Anti-inflammatory and wound healing effect of Copaiba oleoresin on the oral cavity: A systematic review

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ABSTRACT

Copaiba oleoresin has been related to properties including healing and anti-inflammatory effects, making it a potential candidate to treat oral lesions. We aimed to define the benefits related to the anti-inflammatory and healing capacity of Copaiba-based formulations on the oral cavity. This is a systematic review, conducted in PubMed, Web of Science, Scopus, Embase, Scielo, Cochrane Library, BVS, and Google Scholar databases selecting full articles in English, Portuguese, or Spanish, until March 3rd, 2021. Pre-clinical, clinical, or randomized clinical trials, cohort and case-control in vivo studies were included; studies with other designs, in vitro, and those that did not match the PICO question were excluded (PROSPERO: CRD42021244938). Data was collected and synthesized descriptively through a specific form. The risk of bias was evaluated by SYRCLE’s RoB Tool. So, five studies were included. Two reported beneficial wound healing effects, such as early reduction in the wound area and greater immature bone formation in the rats’ mandibles; and two related beneficial anti-inflammatory effects, like reduced acute inflammatory reaction and more advanced tissue repair stage, early formation of collagen fibrils, with greater quantity, thickness and better organization, and more expressive anti-inflammatory activity, reduction of the edema intensity and the CD68+ macrophages concentration. Based on the articles, benefits related to the wound healing and anti-inflammatory effects in the oral cavity of rats treated with Copaiba oleoresin were suggested. However, due to the limited data, future studies are necessary, especially clinical ones.

1. Introduction

Historically, medicinal plants have been used to treat diseases and restore health. Since 2002, the World Health Organization (WHO) has recognized the importance of traditional medicine as part of care (Ricardo et al., 2018). Currently, the use of herbal medicines has grown due to their efficiency, low toxicity, biocompatibility, and low cost (Tobouti et al., 2017).

In the Latin American scenario, including Brazil, the Copaiba tree, of the Copaifera provenance, stands out (da Trindade et al., 2018; Pieri et al., 2009). Copaiba oleoresin can be produced using some of its species (Ames-Sibin et al., 2018); this compound can be used in natura or as an industrialized product, either by oral or by topical application (Dias-da-Silva et al., 2013).

Recent studies demonstrated that Copaiba oleoresin, in addition to its anti-inflammatory popular medicine typical use, has antioxidative, healing, bone formation stimulant, cytotoxic, gastroprotective, nociceptive, antimicrobial, antileishmanial, antiedema, antifungal, antilemmorrhagic, anthelmintic, and antiseptic proprieties (Ames-Sibin et al., 2018; da Trindade et al., 2018; Dalenogare et al., 2019; Dias-da-Silva et al., 2013; Diefenbach et al., 2018; Leandro et al., 2012; Lima et al., 2011; Pfeifer Barbosa, 2018; Valadas et al., 2019; Wagner et al., 2017).

Considering these proprieties of the Copaiba oleoresin, it emerges as a potential candidate to treat lesions on the oral cavity. Thus, this study aimed to define the benefits related to the anti-inflammatory and healing capabilities of Copaiba oleoresin-based formulations on the oral cavity.

2. Material and methods

This systematic review was reported according to the 2020 PRISMA recommendation (Page et al., 2021).

2.1. Eligibility criteria

The PICO question for this review was “What are the benefits related to the anti-inflammatory and healing capabilities (outcome) of Copaiba oleoresin on the oral cavity: A systematic review?”
oleoresin-based formulations (intervention) in lesions in the oral cavity of research subjects who use these formulations (population) compared to those who do not use this substance (comparison)?". Pre-clinical trials, clinical trials, randomized controlled trials (RCT), retrospective or prospective cohort (PC), and case-control studies conducted in vivo, with human or animal subjects, were included. Previous reviews, meta-analyses, case reports, cross-sectional studies, studies conducted in vitro, and those that did not match the PICO question were excluded.

2.2. Information sources and search strategy

A bibliographic search was carried out in PubMed, Web of Science, Scopus, Embase, Scielo, Cochrane Library, BVS and Google Scholar databases selecting full articles published in English, Portuguese or Spanish, until March 3^rd^, 2021, without year limitation. The search was conducted using the keywords "(((fabaceae) OR (copaifera)) OR (copaiba)) AND (oral wound healing') OR (oral anti-inflammatory activity')", "((fabaceae OR copaifera OR copaiba) AND (oral wound healing') OR (oral anti-inflammatory activity') AND (fabaceae) OR copaifera OR copaiba) AND (oral wound healing') OR (oral anti-inflammatory activity')."

Letters, book chapters, and abstracts of meetings were excluded. This systematic review is registered in PROSPERO 2021 as CRD42021244938.

2.3. Selection process

To minimize inadvertent biases, two authors (ACSM and LDBA) conducted the bibliographic search in databases and manual search. All articles were exported from the databases to the Rayyan application (Ouzzani et al., 2016). The identification was based on titles and abstracts obtained via database search.

