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ORIGINAL ARTICLE

# Therapeutic effect of andiroba oil (*Carapa guianensis* Aubl.) against oral mucositis: an experimental study in golden Syrian hamsters

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## Abstract

**Objectives** The aim of this study was to investigate the healing activity of andiroba (*Carapa guianensis* Aubl.) against oral mucositis (OM) induced by 5-fluorouracil in golden Syrian hamsters.
**Materials and methods** A total of 122 animals were randomized and divided into six groups: andiroba oil 100%, andiroba oil 10%, andiroba oil 10% refined, no treatment group, all $n = 28$; and negative control (NC) and cyclophosphamide (CPA) groups, both $n = 5$. OM was induced by intraperitoneal administration of 60 mg/kg 5-FU on days 0, 5 and 10 followed by mechanical trauma on the oral mucosa on days 1 and 2. From day 1 to day 15, the animals of the andiroba group were treated three times a day. On days 4, 8, 12 and 15, the mucosa was photographed and removed for clinical and histopathological analysis. The bone marrow of the femur was removed and the micronucleus test was performed to evaluate the cytotoxicity and genotoxicity. The data were subjected to analysis of variance, followed by the Tukey and Bonferroni test.
**Results** Treatment with 100% andiroba oil reduced the degree of OM compared to that reported in the other groups ($p < 0.05$). Andiroba oil at both concentrations was not cytotoxic, but treatment with 100% andiroba oil showed a genotoxic potential ($p < 0.001$).
**Conclusions** Frequent administration of andiroba oil accelerated the healing process in an experimental model of 5-fluorouracil-induced OM. However, the genotoxicity of andiroba in other cell systems and under other conditions are being tested.
**Clinical relevance** The use of andiroba in topical form may be associated with reduced intensity of OM. Seek therapeutic alternatives to minimize the pain and suffering that these side effects cause cancer patients is an important scientific step.

**Keywords** Oral mucositis · Cancer · Oncology · 5-fluorouracil · *Carapa guianensis* · Phytotherapy

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## Introduction

Oral mucositis is a common and clinically significant side effect of both anticancer chemotherapy (CT) and radiation therapy (RT) [1]. In extremely severe cases, it can significantly affect patient's quality of life, which may result in discontinuation of the antineoplastic treatment [2, 3]. The progression of mucositis can be summarized in 5 stages—initiation, upregulation or activation, signal amplification, ulceration, and healing [4]. Based on the progressive stages of OM development, researchers attempt to minimize the side effect caused by chemotherapy and/or radiotherapy and the Multinational Association for Cancer Support Care in together

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with the International Society of Oral Oncology (MASCC/ISOO) recently published the Clinical Practice Guidelines for mucositis in which it highlights the main scientific evidences of treatments for oral mucositis, including basic oral care (combination of toothbrushing, flossing and mouth rinses); growth factors and cytokines; anti-inflammatory agentes; antimicrobials, coating agents, anesthetics and analgesics; laser and other light therapy; cryotherapy; and natural and miscellaneous agents [5].

Phytotherapy is a novel line of research that has been extensively studied to prevent and treat OM. Chamomile (*Matricaria chamomilla*) [6], turmeric (*Curcuma longa L.*) [7], bee honey [8], cumin (*Carum carvi L.)* [9] and calendula (*Calendula officinalis*) [10] are some of the medicinal plants that have already been tested in an experimental model of oral mucositis, and all have shown significant results. However, all compounds presented limitations, necessitating a further study of the adverse effects, such as drug interaction and toxicity, in addition to clinical trials [11].

*Carapa guianensis* Aubl., popularly known as andiroba, is a tree of the large Meliaceae family, which is found in the Amazon region [12, 13]. Andiroba oil is extracted by seed pressing and has been used in traditional medicine for its anti-inflammatory and analgesic activities, which will both be essential in the treatment of OM. It is also known to have effective antimicrobial, anti-allergic and parasiticidal action on cutaneous and muscular dysfunctions [14].

