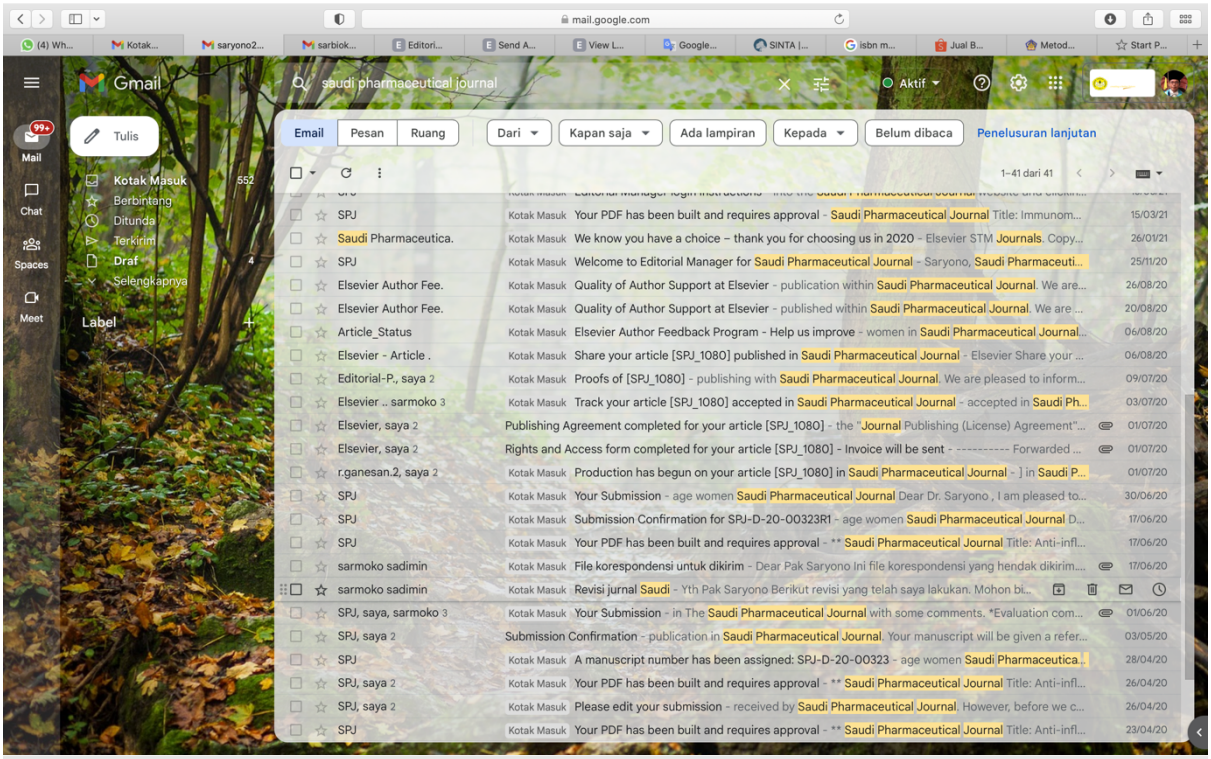
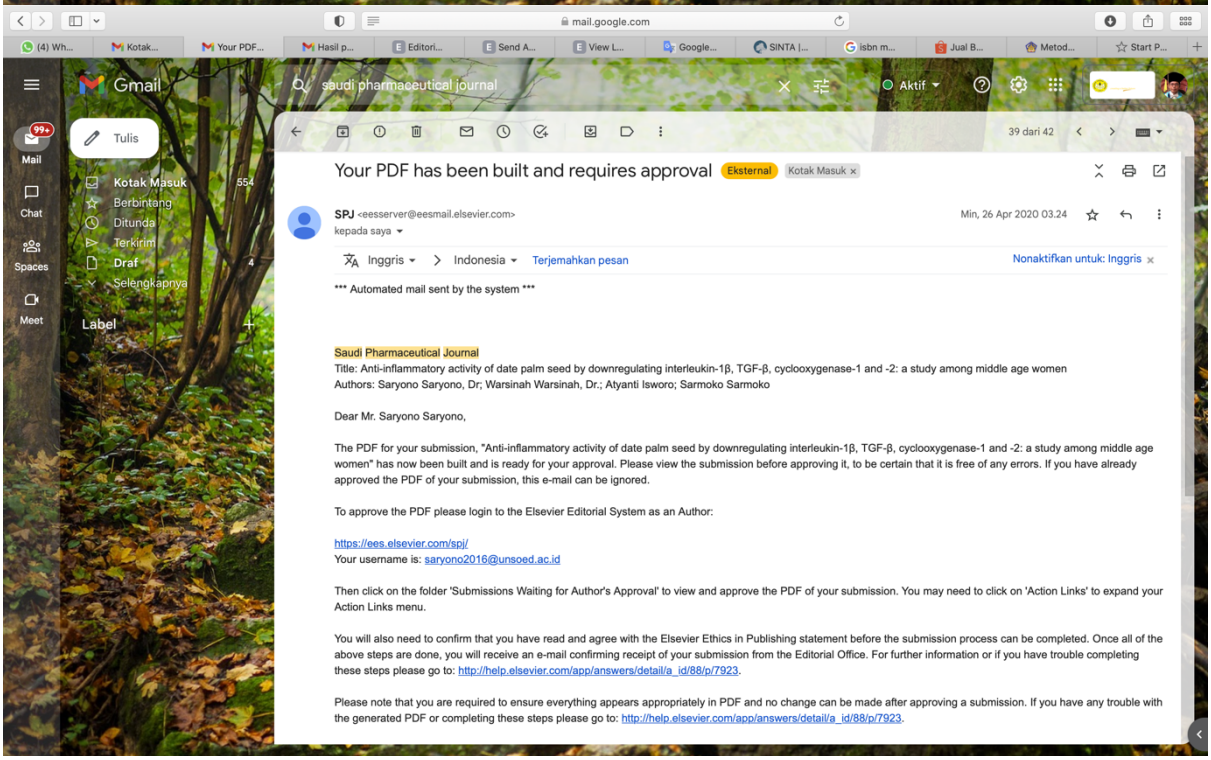
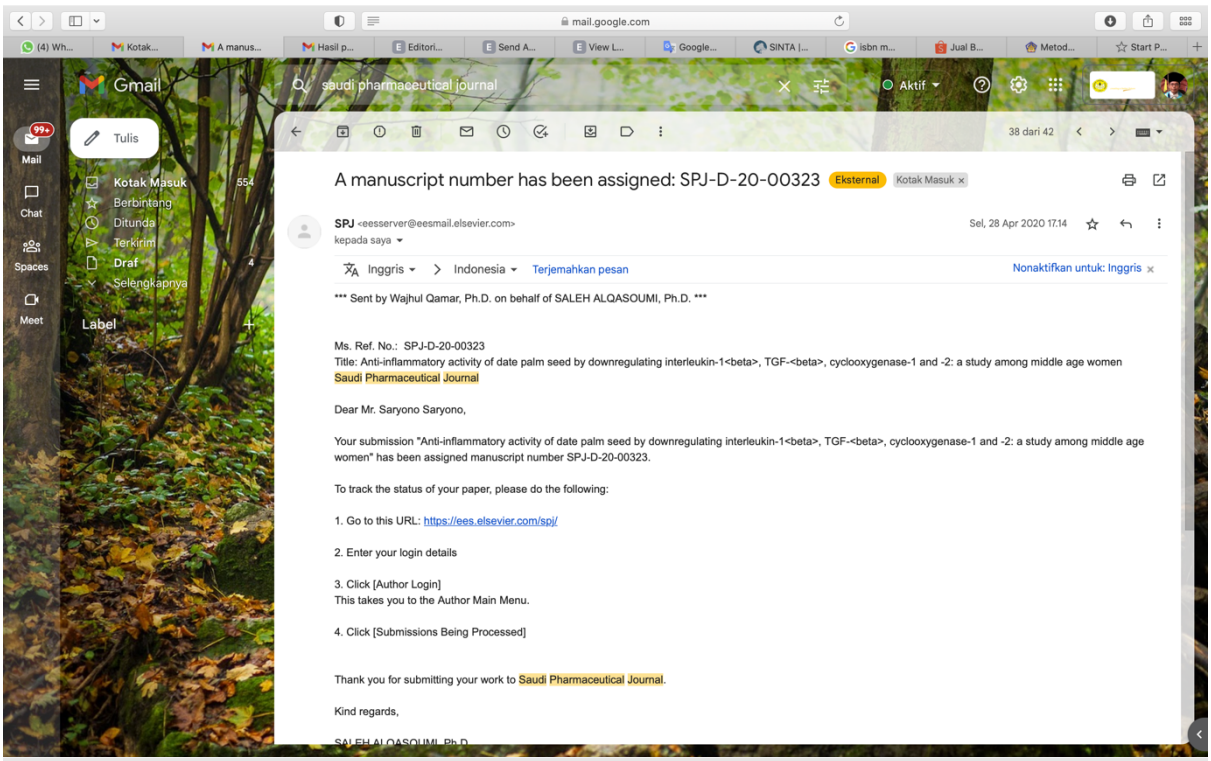
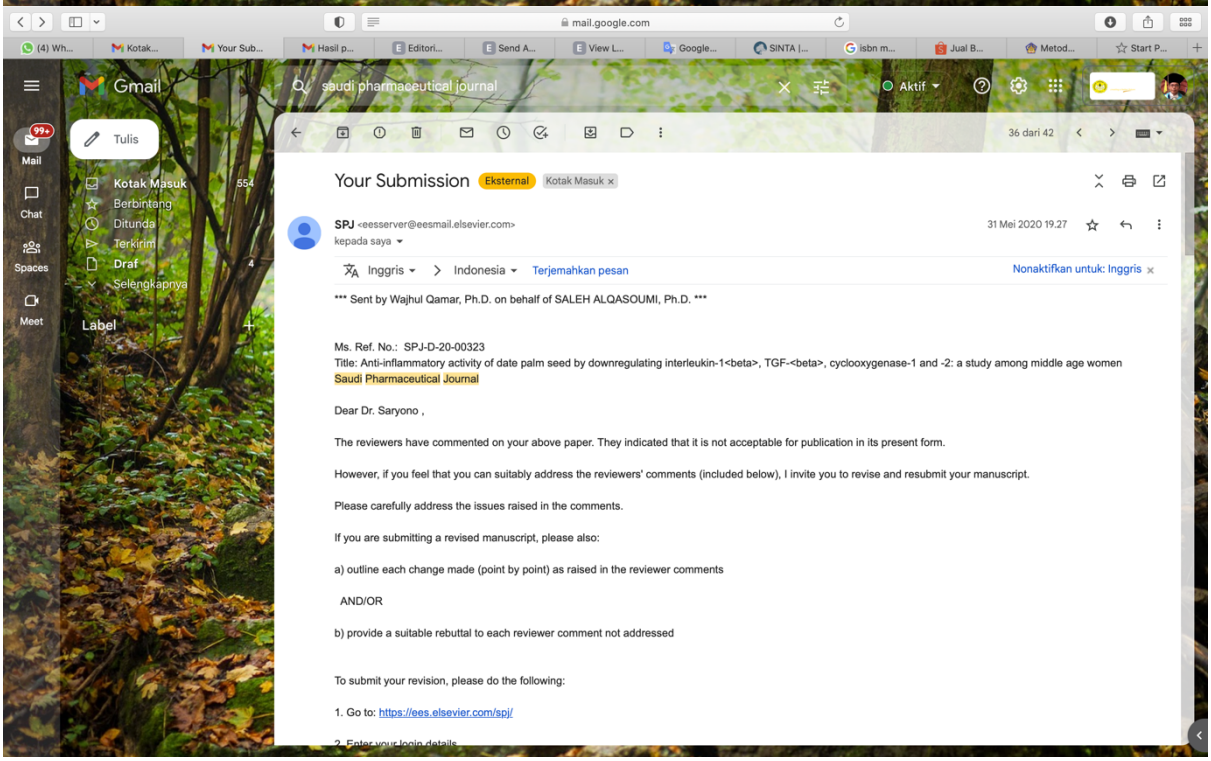
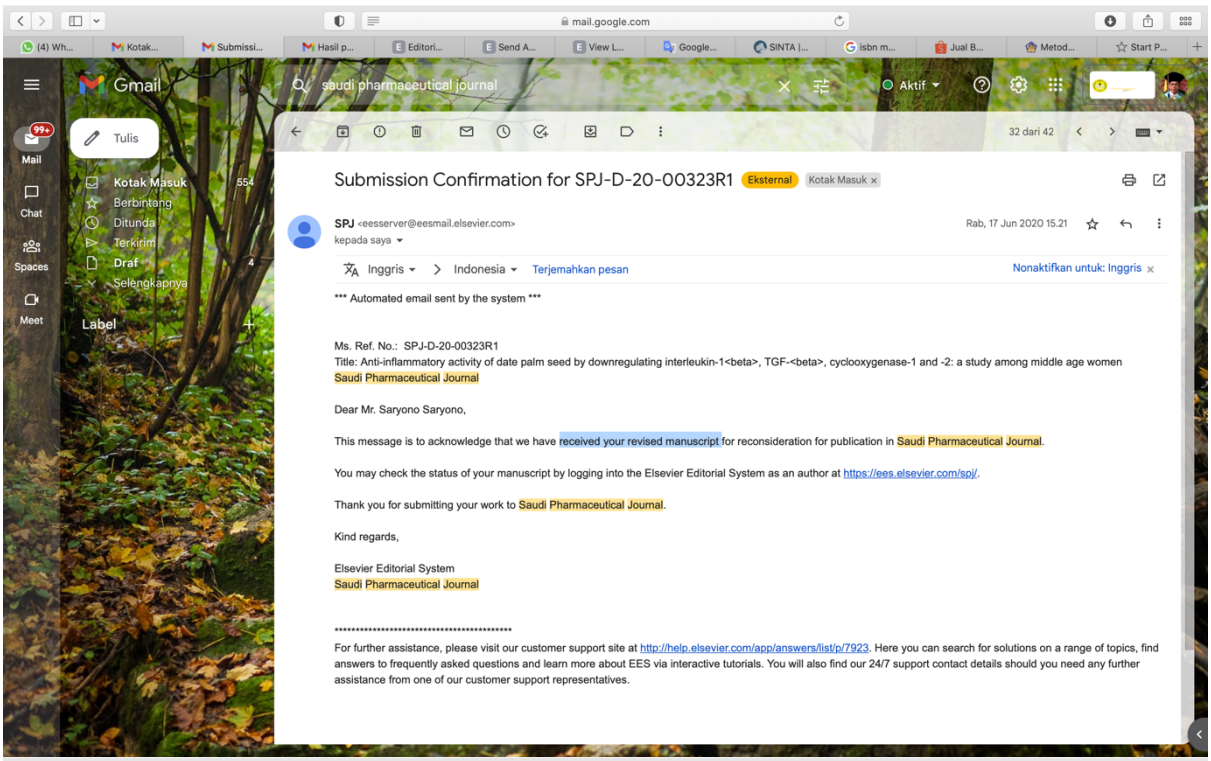
Korespondensi author Saudi Pharmaceutical Journal






