# Systematic Review Protocol for Animal Intervention Studies

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<table>
<thead>
<tr>
<th>Item #</th>
<th>Section/Subsection/Item</th>
<th>Description</th>
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<tbody>
<tr>
<td><strong>A. General</strong></td>
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</table>
| 1. | Title of the review | Natural products for experimental orofacial pain: a systematic review  
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| 3. | Other contributors (names, affiliations, contributions) | |
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| 5. | Funding sources/sponsors | |
| 6. | Conflicts of interest | The authors report no conflict of interest. |
| 7. | Date and location of protocol registration | April, 2019. Syrcle. |
### Background

**What is already known about this disease/model/intervention? Why is it important to do this review?**

Orofacial pain is defined as all pain associated to soft and mineralized tissues of oral cavity and face. Oftentimes, orofacial pain is related to temporomandibular disorders (TMD) (LEEUW, 2010). The treatment of orofacial pain and TMD is done with different drugs, however, these drugs are inefficient to treat many patients (SHEPARD et al., 2013).

Natural products possess biological and pharmacological effects that bring benefits to therapeutic treatment of many diseases (BAKER et al., 2007). Therefore, the use of natural products can be an alternative for orofacial pain and TMD treatment.

The aim this systematic review is to determine the efficacy of natural products and its secondary metabolites in controlling nociceptive and inflammatory reaction in animals with orofacial pain.

### Research question

**Specify the disease/health problem of interest**

Orofacial pain.

**Specify the population/species studied**

Experimental animals submitted to any model of orofacial pain.

**Specify the intervention/exposure**

The use of natural products or its secondary metabolites to reduce nociceptive and inflammatory responses following orofacial pain induction in “in vivo” animal experimentation.

**Specify the control population**

Control group (placebo treatment).

**Specify the outcome measures**

Nociceptive responses.

**State your research question (based on items 11-15)**

Compared to placebo, are natural products efficacious in controlling nociceptive and inflammatory responses of animals submitted to orofacial pain models?

What natural products and secondary metabolites have already been investigated in the treatment of experimental orofacial pain?
| 17. | Identify literature databases to search *(e.g. PubMed, Embase, Web of science)* | ✔ MEDLINE via PubMed  
✔ Web of Science  
✔ SCOPUS  
✔ EMBASE  
✔ SciELO  
✔ LILACS  
☐ Other, namely:  
☐ Specific journal(s), namely:  
When available, please add a supplementary file containing your search strategy: [insert file name]  
Search strategy applied to PubMed and adapted to the other databases:  
**PARTICIPANTS / CONDITION:**  
facial pain OR face pain OR pain, face OR pain, facial OR orofacial pain OR pain, orofacial OR neuralgia facial pain OR facial pain, neuralgia OR pain, neuralgia facial OR craniofacial pain OR pain, craniofacial OR myofacial pain OR pain, myofacial  
**INTERVENTION:**  
ethnobotan* OR Ethnopharmacolog* OR ethnobot* OR caatinga OR inner bark OR traditional chinese medicine OR Chinese medicine OR Chinese medicine OR natural products OR natural product OR plant OR plants OR phytother* OR terpene OR flavonoid OR coumarin OR xanthone OR chromone OR lignan OR neolignan OR tannin OR saponin OR alkaldoid OR xanthine OR methylxanthine  
☐ Reference lists of included studies  
☐ Books  
☐ Reference lists of relevant reviews  
☐ Conference proceedings, namely:  
☐ Contacting authors/ organisations, namely:  
☐ Other, namely: |
| 18. | Define electronic search strategies *(e.g. use the step by step search guide*[^15] and animal search filters[^20-23]*) |  |
| 19. | Identify other sources for study identification | ✔ Reference lists of included studies  
☐ Books  
☐ Reference lists of relevant reviews  
☐ Conference proceedings, namely:  
☐ Contacting authors/ organisations, namely:  
☐ Other, namely: |
| 20. | Define search strategy for these other sources | Google Scholar, Google |
| 21. | Define screening phases *(e.g. pre-screening based on title/abstract, full text screening, both)* | 1. Title/abstract screening.  
2. Full text screening. |
| 22. | Specify (a) the number of reviewers per screening phase | a. Two reviewers will independently screen for relevant studies. |
and (b) how discrepancies will be resolved

*b* Discrepancies will be resolved either by discussion or by a third reviewer (when no agreement is met by the two reviewers).