2.4. Data collection process and synthesis methods

Data collection from the five included articles was performed independently by two authors (ACSM and LDBA) through a specific form designed for this review. The data of interest were characteristics of the study (type, data collection, sampling competence, information about study participants, number of centers involved, confounding factors, main results, anti-inflammatory effects, wound healing capacity, and conclusions) and the use of Copaiba (the species, the formulation, and the concentration of Copaiba compound, the route, the dosage, the frequency and duration of use of the formulation). The extracted data was synthesized descriptively.

2.5. Risk of bias assessment and reporting

Two reviewers (HAS and DCG) independently assessed the risk of bias in the included studies, considering the criteria established by the SYRCLE’s RoB Tool (Hooijmans et al., 2014), a tool designed to assess the methodological quality of animal experiments based on the Cochrane Collaboration RoB Tool for randomized clinical trials and in the QUADAS tool.

In this tool, each animal study was evaluated according to ten entries, and these are related to six types of bias: selection, performance, detections, attrition, reporting, and other bias. For each entry, the reviewers independently assigned a judgment of low, high, or unclear risk of bias. If there is some disagreement between the reviewers regarding the classification of the risk of bias, this was resolved through consensus-oriented discussion. If the discussion is not enough to solve the disagreement a third reviewer was to be consulted.

The risk of bias assessment is presented through a table and a summary containing the risk accessed to all individual studies. This data was generated through Review Manager 5.4 (The Cochrane Collaboration, 2020).

3. Results

The selection process resulted in 613 studies (PubMed = 17, Web of Science = 16, Scopus = 309, Embase = 19, Science Citation Index Expanded = 212, BVS = 17, and Google Scholar = 63). After, the duplicates were removed (n = 91) and four studies that potentially met the inclusion criteria were selected. Another study was identified by cross-reference; thus, five articles were selected for full-text analysis.

The full text of these potentially eligible studies was retrieved and independently assessed for eligibility by the same members of the review team. Any disagreement between them on the eligibility of specific studies was resolved through discussion with a third reviewer (HAS) (Figure 1).

After the complete analysis of the selected research papers, a total of five studies were included in this review. The five studies consisted of prospective preclinical studies, developed by research groups in Brazil and published between 2013 and 2020. Among them, one evaluated only the healing capacity, another one only evaluated the anti-inflammatory capacity, and the other three evaluated both capabilities. Details of the studies are described in Table 1.

3.1. Copaiba oleoresin

Three out of five studies were performed with Copaifera reticulata Ducke (Alvarenga et al., 2020; Teixeira et al., 2017; Wagner et al., 2017); the other two studies did not specify the species (Dias-da-Silva et al., 2013; Silva et al., 2015). As for the standardization of the Copaiba oleoresin, regarding the compound used, there were significant differences between the studies, with three referring the use of Copaiba oleoresin in nature - with Teixeira et al. using saline and tWEEN to facilitate oral gavage - (Silva et al., 2015; Teixeira et al., 2017; Wagner et al., 2017); one specifying that the copaiba oleoresin was dissolved in an emulsion containing saline solution and tWEEN 20 to 5% (Alvarenga et al., 2020); and one did not provide specifications of the formulation (Dias-da-Silva et al., 2013).

The most used route of administration of Copaiba oleoresin in the studies was the systemic one, through oral gavage, which was used in three of the studies (Alvarenga et al., 2020; Silva et al., 2015; Teixeira et al., 2017). The frequency of use of Copaiba oleoresin was once a day in 80% of the studies and the period of use varied between three and 14 days. Among the control groups, there were active controls and placebo; between the four studies that choose active controls groups, three of them chose to do it with corticosteroids, being 2 with dexamethasone (Alvarenga et al., 2020; Teixeira et al., 2017), and 1 with clobetasol (Wagner et al., 2017); Silva et al. chose a non-steroidal anti-inflammatory drug: meloxicam (Silva et al., 2015). None of the studies reported adverse events associated with the use of Copaiba oleoresin and only the study by Silva et al. (2015) reported the death of one rat after the surgical procedure that was not related to the use of Copaiba oleoresin. Other specific information of the five selected studies are described in Table 1.

It is worth noting that, in addition to evaluating the anti-inflammatory capacity, Teixeira et al. performed acute toxicity with Albinino Swiss rats, preceding the study. Five rats were tested with the limit dose of 2000 mg/kg/day and were observed for 48 h. It was proposed that if three consecutive animals survived to the use of the compound at this dose or, if at least four of the five animals survived, the dose prescribed by the study would be defined as 10% of the threshold dose. As in the acute toxicity test none of the tested animals died or showed any signs or symptoms of toxicity, the trial dose was set at 200 mg/kg/day (Teixeira et al., 2017).

3.2. Wound healing effect

The wound-healing effect of Copaiba oleoresin was evaluated in four of the studies included in this review (Alvarenga et al., 2020;
Dias-da-Silva et al., 2013; Silva et al., 2015; Wagner et al., 2017) and information about the evaluation periods, measurements, variations of measures and benefits are described in Table 2. Benefits were reported in two of them (Alvarenga et al., 2020; Dias-da-Silva et al., 2013).

