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Review article

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 benefic 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, antiblennorrhagic, 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

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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 clinical trial (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 3rd, 2021, without year limitation. The search was conducted using the keywords "((((fabaceae) OR (copaifera)) OR (copaíba)) 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 (copaíba) 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, Scielo = 172, Cochrane Library = 0, 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 natura* - 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 Albino 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;

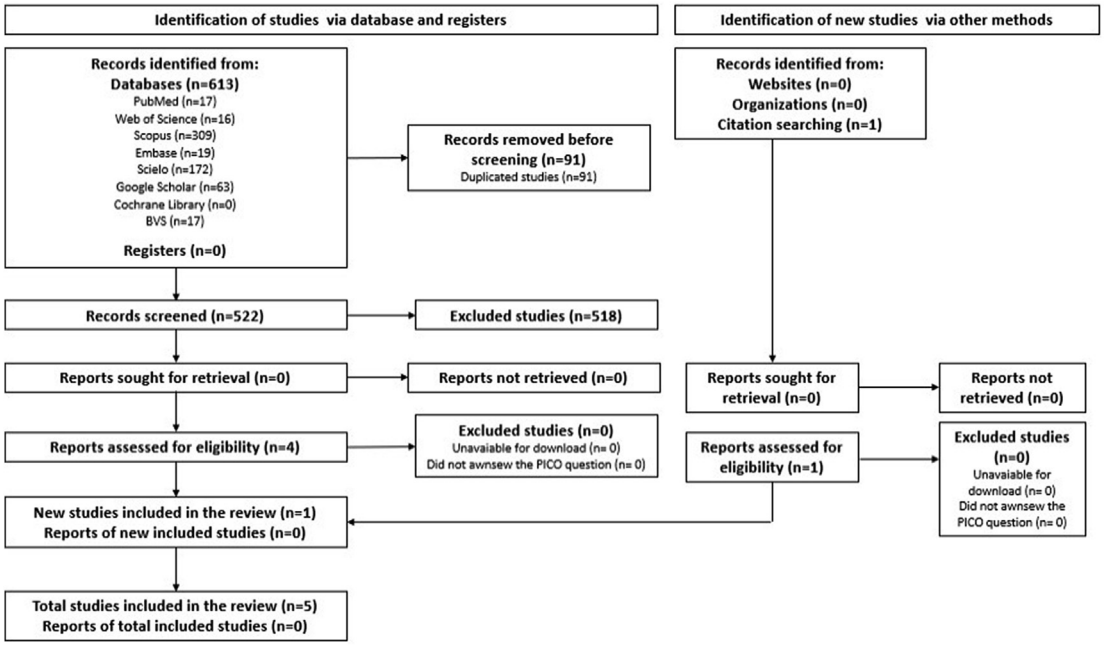

Figure 1. Flowchart of the searching method.

