Author’s response to reviews

Title: Antinociceptive activity of methanolic extract of Muntingia calabura leaves: Further elucidation of the possible mechanisms

Authors:

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Author’s response to reviews:

19th January, 2014

Dear Editor-in-Chief of BMC Complimentary and Alternative Medicine,

The authors have read through the comments/queries made by the honorable reviewers and would like to respond to each of the comment/query. The list of comments/queries and their respective respond by the authors (red coloured) is given below.

Reviewer 1: Lindsay Brown
Reviewer 1 report:
Major compulsory revisions:
1. The major question is – is this study a significant extension of previous studies by the same research group? The authors mention 4 related papers: Sani et al. (2012), Zakaria et al (2006b, 2007a and 2007b). A quick look at these papers shows minor differences, such as aqueous vs methanol extracts, slightly different animal models of nociception, use of the same antagonists with the addition of one or two more. However, the conclusions are remarkably similar. The authors must be able to present solid arguments that the current manuscript represents significant advances in the understanding of the pharmacological responses to this extract.

Respond:
The authors agree that this study is a significant extension of previous studies by the same corresponding author (Dr. Zainul Amiruddin Zakaria) and his colleagues. The authors also agree with the reviewer comment that the differences between the present and previous studies lies in the types of extracts used, and slight addition of animal nociceptive models and antagonists. For the
reviewer information, studies by Zakaria et al. (2006b, 7007a and 2007b) were carried out on the aqueous and chloroform extracts of M. calabura and considered as our preliminary attempt to establish M. calabura antinociceptive activity.

The reason for using aqueous and chloroform extracts were based on the fact that they contained the respective polar and non-polar compounds. Through those studies both types of extracts were found to exert antinociceptive activity and some mechanisms of antinociception involved were also determined.

However, in our attempt to isolate the bioactive compounds responsible for the observed antinocieptive in M. calabura, we decided to use methanol as the solvent for extraction as this solvent can extracted both the polar and non-polar compounds at one time due to its intermediate polarity. If we used only the aqueous extract or chloroform extract, the isolation of bioactive compounds will only represent the respective polar or non-polar compounds, but not both. If we prepared two separate extracts, we have to do two different types of isolation processes. Moreover, using the aqueous or chloroform extracts separately will not permit us to extract out the intermediate compounds, which have both the polar and non-polar portions. It is well known that intermediate solvents like methanol can extract out all the polar, non-polar, as well as the intermediate compounds. With final target of isolating compounds that exhibited antinociceptive activity, therefore, methanol was chosen for the present study.

Since methanol extract all types of compounds, we have decided to evaluate the extract’s antinociceptive mechanisms to establish the mechanism of antinociception profile for future comparison to those obtained using the aqueous and chloroform extracts. In addition, through the study on antinociceptive mechanisms of MEMC, we can study whether the different compounds isolated using methanol did or did not interact with each other to either activate or inactivate some of the nociceptive pathways or systems.

Minor essential revisions:
1. Abstract, Results lines 7-10: Prazosin both reversed and did not reverse the MEMC antinociception – which statement is correct? In section 3.4.3, prazosin reversed the antinociception of phenylephrine but not MEMC.
   
   Respond:
   
The authors have corrected the statement as suggested by the reviewer.

2. Page 2 of Introduction, line 6: “ethnopharmacological”
   
   Respond:
   
The authors have corrected the term as suggested by the reviewer.

3. Introduction page 2 line 10: What are “emmenogogue headaches”? Wikipedia defines emmenogogues as “herbs which stimulate blood flow in the pelvic area and uterus; some stimulate menstruation”.
   
   Respond:
   
The authors have corrected the sentence as suggested by the reviewer.
4. Section 2.4 Animals, line 1: insert “rats” after “Sprague-Dawley”.
Respond:
The authors have added the requested word as suggested by the reviewer.

5. Section 2.5.3: Figure 3 suggests that the four potassium-channel blockers were given to different animals, yet the list in 2.5.3 says “and”. Were the drugs given as one injection, or to separate animals? If separate animals, then use “or” before tetraethylammonium chloride. The same comment applies to the opioid antagonists in section 2.5.5 and figure 9.
Respond:
The authors have corrected the statement as suggested by the reviewer.

6. Section 2.5.7 line 1: Please give reference to “the previous report (2012)”.
Respond:
The authors have added the requested reference as suggested by the reviewer.

7. Sections 3.1 – 3.5 should be combined and shortened as all are referring to the same tests for biological activity.
Respond:
The authors have combined and shortened the suggested section.

8. Section 3.2 line 2 and Discussion paragraph 1 lines 6 and 7: “dose-dependent”.
Respond:
The authors have added the said term as suggested by the reviewer.