Phytotherapeutic studies have identified that the main biologically active substances present in the oil are limonoids and triterpenes (tetranortriterpenoids), which are responsible for the antiseptic, anti-inflammatory, healing and insecticidal activities of andiroba oil and bark [15, 16].

The high availability of the oil throughout the region and low cost contribute to its attractiveness as a therapeutic option; in addition to ameliorating symptoms, costs can also be reduced [12]. Recent experimental studies on the acute and subchronic toxicity of *C. guianesis* Aubl. have concluded that the administration of andiroba seed oil did not produce toxic effects in rats or their offspring [16–19].

This new study proposes to investigate the anti-inflammatory and healing activity of andiroba in golden Syrian hamsters with oral mucositis through a detailed clinical and histopathological evaluation using oral mucositis classification scores. In addition, andiroba oils were analysed by gas chromatography-mass spectrometry (GC-MS) to identify potential bioactive substances. Toxicity caused by phytotherapy was also studied through the micronucleus (MN) test, in order to propose a new therapeutic approach that could combat the deleterious effects of oral mucositis.

## Material and methods

### Ethics

The animals used in this study were treated according to the national legislation enforced by the law on the use and breeding of animals (Federal Law No. 11.794, October 8, 2008), Decree No. 6899, 15 July 2009, and with the norms published by the National Council of Animal Control and Experimentation (CONCEA). The study design was approved by the Ethics Committee for Animal Use (CEUA) of the University Centre of the State of Pará (CESUPA) under protocol number 08/2015.

### Animals

Ninety-day-old male golden Syrian hamsters (*Mesocricetus auratus*), weighing 90–120 g, were obtained from the vivarium of the Evandro Chaga Institute, Belém, PA, Brazil. The hamsters were kept in cages at the CESUPA vivarium under the following controlled conditions: temperature, 20–24 °C; relative air humidity, 40–70% and illumination cycle, 12 h:12 h light/dark. The animals had free access to food and water.

The selection of hamsters for the experiments was based on the ease of observation of their buccal mucosa and their ability to tolerate doses of chemotherapeutic agents capable of inducing OM without significant mortality [20].

All efforts were made to reduce the number of animals used and the pain, suffering and stress to which they were subjected.

### Andiroba collection and analysis by GC-MS

Two types of andiroba oil were used in the research at different concentrations. The first, crude andiroba oil, was collected in the municipality of Tracuateua, in the Northeast region of the State of Pará, Brazil (coordinates: 01° 04′ 19.0″ S and 46° 53′ 49.0″ W) and record OA81P, provided by the Laboratory of Systematic Research in Biotechnology and Molecular Biodiversity of the Federal University of Pará, Brazil (LabISisBio/UFPA).

In one group of animals, 100% in natura oil was used. In another group, the same oil was diluted to a concentration of 10%, where the oil was the only active substance, and the remainder of the composition contained orabase, which consisted of pectin, gelatin, nipagin, EME and purified water.

The second type of andiroba oil used in the study was refined oil, which is sold in many pharmacies. The oil was diluted to 10%, where it was the only active substance, and the remainder of the composition consisted of the same substances reported above.

The oils were analysed by GC-MS following transesterification according to a standard protocol: 10.0 mg oil was weighed in a conical tube (Tb1), 100 μL of dry hexane was added and the mixture was stirred. The oil was transesterified by the addition of 200 μL 2 N KOH solution in methanol, and the mixture was warmed to 55 °C for 20 min under sonication. The esters were extracted three times with 250 μL of hexane. The organic phase was transferred to a second tube (Tb2) and the solvent of Tb1 was evaporated to dryness. HCL 2 N (250 μL) was added to Tb1, and the mixture was heated at 40 °C for 5 min in a water bath. The acidified compounds were extracted three times with 250 μL of hexane and transferred to Tb2. The Tb2 solution was evaporated to dryness in a concentrator (Turbovap) (45 °C, 5 psi, 1 h). One hundred microlitres of the N, O-bis (trimethylsilyl)-trifluoroacetamide derivative (BSTFA) was added to Tb2 and shaken (600 rpm, 1 h, 30 °C) and then 700 μL hexane was added and shaken (45 °C, 5 psi, 1 h). The mixture was centrifuged at 10,000 rpm for 2 min. A 600-μL aliquot of the upper phase was transferred to a 2.0-mL vial and the tube was closed for GC-MS analysis.