mail.google.com
Gmail
saudi pharmaceutical journal
Aktif
Tulis
Kotak Masuk 554
Berbintang
Ditunda
Terkirim
Draft 4
Selengkapnya
Label
38 dari 42
A manuscript number has been assigned: SPJ-D-20-00323 Eksternal Kotak Masuk
SPJ <eesserver@eesmail.elsevier.com>
kepada saya
Sel, 28 Apr 2020 17.14
Inggris Indonesia Terjemahkan pesan
Nonaktifkan untuk: Inggris
*** Sent by Wajhul Qamar, Ph.D. on behalf of SALEH ALQASOUMI, Ph.D. ***
Ms. Ref. No.: SPJ-D-20-00323
Title: Anti-inflammatory activity of date palm seed by downregulating interleukin-1<beta>, TGF-<beta>, cyclooxygenase-1 and -2: a study among middle age women
Saudi Pharmaceutical Journal
Dear Mr. Saryono Saryono,
Your submission "Anti-inflammatory activity of date palm seed by downregulating interleukin-1<beta>, TGF-<beta>, cyclooxygenase-1 and -2: a study among middle age women" has been assigned manuscript number SPJ-D-20-00323.
To track the status of your paper, please do the following:
1. Go to this URL: https://ees.elsevier.com/spj/
2. Enter your login details
3. Click [Author Login]
This takes you to the Author Main Menu.
4. Click [Submissions Being Processed]
Thank you for submitting your work to Saudi Pharmaceutical Journal.
Kind regards,


mail.google.com
Gmail
saudi pharmaceutical journal
Aktif
Tulis
Kotak Masuk 554
Berbintang
Ditunda
Terkirim
Draft 4
Selengkapnya
Label
36 dari 42
Your Submission Eksternal Kotak Masuk
SPJ <eesserver@eesmail.elsevier.com>
kepada saya
31 Mei 2020 19.27
Inggris Indonesia Terjemahkan pesan
Nonaktifkan untuk: Inggris
*** Sent by Wajhul Qamar, Ph.D. on behalf of SALEH ALQASOUMI, Ph.D. ***
Ms. Ref. No.: SPJ-D-20-00323
Title: Anti-inflammatory activity of date palm seed by downregulating interleukin-1<beta>, TGF-<beta>, cyclooxygenase-1 and -2: a study among middle age women
Saudi Pharmaceutical Journal
Dear Dr. Saryono ,
The reviewers have commented on your above paper. They indicated that it is not acceptable for publication in its present form.
However, if you feel that you can suitably address the reviewers' comments (included below), 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
AND/OR
b) provide a suitable rebuttal to each reviewer comment not addressed
To submit your revision, please do the following:
1. Go to: https://ees.elsevier.com/spj/



Gmail

saudi pharmaceutical journal

Tulis

Kotak Masuk 554
Berbintang
Ditunda
Terkirim
Draf 4
Selengkapnya

Label

32 dari 42

Submission Confirmation for SPJ-D-20-00323R1

Eksternal Kotak Masuk ×

SPJ <eesserver@eesmail.elsevier.com> Rab, 17 Jun 2020 15.21
kepada saya

Inggris Indonesia Terjemahkan pesan Nonaktifkan untuk: Inggris ×

*** Automated email sent by the system ***

Ms. Ref. No.: SPJ-D-20-00323R1
Title: Anti-inflammatory activity of date palm seed by downregulating interleukin-1<beta>, TGF-<beta>, cyclooxygenase-1 and -2: a study among middle age women
Saudi Pharmaceutical Journal

Dear Mr. Saryono Saryono,

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 Elsevier Editorial System as an author at https://ees.elsevier.com/spj/.