Alvarenga et al. reported a statistically significant early reduction in the wound area of rats treated with oral gavage of Copaiba oleoresin when compared to the control group (p < 0.05) and the control group (p < 0.01) on the 3rd day after oral injury. They also observed complete healing of the lesions on the 7th day of the rats in the Copaiba group, while in the other groups this only occurred on the 15th day (Alvarenga et al., 2020).

The study conducted by Dias-da-Silva et al. showed greater immature bone formation in the mandibles of rats that received topical Copaiba irrigation when compared to the control. Also, when compared to the placebo group, they reported thicker bone formation in the mandibles that were treated with the systemic one. Although there was a statistically significant increase in bone formation in the two groups treated with Copaiba when compared to the control, when comparing the topical and systemic Copaiba treatment, there was no statistically significant difference between them (27.82 ± 5.71 for topical Copaiba group; 30.27 ± 1.74 for systemic Copaiba group; 20.91 ± 7.53 for topical placebo group; 22.45 ± 7.00 for the systemic placebo group) (Dias-da-Silva et al., 2013).

Nevertheless, the study by Silva et al. evaluated the osteoclasts and osteoblasts activity, the bone formation, and the bone matrix mineralization; the activity of the osteoclasts was observed in four groups (Gcell-Copaiba oleoresin, Gbio-control, Gbio-Copaiba oleoresin, and Gbio-mellox) (P = 0.78), the osteoblast presence was very similar between the groups, except in the Gcell-mellox, that presented a less significant activity (p = 0.009) and the bone matrix mineralization, however, was not different between the groups (p = 0.60) (Silva et al., 2015). Similarly, Wagner et al. did not observe a statistically significant difference (p > 0.05) regarding the percentage of wound closure when comparing the control, placebo, and Copaiba groups. They, however, observed that the corticoid group had a statistically significant slower healing process compared to the others (p = 0.007) (Wagner et al., 2017).

3.3. Anti-inflammatory effect

The anti-inflammatory effect of Copaiba oleoresin was evaluated in four of the studies included in this review (Alvarenga et al., 2020; Silva et al., 2015; Teixeira et al., 2017; Wagner et al., 2017) and information about the evaluation periods, measurements, measurement variations, and benefits are described in Table 3.

In the study by Alvarenga et al., wounds treated with Copaiba oleoresin oral gavage (200 mg/kg/day) once a day for three consecutive days starting in the procedure day showed statistical significance higher inflammatory and reepithelialization scores (p < 0.05) on the 3rd day after the procedure, indicating respectively more advanced inflammatory stage resulting in reduced acute inflammatory reaction and more advanced tissue repair stage. In addition, wounds treated with Copaiba showed the early formation of collagen fibrils, with greater quantity, greater thickness, and better organization when compared to the control and corticoid groups (Alvarenga et al., 2020).

Teixeira et al. reported that the Copaiba and the corticoid group showed more expressive anti-inflammatory activity than the placebo group, with statistical significance (1.2 ± 0.20); regarding edema, Copaiba reduced its intensity, but no statistically significant difference was observed between the other groups (1.8 ± 0.20). A reduction in the concentration of CD68 + macrophages was also observed, with statistical significance (p = 0.0432), when comparing the Copaiba group with the placebo one (Teixeira et al., 2017).

On the other hand, according to the results obtained by Silva et al., when using the Copaiba oleoresin (0.6 mL/kg/day) through oral gavage once a day for seven days starting in the fifth day after the surgical procedure, no benefits related to the anti-inflammatory effect were observed in the rats treated with Copaiba since inflammatory cells were present in all groups, with no statistically significant difference between them (p = 0.52) (Silva et al., 2015). The results of Wagner et al. converge in this sense, and did not observe significant differences related to the inflammatory process between the control, placebo, and Copaiba groups (Wagner et al., 2017).
Table 1. Studies addressing anti-inflammatory and wound healing effect of Copaiba oleoresin on the oral cavity.