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 group treated with corticoid ($p < 0.05$) 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 topic Copaiba group; 30.27 ± 1.74 for systemic Copaiba group; 20.91 ± 7.53 for topic 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 (Gcel-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)	Alvarenga et al. (2020)	Teixeira et al. (2017)	Wagner et al. (2017)	Silva et al. (2015)	Dias-da-Silva et al. (2013)
Study objective	To investigate the therapeutic effects of Copaiba oleoresin (*C. reticulata Ducke*) on 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	To evaluate the anti-inflammatory properties of Copaiba oleoresin (*Copaifera reticulata Ducke*) in a model that transfixes injury in rats' tongues	To evaluate the clinical and histopathological aspects of topical treatment with Copaiba oleoresin (*Copaifera Reticulata Duke*) extract on oral wound healing in an animal model and compared with topical corticosteroids treatment	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	To evaluate the Copaiba oleoresin effects, by topic and systemic administration, on alveolar wound healing in rats
Study type	Randomized, controlled, and blind preclinical study	Randomized, controlled, and blind preclinical study	Preclinical study	Preclinical study	Preclinical study
Animals' specifications	Wistar (200–250 g)	Wistar	Wistar (150–200 g)	Wistar (250–300 g)	Wistar
Data collection	Prospective and simultaneous	Prospective and simultaneous	Prospective and simultaneous	Prospective and simultaneous	Prospective and simultaneous
Sample size	45 rats	15 rats	96 rats	42 rats	28 rats
Number and details of the groups	3 groups (each with 15 rats): • Systemic Copaiba oleoresin/ oral gavage (200 mg/kg/day); • Placebo control (Saline solution and 5% Tween 20, 200 mg/kg/day); • Active control (Dexamethasone 0.5 mg/kg/ day)	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 (Tween 20 200 mg/kg/day)	4 groups (each with 24 rats): • Topical Copaiba oleoresinV/ swab application; • Placebo control (same components of the oil without the Copaiba extract); • Active control (topical 0.05% clobetasol propionate with a hydroxyethylcellulose gel); • Control without treatment	6 groups (each with 7 rats): • Gbio + Systemic Copaiba oleoresin/Oral Gavage (0.6 mL/kg/day); • Gcell + systemic Copaiba oleoresin/oral gavage (0.6 mL/kg/day); • Gbio + placebo control (distilled water - 0.6 mL/kg/ day); • Gcell + placebo control (distilled water - 0.6 mL/kg/ day); • Gbio + active control (Meloxicam 0.25 mg/kg/day diluted in 0.6 mL/kg); • Gcell + active control (Meloxicam 0.25 mg/kg/day diluted in 0.6 ml/kg)	4 groups (6 in each group that used Copaiba and 8 in each control): • Topical Copaiba oleoresin (30ml irrigation); • Systemic Copaiba oleoresin/oral gavage (0.1mL Copaiba/100g body weight); • Topical placebo-control (irrigation with saline); • Systemic placebo-control (gavage with saline)
Intervention frequency	Once a day	Once a day	Twice a day	Once a day	Once a day
Intervention period	3 consecutive days (starting 12 h after the procedure)	7 consecutive days (starting 12 h after the procedure)	14 consecutive days	7 consecutive days (starting on the fifth day after the procedure)	3 consecutive days after the procedure for the topical groups and 7 for the systemic ones
Oral Wound Healing capacity	Yes	No	Yes	Yes	Yes
Oral Anti-inflammatory capacity	Yes	Yes	Yes	Yes	No
Main conclusions	Systemic administration of Copaiba oleoresin has shown to be safe and effective in the healing process of oral wounds compared to steroids therapy, promoted early anti-inflammatory activity, and accelerated wound resolution. Biochemical analyzes showed that the administration of Copaiba oleoresin did not cause kidney or liver damage	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	Topical administration of Copaiba oleoresin did not accelerate the oral healing process and did not promote relevant side effects in this model	Copaiba oleoresin administered through oral gavage did not affect the bone repair of defects in rat jaws 40 days after the procedure	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; Gcell = 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)	Alvarenga et al. (2020)	Wagner et al. (2017)	Silva et al. (2015)	Dias-da-Silva et al. (2013)
Evaluation period	At the procedure day and 3, 7, and 15 days after	3, 5, 10, and 14 days after the procedure	40 days after the procedure	7 days after the procedure
Evaluation criteria	• Wound area (mm^2); • Reepithelialization (Grade 0 – at wound edges, Grade 1 – covering less than half, Grade 2 – covering more than half, Grade 3 – covering the entire wound irregularly and Grade 4 – covering the entire wound evenly); • Collagen – PicroSirius (intensity, arrangement, and arrangement of collagen fibers)/PSR score	• Wound status (open or closed); • Percentage of wound healing; • Wound healing time	• Score of osteoclasts activity (1-inactive, 2 – little, 3 – much activity); • Score of osteoblast presence; • Bone matrix mineralization (1-absence, 2 – 0-50% bone formation, 3- >50% bone formation)	• Area density of the immature bone formed
Copaiba group main results	• Wound area: D0 = 7mm2, D3 = 2mm2, D7 = 0mm2; • Reepithelialization: D3 = 2.5, D7 = 4; • Collegen/PSR score: D3 = 2.5, D7 = 2.8	• Wound status: D3 and D5 = open in all animals; D10 and D14 = closed in all animals ($p > 0,05$ – teste de Kruskal-Wallis); • Percentage of wound healing *: D3 ≅ 75%; D5 ≅ 75%; D10 = 100%; D14 = 100%; • Wound healing time: did not differ from the control without treatment group regarding wound closure time ($p > 0.05$—Log-rank test)	Gbio + Systemic Copaiba oleoresin/Oral Gavage • Score of osteoclasts activity* ≅ 0.1; • Score of osteoblast presence = 1; • Bone matrix mineralization* ≅ 1.55 Gcell + Systemic Copaiba oleoresin/Oral Gavage • Score of osteoclasts activity = 0.4; • Score of osteoblast presence = 1; • Bone matrix mineralization* ≅ 1.3	Topical Copaiba oleoresin • Area density of the immature bone formed: Relative frequency of bone formation = 27.82 ± 5.71 (27%); 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 = 30.27 ± 1.74 (30%); thicker bony trabeculate
Control(s) group(s) main results	Placebo control • Wound area: D0 = $7mm^2$, D3 = $6mm^2$, D7 = $2mm^2$, D15 = $0mm^2$; • Reepithelialization: D3 = 1, D7 = 3; • Collegen/PSR score: D3 = 1, D7 = 1.7 Active control • Wound area: D0 = $7mm^2$, D3 = $5mm^2$, D7 = $1.8mm^2$, D15 = $0mm^2$; • Reepithelialization: D3 = 0.8, D7 = 1.9; • CollegenPSR score: D3 = 1, D7 = 1.5	Placebo control • Wound status: D3 and D5 = open in all animals; D10 and D14 = closed in all animals; • Percentage of wound healing*: D3 ≅ 60%; 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) Active control • Wound status: D3 and D5 = open in all animals; D10 = closed in all animals; D14 = open in 1 animal and closed in the others; • Percentage of wound healing*: D3 ≅ 70%; D5 ≅ 72 %; D10 = 100%; D14 = 100%; • Wound healing time: a significantly slower process of wound closure compared with the control without treatment group ($p = 0.007$—Log-rank test) Control without treatment • Wound status: D3 and D5 = open in all animals; D10 and D14 = closed in all animals; • Percentage of wound healing*: D3 ≅ 65 %; D5 ≅ 80 %; D10 = 100%; D14 = 100%; • Wound healing time: no sign of healing until D6; did not differ from the control without treatment, placebo, and the Copaiba oleoresin group regarding wound closure time ($p > 0.05$—Log-rank test)	Gbio + Placebo Control • Score of osteoclasts activity* ≅ 0.1; • Score of osteoblast presence = 1; • Bone matrix mineralization* ≅ 1.75 Gcell + Placebo Control • Score of osteoclasts activity = 0; • Score of osteoblast presence = 1; • Bone matrix mineralization* ≅ 1.35 Gbio + active control • Score of osteoclasts activity* ≅ 0.1; • Score of osteoblast presence = 1; • Bone matrix mineralization* ≅ 1.75 Gcell + active control • Score of osteoclasts activity = 0; • Score of osteoblast presence* ≅ 0.6; • Bone matrix mineralization* ≅ 1.6	Topical placebo control • Area density of the immature bone formed: Relative frequency of bone formation = 20.91 ± 7.53 (21%) Systemic placebo control • Area density of the immature bone formed: Relative frequency of bone formation = 22.45 ± 7.00 (22%)
Benefits associated with Copaiba oleoresin use	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	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	Osteoclast activity was observed only in four groups and was more expressive in oil-Gcell ($p = 0.78$), but it was not statistically significant; regarding the presence of osteoblasts, Gcell-melox ($p = 0.009$), had lower osteoblastic activity compared to the other	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