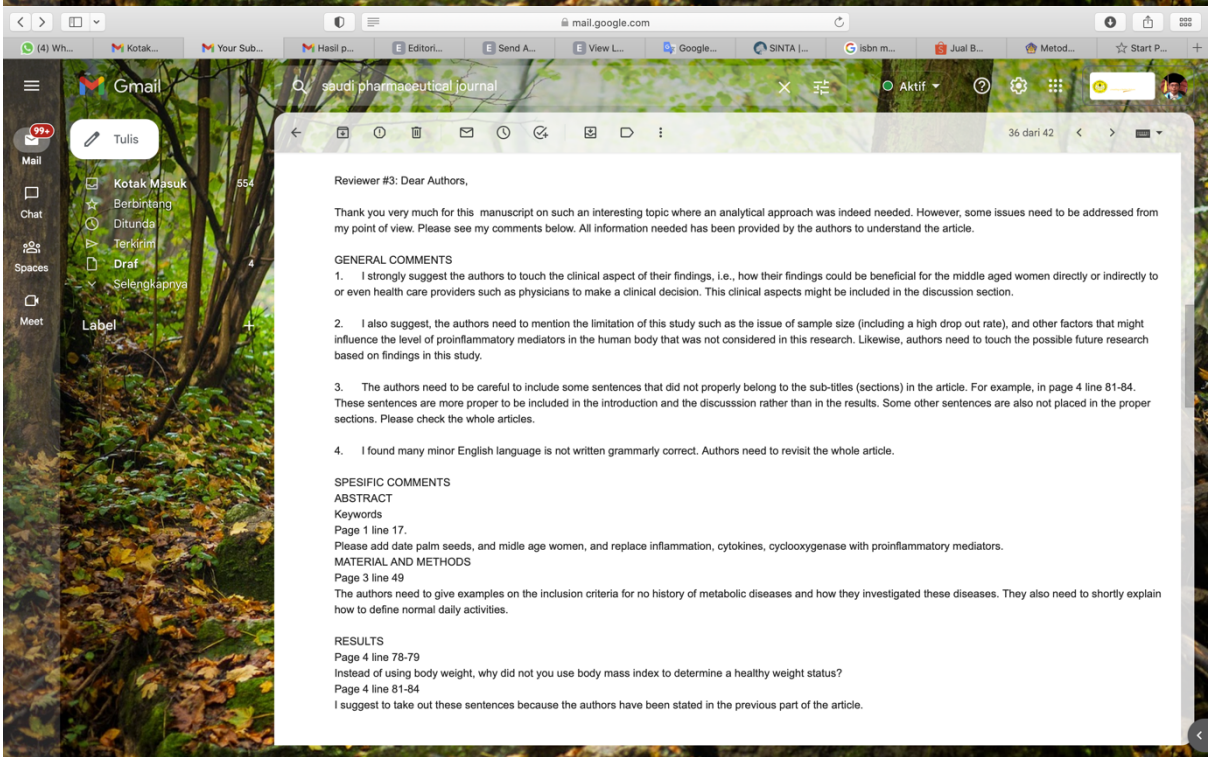
Thank you for submitting your work to Saudi Pharmaceutical Journal.

Kind regards,

Elsevier Editorial System
Saudi Pharmaceutical Journal

****

For further assistance, please visit our customer support site at http://help.elsevier.com/app/answers/list/p/7923. Here you can search for solutions on a range of topics, find answers to frequently asked questions and learn more about EES via interactive tutorials. You will also find our 24/7 support contact details should you need any further assistance from one of our customer support representatives.



Gmail

saudi pharmaceutical journal

Tulis

Kotak Masuk 554
Berbintang
Ditunda
Terkirim
Draf 4
Selengkapnya

Label

36 dari 42

Reviewer #3: Dear Authors,

Thank you very much for this manuscript on such an interesting topic where an analytical approach was indeed needed. However, some issues need to be addressed from my point of view. Please see my comments below. All information needed has been provided by the authors to understand the article.

GENERAL COMMENTS

1. I strongly suggest the authors to touch the clinical aspect of their findings, i.e., how their findings could be beneficial for the middle aged women directly or indirectly to or even health care providers such as physicians to make a clinical decision. This clinical aspects might be included in the discussion section.

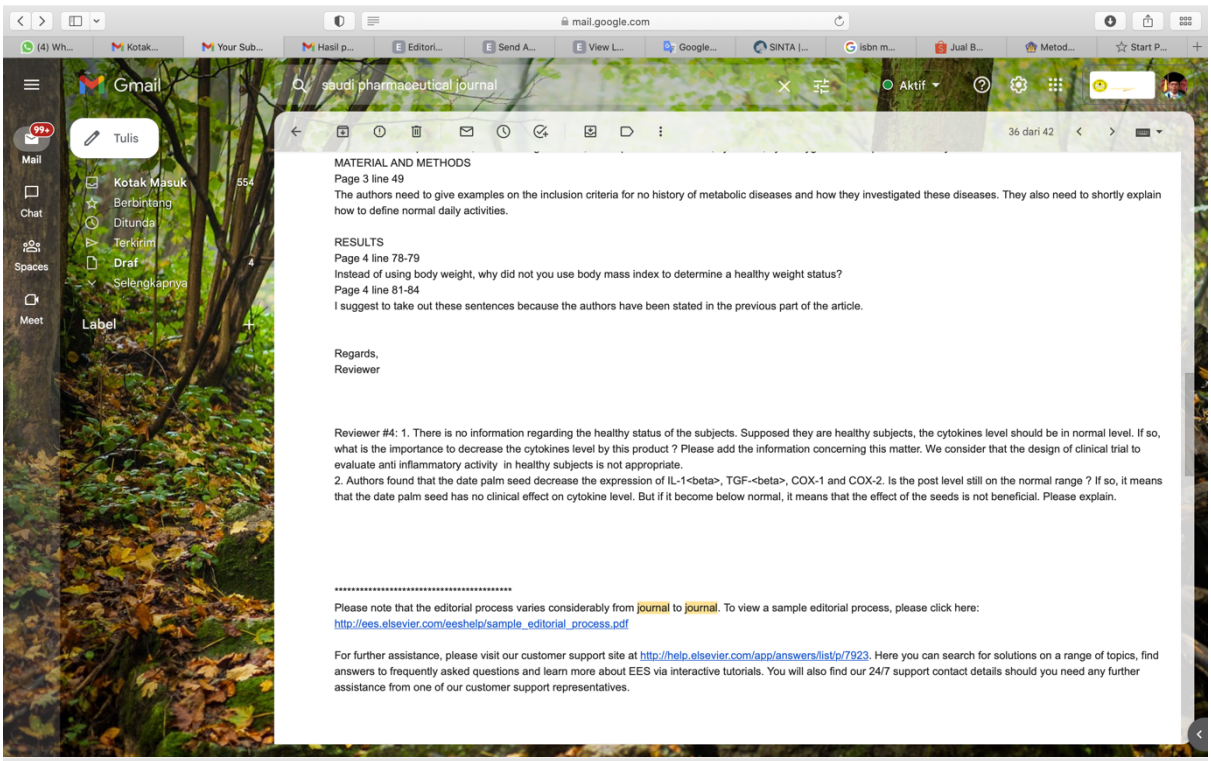
2. I also suggest, the authors need to mention the limitation of this study such as the issue of sample size (including a high drop out rate), and other factors that might influence the level of proinflammatory mediators in the human body that was not considered in this research. Likewise, authors need to touch the possible future research based on findings in this study.

3. The authors need to be careful to include some sentences that did not properly belong to the sub-titles (sections) in the article. For example, in page 4 line 81-84. These sentences are more proper to be included in the introduction and the discusssion rather than in the results. Some other sentences are also not placed in the proper sections. Please check the whole articles.

4. I found many minor English language is not written grammarly correct. Authors need to revisit the whole article.

SPESIFIC COMMENTS
ABSTRACT
Keywords
Page 1 line 17.
Please add date palm seeds, and midle age women, and replace inflammation, cytokines, cyclooxygenase with proinflammatory mediators.
MATERIAL AND METHODS
Page 3 line 49
The authors need to give examples on the inclusion criteria for no history of metabolic diseases and how they investigated these diseases. They also need to shortly explain how to define normal daily activities.

RESULTS
Page 4 line 78-79
Instead of using body weight, why did not you use body mass index to determine a healthy weight status?
Page 4 line 81-84
I suggest to take out these sentences because the authors have been stated in the previous part of the article.