| Author (year)               | Study objective                                                                 | Study type                                | Animals' specifications | Data collection          | Sample size | Number and details of the groups | Intervention frequency | Intervention period | Oral Wound Healing capacity | Oral Anti-inflammatory capacity | Main conclusions                                                                 |
|-----------------------------|---------------------------------------------------------------------------------|-------------------------------------------|-------------------------|--------------------------|-------------|---------------------------------|------------------------|------------------------|-------------------------------|---------------------------------|----------------------------------------------------------------------------------|
| Alvarenga et al. (2020)     | To investigate the therapeutic effects of Copaiba oleoresin (C. reticulata Ducke) on oral reepithelization by decreasing inflammatory response in an animal model of traumatic ulcer induced in the tongue of rats. To analyze the safety of the dosage used in this experiment through analyses of biochemical parameters of liver and kidneys functions to introduce the oleoresin as an alternative therapy in oral lesions. | Randomized, controlled, and blind preclinical study | Wistar (200–250 g)       | Prospective and simultaneous | 45 rats     | 3 groups (each with 15 rats): Systemic Copaiba oleoresin/oral gavage (200 mg/kg/day); Active control (Dexamethasone 0.5 mg/kg/day); Active control (Dexmethasone 0.5 mg/kg/day) | Once a day              | 3 consecutive days (starting 12 h after the procedure) | Yes                           | Yes                             | Copaiba oleoresin is a natural product effective in reducing chronic inflammation and inhibiting macrophage activity; about the lack of effective capacity to reduce edema, the data suggest further research to investigate the role of this oil in the modulation of the inflammation process. |
| Teixeira et al. (2017)      | To evaluate the anti-inflammatory properties of Copaiba oleoresin (Copaifera reticulata Ducke) in a model that transfixes injury in rat tongues. To evaluate the clinical and histopathological aspects of topical treatment with Copaiba oleoresin (Copaifera Reticulata Ducke) extract on oral wound healing in an animal model and compared with topical corticosteroids treatment. | Preclinical study                          | Wistar (150–200 g)       | Prospective and simultaneous | 15 rats     | 3 groups (each with 5 rats): Systemic Copaiba oleoresin/oral gavage (200 mg/kg/day); Active control (Dexamethasone 0.5 mg/kg/day); Placebo control (Twe 20 200 mg/kg/day) | Twice a day             | 7 consecutive days (starting 12 h after the procedure) | No                            | Yes                             | Topical administration of Copaiba oleoresin did not accelerate the oral healing process and did not promote relevant side effects in this model. |
| Wagner et al. (2017)        | To evaluate the Copaiba oleoresin influence in experimental bone defects filled with two bone substitutes in rat's jaw by evaluating histologically the composition of formed bone tissue. | Preclinical study                          | Wistar (250–300 g)       | Prospective and simultaneous | 96 rats     | 4 groups (each with 24 rats): Systemic Copaiba oleoresin/oral gavage (0.6 mL/kg/day); Active control (same components of the oil without the Copaiba extract); Active control (topical 0.05% clohexadolin propionate with a hydroxyethylcellulose gel); Control without treatment | Once a day              | 7 consecutive days (starting on the fifth day after the procedure) | Yes                           | Yes                             |考上和syepia。 |
| Silva et al. (2015)         | To evaluate the Copaiba oleoresin effects, by topical and systemic administration, on alveolar wound healing in rats. | Preclinical study                          | Wistar                  | Prospective and simultaneous | 42 rats     | 6 groups (each with 7 rats): Gbio + Systemic Copaiba oleoresin/Oral Gavage (0.6 mL/kg/day); Gccl + Systemic Copaiba oleoresin/oral gavage (0.6 mL/kg/day); Gbio + placebo control (distilled water - 0.6 mL/kg/day); Gccl + placebo control (distilled water - 0.6 mL/kg/day); Gbio + active control (Meloxicam 0.25 mg/kg/day diluted in 0.6 mL/kg); Gccl + active control (Meloxicam 0.25 mg/kg/day diluted in 0.6 mL/kg) | Once a day              | 14 consecutive days            | Yes                           | Yes                             | Copaiba oleoresin administered through oral gavage did not affect the bone repair of defects in rat's jaws 40 days after the procedure. |
| Dias-da-Silva et al. (2013) | To evaluate the Copaiba oleoresin effects, by topical and systemic administration, on alveolar wound healing in rats. | Preclinical study                          | Wistar                  | Prospective and simultaneous | 28 rats     | 4 groups (6 in each group that used Copaiba and 8 in each control): Topical Copaiba oleoresin (30 ml irrigation); Systemic Copaiba oleoresin/oral gavage (0.1 ml Copaiba/100 g body weight); Topical placebo-control (irrigation with saline); Systemic placebo-control (gavage with saline) | Once a day              | 3 consecutive days after the procedure for the topical groups and 7 for the systemic ones | Yes                           | Yes                             | Topical and systemic administration of Copaiba oleoresin promotes better results after oral surgical procedures due to greater bone neoformation when compared to the control group. |

Abbreviations: Gbio = group in which the bone defects were filled with bioglass; Gccl = group in which the bone defects were filled with adipose tissue.

3.4. Bias analysis

Bias analysis of the five articles showed that all the articles (100%) presented a low risk of bias for random sequence generation, baseline characteristics, random housing, and selective outcome reporting; four of them (80%) for allocation concealment, blinding of participants and personnel, random of outcome assessment, and other source of bias; and three of them (60%) for incomplete outcome data. For more information, consult Figure 2 and Table 4.
Table 2. Evaluation of the wound healing effect of Copaiba oleoresin in the oral cavity.