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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

Table 3. Evaluation of the anti-inflammatory effect of Copaiba oleoresin in the oral cavity.

Author (year)	Alvarenga et al. (2020)	Teixeira et al. (2017)	Wagner et al. (2017)	Silva et al. (2015)
Evaluation period	3 and 7 days after the procedure	7 days after the procedure	3, 5, 10, and 14 days after the procedure	40 days after the procedure
Evaluation criteria	• Inflammatory Score; • Inflammatory response intensity (descriptive histopathological analysis); • PSR score (+1 = thin, delicate, loosely arranged collagen fibers seen throughout the wound area, +2 = thin, delicate, loosely arranged collagen fibers in some areas and thicker and gross fibers in other areas of the wound, +3 = thick, gross, densely arranged collagen fibers seen throughout the wound area)	• Evaluation of edema, inflammatory cells, angiogenesis, and muscle fibers (descriptive histopathological analysis); • Edema score (0- absent, 1- mild, 2- moderate or 3 - severe); • Inflammatory infiltrate score (0- absent, 1- mild, 2- moderate or 3 - severe); • CD68 + macrophages concentration (descriptive immunohistochemical analysis)	• Descriptive histopathological analysis	• Inflammatory infiltrate score (1- absent, 2 – mild, 3 – moderate, 4 – intense)
Copaiba group main results	• Inflammatory Score: D3 = 2.5, D7 = 3.5 • Inflammatory response intensity: D3 = predominance of granulation tissue, with macrophage lymphocytes, collagen, and new vessels, D7 = complete wound closure uniformly in most of the samples; • PicroSirius red staining. score (PSR score): D3 = +2, D7* = +3	• 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; • Edema score: 1.8 ± 0.20; • Inflammatory infiltrate score: 1.2 ± 0.20; • CD68 + macrophages concentration*: 170	• Descriptive histopathological analysis: D3 = discrete or no migration of epithelial cells towards the center of the wound, intense and diffuse chronic inflammatory infiltrate; D5 = slight progress in the wound healing process, with few animals presenting a more pronounced migration of epithelial cells, but all animals still presented some degree of exposed connective tissue and the inflammatory infiltrate remained nearly unchanged, with chronic and diffuse inflammatory cells were still present, with an increase in neovascularization and fibroblast proliferation was observed; D10 = full reepithelialization varying from irregular to normal thickness and resolution of the inflammatory process, formation of granulation with rare polymorphonuclear cells; D14 = similar to D10	Gbio + Systemic Copaiba oleoresin/Oral Gavage • Inflammatory infiltrate score = 1.5 Gcell + systemic Copaiba oleoresin/oral gavage • Inflammatory infiltrate score* ≅ 1.8;