MATERIAL AND METHODS
Page 3 line 49
The authors need to give examples on the inclusion criteria for no history of metabolic diseases and how they investigated these diseases. They also need to shortly explain how to define normal daily activities.

RESULTS
Page 4 line 78-79
Instead of using body weight, why did not you use body mass index to determine a healthy weight status?
Page 4 line 81-84
I suggest to take out these sentences because the authors have been stated in the previous part of the article.

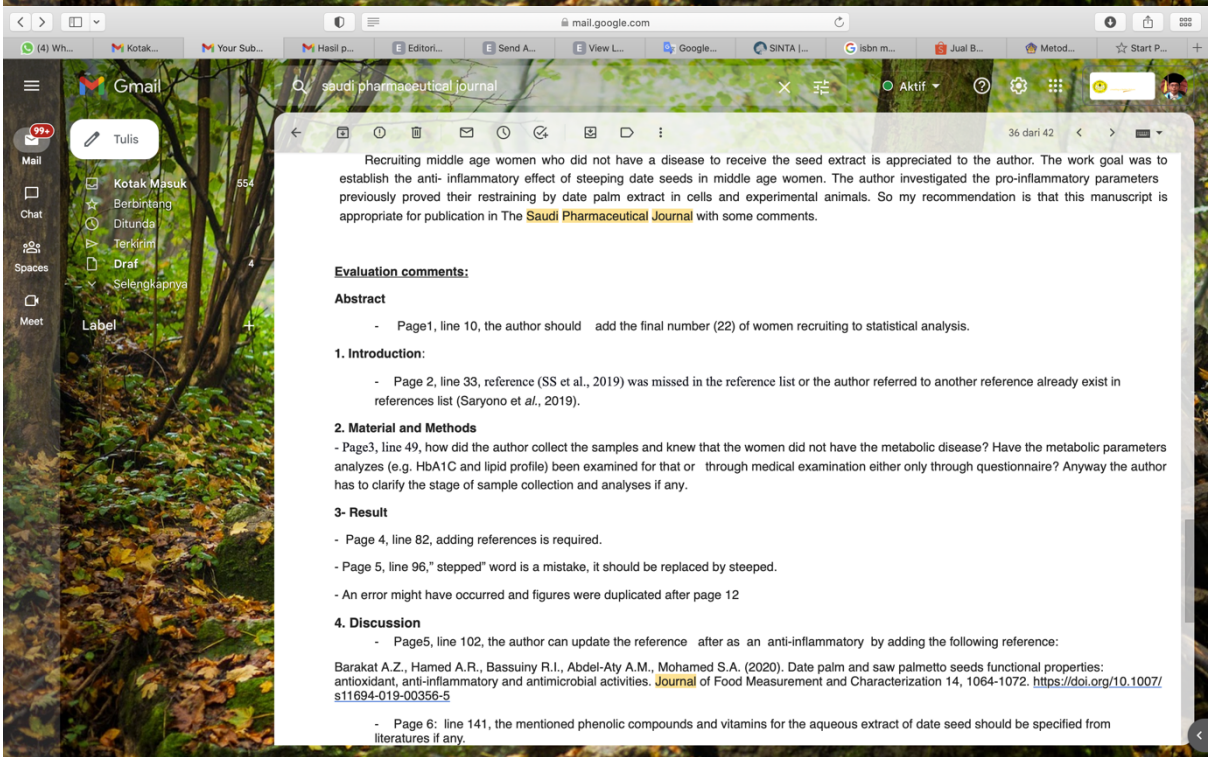
Regards,
Reviewer

Reviewer #4: 1. There is no information regarding the healthy status of the subjects. Supposed they are healthy subjects, the cytokines level should be in normal level. If so, what is the importance to decrease the cytokines level by this product ? Please add the information concerning this matter. We consider that the design of clinical trial to evaluate anti inflammatory activity in healthy subjects is not appropriate.
2. Authors found that the date palm seed decrease the expression of IL-1<beta>, TGF-<beta>, COX-1 and COX-2. Is the post level still on the normal range ? If so, it means that the date palm seed has no clinical effect on cytokine level. But if it become below normal, it means that the effect of the seeds is not beneficial. Please explain.

****

Please note that the editorial process varies considerably from journal to journal. To view a sample editorial process, please click here:
http://ees.elsevier.com/eeshelp/sample_editorial_process.pdf

For further assistance, please visit our customer support site at http://help.elsevier.com/app/answers/list/p/7923. Here you can search for solutions on a range of topics, find answers to frequently asked questions and learn more about EES via interactive tutorials. You will also find our 24/7 support contact details should you need any further assistance from one of our customer support representatives.



Recruiting middle age women who did not have a disease to receive the seed extract is appreciated to the author. The work goal was to establish the anti- inflammatory effect of steeping date seeds in middle age women. The author investigated the pro-inflammatory parameters previously proved their restraining by date palm extract in cells and experimental animals. So my recommendation is that this manuscript is appropriate for publication in The Saudi Pharmaceutical Journal with some comments.

Evaluation comments:

Abstract

- Page1, line 10, the author should add the final number (22) of women recruiting to statistical analysis.

1. Introduction:

- Page 2, line 33, reference (SS et al., 2019) was missed in the reference list or the author referred to another reference already exist in references list (Saryono et *al.*, 2019).

2. Material and Methods

- Page3, line 49, how did the author collect the samples and knew that the women did not have the metabolic disease? Have the metabolic parameters analyzes (e.g. HbA1C and lipid profile) been examined for that or through medical examination either only through questionnaire? Anyway the author has to clarify the stage of sample collection and analyses if any.

3- Result

- Page 4, line 82, adding references is required.

- Page 5, line 96," stepped" word is a mistake, it should be replaced by steeped.

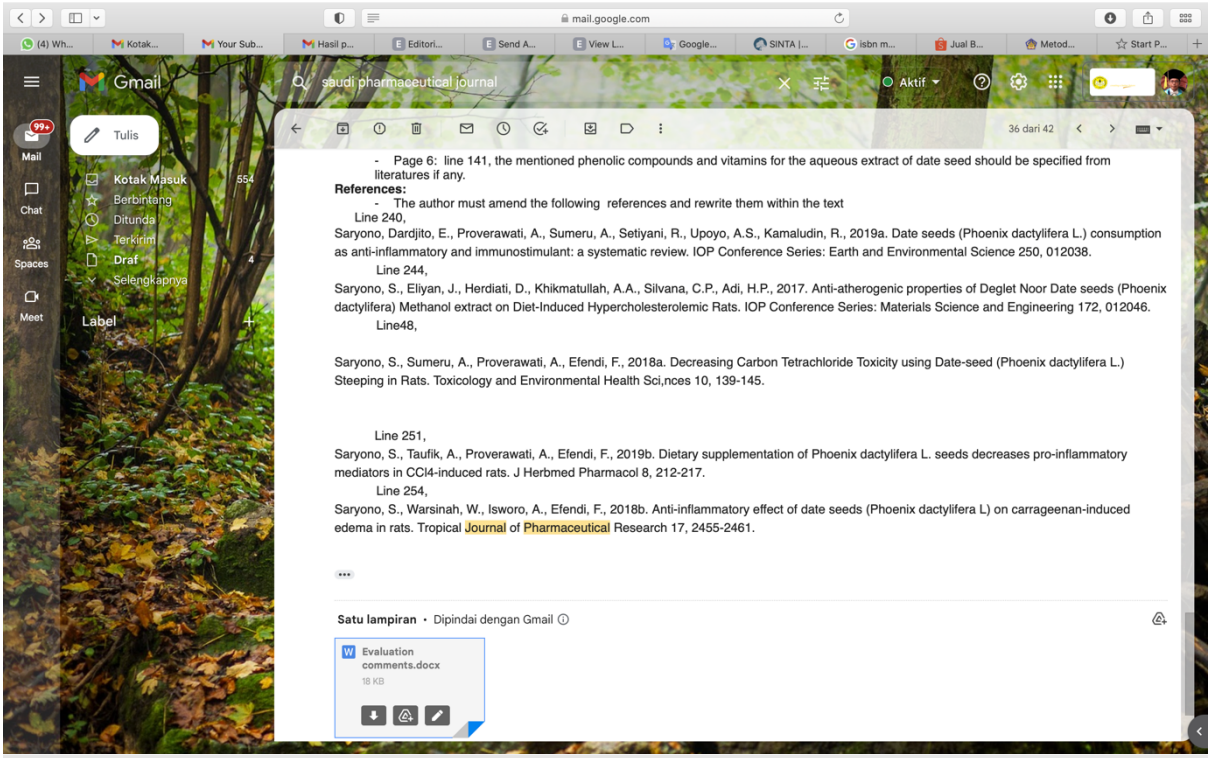
- An error might have occurred and figures were duplicated after page 12