**Define all inclusion and exclusion criteria based on:**

| 23. | Type of study (design) | Inclusion criteria: Pre-clinical study with intervention and control group. Exclusion criteria: N/A |
| 24. | Type of animals/population (*e.g.* age, gender, disease model) | Inclusion criteria: Laboratory animals submitted to orofacial pain. Exclusion criteria: Dental comorbidity and TMD model |
| 25. | Type of intervention (*e.g.* dosage, timing, frequency) | Inclusion criteria: Natural product or its secondary metabolites, any dosage, timing, frequency or via administration. Exclusion criteria: Mixture of treatment |
| 26. | Outcome measures | Inclusion criteria: Orofacial nociception, immunohistochemistry, inflammatory parameters. Exclusion criteria: N/A |
| 27. | Language restrictions | Inclusion criteria: No language restrictions. Exclusion criteria: N/A. |
| 28. | Publication date restrictions | Inclusion criteria: Studies published up to search date. Exclusion criteria: No past date restriction |
| 29. | Other | Inclusion criteria: N/A Exclusion criteria: N/A |

**Sort and prioritize your exclusion criteria per selection phase**

Selection phase: Title and abstract screening
1. Type of study
2. Type animals
3. Type of intervention
Selection phase: Full text screening
1. Reviews or non-original papers
2. Type of study
3. Type animals
4. Type of intervention
5. Outcome measure