(*continued on next page*)

Table 3 (*continued*)

Author (year)	Alvarenga et al. (2020)	Teixeira et al. (2017)	Wagner et al. (2017)	Silva et al. (2015)
Control(s) group(s) main results	Placebo control • Inflammatory Score: D3 = 2, D7 = 3.5; • Inflammatory response intensity: D3 = predominance of neutrophils (acute inflammation), D7 = incomplete closure, smaller amount of collagen deposition, and disappearance of chronic inflammation; • PSR score: D3 = +1, D7* = + 1.7 Active control • Inflammatory Score: D3 = 2, D7 = 2.9; • Inflammatory response intensity: D3 = predominance of neutrophils (acute inflammation), D7 = incomplete closure, smaller amount of collagen deposition and disappearance of chronic inflammation; • PSR score: D3 = +1, D7* = 1.5	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; • Inflammatory infiltrate score: 2.0 ± 0.0; • CD68 + macrophages concentration*: 95 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; • Inflammatory infiltrate score: 1.0 ± 0.0; • CD68 + macrophages concentration*: 0,7	Placebo control group: • Descriptive histopathological analysis: similar to Copaiba oleoresin group in all days Active control group: • Descriptive histopathological analysis: similar do Copaiba oleoresin group in all days, but on D10 one animal presented intense acute inflammatory infiltrate, compatible with an abscess. And on D14, presented chronic inflammatory infiltrate and one, a discrete strip of exposed connective tissue, compatible with the open wound observed in the clinical analysis Control without treatment: • Descriptive histopathological analysis: similar to Copaiba oleoresin group in all days	Gbio + placebo control • Inflammatory infiltrate score* ≅ 1.6 Gcell + placebo control • Inflammatory infiltrate score = 1.5 Gbio + active control • Inflammatory infiltrate score* ≅ 1.4 Gcell + active control • Inflammatory infiltrate score* ≅ 1.9
Benefits associated with Copaiba oleoresin use	Wounds treated with Copaiba oleoresin showed significantly higher inflammatory score (more advanced inflammatory stage, indicating reduced acute inflammatory reaction) in D3 and reepithelialization (more advanced stage of tissue repair) in D3 and D7 when compared to the other groups, with a statistically significant difference between the groups in D3 and not significantly statistical in D7; wounds treated with Copaiba oleoresin show the early formation of collagen fibrils, a greater quantity of them, greater thickness and better organization when compared to the control and corticoid groups	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 = 0,0432); Copaiba oleoresin can modulate the inflammatory response by reducing the recruitment of inflammatory cells after 7 days of oral treatment in a similar way to dexamethasone, and with the advantage of the absence of side effects associated with the use of dexamethasone	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. Inflammatory cells were present in all groups, but there was no statistical significance (p = 0.52)

* 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, consequent, reducing the secretion of

