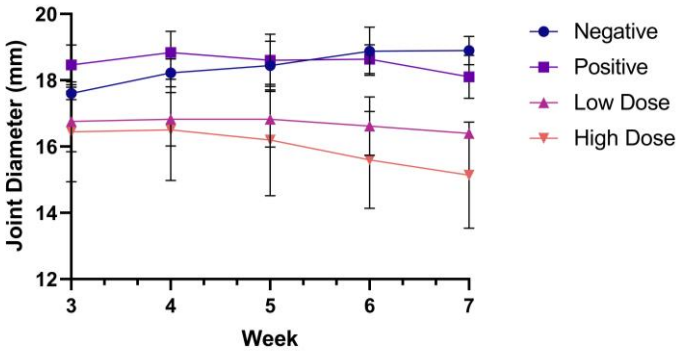

Figure 4. Rat's joint diameter during therapy was significantly decreased after treatment with deer antler extract as compared to the negative group. Data are present as Mean ± SD based on n=5 for all groups.

The negative, extract, and positive groups also showed significant differences ($p<0.05$) from weeks 4 to 7 during therapy in rat heat resistance time (Figure 5).

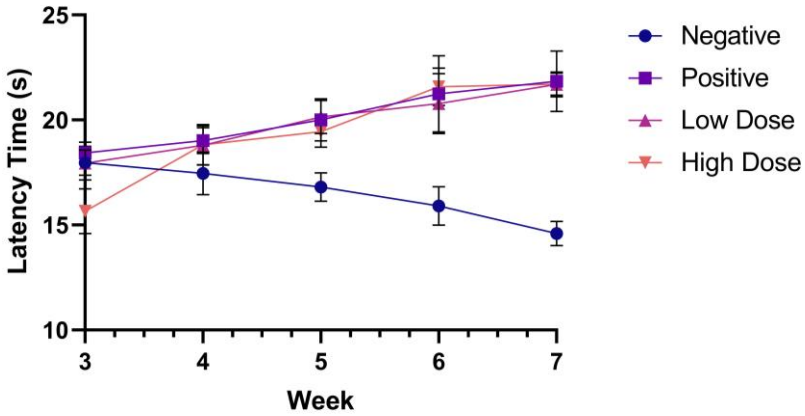

Figure 5. Rat's latency time during therapy was significantly increased after the treatment with deer antler extract as compared to the negative group. Data are present as Mean ± SD based on n=5 for all groups.

In week 7, the rat blood of the extract group was drawn to evaluate altered pro-inflammatory cytokine IL-Iβ levels. The result has shown a significant difference between cytokine IL-Iβ levels before (pre-test) and after therapy (post-test) ($p<0.05$) in the extract group (see Figure 6). Then, it was analyzed using an LSD test that showed the negative and extract groups had a significant decrease in cytokine IL-Iβ levels between the pre-test and post-test.

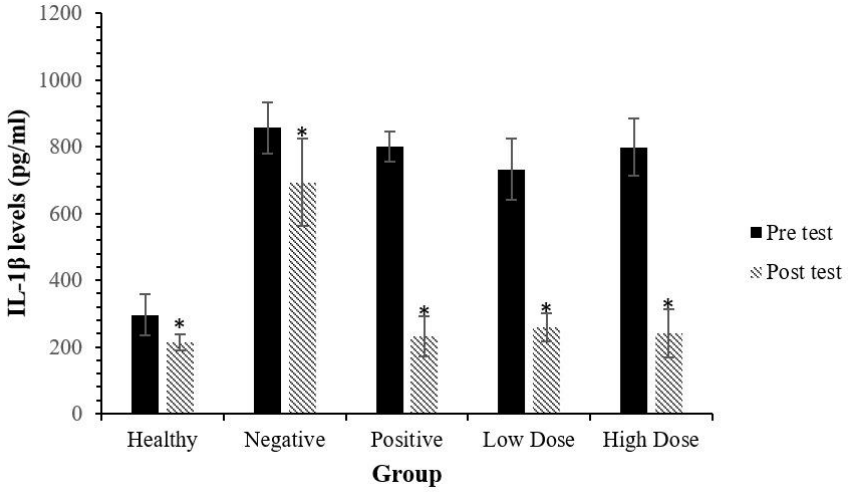

Figure 6. The pro-inflammatory cytokine IL-Iβ levels in rats before and after therapy. The IL-Iβ levels were decreased after treatment as compared with the negative group ($p<0.05$). Data are present as Mean ± SD based on n=5 for all groups. *$p<0.0$