| Author (year) | Evaluation period | Control(s) group(s) main results | Copaiba group main results | Benefits associated with Copaiba oleoresin use |
|---------------|-------------------|---------------------------------|---------------------------|---------------------------------------------|
| Alvarenga et al. (2020) | At the procedure day and 3, 7, and 15 days after | Placebo control | ● Wound area: D0 = 7mm², D3 = 2mm², D7 = 0mm²; ● Reepithelialization: D3 = 2.5, D7 = 0.8; ● Collagen/PSR score: D3 = 1, D7 = 1.5 | Early reduction in wound area compared to the steroid group and the control group on D3, with a statistically significant difference when compared to the steroid group (p < 0.05) and control (p < 0.01); mandibles in the Copaiba group had complete healing of the wounds; |
| Wagner et al. (2017) | 3, 5, 10, and 14 days after | Placebo control | ● Wound status: D3 and D5 – open in all animals; D10 and D14 – closed in all animals; ● Percentage of wound healing: D3 ≥ 75%; D5 ≥ 75%; D10 = 100%; D14 = 100%; ● Wound healing time: no sign of scarring until D6; did not differ from the control without treatment group regarding wound closure time (p > 0.05—Log-rank test) | There was no statistically significant difference in the percentage of wound closure when comparing the control, placebo, and Copaiba groups, however, the corticoid group showed a slower healing process |
| Silva et al. (2015) | 40 days after the procedure | Active control | ● Wound status: D3 and D5 – open in all animals; D10 and D14 – closed in all animals; ● Percentage of wound healing: D3 ≥ 70%; D5 ≥ 70%; D10 = 100%; D14 = 100%; ● Wound healing time: no sign of scarring until D6; did not differ from the control without treatment group regarding wound closure time (p > 0.05—Log-rank test) | Osteoclast activity was observed only in four groups and was more expressive in oil-Gcell (p = 0.009), but it was not statistically significant; regarding the presence of osteoblasts, Gcell-melox (p = 0.78), had lower osteoblastic activity compared to the other |
| Dias-da-Silva et al. (2013) | 0.009), had lower osteoblastic activity compared to the other | Systemic placebo control | ● Wound status: D3 and D5 – open in all animals; D10 and D14 – closed in all animals; ● Percentage of wound healing: D3 ≥ 0%; D5 ≤ 0%; D10 = 100%; D14 = 100%; ● Wound healing time: no sign of scarring until D6; did not differ from the control without treatment group regarding wound closure time (p > 0.05—Log-rank test) | The group reported greater immature bone formation in the mandibles of rats that received topical irrigation with Copaiba when compared to the control, thicker bone formation in the mandibles that received systemic Copaiba compared to placebo, and thinner bone trabeculate |

| | | Topical Copaiba oleoresin | | |
| | | ● Area density of the immature bone formed: Relative frequency of bone formation = 20.91 ± 7.53 (21%); discrete formation of immature bone irregularly distributed in thin trabeculae | |
| | | Systemic Copaiba oleoresin | | |
| | | ● Area density of the immature bone formed: Relative frequency of bone formation = 22.45 ± 7.60 (22%) | |
Table 2 (continued)

| Author (year) | Alvarenga et al. (2020) | Wagner et al. (2017) | Silva et al. (2015) | Dias-da-Silva et al. (2013) |
|---------------|-------------------------|---------------------|--------------------|-----------------------------|
| lesion on D7 while in the other groups this only occurred on D15 | groups; bone formation was observed in all groups and only two animals did not show bone formation even after 40 days; more than 50% of bone matrix mineralization was observed in 56% (23 animals) of the analyzed areas and bone matrix mineralization was not different between groups \( (p = 0.60) \) | a statistically significant increase in bone formation in the two groups treated with Copaiba when compared to the control, but there was no statistically significant difference between topical and systemic treatment with Copaiba |

* Values estimated according to the graphs present in the studies; the authors did not define the exact values in the results.

4. Discussion

Although the extensive bibliographic search, few articles were included. This occurs due to the small number of studies with Copaiba. Considering that the first article included was published in 2013 (Dias-da-Silva et al., 2013) and the last in 2020 (Alvarenga et al., 2020), it is observed that within 7 years, only five research papers on this topic were published, highlighting a gap in the literature related to this subject.

Regarding the included studies, there is uniformity to the species of rat used - unanimity regarding male Wistar rats (Alvarenga et al., 2020; Dias-da-Silva et al., 2013; Silva et al., 2015; Teixeira et al., 2017; Wagner et al., 2017). Furthermore, we can observe that all projects were developed in the Brazilian research scenario, which is explained by the typical Latin American origin of Copaiba (da Trindade et al., 2018; Pieri et al., 2009).

On the other hand, the studies differ significantly regarding the standardization of the Copaiba compound used, the proposed methodologies, and the type of control. Also in this sense, concerning the healing effect, two studies address the effect on mineralized tissues (Dias-da-Silva et al., 2013; Wagner et al., 2017) and two on mucous membranes (Alvarenga et al., 2020; Silva et al., 2015). Such factors make it difficult to directly compare the results presented by them.