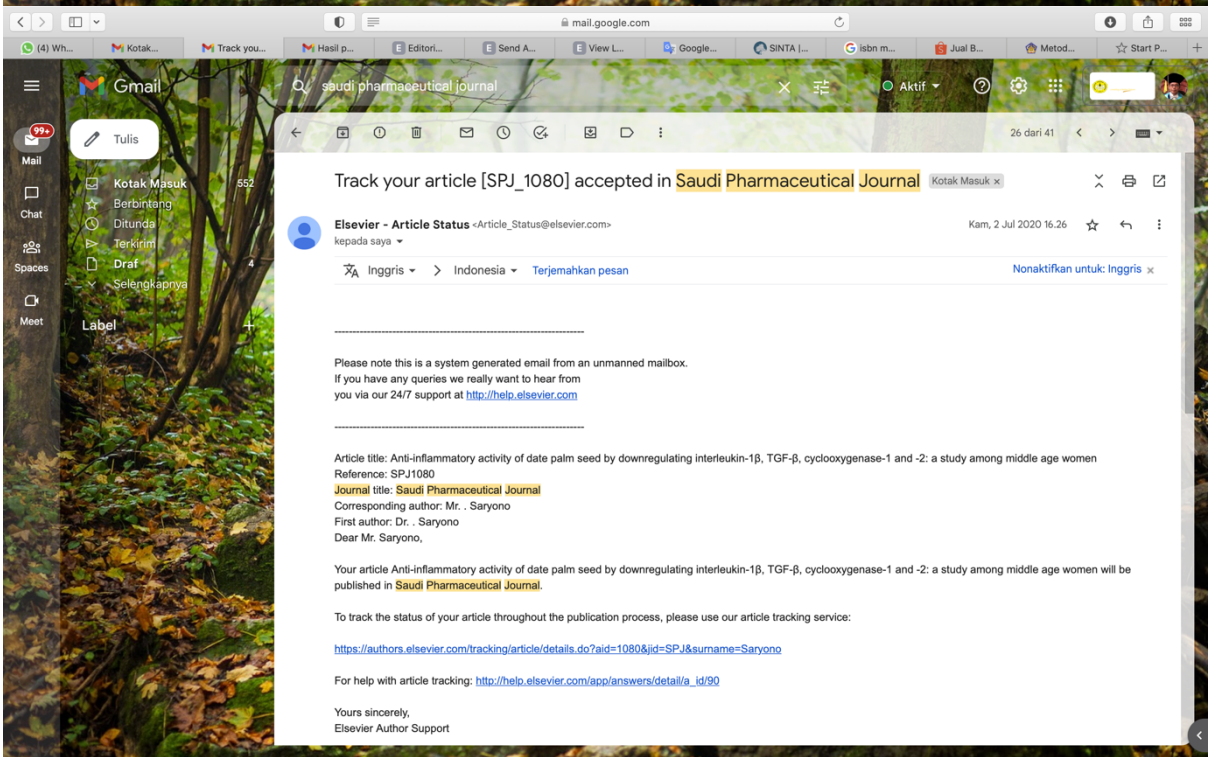
4. Discussion

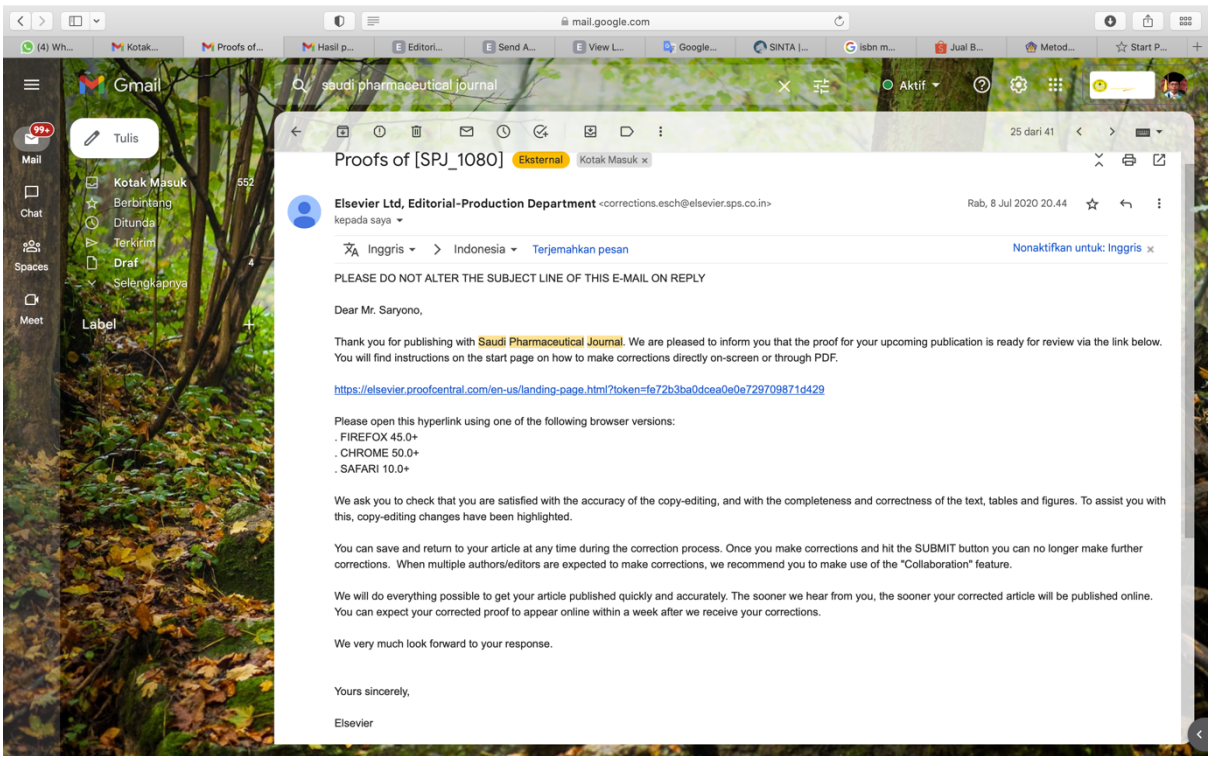
- Page5, line 102, the author can update the reference after as an anti-inflammatory by adding the following reference:

Barakat A.Z., Hamed A.R., Bassuiny R.I., Abdel-Aty A.M., Mohamed S.A. (2020). Date palm and saw palmetto seeds functional properties: antioxidant, anti-inflammatory and antimicrobial activities. Journal of Food Measurement and Characterization 14, 1064-1072. https://doi.org/10.1007/s11694-019-00356-5

- Page 6: line 141, the mentioned phenolic compounds and vitamins for the aqueous extract of date seed should be specified from literatures if any.


mail.google.com
Gmail
saudi pharmaceutical journal
Aktif
Tulis
Kotak Masuk 554
Berbintang
Ditunda
Terkirim
Draf 4
Selengkapnya
Label
Mail
Chat
Spaces
Meet
36 dari 42
- Page 6: line 141, the mentioned phenolic compounds and vitamins for the aqueous extract of date seed should be specified from literatures if any.
References:
- The author must amend the following references and rewrite them within the text
Line 240,
Saryono, Dardjito, E., Proverawati, A., Sumeru, A., Setiyani, R., Upoyo, A.S., Kamaludin, R., 2019a. Date seeds (Phoenix dactylifera L.) consumption as anti-inflammatory and immunostimulant: a systematic review. IOP Conference Series: Earth and Environmental Science 250, 012038.
Line 244,
Saryono, S., Eliyan, J., Herdiati, D., Khikmatullah, A.A., Silvana, C.P., Adi, H.P., 2017. Anti-atherogenic properties of Deglet Noor Date seeds (Phoenix dactylifera) Methanol extract on Diet-Induced Hypercholesterolemic Rats. IOP Conference Series: Materials Science and Engineering 172, 012046.
Line48,
Saryono, S., Sumeru, A., Proverawati, A., Efendi, F., 2018a. Decreasing Carbon Tetrachloride Toxicity using Date-seed (Phoenix dactylifera L.) Steeping in Rats. Toxicology and Environmental Health Sci,nces 10, 139-145.
Line 251,
Saryono, S., Taufik, A., Proverawati, A., Efendi, F., 2019b. Dietary supplementation of Phoenix dactylifera L. seeds decreases pro-inflammatory mediators in CCl4-induced rats. J Herbmed Pharmacol 8, 212-217.
Line 254,
Saryono, S., Warsinah, W., Isworo, A., Efendi, F., 2018b. Anti-inflammatory effect of date seeds (Phoenix dactylifera L) on carrageenan-induced edema in rats. Tropical Journal of Pharmaceutical Research 17, 2455-2461.
Satu lampiran • Dipindai dengan Gmail
Evaluation comments.docx
18 KB


