Author's response to reviews

Title: Anti-inflammatory effects in muscle injury by transdermal application of gel with Lychnophora pinaster aerial parts using phonophoresis in rats

Authors:

Viviane GC Abreu VGCA (vivianegca@ufmg.br)
Geone M Correa GMC (geonemaia@ufam.edu.br)
Thiago M Silva TMS (silvatm@ufmg.br)
Humberto S Fontoura HSF (humbertofisio@hotmail.com)
Denise C Cara DCC (deniseccm@gmail.com)
Dorila Piló-Veloso DPV (dorila@zeus.qui.ufmg.br)
Antônio FC Alcântara AFCA (aalcantara@zeus.qui.ufmg.br)

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Author's response to reviews: see over
Dear Editor,

The suggestions of the referee were evaluated and considered.

**REFEREE 1:**

**Question:** Title: write “aerial parts”.

**Answer:** The text was changed as suggested by the referee.

**Question:** The Background section should be written illustrating the research and its aims. Add information about popular use of the plant as an anti-inflammatory agent. The section should end with a brief description of what is being reported in the article. Re-write this section.

In the conclusion section of the abstract, change quercetin attenuates the inflammatory by “quercetin attenuates the inflammation”.

**Answer:** The Background was rewritten as suggested and the word inflammatory was changed by “inflammation”.

**Question:** The authors affirm that “This species exhibits antitumor, antimicrobial, antipyretic, analgesic, anticonvulsant, anti-inflammatory and antioxidant activities”. My question for the authors is: these activities are attributed specifically to L. pinaster or to species of the genus *Lychnophora* in general?

**Answer:** These activities are attributed to the genus. So, the phrase was rewritten to “These species exhibit antitumor, antimicrobial…”

**Question:** write the word Herbarium without italic

**Answer:** the italic form of Herbarium was withdrawn.
Question: in lines 3 and 5 of this section write “3:1 ethyl acetate:ethyl ether solution”.

Answer: the term 3:1 ethyl acetate-ethyl ether solution was changed to “3:1 ethyl acetate:ethyl ether solution”

Question: In this section, the authors affirm that “the aqueous phase provided the alkaloid fraction”. The phytochemistry of this genus is well characterized by the presence of caffeoylquinic acid derivatives, flavonoids, sesquiterpenes and sesquiterpene lactones. To our knowledge, there is no occurrence of alkaloids in the genus Lychnophora. If there are reports, the authors should cite this work and then explain how it was made to characterize the presence of alkaloids in this fraction. It was used some reagent of identification (Dragendorf for example) or some other chromogenic agent? I wait for a response.

Answer: We have not references in the literature reporting the presence of the alkaloids in Lychnophora. However, chemical identification tests indicated the presence of phenolic compounds and alkaloids in the corresponding fractions.

Question: (1:4 methanol:water solution)

Answer: 1:4 methanol-water solution was changed to “1:4 methanol:water solution”

Question: The numeration of the structure of quercetin in Figure 1 is not correct. The B ring of this flavonoid has a catechol nucleus, which is 3’-4’ dihidroxibenzene. Thus, the correct numeration of the B ring in this structure is:

Answer: The numeration of the structure of quercetin in figure 1 was corrected, as suggested by referee.
**Question:** In page 6 change the hydrogens H-6’ by H-2’; H-2’ by H-6’ and H-3’ by H-5’. In this form of numeration, the chemical shifts of the carbons presented in the paper are correct.

**Answer:** The attribution of the hydrogen atoms were changed as suggested by the referee.

**Question:** Groups of rats (n=36). The total number of animals is 36 per group (360 animals were used in total?) or 36 animals in all study? What is the number of animals per group?

**Answer:** The tests were made for 12 groups of animals, containing 3 animals for each group.

**Question:** “The low inflammatory activity suggested for HE may be related to a low concentration of triterpenes in this extract, mainly the triterpene lupeol, which exhibits significant activity”. Inflammatory of anti-inflammatory activity???

**Answer:** The correct is anti-inflammatory and was changed in the text.

**Question:** Correct the structure of quercetin and add the number (4) below the structure of 3-O-acetyllupeol. Correct structures 4 and 3, add the group acetyl with

**Answer:** This figure was corrected as suggested by referee.

**EDITORIAL COMMENTS:**

**Question:** If possible, we would ask you to provide more evidence in your background section to indicate why you would expect your treatment to have an anti-
inflammatory effect in an animal model. Ideally this evidence should come from previous in vitro studies.

**Answer:** The anti-inflammatory tests performed in this work were based on ethnopharmacologic information related by popular Brazilian medicine.

**Question:** Please provide details in your methods section on who formally identified the plant material used in your study.

**Answer:** The responsible for the identification: Dr. J. Semir (Departamento de Botânica, Universidade Estadual de Campinas). This information was described in the text.

We thank the contributions of the referees and hope a positive appreciation to publication of this paper, as soon as, in BMC: Complementary & Alternative Medicine.