Several administration routes were proposed in these studies, and the results obtained by Alvarenga et al., and Dias-da-Silva et al. were... (continued on next page)
Table 3 (continued)

| Author (year) | Placebo control | Teixeira et al. (2017) | Wagner et al. (2017) | Silva et al. (2015) |
|---------------|-----------------|------------------------|----------------------|-------------------|
| **Control(s) group(s) main results** | | | | | |
| Placebo control | • Evaluation of edema, inflammatory cells, angiogenesis, and muscle fibers; moderate chronic inflammatory infiltrate, with presence of lymphocytes, plasma cells, and macrophages and accompanied by extensive edema. The angiogenesis process was also observed along with little formations of immature muscle fibers; | • Edema score: 2.4 ± 0.24; | • Immune infiltrate score: 2.0 ± 0.3; | • CD68 + macrophages concentration*: 95 |
| | • Edema score: 0.25 ± 0.25; | • Immune infiltrate score: 1.0 ± 0.3; | • CD68 + macrophages concentration*: 0.7 |
| Active control | • Evaluation of edema, inflammatory cells, angiogenesis, and muscle fibers; less chronic inflammatory infiltrate and greater formation of muscle fibers in the injury area when compared to the placebo control group and less edema | | | |
| | • Edema score: 2.4 ± 0.24; | • Immune infiltrate score: 2.0 ± 0.3; | • CD68 + macrophages concentration*: 95 |
| Benefits associated with Copaiba oleoresin use | Both Copaiba and corticoid group showed more expressive anti-inflammatory activity and accelerated repair of the area when compared to the placebo group with statistical significance, associated with a reduction in the intensity of the chronic inflammatory infiltrate; concerning edema, Copaiba reduced the intensity of the edema, but no statistically significant difference was observed when compared to placebo or the corticoid group; reduction in the concentration of CD68 + macrophages in both the corticoid and Copaiba groups, but the reduction was significant only when comparing the Copaiba group with the placebo (p = 0.0439); | No statistically significant difference in the inflammatory process was observed when comparing the control, placebo, and Copaiba groups, however, the corticoid group showed a more intense inflammatory process in the histopathological analysis | No benefits were observed. | |

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| Active control | • Evaluation of edema, inflammatory cells, angiogenesis, and muscle fibers; less chronic inflammatory infiltrate and greater formation of muscle fibers in the injury area when compared to the placebo control group and less edema | • Edema score: 0.25 ± 0.25; | • Immune infiltrate score: 1.0 ± 0.3; | • CD68 + macrophages concentration*: 0.7 |
| Benefits associated with Copaiba oleoresin use | Both Copaiba and corticoid group showed more expressive anti-inflammatory activity and accelerated repair of the area when compared to the placebo group with statistical significance, associated with a reduction in the intensity of the chronic inflammatory infiltrate; concerning edema, Copaiba reduced the intensity of the edema, but no statistically significant difference was observed when compared to placebo or the corticoid group; reduction in the concentration of CD68 + macrophages in both the corticoid and Copaiba groups, but the reduction was significant only when comparing the Copaiba group with the placebo (p = 0.0439); | No statistically significant difference in the inflammatory process was observed when comparing the control, placebo, and Copaiba groups, however, the corticoid group showed a more intense inflammatory process in the histopathological analysis | No benefits were observed. | |

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* Values estimated according to the graphs present in the studies; the authors did not define the exact values in the results.

Associated with greater benefit related to the oral wound healing process (Alvarenga et al., 2020; Dias-da-Silva et al., 2013) and, anti-inflammatory activity (Alvarenga et al., 2020; Teixeira et al., 2017). Regarding oral wound healing effect, Alvarenga et al. showed an early reduction in the wound area due to early re-epithelialization and early formation of collagen fibrils in greater quantities, thicker and more organized (Alvarenga et al., 2020), while Dias-da-Silva et al. reported the greater formation of immature bone and thicker bone formation (Dias-da-Silva et al., 2013). When it comes to the anti-inflammatory effect, Alvarenga et al. showed a reduction in the acute inflammatory response (Alvarenga et al., 2020) and Teixeira et al. demonstrated a reduction in edema and in the concentration of CD68 + macrophages (Teixeira et al., 2017). Considering that these studies were conducted indicating systemic use of Copaiba, through oral gavage, its seems that this route is more effective when compared to topical use.

The anti-inflammatory effect of Copaiba oleoresin, demonstrated in the studies by Alvarenga et al. and Teixeira et al. (Alvarenga et al., 2020; Teixeira et al., 2017) was previously suggested by several authors (Ames-Sibin et al., 2018; Basile et al., 1988; da Trindade et al., 2018; Ferro et al., 2018; Gelmini et al., 2013; Gomes et al., 2010; Veiga et al., 2007). It probably results from the presence of β-caryophyllene, which reduces the production of metalloproteinases in the liver, the number of leukocytes in the blood, and their recruitment to the area of inflammation by blocking receptors and, consequently, reducing the secretion of...
pro-inflammatory mediators (Ames-Sibin et al., 2018; da Trindade et al., 2018; Gomes et al., 2010). This effect is also related to inhibition of nuclear factor-kappa-β translocation, and, consequently, inhibition of pro-inflammatory cytokine secretion (Gelmini et al., 2013).

Regarding the healing capacity demonstrated by Alvarenga et al. and Dias-da-Silva et al. (Alvarenga et al., 2020; Dias-da-Silva et al., 2013), it is suggested that the use of Copaiba oleoresin is associated with an increase of vascularization, the capacity to form granulation tissue and the population of fibroblasts, therefore favoring the second phase of the healing process (Esteves et al., 2013; Paiva et al., 2002).