**Study characteristics to be extracted (for assessment of external validity, reporting quality)**

<p>| 31. | Study ID (<em>e.g.</em> authors, year) | Authors, title, year of publication, language, contact author e-mail |
| 32. | Study design characteristics (<em>e.g.</em> experimental groups, number of animals) | Experimental groups Number of animals per group |
| 33. | Animal model characteristics (<em>e.g.</em> species, gender, disease induction) | Animal species, Strain, Age, Weight, Orofacial pain induction technique |
| 34. | Intervention characteristics (<em>e.g.</em> intervention, timing, duration) | Type of drug, type of natural product, dosage of drug, duration of treatment, frequency of drug administration, route of administration, timing relative orofacial pain induction, type of control group |</p>
<table>
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<tr>
<th>No.</th>
<th>Section</th>
<th>Details</th>
</tr>
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<tbody>
<tr>
<td>35.</td>
<td>Outcome measures</td>
<td>All</td>
</tr>
<tr>
<td>36.</td>
<td>Other (e.g. drop-outs)</td>
<td>Age of sacrificing animals, form of sacrifice, side effects of drug (weight loss, death, etc.)</td>
</tr>
</tbody>
</table>
| 37. | Assessment risk of bias (internal validity) or study quality                                                          | a. Two reviewers will independently assess risk of bias of included studies.  
|     | Specify (a) the number of reviewers assessing the risk of bias/study quality in each study and (b) how discrepancies will be resolved | b. Discrepancies will be resolved either by discussion or by a third reviewer (when no agreement is met by the two reviewers).          |
| 38. | Define criteria to assess (a) the internal validity of included studies (e.g. selection, performance, detection and attrition bias) and/or (b) other study quality measures (e.g. reporting quality, power) | a. By use of SYRCLE’s Risk of Bias tool  
|     |                                                                                                                                 | b. By use of CAMARADES’ study quality checklist, e.g.  
|     |                                                                                                                                 | c. By use of CAMARADES’ study quality checklist, adapted as follows:  
|     |                                                                                                                                 | d. Other criteria, namely: |
| 39. | Collection of outcome data                                                                                                | Nociception: stimuli threshold (g); continuous. Histological analysis: histological scores; discrete. Myeloperoxidase activity: unit of MPO/mg protein, units of MPO/mg tissue or optical density; continuous. Orofacial nociception (time of behavior, percentage)  
|     | For each outcome measure, define the type of data to be extracted (e.g. continuous/dichotomous, unit of measurement)    | 1) Extract data from text or tables  
|     | Methods for data extraction/retrieval (e.g. first extraction from graphs using a digital screen ruler, then contacting authors) | 2) Extract data from figures  
|     |                                                                                                                                 | 3) Contact authors for data not presented in paper  
|     | If no answer is obtained within a week or there is no contact information, other authors will be randomly contacted. After three weeks, if no answer is received, the study will be excluded from analysis. |
| 40. | Specify (a) the number of reviewers extracting data and (b) how discrepancies will be resolved                         | a. Two reviewers will independently assess risk of bias of included studies.  
|     |                                                                                                                                 | b. Discrepancies will be resolved either by discussion or by a third reviewer (when no agreement is met by the two reviewers).          |
| 41. | Data analysis/synthesis                                                                                                 | To all outcomes network meta-analysis is intended.                                                                                           |
| 42. | Specify (per outcome measure) how you are planning to combine/compare the data (e.g. descriptive summary, meta-analysis) | To all outcomes:  
|     |                                                                                                                                 | - at least two studies;  
<p>| 43. | Specify (per outcome measure) how it will be decided whether a meta-analysis will be performed                           | - same specie investigated.                                                                                                                  |</p>
<table>
<thead>
<tr>
<th><strong>If a meta-analysis seems feasible/sensible, specify (for each outcome measure):</strong></th>
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<tbody>
<tr>
<td><strong>44.</strong> The effect measure to be used <em>(e.g. mean difference, standardized mean difference, risk ratio, odds ratio)</em></td>
</tr>
</tbody>
</table>
| **45.** The statistical model of analysis *(e.g. random or fixed effects model)* | To all outcomes:  
- Random effects model  
- Markov Chain Monte Carlo |
| **46.** The statistical methods to assess heterogeneity *(e.g. $I^2$, $Q$)* | I-square. |
| **47.** Which study characteristics will be examined as potential source of heterogeneity *(subgroup analysis)* | Animal species.  
Gender.  
Orofacial pain models.  
Natural plant.  
Dose. |
| **48.** Any sensitivity analyses you propose to perform  
Other details meta-analysis *(e.g. correction for multiple testing, correction for multiple use of control group)* | Risk of bias of included studies  
Correction for multiple use of control group. |
| **49.** The method for assessment of publication bias | Funnel plot, if applicable *(i.e. 10+ studies included).* |

**Final approval by (names, affiliations):**

| Date: |

**Notes:**
The results of initial database research proposed for the protocol offered material for confection of two independent systematic reviews. Then, we decided divide it. Due to this protocol need to be altered for update the methods of search.  
The other review, about the use of natural products in laboratory animals submitted to TMD models, was done using the same methodology, except the search strategy, and the condition (TMD). This strategy used was:

(temporomandibular joint disorders OR disorder, temporomandibular joint OR disorders, temporomandibular OR temporomandibular OR joint disorder, temporomandibular OR joint disorders, temporomandibular OR temporomandibular OR tmj disorders OR disorder, tmj OR disorders, tmj OR tmj disorder OR temporomandibular disorders OR disorder, temporomandibular OR disorders, temporomandibular OR temporomandibular disorder OR temporomandibular joint diseases OR disease, temporomandibular joint OR diseases, temporomandibular joint OR joint disease, temporomandibular OR joint diseases, temporomandibular OR temporomandibular OR tmj diseases OR disease, tmj OR diseases, tmj OR tmj disease) AND (ethnobotan* OR Ethnopharmacolog* OR ethno botan* OR caatinga OR inner bark OR traditional chinese medicine OR chinese medicine OR chinese medicine OR natural products OR natural product OR plant OR plants OR phytother* OR terpene OR flavonoid OR coumarin OR xanthone OR chromone OR lignan OR neolignan OR tannin OR saponin OR alkaloïd OR xanthine OR methylxanthine)
Referências Bibliográficas:

