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OPEN PEER REVIEW

Anti-inflammatory and Analgesic Activity of *Cleome brachycarpa* Ethanolic Extract in Lower Mammals and Effects on Blood and Liver Enzymes

Hira Naeem, Somia Gul, Maria Khan, Shaista Hamid, Qurratul Ain Leghari, Hina Yasin, Rehana Perveen

SUBMITTED	REVISED	ACCEPTED	DOI
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About this report

This open peer review (OPR) report shows the public comments made on this article during peer review, by the reviewers, the associate editor and the editor-in-chief, for every revision round, together with the authors' replies. Comments appear only with the consent of the people who wrote them. Reviewers who chose to sign their report are named; the others are shown as "Referee". Comments are edited for language only, and confidential remarks to the editors are not included.

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1 Original submission

2 comments · 5 Dec 2022 – 8 Dec 2022

Reza Azadnasab Reviewer

MAJOR REVISION 5 Dec 2022

Dear Prof.Ramezani
Editor-in-chief Jundishapur Journal of Natural Pharmaceutical Products

The title of this article is well chosen according to other studies.

Abstract
There are many spelling mistakes in the abstract section; for example, I will mention some of them:
"Wistar" rats, not "wister" rats.
In line 15, the manuscript states Wistar "mice". I don't think "Wistar" mice exist. Because the Wistar strain has only been reported in rats, please specify or correct the source for this breed.
To evaluate "the" anti-inflammatory instead of evaluate anti-inflammatory.
Compute "the" number of platelets instead of compute number of platelets.
Hematological data "were" analyzed statically instead of hematological data analyzed statically.
"Which" means no prominent change "was" seen in hematological parameters.
And so on.

Background
Page 4 lines 48 and 42
The author(s) have mentioned various properties of medicinal plants from the perspective of medical importance, which requires an appropriate reference.
Page 4 line 50
The author(s) have mentioned the word " Cleome", and it is necessary to provide the name of this plant along with its genus and species in italics.
Pages 4 and 5
Regarding the picture introduced on these two pages:
Because it is introduced in the text as Fig 1, the label under the figure should read Fig 1, and the 0 before the 1 should be removed.
If possible, it is suggested that a suitable reference be provided for this photo.
Experimental Methods
Page 5 line 89
The title Experimental "Method" should be replaced by Experimental Methods.
Page 6 line 102
Please note the age of the animals in the manuscript. How many weeks old were they?
Page 6 lines 114-131
Please provide the appropriate reference for the selected dosage of the substances used in this study (Diclofenic sodium, Cleome brachycarpa and morphine).

Discussion
Pages 8-9 Lines 205-210
It is suggested that the authors refrain from mentioning test methods in the discussion section.
Pages 10 Lines 245-252
The author(s) of the study claimed that Cleome brachycarpa has effects against bleeding disorders and viral diseases such as dengue fever, as well as hematopoietic effects. Please use the appropriate reference for this claim.

Page 10 Lines 267-276
The author(s) in this study claimed that Cleome brachycarpa could be a good candidate for the prevention of atherosclerosis due to the reduction of cholesterol levels. Has this effect been reported in other studies? If so, please mention them.
Page 10 Lines 253-256
The author(s) in this study claimed that Cleome brachycarpa has analgesic and anti-inflammatory effects. Have other studies reported on the analgesic and anti-inflammatory properties of this plant? Please mention them if they exist.

References:
In general, it is recommended that more recent studies be cited in this section, as this will help to make the study stronger.

Pages 7 line 165, page 8 line 192 and page 21 line 497
"RESULT AND OBSERVARTIONS", "DISCUSSION" and "REFERENCES"
All the letters in these headings were capitalized, while the titles of the other sections were written with only the first letter capitalized and the rest in lowercase. It is recommended that the authors refer to the format requested by the journal to correct this part.

This manuscript needs major improvement in English syntax and word usage. Spelling mistakes are evident in all parts of this manuscript. Authors should seek help in writing scientific articles in English from a person or service specializing in writing scientific articles in English to make the necessary improvements to meet the standards of the journal.

In my view, this manuscript needs major revision.

Editor-in-Chief

MAJOR REVISION 8 Dec 2022

Dear Authors,
The review of your manuscript is now complete. The referees suggested major revisions. Please revise your manuscript according to the provided comments, and upload a point-by-point answer or any rebuttal to the comments along with the revised manuscript. We will reconsider it for possible publication in JJNPP.

Kind Regards,
JJNPP EIC

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Revision 1

AUTHORS' RESPONSE TO REVIEWERS

Authors' reply to reviewers

The next pages contain the authors' point-by-point reply to the comments of this revision round, as submitted with the revised manuscript. It is published as the authors wrote it.

Manuscript Title: Assessment of Anti-Inflammatory and Analgesic Activity of *Cleome Brachycarpa* Ethanolic Extract on Lower Mammals along its Effects on Blood and Liver Enzymes
Manuscript ID: 132712
Journal Name: Jundishapur Journal of Natural Pharmaceutical Products

Reviewers' Comments:

Reviewer 1:

Comment:
The title of this article is acceptable compared to other studies considering the factors examined in this study.
According to the format of the journal, scientific names should be in italics, which fortunately is well respected in this article.
Response:
Thank you so much for your generous comments.

Comment:
It is suggested to add "Lower Mammals" to the keywords section.
Response:
It is added as per suggestion!

Comment:
Background
It is suggested to explain more about the side effects of non-steroidal anti-inflammatory drugs in this section.
Response:
According to your suggestion, more side effects of NSAIDS are added as reference no. 59.

Comment:
Extraction of Plant Material
Considering that this type of plant has a special innovation, it is suggested to specify the different fractions of the sieved extract
Response:
Yes, it should be! but current manuscript is focusing on pharmacological activities of this special plant only.

Comment:
Methodology
Please explain more about your methodology.
Please explain about the ethical factors you did in using animals in this study.
Response:
“All procedures performed in the study were in accordance with Helsinki declaration (1964)” statement is added while all ethical protocols were followed and the entire investigational trial was officially permitted by Ethics Committee of Baqai Medical University.

Comment:
Please state the age of the rats, mice and rabbits in the manuscript?
Response:
According to your suggestion, ages of mice, rats and rabbits has been added in the manuscript under the heading of experimental animals. The part of experimental is already lengthy as compare to usual……..

Comment:
Discussion
You've better explain more about the "limitations" of the study at "Discussion" section.
This manuscript still needs minor improvement in English syntax and word usage.
In my perspective, this manuscript needs minor revision.
Response:
Thank you for comments! English syntax and word usage revised as per instructions.

Reviewer 2:

Comment:
1. What is the reference for the therapeutic doses used in the study? Please mention the valid reference.
Response:
According to your suggestion references for Cleome brachycarpa dose are added under the heading of test doses (31, 32, 33). References are also mentioned below:

- Sarfaraz, S., Ikram, R., Osama, M. And Gul, S., 2018. Evaluation Of Hematopoeitic Effects Of Cleome Brachycarpa In Albino Rabbits. Pakistan journal of pharmacology.35,1 and 2, pp:13-19.
- Malik, A., Arooj, M., Butt, T.T., Zahid, S., Zahid, F., Jafar, T.H., Waquar, S., Gan, S.H., Ahmad, S. and Mirza, M.U., 2018. In silico and in vivo characterization of cabralealactone, solasodin and salvadorin in a rat model: Potential anti-inflammatory agents. *Drug design, development and therapy*, *12*, p.1431.
- Naeem, H., Perveen, R., Zaidi, S.S.M., Zia, Z., Fatima, K., Akram, Z., Hussain, M. and Ishaque, F., 2019. Cleome brachycarpa: A review on ethnobotany, phytochemistry, and pharmacology. *RADS Journal of Pharmacy and Pharmaceutical Sciences*, *7*(2), pp.107-111.

Comment:
2. A valid letter for the ethical code should be sent.
Response:
The entire investigational trial was officially permitted by Ethics Committee of Baqai Medical University with letter no. BMU-EC/05-2022.

Comment:
3. Many of the contents given in the discussion of the manuscript are without reference.
Response:
All the required references are already mentioned while other parts in discussion are our own interpretations and discussion on our own obtained results.

Comment:
4. What is the reference of the plant structure in the manuscript (figure 1)? Did you get permission from the owner?
Response:
Permission from owner was not required and reference no. 14 was there in text for it. Following reference has been added as per suggestion.