mail.google.com
Gmail
saudi pharmaceutical journal
Aktif
Tulis
Kotak Masuk 552
Berbintang
Ditunda
Terkirim
Draf 4
Selengkapnya
Label
Mail
Chat
Spaces
Meet
26 dari 41
Track your article [SPJ_1080] accepted in Saudi Pharmaceutical Journal
Kotak Masuk
Elsevier - Article Status <Article_Status@elsevier.com>
kepada saya
Kam, 2 Jul 2020 16.26
Inggris
Indonesia
Terjemahkan pesan
Nonaktifkan untuk: Inggris
Please note this is a system generated email from an unmanned mailbox.
If you have any queries we really want to hear from
you via our 24/7 support at http://help.elsevier.com
Article title: Anti-inflammatory activity of date palm seed by downregulating interleukin-1β, TGF-β, cyclooxygenase-1 and -2: a study among middle age women
Reference: SPJ1080
Journal title: Saudi Pharmaceutical Journal
Corresponding author: Mr. . Saryono
First author: Dr. . Saryono
Dear Mr. Saryono,
Your article Anti-inflammatory activity of date palm seed by downregulating interleukin-1β, TGF-β, cyclooxygenase-1 and -2: a study among middle age women will be published in Saudi Pharmaceutical Journal.
To track the status of your article throughout the publication process, please use our article tracking service:
https://authors.elsevier.com/tracking/article/details.do?aid=1080&jid=SPJ&surname=Saryono
For help with article tracking: http://help.elsevier.com/app/answers/detail/a_id/90
Yours sincerely,
Elsevier Author Support


mail.google.com
Gmail
saudi pharmaceutical journal
Aktif
Tulis
Kotak Masuk 552
Berbintang
Ditunda
Terkirim
Draf 4
Selengkapnya
Label
Mail
Chat
Spaces
Meet
25 dari 41
Proofs of [SPJ_1080] Eksternal Kotak Masuk ×
Elsevier Ltd, Editorial-Production Department <corrections.esch@elsevier.sps.co.in>
kepada saya
Rab, 8 Jul 2020 20.44
Inggris Indonesia Terjemahkan pesan
Nonaktifkan untuk: Inggris
PLEASE DO NOT ALTER THE SUBJECT LINE OF THIS E-MAIL ON REPLY
Dear Mr. Saryono,
Thank you for publishing with Saudi Pharmaceutical Journal. We are pleased to inform you that the proof for your upcoming publication is ready for review via the link below. You will find instructions on the start page on how to make corrections directly on-screen or through PDF.
https://elsevier.proofcentral.com/en-us/landing-page.html?token=fe72b3ba0dcea0e0e729709871d429
Please open this hyperlink using one of the following browser versions:
. FIREFOX 45.0+
. CHROME 50.0+
. SAFARI 10.0+
We ask you to check that you are satisfied with the accuracy of the copy-editing, and with the completeness and correctness of the text, tables and figures. To assist you with this, copy-editing changes have been highlighted.
You can save and return to your article at any time during the correction process. Once you make corrections and hit the SUBMIT button you can no longer make further corrections. When multiple authors/editors are expected to make corrections, we recommend you to make use of the "Collaboration" feature.
We will do everything possible to get your article published quickly and accurately. The sooner we hear from you, the sooner your corrected article will be published online. You can expect your corrected proof to appear online within a week after we receive your corrections.
We very much look forward to your response.
Yours sincerely,
Elsevier

Anti-inflammatory activity of date palm seed by downregulating interleukin-1β, TGF-β, cyclooxygenase-1 and -2: a study among middle age women

Abstract

The prevalence of degenerative diseases increases with age. Various factors can trigger cells injury and causing inflammation. This study aims to examine the anti-inflammatory mechanisms of steeping date seeds in middle age women. A quasi-experimental design with a pre- and post-test approach was conducted in 30 healthy middle-aged women to evaluate anti-inflammatory effect of the steeped of date palm seed, consumed 2.5 grams per day (in 250 mL water) for 14 days. The final number (22 subjects) of recruited women were included to statistical analysis. The level of IL-1β, TGF-β, IL-6, TNF-α, IL-12, COX-1, COX-2, PGE2 was determined by using ELISA. The results showed that the expression of IL-1β, TGF-β, COX-1, COX-2 had significantly decreased after consuming date palm seed. Steeped of date seed acts as an anti-inflammatory by downregulating the expression of key proinflammatory mediators.

Keywords: date palm seeds, middle age women, proinflammatory mediators

1. Introduction

Chronic and degenerative diseases in middle-aged women are increasing, such as cancer, cardiovascular disease, and metabolic disease. These diseases are the leading cause of death and disability disorders in developing countries. The current research showed that large changes in behavior patterns in decreasing consumption of vegetables

and fruits plays an important role in increasing the incidence of chronic and degenerative diseases (Kim et al., 2018). Bioactive compounds in fruits and vegetables such as polyphenols, flavonoids, anthocyanins, micronutrients, minerals and vitamins are known to have antioxidant and anti-inflammatory activities (Sofi and Dinu, 2016). Therefore, they have preventive and therapeutic potencies against the disease (Aguilera et al., 2016; Wang et al., 2016).

Date palm (*Phoenix dactilyfera* L.) seed is one of the rich source of polyphenols and flavonoids (Djaoudene et al., 2019). Date seeds have been extensively investigated for pharmacological activities such as anti-inflammatory (Saryono et al., 2018), immunostimulant (Saryono et al., 2019), antidiabetic (El-Fouhil et al., 2010), antibacterial (Chinelo et al., 2019), antiviral (Jassim and Naji, 2010), antioxidant (Bouhlali et al., 2015; Djaoudene et al., 2019; Habib and Ibrahim, 2011; Platat et al., 2019). In previous studies, we showed that that date palm seeds are proven to work as anti-inflammatory (Saryono et al., 2019; Saryono et al., 2019b, 2019a), and antiatherogenic (Saryono et al., 2017). Recent study showed that various compounds act as anti-inflammatory by metabolomic approach (Abdul-Hamid et al., 2019). Anti-inflammatory effect of date seeds in human has not been much studied, especially in middle-aged women. This study aims to examine the anti-inflammatory mechanisms of steeped date palms in middle-aged women.

2. Material and Methods

2.1. Study Design and Participants

The study was a quasi-experimental design with a pre and post-test without control group design. A total of 30 randomly selected middle-aged women living at Gununglurah, Cilongok District, Banyumas Regency was recruited in this study. Inclusion criteria were

age between 45-60, no history of metabolic disease (by measuring blood glucose and total cholesterol), and having normal daily activities. They are living in farming community that perform domestic tasks as a housewife (*this sentence to explain normal daily activities, because the reviewer ask how to define normal daily activity*). In this study, each subject received a single dose (2.5 grams seed powder) per day for 14 days. Seed powder was consumed by steeping them with 250 mL boiling water. Subjects signed an informed consent before participate this study. The study was conducted after obtaining approval of ethical clearance from the medical research ethics committee of the Faculty of Medicine University of Negeri Sebelas Maret Surakarta, number: 541/IV/HREC/2019.

2.2. Preparation of date seeds powder

Deglet Nour dates (collected from Tunisia) were obtained from market at Purwokerto, Banyumas Regency, Indonesia. Seeds were manually separated from the date flesh, cleaned with water, selected for the good seed, then dried for one day in sunlight. Dried seeds were roasted at medium temperature, then crushed in a blender and filtered to obtain a fine powder.

2.3 Measurement pro-inflammatory mediators

A 3 ml blood sample was taken through the median cubital vein before and after treatment. The level of IL-1β, TNF-α, IL-6, IL-12, TGF-β, COX-1, COX-2, and prostaglandin E2 were examined by ELISA kit (BT Laboratories, Shanghai) based on the manufacturer's protocol, using an ELISA machine Reader (Labotrone, Germany).

2.4. Statistical analysis

The mean score was compared between before and after treatment. All data were presented as mean ± SEM. Differences in scores before and after treatment were analyzed

by student t-test. The graph pad software (GraphPad Prism, San Diego, CA) was used to analyze statistical and graphical data. A p value < 0.05 was considered significant.

3. Results

3.1. Characteristics of participants

Thirty subjects were initially recruited. However, eight subjects cannot take the treatment completely. Finally, 22 subjects were included in the statistical analysis. The age of subjects ranged was from 51-57 years. They had a healthy weight status with an average body mass index of 24 kg/m^2 (Table 1), blood glucose below 140 mg/dL, and total cholesterol below 200 mg/dL.

3.2 Expression of proinflammatory cytokines

~~Date palm seeds have been investigated as anti-inflammatory activities both *in vitro* and *in vivo*. In this study, we evaluated the anti-inflammatory effects of date palm seeds on humans by observing the cytokines involved in inflammation, namely IL-1β, TNF-α, IL-6, IL-12, and TGF-β.~~ We found that expression of IL-1β and TGF-β reduced significantly after consumption of date palm seeds compared to pretreatment, whereas expression of TNF-α, IL-12, and IL-6 did not show significant changed (Figure 1). This result suggests the date palm seed can reduce IL-1β and TGF-β that produced in healthy middle-aged women.

3.3. The role of dates palm seed on cyclooxygenase pathway

The cyclooxygenase (COX) pathway is important in arachidonic acid metabolism related to the inflammatory process (Levick et al., 2007). Therefore, we checked the expression of COX-1, COX-2, as well as prostaglandin E2 level that those mediators are

important markers on COX pathway. A significant decrease in the level of COX-1, COX-2 and PGE2 was observed in post-treatment compared with pre-treatment of steeped of date palm seeds (Figure 2). Interestingly, the expression of COX-2 was substantially decreased ($p < 0.001$), which indicates that the inducible expression of COX-2 in middle-aged women was downregulated by consumption of date palm seeds.