4. Discussion

In this study, we determined pro-inflammatory cytokine IL-Iβ alteration levels in OA rat models that had received deer antler extract therapy. This parameter is one of the main pro-inflammatory cytokines that have an important role in OA (Mahajan et al., 2005). The increased sensitivity of chondrocytes to the release of IL-Iβ makes the OA inflammatory worse condition (Melo-florián, 2011). Cytokines IL-Iβ can be combined by mononuclear cells in excited joints as a major mediator in cartilage matrix reduction and stimulate secretion and synthesis of various degradative enzymes in cartilage.

OA induction was carried out by MIA injection intra-articularly to the right knee of rats. MIA is one of the metabolic inhibitors that damage the vigorous glycolysis pathway to incite cell death and also repress glyceraldehyde-3-phosphate dehydrogenase causing disruption of chondrocyte digestion consequently influencing the cartilage matrix catabolism and responsive oxygen species (ROS) production (Moilanen et al., 2015). Giving MIA injection causes a decrease in proteoglycans, and osteophyte formation, increases cartilage degradation (Nagy et al., 2017), and induces an increase of pro-inflammatory cytokines as well as TNF-α, IL-1β, and matrix metalloproteinases (MMPs) have an active role in the inflammatory process (Moilanen et al., 2015; Pitcher et al., 2016). Osteoarthritis generally occurs within 2-8 weeks after being induced by MIA and it depends on the dose of MIA (1-4 mg). The condition occurs progressively and is similar to lesion osteoarthritis in humans.

The measurement of the right rat joint aims to determine the occurrence of swelling in the tissue as an indicator of inflammation that occurs due to injection by MIA. Based on the analysis result (see

Figure 1), it was shown that there was an increase in the joint diameter of rats injected with MIA which was marked by swelling ($p<0.05$). Swelling can occur due to the mechanism of MIA that can cause an inflammatory process in the form of cell migration, vascular permeability, and capillary extravasation. The appearance of swelling also indicates that the occurrence of inflammation in the synovial is correlated with the occurrence of osteoarthritis and can reduce joint movement in rats (Yamada et al., 2019). The time resistance parameter of the negative has been shown at weeks 1 to 3 after induced MIA. It indicates a sign of soreness development in rats induced by osteoarthritis.

There is a significant difference between the healthy and negative control group ($p<0.05$) in the pro-inflammatory cytokine IL-1β levels in the rats' blood serum (see Figure 3). It happens because inducing MIA has a mechanism of action in synovial cells and chondrocytes to respond to intracellular signals and produce pro-inflammatory mediators as well as proteinases and cytokines (Orita et al., 2011).

Deer antlers are assessed as a valuable traditional Chinese restorative material and have been popular to reinforce the kidney's yang, provide a gist, and fortify the bone capacity (Chen et al., 2015; Widyowati et al., 2020). The leading bioactive constituents in deer antlers are water-soluble proteins that present potency roles in repair and bone formation (REN et al., 2019; Yu et al., 2017). Deer antler extract also has a function as a nominee supplement to inhibit cartilage inflammation and degeneration, as well as repair cartilage homeostasis. This effect can be accomplished by stimulating the useful gene expression included in the arrangement, development, and repair of cartilage, including suppressing the expression of helplessness qualities involved within the osteoarthritis pathophysiology (Yao et al., 2021). Glucosamine has the function to restore components that produce the extracellular matrix of cartilage and prevent further cartilage degradation along with activating IL-1β and chondrocytes activation (Fajardo & Di Cesare 2005).