- Ahmad, VU, KA and Khan, MA. 1986. The Molecular Structure and Absolute Configuration of Brachycarpone, a New Trinortriterpenoid Dilactone from Cleome brachycarpa. *J. Nat. Prod.* 1986, 49, 2, 249–252

Reviewer 3:

Comment:
The title of this article is well chosen according to other studies.
Response:
Thank you for your appreciation!

Comment:
Abstract
There are many spelling mistakes in the abstract section, for example, I will mention some of them:
"Wistar" rats not "wister" rats.
In line 15, you wrote Wistar "mice". I don't think "Wistar" mice exist. Because the Wistar strain has only been reported in rats please specify or correct your source for this breed.
To evaluate "the" anti-inflammatory instead of evaluate anti-inflammatory.
Compute "the" number of platelets instead of compute number of platelets.
Hematological data "were" analyzed statically instead of hematological data analyzed statically.
"Which" means, no prominent change "was" seen in hematological parameters.
And so on.
Response:
Please note that new versions of MS Word marked “grammatical error” if these “the” or such words add in manuscript….anyways! all required corrections has been done as per suggestions.

Comment:
Background
Page 4 lines 48 and 42
Author(s) have mentioned various properties of medicinal plants from the perspective of medical importance, which needs to mention the appropriate reference.
Page 4 line 50
The author(s) have mentioned the word "Cleome" and it is necessary to mention name of this plant along with its genus and species in italics.
Pages 4 and 5
Regarding the picture introduced in these two pages:
Because it is introduced in the text as Fig 1, it should be written under the figure of Fig 1 and the 0 before the 1 should be removed.
If possible, it is suggested to mention a suitable reference for this photo
Response:
Please note that all required references are already mentioned regarding medical uses of plant as mentioned below from manuscript:

- *Cleome brachycarpa* is a woody herb with yellow flowers (6), mostly cultivated in different areas of India, Iran and Pakistan. It has miscellaneous usages including in joint pain (7), soreness, tenderness, itching, leprosy, leucoderma, scabies, intestinal pain (8), swelling, anti-rheumatic (9, 10), vermicidal and anthelmintic (11) anti-oxidant (12) and anti-emetic (13).

- *Cleome brachycarpa* have exceptional chemical composition like triterpenoid dilactone, deacetoxy-brachycarpone (Fig 1), triterpenoid cleocarbpone, ursolic acid and cabralealactone (14). Presence of these compounds make the whole plant medicinal and resulted its usefulness in bronchitis, diarrhea, malaria, heart failure, seizures, immune system deficiencies, jaundice, fever chills and in treating swollen body parts (15, 16, 17). Likewise, sesquiterpenes and flavonoids make *Cleome* more anti-fungal, anti-bacterial and antitumor with good cytotoxic effects (18). It strongly kills plant pests, microbes (19) and also uses as tranquillizer (20). Hence, this specie of Cleome is vital in its properties and composition (21, 22, 23).

While all other suggested changes has been incorporated in manuscript and are highlighted.

Comment:
Experimental Methods
Page 5 line 89
The title Experimental "Method" should be replaced by Experimental Methods
Page 6 line 102
Please note the age of the animals in the manuscript. How many weeks were they?
Page 6 lines 114-131
Response:
All suggested changes have been incorporated and highlighted in manuscript in experimental part.

Comment:
Please provide the appropriate reference for the selected dosage of the substances used in this study (Diclofenic sodium, Cleome brachycarpa and morphine).
Response:

Following suggested references have been incorporated and highlighted in manuscript text as well as in reference section under the heading of test doses. *Cleome brachycarpa* 200mg/kg/body weight (31, 32, 33), morphine (34) and diclofenac sodium (35).