4. Discussion

Several studies have been conducted to examine the potential of date palm seeds as an anti-inflammatory (Barakat et al., 2020) *in vitro* and *in vivo* (Djaoudene et al., 2019; Maqsood et al., 2020; SY et al., 2018); however, there is no anti-inflammatory activity studies of date palm seeds in humans, especially in women middle age. In this study, we found that the expression of IL-1β, TGF-β, COX-1 and COX-2 decreased after administration of date palm seeds to women of middle age.

Towards the elderly, more middle age women experience an increase in free radicals due to various factors such as aging, food, pollution and excessive activity. Free radicals in the body will cause oxidation in normal cells, causing inflammation and disease (Sies, 2018; Suleman, 2018). Lymphocytes and other immune cells will produce cytokines such as IL-1β and TGF-β when there are inflammatory stimuli.

Interleukin-1β is highly elevated in chronic diseases such as obesity, osteoarthritis, and gout (Dinarello, 2011). IL-1β affects lymphocytes and macrophages, induces the formation of prostaglandins, colony stimulating factors and other cytokines (Wojdasiewicz et al., 2014). IL-1β expression is stimulated by various stimuli including sterile stimulus through the formation of inflammasomes (Shi et al., 2015). Since middle age women have developed an accumulation of agents which can activate the

inflammasome formation, such as cholesterol and uric acid (Qin et al., 2014), it is interesting finding that date palm seed can reduce the expression of IL-1β. Another interesting aspect is to reveal the role of date palm seeds in inhibiting IL-1β expression, which can be further investigated in the future.

Although TGF-β initially was believed as anti-inflammatory cytokines, this cytokine also has known involved in inducing inflammatory Th17 cells differentiation (Yoshimura et al., 2010). While the role TGF-β in inflammation is still unclear, the date palm seed showed ability to decrease TGF-β expression.

Cyclooxygenase pathway has been well-established for their role in inflammation (Hanna and Hafez, 2018). Conversion of phospholipids to arachidonic acid is mediated by the enzyme cyclooxygenase. While COX-1 is expressed constitutively and plays a role in the protection of the gastric mucosa, COX-2 is expressed inducibly by an inflammatory stimulus (Urban, 2000). Various stimuli can induce COX-2 expression, which may occur with increasing age. In this study, COX-2 expression greatly decreased after administration of date palm seeds to middle age women. Both enzymes induce the conversion of arachidonic acid into prostaglandins, e.g. PGE2 which plays a role in vasodilation of blood vessels and increased vascular endothelial permeability (Kawahara et al., 2015). The expression of both COX enzymes is inhibited by date palm seeds, thereby decreasing the production of PGE2.

The limitation of this study is high of dropping out because the subject did not complete the consumption of date palm seed powder as assigned. In addition, we did not record the food that subjects consumed in detail, which might affect the level of proinflammatory mediators in the human body. In this study, consumption of date palms seed powder is significantly decreased IL-1β levels. Meanwhile, IL-1β formation is

mediated by inflammasome activation (Lopez-Castejon and Brough, 2011). Research to determine whether date palms can inhibit the formation of inflammasome will be an interesting subject for further research.

Date palm seeds may work as an anti-inflammatory and improve the performance of the immune system (Saryono et al., 2019). Date palm seeds can work to suppress NF-κB, COX-1 and COX-2 enzymes thus suppressing pro-inflammatory mediators (Rahmani et al., 2014). The anti-inflammatory activity of the aqueous extract of date palm seeds is related to components of polyphenols such as caffeoyl hexoside, 5-O-caffeoyl shikimic acid isomers, hydrocaffeic acid, and isorhamnetin (Thouri et al., 2017; John and Shahidi, 2019). This ingredient has also been proven safe for liver and kidney (El Fouhil et al., 2011), thus it can be used as regular consumption in middle age women to maintain health status, to improve immune systems, and to prevent chronic diseases. Clinical implications obtained from this study are the physicians may suggest the steeped of palm seed powder as a functional beverage.

5. Conclusion

Dates seeds can act as an anti-inflammatory by reducing interleukin-1β, TGF-β, cyclooxygenase-1 and -2 expression. Consumption of date palm seeds regularly can increase the body's immunity as well as to prevent chronic diseases.

APPENDICES

Availability of Data and Materials

All data generated or analyzed during this study are included in this manuscript. Raw data are available from the corresponding author on a reasonable request.

Acknowledgments

The authors are grateful to Dr. Hernayanti for her laboratory support; to Lita Kusuma and Nina Setiowati for critically reviewing the manuscript.

Funding

This study was supported by The Ministry of Research Technology and Higher Education of Indonesia through PDUPT funding.

Conflict of interest

The authors declare that they have no conflict of interest.

Authors' contributions

SY, W, AI conceived and designed the experiments; SY, W, AI performed the experiments; SM analyzed the data; SY, W, AI contributed chemicals/reagents/materials/analysis tools; SY and SM wrote the paper. The authors read and approved the final manuscript.

References

Abdul-Hamid, N.A., Abas, F., Ismail, I.S., Tham, C.L., Maulidiani, M., Mediani, A., Swarup, S., Umashankar, S., 2019. 1H-NMR-based metabolomics to investigate the effects of Phoenix dactylifera seed extracts in LPS-IFN-γ-induced RAW 264.7 cells. Food Res. Int. 125, 108565. doi:10.1016/j.foodres.2019.108565

Aguilera, Y., Martin-Cabrejas, M.A., González de Mejia, E., 2016. Phenolic compounds in fruits and beverages consumed as part of the mediterranean diet: their role in prevention of chronic diseases. Phytochem. Rev. 15, 405–423. doi:10.1007/s11101-015-9443-z

Barakat A.Z., Hamed A.R., Bassuiny R.I., Abdel-Aty A.M., Mohamed S.A., 2020. Date palm and saw palmetto seeds functional properties: antioxidant, anti-inflammatory and antimicrobial activities. Journal of Food Measurement and Characterization 14, 1064-1072. doi:10.1007/s11694-019-00356-5

Bouhlali, E. dine T., Alem, C., Ennassir, J., Benlyas, M., Mbark, A.N., Zegzouti, Y.F.,

2015. Phytochemical compositions and antioxidant capacity of three date (Phoenix dactylifera L.) seeds varieties grown in the South East Morocco. Journal of the Saudi Society of Agricultural Sciences. doi:10.1016/j.jssas.2015.11.002

Chinelo, C.E., Ezinwanne, N.E., Chizoba, A.O., Martina, C.A., Chineye, N.U., Somtochukwu, A.E., Uchechi, L.I., 2019. Evaluation of Antimicrobial Activities of Crude Methanol Extract of Phoenix dactylifera Seeds on Clinical Isolates of Different Strains of E. coli. Int. J. Biochem. Res. Rev. 1–7. doi:10.9734/ijbcrr/2019/v25i130066

Dinarello, C.A., 2011. Interleukin-1 in the pathogenesis and treatment of inflammatory diseases. Blood 117, 3720–3732. doi:10.1182/blood-2010-07-273417

Djaoudene, O., López, V., Cásedas, G., Les, F., Schisano, C., Bachir Bey, M., Tenore, G.C., 2019. Phoenix dactylifera L. seeds: a by-product as a source of bioactive compounds with antioxidant and enzyme inhibitory properties. Food Funct 10, 4953–4965. doi:10.1039/c9fo01125k

El Fouhil, A.F., Ahmed, A.M., Darwish, H.H., Atteya, M., Al-Roalle, A.H., 2011. An extract from date seeds having a hypoglycemic effect. Is it safe to use? Saudi Med J 32, 791–796.

El-Fouhil, A.F., Ahmed, A.M., Darwish, H.H., 2010. Hypoglycemic effect of an extract from date seeds on diabetic rats. Saudi Med J 31, 747–751.

Habib, H.M., Ibrahim, W.H., 2011. Effect of date seeds on oxidative damage and antioxidant status in vivo. J. Sci. Food Agric. 91, 1674–1679. doi:10.1002/jsfa.4368

Hanna, V.S., Hafez, E.A.A., 2018. Synopsis of arachidonic acid metabolism: A review. J. Advanc. Res. 11, 23–32. doi:10.1016/j.jare.2018.03.005

Jassim, S.A.A., Naji, M.A., 2010. In vitro Evaluation of the Antiviral Activity of an Extract of Date Palm (Phoenix dactylifera L.) Pits on a Pseudomonas Phage. Evid. Based Complement. Alternat. Med. 7, 57–62. doi:10.1093/ecam/nem160

John, J.A., Shahidi, F., 2019. Phenolic content, antioxidant and anti-inflammatory activities of seeds and leaves of date palm (Phoenix dactylifera L.). Journal of Food Bioactives. 5:120-130. doi:10.31665/JFB.2019.5179.