In the study designed by Silva et al., the graft used in rats was composed of, in addition to Copaiba oleoresin (in the test groups), distilled water (in the placebo groups), and meloxicam (in the active control groups), bioglass, or adipose tissue. It is, therefore, possible that these had some influence on the anti-inflammatory and healing effects observed, thus constituting a confounding factor in the study (Silva et al., 2015).

Likewise, Wagner et al. suggest that the immunosuppressive effect associated with corticosteroids may have contributed to the growth of opportunistic microorganisms in the lesion from rats treated in the corticosteroid group. This could explain the more acute inflammatory infiltrate and may have contributed to a slower healing process, also representing a confounding factor of the study (Silva et al., 2015).

Procedures to induce oral lesions in animals, Alvarenga et al. performed traction of the animal’s tongue with exposure of the ventral surface for induction of a traumatic ulcer of 3 mm on the ventral surface, with a biopsy punch, 5 mm from the apex and in the midline region of the tongue; the punch was pressed into the tissue to penetrate 2mm, without crossing the muscle plane. Procedures were performed in the supine position, after anesthesia with ketamine hydrochloride (90 mg/kg) and xylazine hydrochloride (10 mg/kg), the region was previously cleaned with 2% chlorhexidine before the procedure and all the procedures were performed by the same operator, trained in a pilot study (Alvarenga et al., 2020). On the other hand, Teixeira et al. performed the traumatic injuries by immobilizing the tongue of the animals and inducing perforations with the Perry forceps, in pairs: one in the right lobe and one in the left. Procedures were performed in dorsal decubitus after anesthesia with ketamine hydrochloride (90 mg/kg) and xylazine hydrochloride (10 mg/kg) (Teixeira et al., 2017).

Comparing the two models of oral wound induction, it is clear that the one proposed by Alvarenga et al. was more standardized, as the wounds were all performed by the same operator, using a biopsy punch in a determinate depth and positioning of placement, making the lesions generated reproducible; in addition, there is a previous cleaning of the area in with the wound will be performed, reducing the risk of contamination of the traumatic injury, and also the model was previously tested in a pilot study (Alvarenga et al., 2020). The process proposed by Teixeira et al. is more susceptible to bias as it is not clear whether the same operator was responsible for inducing all the injuries, there is no related standardization regarding the dimensions of the injury and, there is no

Figure 2. Risk of bias of the selected articles. If the item was considered present in the article, it was judged as “low risk of bias” (green square). If it was not, the paper was classified as “high risk of bias” (red square). If this information was not available, the paper was classified as “undefined risk of bias” (yellow square) in this specific item.
report of cleaning of the area previously of the procedure, increasing the risk of contamination and, therefore, inflammatory reaction; additionally, although suggested by the position of the animals for the procedure, there is no specification if the injuries were all performed in the ventral surface of the tongue (Teixeira et al., 2017). Thus, considering the bias analysis, the study by Teixeira et al. was the only one in which high risk of other sources bias was found.

Although the lack of uniformity regarding the results of the studies towards the beneficial anti-inflammatory and healing effects of Copaiba oleoresin, it is worth emphasizing that none of them found harm for the groups that used Copaiba. The only adverse event reported was not related to the use of Copaiba (Silva et al., 2015), suggesting, therefore, that the use of this compound is safe, being related to a lower presence of associated side effects when compared to corticoids.

The current study has several limitations. The number of studies included was small, all of them were preclinical studies carried out in animal models, and the scenarios in which the effects, mainly the healing ones, were tested, varied between the studies, making comparisons between them exceedingly difficult. Therefore, the need to conduct novel studies, in humans, in more faithfully defined scenarios (especially in mucous lesions such as radio and/or chemo-induced oral mucositis or aphthous lesions) is highlighted, aiming to prove and validate the preliminary results observed in this review.

5. Conclusions

Based on the five articles included in this systematic review, regarding the four that analyzed the wound-healing effects, two studies suggested benefits; considering the four that analyzed anti-inflammatory activity, two suggested benefits in the oral cavity of rats treated with Copaiba oleoresin. Among the wound-healing effects, early reduction in the wound area and greater immature bone formation in the rats’ mandibles were reported. As for the anti-inflammatory effects, reduced acute inflammatory reaction and more advanced tissue repair stage, the early formation of collagen fibrils, with greater quantity, thickness, and better organization, and more expressive anti-inflammatory activity, reduction of the edema intensity and the CD68 + macrophages concentration was reported. However, although the results are promising, due to the limited number of studies on the subject, we emphasize the need for future studies, especially clinical ones, so that such benefits can be better analyzed.

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References

Alvarenga, M.O.P., et al., 2020. Safety and effectiveness of copaiba oleoresin (C. reticulata duke) on inflammation and tissue repair of oral wounds in rats. Int. J. Mol. Sci. 21 (10), 1–14.

Ames-Sibin, A.P., et al., 2018. β-Caryophyllene, the major constituent of copaiba oil, reduces systemic inflammation and oxidative stress in arthritic rats. J. Cell. Biochem. 119 (12), 10262–10277.

Basile, A.C., et al., 1988. Anti-inflammatory activity of oleoresin from Brazilian Copaifera. J. Ethnopharmacol. 22, 101–109.