- Sarfaraz, S., Ikram, R., Osama, M. And Gul, S., 2018. Evaluation Of Hematopoeitic Effects Of Cleome Brachycarpa In Albino Rabbits. Pakistan journal of pharmacology.35,1 and 2, pp:13-19.
- Malik, A., Arooj, M., Butt, T.T., Zahid, S., Zahid, F., Jafar, T.H., Waquar, S., Gan, S.H., Ahmad, S. and Mirza, M.U., 2018. In silico and in vivo characterization of cabralealactone, solasodin and salvadorin in a rat model: Potential anti-inflammatory agents. *Drug design, development and therapy*, *12*, p.1431.
- Naeem, H., Perveen, R., Zaidi, S.S.M., Zia, Z., Fatima, K., Akram, Z., Hussain, M. and Ishaque, F., 2019. Cleome brachycarpa: A review on ethnobotany, phytochemistry, and pharmacology. *RADS Journal of Pharmacy and Pharmaceutical Sciences*, *7*(2), pp.107-111.
- Gades, N.M., Danneman, P.J., Wixson, S.K. and Tolley, E.A., 2000. The magnitude and duration of the analgesic effect of morphine, butorphanol, and buprenorphine in rats and mice. Journal of the American Association for Laboratory Animal Science, 39(2), pp.8-13.
- Hajzadeh, M.R., Rakhshandeh, H., Esmaeilzadeh, M. and Ghorbani, A., 2004. Analgesic and anti-inflammatory effects of Portolaca oleracea extracts in mice & rat. *J. Semnan University of Medical Sciences. 5 (3-4): 113-120.*

Comment:
Discussion
Pages 8-9 Lines 205-210
It is suggested to refrain from mentioning test methods in the discussion section.
Response:
Your suggestion is absolute right and we refrained this in discussion portion…….only a small paragraph is there about anti-inflammatory activity which was needed to elaborate the discussion in better way!

Comment:
Pages 10 Lines 245-252
The author(s) of the study claimed that Cleome brachycarpa has anti-bleeding disorder effects, Anti-viral diseases such as dengue fever, and hematopoietic effects. Please use the appropriate reference for this claim
Response:
In our current study, results indicated little increase (non significant) in platelet count so it may play its part in preventing bleeding disorders. Similarly, platelet count plays a role in dengue fever so we are suggesting and predicting for future possibilities. Reference is already incorporated in main text and in reference portion (reference 54) as mention below!

- Sarfaraz S, Ikram R, Osama M, Gul S. 2018. Evaluation Of Hematopoeitic Effects Of *Cleome* Brachycarpa In Albino Rabbits. Pak. J. Pharmacol: 35(1 & 2), Pp.13-19.

Comment:
Page 10 Lines 267-276
The author(s) in this study claimed that Cleome brachycarpa could be a good candidate for the prevention of atherosclerosis due to the reduction of cholesterol levels. Has this effect been reported in other studies? If there is, please mention it.
Response:
Reference is also incorporated in main text and in reference portion as mention below(reference 55)

- Sarfaraz, S., Najam, R., Azhar, I., Ahmed, S. and Sarwar, G., 2017. Evaluation of hypolipidemic and hepatoprotective effects of ethanolic extract of Cleome brachycarpa on Albino rabbits. *J. Anal. Pharm. Res*, *5*(6), p.00162. DOI: 10.15406/japlr.2017.05.00162.)

Comment:
Page 10 Lines 253-256
The author(s) in this study claimed that Cleome brachycarpa has analgesic and anti inflammatory effects. Have other studies been reported on the analgesic and anti-inflammatory properties of this plant? Please mention them if they are exist
Response:
Inflammation and pain are the vital signs of any disease and required antioxidants to manage these conditions; several studies have been reported as mention in reference no. 21, 22 and 41.

Comment:
Pages 7 line 165, page 8 line 192 and page 21 line 497
RESULT AND OBSERVARTIONS, DISCUSSION and REFERENCES
All the letters of these words were written in capital letters. While the title of the other sections is written with only the first letter in capital letters and the rest in small letters. It is recommended to refer to the

format requested in the journal to correct this part.

Response:

All required corrections in headings have been done as per format of journal.

Comment:

This manuscript needs major improvement in English syntax and word usage. Spelling mistakes are evident in all parts of this manuscript. Authors should seek help in writing scientific articles in English from a person or service specializing in writing scientific articles in English to make the necessary improvements to meet the standards of the journal.

Response:

Please note that new versions of MS Word marked “grammatical error” if these “the” or such words add in manuscript….anyways! All required corrections have been done as per suggestions.