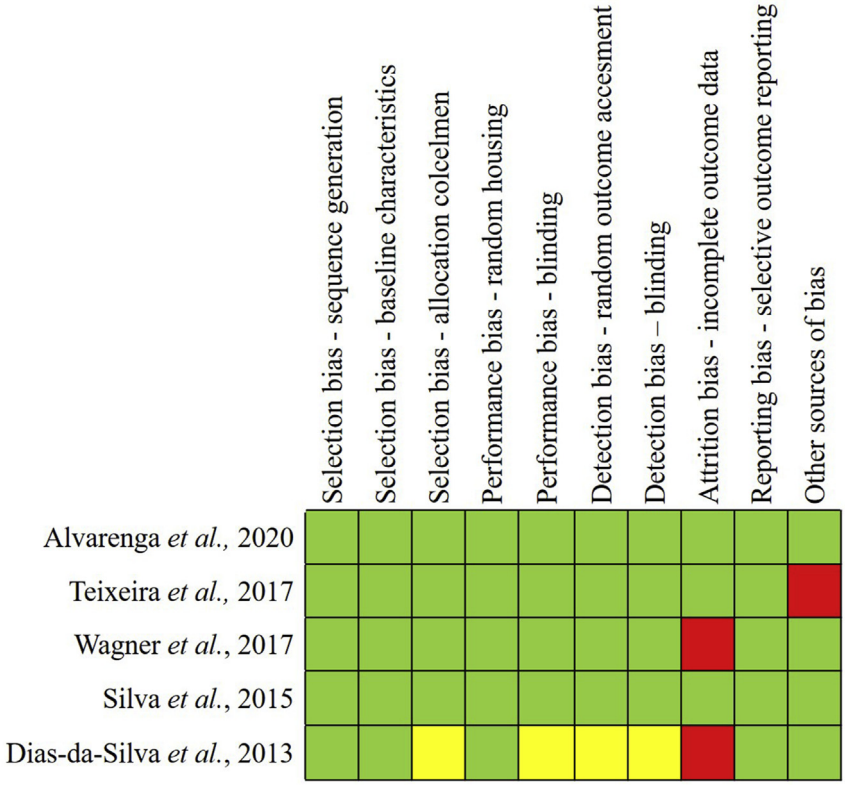

Figure 2. Risk of bias of the selected articles. If the item was considered present in the article, it was judged as "low risk of bias" (green square). If it was not, the paper was classified as "high risk of bias" (red square). If this information was not available, the paper was classified as "undefined risk of bias" (yellow square) in this specific item.

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 (Estevão 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. This group also performed a weight analysis of the study subjects and found that on the tenth day, the corticoid group showed greater weight loss than the control and placebo group; on the fourteenth day, they showed greater weight loss when compared to the Copaiba group. They suggest that this greater weight loss may be associated with anorexia, which is a side effect of corticosteroids (Wagner et al., 2017).

Alvarenga et al. also performed a biochemical evaluation in rats submitted to the research. They detected decreased alanine aminotransferase levels on the seventh day in the Copaiba and the corticoid group and increased direct bilirubin values in the corticoid group when compared to the others. There was no difference in serum levels of urea and creatinine between the groups (therefore indicating the absence of signs of kidney and liver damage) and similar gamma-glutamyl transferase in the groups (Alvarenga et al., 2020).

Regarding the 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

Table 4. SYRCLE's tool for assessing the risk of bias: Type of bias, domain and signaling questions.

	Alvarenga et al. (2020)	Teixeira et al. (2017)	Wagner et al. (2017)	Silva et al. (2015)	Dias-da-Silva et al. (2013)
Question 1 - Selection bias/Sequence generation: Was the allocation sequence adequately generated and applied?	Yes	Yes	Yes	Yes	Yes
Question 2 - Selection bias/Baseline characteristics: Were the groups similar at baseline or were they adjusted for confounders in the analysis?	Yes	Yes	Yes	Yes	Yes
Question 3 - Selection bias/Allocation concealment: Was the allocation to the different groups adequately concealed during?	Yes	Yes	Yes	Yes	Unclear
Question 4 - Performance bias/Random housing: Were the animals randomly housed during the experiment?	Yes	Yes	Yes	Yes	Yes
Question 5 - Performance bias/Blinding: Were the caregivers and/or investigators blinded from knowledge which intervention each animal received during the experiment	Yes	Yes	Yes	Yes	Unclear
Question 6 - Detection bias/Random outcome assessment: Were animals selected at random for outcome assessment?	Yes	Yes	Yes	Yes	Unclear
Question 7 - Detection bias/Blinding: Was the outcome assessor blinded?	Yes	Yes	Yes	Yes	Unclear
Question 8 - Attrition bias/Incomplete outcome data: Were incomplete outcome data adequately addressed?	Yes	Yes	No	Yes	No
Question 9 - Reporting bias/Selective outcome reporting: Are reports of the study free of selective outcome reporting?	Yes	Yes	Yes	Yes	Yes
Question 10 - Other/Other sources of bias: Was the study apparently free of other problems that could result in high risk of bias?	Yes	No	Yes	Yes	Yes

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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Additional information

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