Reviewer's report

Title: TRPV1 channel inhibition contributes to the antinociceptive effects of Croton macrostachyus extract in mice

Version: 2 Date: 6 April 2015

Reviewer: Vinicius M Gadotti

Reviewer's report:

The present work investigates the antinociceptive action of the methanol/methylene chloride extract of the stem bark from Croton macrostachyus (MECM) in models of acute and persistent pain in mice. Overall, the results convincingly showed that treatment with MECM reduced pain behavior of mice against the different animal models applied in this study. Despite, the present manuscript is well-written, concise, and the topic is appropriate for publication in BMC Complement. Altern. Med. Molecular, the implication for MECM or its active compounds as a therapy for pain is still far away from became a real possibility. The lack of an experiment demonstrating that the MECM specifically interferes with pain pathways and do not produce sedation or locomotor activity impairment represents a down point unless the authors can provide concise explanation or additional experimental support. Also, many pharmacology terms are misused and need to be substituted by the proper terminology.

Major comments:

1) Von Frey measurements starting 1h after CFA injection seems a bit awkward in terms of kinetics for CFA to induce inflammatory process. Measurements are often done 24 – 72h after CFA injection. Proper background and references are needed to justify why measurements started as soon as 1h after CFA delivery.

2) On figure 1 and on page10, lines 206-207 authors mentioned that treatment induced an progressive and long lasting effect, however it took 2 days to watch effect again after the daily treatment stated (no effect on 7 and 8 days), indicating development of a tolerance phenomena what can be seem on the graphic. Mention about this phenomena must be added to the section, with does not mean that it will compromise the publication of the manuscript, in fact the lack of such info might be an issue. This should be also mentioned in the discussion section.

3) Were statistical differences between baselines and control groups detected in every experiment? This need to be clarified and stated in all result sections of the manuscript.

Minor comments:

1) Pg2, line 29-30: rewrite “… (CFA)-induced persistent thermal and mechanical…”
2) Pg5, lines 94-98: it is confusing the two sentences stating different animal protocols numbers, this paragraph needs to be reworded to make better sense.

3) It not clear to me why the authors have used gabapentin as a positive control for an inflammatory pain model (CFA), gabapentin is known to be used to treat neuropathic pain states in patients and not inflammatory conditions, a NSAID seems more appropriate for control in this model. Proper justification is needed.

4) Pg6, lines 117-119. Shouldn’t the aim of the daily treatment with MECM on CFA test be done to watch development of tolerance caused by MECM treatment?

5) Pg7, lines 146-147: Glibenclamide is a inhibitor of ATP-sensitive potassium channels and not an antagonist, proper terminology must be used all along the manuscript.

6) Pg7, line 151: intensity of 15 what? The proper unit is missing, is it 15% of total power?

7) Pg7, line 155: info missing, measurements were taken up to 14 days after CFA injection, this info need to be add to the section.

8) Pg8, lines 168-169: again proper terminology must be used, rimonabant is not a CB1 antagonist, due to its electrophysiological properties rimonabant is an Inverse Agonist of CB1 receptors, despite its interaction with CB1 receptors shows a functionality antagonistic-like action specially when evaluated in vivo, this drug is not an antagonist, but an Inverse agonist. Proper terminology must be used.

9) Pg9, line 195: rewrite “… post hoc test or two-way ANOVA followed …”

10) Pg10. line 218: change “… both extract and gabapentin…” by “… either the extract or gabapentin…”, once this seems is not a co-administration protocol.

11) Pg10, line 227: Author state that glibenclamide failed to revers effect of MECM, but the result show it does at 4 hours. Clarification is needed.

12) Pg11. Line 240: Effect lasted for up to 4h, and not up to 6h as authors states.

13) Pg12, line 252: rewrite “… in models of persistent inflammatory and neuropathic pain…”

14) Pg13, line 282: Well stablished chronic pain after only 5 days from nerve injury? Not likely once chronic pain is observed in sciatic nerve injury models after 10 days from surgery. Reword is needed.

**Level of interest:** An article whose findings are important to those with closely related research interests

**Quality of written English:** Acceptable
Statistical review: No, the manuscript does not need to be seen by a statistician.

Declaration of competing interests:

I declare that I have no competing interests' below.