9. Section 3.6: What are the identities of the 4 major peaks from HPLC? If they are flavonoids as suggested by previous reports from this group, what is the advantage of this plant extract over use of the pure flavonoids such as rutin and quercetin, which are widely available and relatively cheap? What is the uniqueness of this plant extract, as flavonoids are remarkably widespread throughout the plant kingdom?
Respond:
For the reviewer information, Section 3.6 has been shifted to Section 3.1 (page 10). The UV-Vis of several major peaks detected in the chromatogram of MEMC was found to fall within the #max range that represents flavonoid-type of compounds. Moreover, the authors have also compared the chromatogram of MEMC with several pure flavonoids and some of the peaks have been detected to show the presence of, at least, rutin, fisetin and quercitrin, and further confirmed our postulation based on the #max value. The authors agree with the reviewer opinion that flavonoids are widely spread throughout the plant kingdom and that pure flavonoids are widely available and relatively cheap.
However, preliminary comparison on the total flavonoids content (TFC), total phenolic content (TPC) and antioxidant activity of M. calabura against several other plants that are also being studied in our laboratory for their potential anti-ulcer, hepatoprotective, antinociceptive, anti-inflammatory etc., have demonstrated that M. calabura, as well as MEMC, possessed the highest TFC and TPC contents and antioxidant activity. This is the unique part of M. calabura in comparison to other plants, including Melastoma malabathricum, which is a famous Malaysian herb.

The advantage of using plant extract over pure flavonoids includes synergistic action among or between the bioactive compounds, which could not be seen if a pure compound is used. M. calabura, other than contains flavonoids, were also demonstrated to contain saponins, tannins and triterpenes. Therefore, the synergistic action are postulated to occur between the flavonoids themselves or between the different phytoconstituents classes. Due to the synergistic action also, the concentration of each phytoconstituents required to modulate any pharmacological activity, including antinociceptive activity, will be lower than the concentration required by the pure compound alone. This phenomenon has been reported for antimalarial studies as cited by Rasoanaivo et al. (2011) (P. Rasoanaivo, C.W. Wright, M.L Willcox and B. Gilbert (2011). Whole plant extracts versus single compounds for the treatment of malaria: synergy and positive interactions. Malaria Journal 2011, 10(Suppl 1):S4).

According to Rasoanaivo et al. (2011) pure drugs, either industrially produced or isolated from plants have several disadvantages. Firstly, they rarely have the same level of effectiveness as the untreated extract at comparable concentrations/doses of the pure compound. This might be attributed to the absence of interacting substances present in the extract. Secondly, despite being cheaper in the develop countries, pure compounds, including those of flavonoid-based, are often more expensive to produce and distribute especially in the third-world countries. Thus, are so often unavailable and/or unaffordable to the poorest populations in remote areas. On the contrary, plant-based or herbal medicines can be cultivated and produced in the vicinity, at lower cost, by or close to those who require them.

Lastly, the authors did not attempt to promote the uniqueness of flavonoids over other phytoconstituents. The reason for highlighting flavonoids over other phytoconstituents is because flavonoids are the easiest constituents to be detected using HPLC analysis as they are presence in most plants and the pure standard are easily obtained. Therefore, the proposed mechanisms are discussed partly via the presence of those flavonoids in the extract.

Reviewer 2:Niraldo Paulino

Reviewer 2 report:
The authors present additional results previously published: Antinociceptive Activity of Methanol Extract of Muntingia calabura Leaves and the Mechanisms of Action Involved, in another journals.
Respond:
The present study is considered as our continuous study on the mechanisms of antinociception of MEMC and covered other nociceptive pathways modulated by the extract, which have not been published in the earlier report (Antinociceptive Activity of Methanol Extract of Muntingia calabura Leaves and the Mechanisms of Action Involved)

1. Abstract
In abstract, the authors present the description of the methodological work properly, represent the numerical results obtained in experiments, but not demonstrate the statistical method used for the evaluation of experimental data.
Respond:
The authors have added the statistical method used as requested by the reviewer.

2. Introduction
The authors present a review with references to antinociceptive activity of Muntingia calabura, however there are at least 06 other references that could complement the literature review of the subject in research.
Respond:
The authors have added the other references related to Muntingia calabura as requested by the reviewer. Moreover, the Introduction section was also re-written as can be seen in page 4-5 (Paragraph 2).

3. Material and methods
The authors present the experimental methodology adequately
Preparation of extract:
- The authors used the correct methodology in sample preparation.
- Shown the number of protocol to Ethical committee for use of animals.
Respond:
Based on the reviewer 2 comments no changes were made to the Materials and Methods section.

4. Results
I suggest show the chemical composition of extract in the first figure in the results.
Respond:
The authors have made the necessary changes to the figure as requested by the reviewer.

5. Graphs should show the units of dose applied to the animals
Respond:
The authors have added the units of dose as requested by the reviewer.

6. Discussion
The discussion and conclusions demonstrate adequately that treatment with Muntingia calabura can produce antinociceptive activity and show that activity could be mediated by inhibition of PKC pathway and bradykinin receptor as well as through the activation of K+ channels, adrenergic, serotonergic and adenosinergic receptor systems.

Respond:
Based on the reviewer 2 comments no changes were made to the Discussion section.

6. Reference
References are actual and contribute to discussion.

Respond:
Based on the reviewer 2 comments no changes were made to the Reference section.

That’s all for now and thank you for the opportunity to provide rebuttal to each comments by the reviewer.

Best regards,
Zainul Amiruddin Zakaria (PhD.)
Associate Professor