Kawahara, K., Hohjoh, H., Inazumi, T., Tsuchiya, S., Sugimoto, Y., 2015. Prostaglandin E2-induced inflammation: Relevance of prostaglandin E receptors. Biochim. Biophys. Acta 1851, 414–421. doi:10.1016/j.bbalip.2014.07.008

Kim, E.K., Kim, H., Kwon, O., Chang, N., 2018. Associations between fruits, vegetables, vitamin A, β-carotene and flavonol dietary intake, and age-related macular degeneration in elderly women in Korea: the Fifth Korea National Health and Nutrition Examination Survey. Eur. J. Clin. Nutr. 72, 161–167. doi:10.1038/ejcn.2017.152

Levick, S.P., Loch, D.C., Taylor, S.M., Janicki, J.S., 2007. Arachidonic acid metabolism as a potential mediator of cardiac fibrosis associated with inflammation. J. Immunol. 178, 641–646. doi:10.4049/jimmunol.178.2.641

Lopez-Castejon G, Brough D. 2011. Understanding the mechanism of IL-1β secretion.

239 Cytokine Growth Factor Rev. 22(4):189-195. doi:10.1016/j.cytogfr.2011.10.001
240 Maqsood, S., Adiamo, O., Ahmad, M., Mudgil, P., 2020. Bioactive compounds from date
241 fruit and seed as potential nutraceutical and functional food ingredients. Food
242 Chem. 308, 125522. doi:10.1016/j.foodchem.2019.125522
243 Platat, C., Hillary, S., Tomas-Barberan, F.A., Martinez-Blazquez, J.A., Al-Meqbali, F.,
244 Souka, U., Al-Hammadi, S., Ibrahim, W., 2019. Urine Metabolites and
245 Antioxidant Effect after Oral Intake of Date (Phoenix dactylifera L.) Seeds-Based
246 Products (Powder, Bread and Extract) by Human. Nutrients 11.
247 doi:10.3390/nu11102489
248 Qin, L., Yang, Z., Gu, H., Lu, S., Shi, Q., Xing, Y., Li, X., Li, R., Ning, G., Su, Q., 2014.
249 Association between serum uric acid levels and cardiovascular disease in middle-
250 aged and elderly Chinese individuals. BMC Cardiovasc. Disord. 14, 26.
251 doi:10.1186/1471-2261-14-26
252 Rahmani, A.H., Aly, S.M., Ali, H., Babiker, A.Y., Srikar, S., Khan, A.A., 2014.
253 Therapeutic effects of date fruits (Phoenix dactylifera) in the prevention of
254 diseases via modulation of anti-inflammatory, anti-oxidant and anti-tumour
255 activity. Int J Clin Exp Med 7, 483–491.
256 Saryono, Dardjito, E., Proverawati, A., Sumeru, A., Setiyani, R., Upoyo, A.S.,
257 Kamaludin, R., 2019. Date seeds (*Phoenix dactylifera* L.) consumption as anti-
258 inflammatory and immunostimulant: a systematic review. IOP Conf. Ser.: Earth
259 Environ. Sci. 250, 012038. doi:10.1088/1755-1315/250/1/012038
260 Saryono, S., Eliyan, J., Herdiati, D., Khikmatullah, A.A., Silvana, C.P., Adi, H.P., 2017.
261 Anti-atherogenic properties of Deglet Noor Date seeds (*Phoenix dactylifera*)
262 Methanol extract on Diet-Induced Hypercholesterolemic Rats. IOP Conf. Ser.:
263 Mater. Sci. Eng. 172, 012046. doi:10.1088/1757-899X/172/1/012046
264 Saryono, S., Sumeru, A., Proverawati, A., Efendi, F., 2018. Decreasing Carbon
265 Tetrachloride Toxicity using Date-seed (Phoenix dactylifera L.) Steeping in Rats.
266 Toxicol. Environ. Health Sci. 10, 139–145. doi:10.1007/s13530-018-0357-1
267 Saryono, S., Taufik, A., Proverawati, A., Efendi, F., 2019a. Dietary supplementation of
268 Phoenix dactylifera L. seeds decreases pro-inflammatory mediators in CCl4-
269 induced rats. J. Herbmed Pharmacol. 8, 212–217. doi:10.15171/jhp.2019.31
270 Saryono, S., Warsinah, W, Isworo., A., Efendi, F., 2019b. Anti-inflammatory effect of
271 date seeds (*Phoenix dactylifera* L) on carrageenan-induced edema in rats. Trop.
272 J. Pharm Res 17, 2455. doi:10.4314/tjpr.v17i12.22
273 Shi, G., Chen, S., Wandu, W.S., Ogbeifun, O., Nugent, L.F., Maminishkis, A., Hinshaw,
274 S.J.H., Rodriguez, I.R., Gery, I., 2015. Inflammasomes Induced by 7-
275 Ketocholesterol and Other Stimuli in RPE and in Bone Marrow-Derived Cells
276 Differ Markedly in Their Production of IL-1β and IL-18. Invest. Ophthalmol. Vis.
277 Sci. 56, 1658–1664. doi:10.1167/iovs.14-14557
278 Sies, H., 2018. On the history of oxidative stress: Concept and some aspects of current
279 development. Curr. Opin. Toxicol. 7, 122–126. doi:10.1016/j.cotox.2018.01.002
280 Sofi, F., Dinu, M.R., 2016. Nutrition and Prevention of Chronic-degenerative Diseases.

Agriculture and Agricultural Science Procedia 8, 713–717. doi:10.1016/j.aaspro.2016.02.052
Suleman, M., 2018. Antioxidants, its role in preventing free radicals and infectious diseases in human body. PAB 7. doi:10.19045/bspab.2018.700197
Thouri, A., Chahdoura, H., El Arem, A., Omri Hichri, A., Ben Hassin, R., Achour, L., 2017. Effect of solvents extraction on phytochemical components and biological activities of Tunisian date seeds (var. Korkobbi and Arechti). BMC Complement Altern Med 17, 248. doi:10.1186/s12906-017-1751-y
Urban, M.K., 2000. COX-2 specific inhibitors offer improved advantages over traditional NSAIDs. Orthopedics 23, S761-4.
Wang, P.-Y., Fang, J.-C., Gao, Z.-H., Zhang, C., Xie, S.-Y., 2016. Higher intake of fruits, vegetables or their fiber reduces the risk of type 2 diabetes: A meta-analysis. J. Diabetes Investig. 7, 56–69. doi:10.1111/jdi.12376
Wojdasiewicz, P., Poniatowski, Ł.A., Szukiewicz, D., 2014. The role of inflammatory and anti-inflammatory cytokines in the pathogenesis of osteoarthritis. Mediators Inflamm. 2014, 561459. doi:10.1155/2014/561459
Yoshimura, A., Wakabayashi, Y., Mori, T., 2010. Cellular and molecular basis for the regulation of inflammation by TGF-beta. J. Biochem. 147, 781–792. doi:10.1093/jb/mvq043

FIGURES NOTES

Figure 1. Treatment date palm seed decreases IL-1β and TGF-β expression. The cytokines levels were measured at day 0 (pre-treatment) and day 15 (post-treatment) by enzyme-linked immunosorbent assay (ELISA). Mean ± SE are presented (n = 22). Asterisks indicate student t-test significance values; **P<0.01, *P<0.05. ns = not significant.

Figure 2. Treatment date palm seed decreases COX-1, COX-2, PGE2 level. The enzyme/mediator levels were measured at day 0 (pre-treatment) and day 15 (post-

treatment) by enzyme-linked immunosorbent assay (ELISA). Mean ± SE are presented (n = 22). Asterisks indicate student t-test significance values; **P<0.01, ****P<0.001.

Reviewer #4:

1. There is no information regarding the healthy status of the subjects. Supposed they are healthy subjects, the cytokines level should be in normal level. If so, what is the importance to decrease the cytokines level by this product ? Please add the information concerning this matter. We consider that the design of clinical trial to evaluate anti inflammatory activity in healthy subjects is not appropriate.

We included the healthy status of the subject by add information about BMI, blood glucose level, and total cholesterol level. By increasing of the age, middle-age women tend to increase inflammation process that develop to chronic disease. The consumption of palm date seed powder showed that will prevent inflammation process, ultimately prevent development of chronic disease.

In clinical trial there are several phase for example phase that involved normal subjects to evaluate efficacy and safety of the agent. In addition, the main goal of the study is to evaluate the health benefit of consuming date seeds products in daily basis, therefore it can be used as functional beverage to prevent chronic disease.

2. Authors found that the date palm seed decrease the expression of IL-1<beta>, TGF-<beta>, COX-1 and COX-2. Is the post level still on the normal range ? If so, it means that the date palm seed has no clinical effect on cytokine level. But if it become below normal, it means that the effect of the seeds is not beneficial. Please explain.

Cytokines are produced inducible manner that we cannot conclude those levels are in above, normal, or below normal. Here, we are interested to know the ability of palm seed consumption on decreasing pro-inflammatory mediators.
Cytokines are important mediator in communication among immune cells and other cells. Although their levels are increased in inflammatory condition, they still have physiological action for example to fight infection. Therefore, the best agents that can decrease inflammatory mediators will reduce the mediator until certain level that are needed to physiological action.