2 Revision 1

2 comments · 7 Mar 2023 – 13 Mar 2023

Referee 1 Reviewer

MAJOR REVISION 7 Mar 2023

1- How did you prove that the edema was related to vascular changes and inflammatory cells?
2- A decrease in blood proteins, including albumin, can also be one of the causes of edema.
3- Why have inflammatory factors, such as tumor necrosis factor alpha or interleukin-1 beta, not been measured to prove the anti-inflammatory effects of the tested drug?
4- In the conclusion, it is mentioned that the reduction in edema is due to the inhibition of Cyclooxygenases. How do you prove this if gene expression has not been checked?
5- Why were specific liver parameters not used to assess the activity and effect of the studied drug? Measurements of serum albumin, sorbitol dehydrogenase, and aspartate transaminase could be useful for your research results.
6- In the results section, you reported that the studied drug is effective for the treatment of all types of anemia. Is the cause of all anemias the same? And by what mechanism does this drug increase hematopoiesis? Has the bone marrow been investigated? How do you prove this statement?
7- It is mentioned in the text of the article that the mean corpuscular hemoglobin concentration decreases, but you showed a slight increase in the amount of hemoglobin. Don't you think this is a contradiction? How is it possible that the amount of hemoglobin increases, but the mean corpuscular hemoglobin concentration decreases?
8- If histopathology has been done, photos of the histopathology slides showing the presence or absence of lesions should be included in the article.

Editor-in-Chief

MAJOR REVISION 13 Mar 2023

Dear Authors,
Your manuscript has been scrutinized by our experts again. They believe it needs significant changes before a final decision can be made. Please revise the manuscript according to the provided comments. Upload a list of changes with the revised manuscript and highlight the changes.
We look forward to receiving the revised version as soon as possible.

Best wishes,
JJNPP EIC

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Revision 2

AUTHORS' RESPONSE TO REVIEWERS

Authors' reply to reviewers

The next pages contain the authors' point-by-point reply to the comments of this revision round, as submitted with the revised manuscript. It is published as the authors wrote it.

Manuscript Title: Assessment of Anti-Inflammatory and Analgesic Activity of *Cleome Brachycarpa* Ethanolic Extract on Lower Mammals along its Effects on Blood and Liver Enzymes
Manuscript ID: 132712
Journal Name: Jundishapur Journal of Natural Pharmaceutical Products

Reviewers' Comments:

Comment:

1- *How did you prove that the edema was related to vascular changes and inflammatory cells?*
2- *A decrease in blood proteins, including albumin, can also be one of the causes of edema.*

Response:

As in our experimentation, *Group B:* Positive Control, Wister Strain rats were administered with acetic acid (2%) in plantar surface of right hind paw to develop inflammation at the time of trial.
According to the literature review, acetic acid–induced writhing test has been used as a screening tool for assessing analgesic or anti-inflammatory agents. Pain is induced by injection of irritants into the peritoneal cavity of mice. Acetic acid induces an inflammatory response, with subsequent activation of nociceptors.

Moreover, Acetic acid-induced writhing test is widely used model to evaluate peripheral antinociceptive effects. In this model, pain is generated by PGE2 and PGE2 alpha, histamine, serotonin, bradykinin, and cytokines (TNF-α, IL-1β, IL-6, and IL-8). These inflammatory mediators also increase vascular capillary permeability, decrease pain threshold, and increase the sensitivity of nerve terminals of nociceptive fibers.

Reference is mention for your convenience!

- https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/writhing-test#:~:text=Acetic%20acid%20induces%20an%20inflammatory,emerge%20in%20the%20peritoneal%20area.

- Anti-nociceptive and Anti-inflammatory Activities of Asparacosin A Involve Selective Cyclooxygenase 2 and Inflammatory Cytokines Inhibition: An *in-vitro*, *in-vivo*, and *in-silico* Approach. Available at https://www.frontiersin.org/articles/10.3389/fimmu.2019.00581/full

Further, it is also being a known fact that when the level of protein in the blood is low, water may leave the blood vessels and collect in the tissues. Water in the tissues is called "edema". In our case, animals are young and healthy and their liver and kidney were not compromised as were also evident by their histological slides (already published and reference is mention below). But, for the next time, we will keep this parameter too for evaluation.