According to Lee et al., (2014), the administration of deer bone extract effectively protects against bone damage and reduces the number of void erosion. Then Choi et al., (2016) concluded that the administration of deer bone oil extract in the treatment of OA rats has a good effect and recommend it for osteoarthritis repair. Widyowati et al., (2020) reported that 70% ethanol extract of deer antlers showed higher NO inhibitory activity than aqueous extract with a 40% inhibition value. However, both had no cytotoxic effect on LPS 264.7 stimulated RAW. The 70% ethanol extract of deer antlers can stop NO production up to 40% in a concentration of 10 µg/mL. Likewise, aqueous extract of deer antlers can inhibit up to 80% in the same concentration. Therefore, the extract can be considered successful in reducing the expression of inflammatory markers in osteoblasts and maintain osteoblast function. In addition, deer antler extract *in vitro* reduced the pro-inflammatory impact of TNF-stimulated MH7A RA-FLS cells and *in vivo* treatment with deer antler extract reduced clinical arthritis scores and offered protection against synovial and cartilage damage induced by cells. Cytokine-mediated immunity in mice induced by zymosan (Cheng et al., 2022).

Based on these results, there are cytokine IL-Iβ levels decreation after therapy due to the mechanism of deer antler extract and glucosamine sulfate by inhibiting pro-inflammatory cytokine IL-Iβ release. The IL-1β is a major pro-inflammatory cytokine that is yielded in large quantities in osteoarthritis that can cause the expression of factors as well as iNOS, IL-6, and COX-2 (Kucharz et al., 2016). So, we conclude that oral administration of deer antler extract effectively reduces levels of the pro-inflammatory cytokine IL-I β induced by MIA injection in animal models. Hence, the deer antler extract is a potential agent for OA therapy.

5. Conclusion

This study showed that 70% ethanol extract from deer antlers reduced levels of the pro-inflammatory cytokine IL-1β, reduced joint swelling, and increased latency time by MIA injection in animal models. These findings suggest that deer antler extracts 250 and 500 mg/kg BW may be potential treatments for OA as well as glucosamine sulfate.

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Conflict of Interest Statement

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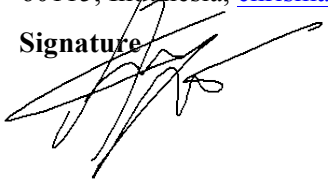

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Kind regards,
Editorial Office
Saudi Pharmaceutical Journal

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rr retno widyowati <rr-retno-w@ff.unair.ac.id>

Submission Confirmation for SPJ-D-22-00677R1

1 message

SPJ <em@editorialmanager.com> Tue, Feb 28, 2023 at 2:13 PM
Reply-To: SPJ <support@elsevier.com>
To: Retno - Widyowati <rr-retno-w@ff.unair.ac.id>

Ms. Ref. No.: SPJ-D-22-00677R1
Title: The Pro-Inflammatory Cytokine IL-Iβ Alteration by Deer (Rusa unicolor) Antler Extract on Osteoarthritis Rat Model
Saudi Pharmaceutical Journal

Dear Retno,

This message is to acknowledge that we have received your revised manuscript for reconsideration for publication in Saudi Pharmaceutical Journal.

You may check the status of your manuscript by logging into the Editorial Manager as an author at https://www.editorialmanager.com/spj/.

Thank you for submitting your work to Saudi Pharmaceutical Journal.

Kind regards,

Editorial Manager
Saudi Pharmaceutical Journal

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Your Submission

1 message

SPJ <em@editorialmanager.com> Wed, Mar 29, 2023 at 4:09 PM
Reply-To: SPJ <support@elsevier.com>
To: Retno - Widyowati <rr-retno-w@ff.unair.ac.id>

Ms. Ref. No.: SPJ-D-22-00677R1
Title: The Pro-Inflammatory Cytokine IL-Iβ Alteration by Deer (Rusa unicolor) Antler Extract on Osteoarthritis Rat Model
Saudi Pharmaceutical Journal

Dear Retno,

I am pleased to inform you that your paper "The Pro-Inflammatory Cytokine IL-Iβ Alteration by Deer (Rusa unicolor) Antler Extract on Osteoarthritis Rat Model" has been accepted for publication in Saudi Pharmaceutical Journal.