Da Trindade, R., Da Silva, J.K., Setzer, W.N., 2018. Copaifera of the neotropics: a review of the phytochemistry and pharmacology. Int. J. Mol. Sci. 19 (5), 1096–1096.

Dalenogare, D.P., et al., 2019. Antiinociceptive activity of Copaifera officinalis Jacq. L oil and kaurenoic acid in mice. Inflammopharmacology 27 (4), 829–844. Disponível em: Dias-Da-Silva, M.A., et al., 2013. The influence of topic and systemic administration of copaiba oil on the alveolar wound healing after tooth extraction in rats. J. Clin. Experim. Dentist 5 (4), 169–173.

Diefenbach, A.L., et al., 2018. Antimicrobial activity of copaiba oil (Copaifera sp.) on oral pathogens: systematic review. Phytother Res. 32 (4), 586–596.
Estevão, L.R.M., et al., 2013. Effects of the topical administration of copaiba oil ointment (Copaifera langsdorffii) in skin flaps viability of rats. Acta Cir. Bras. 28 (12), 863–869.

Ferro, M., et al., 2018. Meta-analysis on copaiba oil: its functions in metabolism and its properties as an anti-inflammatory agent. J. Morpholog. Sci. 35 (3), 161–166.

Gelmini, F., et al., 2013. GC-MS profiling of the phytochemical constituents of the oleoresin from Copaifera langsdorffii Desf. and a preliminary in vivo evaluation of its antipsoriatic effect. Int. J. Pharm. 440 (2), 170–178. Disponível em:

Gomes, N. De M., et al., 2010. Characterization of the noninociceptive and anti-inflammatory activities of fractions obtained from Copaifera multijuga Hayne. J. Ethnopharmacol. 128 (1), 177–183.

Hooijmans, C.R., et al., 2014. SYRCLE’s risk of bias tool for animal studies. BMC Med. Res. Methodol. 14 (1), 43. Disponível em:

Leandro, L.M., et al., 2012. Chemistry and biological activities of terpenoids from copaiba (Copaifera spp.) oleoresins. Molecules 17 (4), 3866–3889.

Lima, C.S., et al., 2011. Pre-clinical validation of a vaginal cream containing copaiba oil (reproductive toxicology study). Phytomedicine 18 (12), 1013–1023. Disponível em:

Ouzzani, M., et al., 2016. Rayyan—a web and mobile app for systematic reviews. Syst. Rev. 5 (1), 210, 5 dez. 2016. Disponível em: http://systematicreviewjournal.biomedcentral.com/articles/10.1186/s13643-016-0384-4.

Page, M.J., et al., 2021. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. BMJ 371, 29 mar. Disponível em:

Paiva, L.A.F., et al., 2002. Investigation on the wound healing activity of oleo-resin from copaíba langsdorffi in rats. Phytother Res. 16 (8), 737–739.

Pfeifer Barbosa, A.L., et al., 2018. Antimicrobial and cytotoxic effects of the Copaifera reticulata oleoresin and its main diterpene acids. J. Ethnopharmacol. 233 (November 2018), 94–100. Disponível em:

Pieri, F.A., Mussi, M.C., Moreira, M.A.S., 2009. Óleo de copaíba (Copaifera sp.): histórico, extração, aplicações industriais e propriedades medicinais. Rev. Bras. Plantas Med. 11 (4), 465–472.

Ricardo, L.M., et al., 2018. Evidence of traditionality of Brazilian medicinal plants: the case studies of Stryphnodendron adstringens (Mart.) Coville (barbatimão) barns and Copaifera spp. (copaíba) oleoresin in wound healing. J. Ethnopharmacol. 219 (March), 319–336. Disponível em:

Silva, P.P., et al., 2015. Copaiba oil effect on experimental jaw defect in Wistar rats. Acta Cir. Bras. 30 (2), 120–126 fev. 2015. Disponível em: http://www.scielo.br/scielo.php?script=sci_arttext&pid=S0102-86502015000200120&lng=en&tlng=en.

Teixeira, F.B., et al., 2017. Copaiba oil-resin (Copaifera reticulata Ducke) modulates the inflammation in a model of injury to rats’ tongues. BMC Compl. Alternative Med. 17 (1), 1–8.

The Cochrane Collaboration. 2020. Review Manager (RevMan) Version 5.4 [S.]: n.s.,

Tobouti, P.L., et al., 2017. Antimicrobial activity of copaiba oil: a review and a call for further research. Biomed. Pharmacother. 94, 93–99. Disponível em:

Valadas, L.A.R., et al., 2019. Dose-response evaluation of a copaiba-containing varnish against streptococcus mutans in vivo. Saudi Pharmacuet. J. 27 (3), 363–367.

Veiga, V.F., et al., 2007. Chemical composition and anti-inflammatory activity of copaiba oils from Copaifera cearensis Huber ex Ducke, Copaifera reticulata Ducke and Copaifera multijuga Hayne-A comparative study. J. Ethnopharmacol. 112 (2), 248–254.

Wagner, V.P., et al., 2017. Effects of copaiba oil topical administration on oral wound healing. Phytother Res. 31 (8), 1283–1288.