- Effect of Cleome Brachycarpa Ethanol Extract On Liver and Kidney Tissues of Rats: A Prospective Study Focusing On Histopathological Variations. 2017. Hira Naeem, RehanaParveen, IjazHussain Zaidi and Mohammad Mahmood. Med.Forum.Vol. 28 (7), Pp:110-114.

Comment:
3- Why have inflammatory factors not been measured to prove the anti-inflammatory effects of the tested drug? Like tumor necrosis factor alpha or interleukin-1 beta.

Response:
Thanks for pointing; We will plan this for our next projects. For now, we were unable to perform these tests because of limited budget.

Comment:
4- In the conclusion, it is mentioned that the reason for the reduction of edema is due to the inhibition of Cyclooxygenases. How do you prove this? If the gene expression has not been checked?

Response:
Dual COX inhibition is required to inhibit acetic acid-evoked irritation. This is supported by the effects of dual, and not selective COX-1 or COX-2 inhibitors. Diclofenac inhibits cyclooxygenase-1 and -2, the enzymes responsible for production of prostaglandin (PG) G_2 which is the precursor to other PGs. These molecules have broad activity in pain and inflammation and the inhibition of their production is the common mechanism linking each effect of diclofenac.
We compared our test drug with that of standard used i.e. diclofenac that follow COX path so it is hypothesized that test drug follow the same path. Gene expression will be planned for next project, Thanks for suggestion.
Further, corrections in reporting result is done now as you highlighted and now it is stated that
"Further, result revealed anti-inflammatory effect of extract **which may be due to** inhibiting both leukocyte migration and COX-1 and COX-2 enzyme."
Other prospective may be that, any of components of *Cleome brachycarpa* has the therapeutic and healing property for inflammation. However, further advanced studies (clinical) are compulsory to approve theses effects.

Comment:
5- Why were specific liver parameters not used for the activity and effect of the studied drug? Such as serum albumin, sorbitol dehydrogenase, and aspartate transaminase measurements could be useful for your research results.

Response:
Thanks for your suggestions! Please note that effect of studied drug on ALP and SGPT has been evaluated while other enzymes like mention in comments were not possible because of very limited budget for the project.

Comment:
6- In the results section, you reported that this studied drug is effective for the treatment of all types of anemia. Is the cause of all anemias the same? And by what mechanism does this drug increase hematopoiesis? Has the bone marrow been investigated? How do you prove this statement?

Response:
Actually, test drug reported increase in hemoglobin so we hypothesized that it could be a source to treat anemia. But this was just a hypothesis, requiring further studies to evaluate this effect.

Comment:
7- It is mentioned in the text of the article that the Mean corpuscular hemoglobin concentration decreases, but you showed a slight increase in the amount of hemoglobin, don't you think this is a contradiction? How is it possible that the amount of hemoglobin increases, but the Mean corpuscular hemoglobin concentration decreases?

Response:

Variables	**Control**	**Treated**	**t. test**	**Sig (P value)**
Hb	10.9±0.186	13.10±0.242	0.655	0.527
MCHC	29.85±0.299	29.00±0.426	0.608	0.557

Actually the decrease is of only 0.85 as shown in table no 5 above which is insignificant at all or I can say that the amount of MCHC remain same.

Comment:
8- If histopathology has been done, the photos of the histopathology slides with the presence or absence of lesions should be included in the article.

Response:
Yes, histopathological evaluation of liver and kidney was done and that part is already published and reference is mention below for your convenience!

- Hira Naeem, Rehana Parveen, Ijaz Hussain Zaidi and Mohammad Mahmood (2017), Effect of Cleome Brachycarpa Ethanol Extract On Liver and Kidney Tissues of Rats: A Prospective Study Focusing On Histopathological Variations. Med.Forum.Vol. 28 (7), Pp:110-114.

Regards,

3 Revision 2

2 comments · 8 Apr 2023 – 9 Apr 2023

Reza Azadnasab Reviewer

ACCEPT 8 Apr 2023

The requested corrections were made by the author(s). Best of luck to them in their other research. In my opinion, this manuscript is suitable and is recommended for publication in this journal.

Editor-in-Chief

ACCEPT 9 Apr 2023

Dear Authors,
I am glad to inform you that your manuscript has been accepted for publication in JJNPP in its current form. We will send it to the publisher for further processing.
Best regards,
JJNPP EIC