Below are comments from the editor and reviewers.

Thank you for submitting your work to Saudi Pharmaceutical Journal.

Yours sincerely,

Aws Alshamsan, Ph.D
Editor-in-Chief
Saudi Pharmaceutical Journal

Comments from the editors and reviewers:

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Re: Author query SPJ SPJ-D-22-00677R1 [230330-008510]

2 messages

Researcher Support <support@elsevier.com> Thu, Mar 30, 2023 at 11:49 AM
Reply-To: Researcher Support <support@elsevier.com>
To: rr-retno-w@ff.unair.ac.id

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Regards,

Elsevier Researcher Support

From: Retno Widyowati
Date: 30/03/2023 04.49 AM

Please enter your name, e-mail address and query below, then click 'Preview and Send'.

Name: Retno Widyowati
E-mail address: rr-retno-w@ff.unair.ac.id

Query:

Hello good afternoon I want to ask, yesterday I received a notification that the article with id manuscript number SPJ-D-22-00677 was declared accepted for publication in this journal. then what is the payment procedure for the publication fee of the article and What is the account number for APC payments? thank you.

Please do not modify the text below this line.

From Corresponding author: Dr Retno - Widyowati

Journal title: Saudi Pharmaceutical Journal

Article title: The Pro-Inflammatory Cytokine IL-Iβ Alteration by Deer (Rusa unicolor) Antler Extract on Osteoarthritis Rat Model

Manuscript number: SPJ-D-22-00677R1

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rr retno widyowati <rr-retno-w@ff.unair.ac.id> Thu, Mar 30, 2023 at 1:21 PM
To: irawaty sholikhah <irawatysholikhah55@gmail.com>

Dikirim dari iPhone saya

Awal pesan yang diteruskan:

> **Dari:** Researcher Support <support@elsevier.com>
> **Tanggal:** 30 Maret 2023 11.49.06 WIB
> **Kepada:** rr-retno-w@ff.unair.ac.id
> **Subjek: Balasan: Author query SPJ SPJ-D-22-00677R1 [230330-008510]**
> **Balas-Ke:** Researcher Support <support@elsevier.com>
>
> [Quoted text hidden]

rr retno widyowati <rr-retno-w@ff.unair.ac.id>

IMPORTANT PLEASE TAKE ACTION, Production has begun on your article [SPJ_1611] in Saudi Pharmaceutical Journal

1 message

r.ganesan.2@elsevier.com <r.ganesan.2@elsevier.com> Fri, Mar 31, 2023 at 2:46 PM
To: rr-retno-w@ff.unair.ac.id

Our reference: SPJ 1611
Article reference: SPJ_SPJ-D-22-00677
Article title: The Pro-Inflammatory Cytokine IL-Iβ Alteration by Deer (Rusa unicolor) Antler Extract on Osteoarthritis Rat Model
To be published in: Saudi Pharmaceutical Journal

Dear Dr. Widyowati,

Congratulations on your accepted paper! Thank you for choosing to publish in Saudi Pharmaceutical Journal. Please read this e-mail carefully as it contains important information.

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CHECK YOUR CONTACT DETAILS:

Please check that your details listed below are correct so we can contact you if needed:

Dr. Retno Widyowati
Department of Pharmaceutical Sciences
Faculty of Pharmacy
Nanizar Zaman Joenoes Building
Campus C, Universitas Airlangga
Surabaya, East Java 60115
Indonesia
Phone: +6281615886978
Fax: not available
E-mail: rr-retno-w@ff.unair.ac.id

YOUR REFERENCE NUMBER:

To help us provide you with the best service, please make a note of your article's reference number SPJ 1611 and quote it in all of your messages to us.

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Thank you for your cooperation.

Kind regards,

Mr. Raja G. Soundhar
DA
Elsevier
E-Mail: r.ganesan.2@elsevier.com

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