GC-MS analysis was performed using a Thermo Scientific Trace 1300 GC coupled to a Thermo Scientific MS-ISQ Single Quadrupole attached to a mass spectrometer with an AI 1310 autosampler equipped with a RTX-65 TG (15 m × 0.25 mm × 0.1 μm). Substance identification was conducted by comparison of the mass spectra with WILEI2009 software data. The concentration of free fatty acids (FFA) was determined by normalization of the peak area.

## Experimental groups

A total of 122 animals were randomized and divided into six groups. The test compound groups were 100% andiroba oil group (Ol 100%), 10% andiroba oil group (Ol 10%) and refined andiroba oil group 10% (Ref 10%) (all $n = 28$). From day 1 to day 15, andiroba was administered three times daily with the aid of a swab. After application, the animals' access to food and water was restricted for 1 h.

The no treatment group (NT; $n = 28$) received the OM induction protocol, but did not receive any treatment. The negative control group (NC; $n = 5$) did not receive the oral mucositis induction protocol. Their buccal mucosa served as the standard and was used as a negative control for the MN test. A cyclophosphamide group ($n = 5$) was also used for the MN test.

## Experimental protocol

Oral mucositis similar to that in humans was induced using the protocol of Sonis et al. [20]. It was induced by intraperitoneal injection of the chemotherapeutic 5-fluorouracil (5-FU) (Fluoro-Uracil® 25 mg/mL, ICN Pharmaceuticals Ltd) on days 0, 5 and 10 of the experiment at a dose of 60 mg/kg body weight.

To reproduce the effect of chronic irritation, the animals were fixed to the surgical table, anaesthetised, and using sterile 18 gauge needles, the everted buccal mucosa was linearly superficially scarified twice on days 1 and 2 of the experiment. This procedure was performed by a single-trained researcher.

On days 4, 8, 12 and 15, seven animals from each andiroba group and the NT group were randomly selected to be photographed and excisional biopsy of the right and left buccal mucosa was performed. The removed tissue was immediately immersed in 10% formalin solution for a period of 72 h.

For all operative procedures (scarification of the mucosa, photography and performance of the biopsies), the animals were anesthetized by intraperitoneal injection of the general anaesthetic ketamine (Francotar ®—10 mL—veterinary use. Virbac do Brasil Ind. Co. Ltd), at a dose of 150 mg/kg, in combination with xylazine hydrochloride (10 mL—veterinary use. Virbac do Brasil Ind. Co. Ltd.) at a dose of 10 mg/kg.

After the excisional biopsy of the buccal mucosa, the animals were euthanised by anaesthetic overdose. The femoral medulla of the animals that was euthanised on day 15 was removed, in addition to animals from the NC, and subjected to the MN test.

## Clinical evaluation of the buccal mucosa

The clinical evaluation of the severity of oral mucositis was determined from the observation of the photographs, which had been previously disordered, coded by an examiner and analysed blind by a second examiner. This analysis was performed twice by the same examiner, with an interval of 2 weeks between each analysis. Each photograph was classified on the 4-point scale recommended by [21] with the following scores: 0, absent or discrete hyperaemia and erythema, absent haemorrhage and absence of ulcers and abscesses; 1, moderate hyperaemia and erythema, absent haemorrhage, absence of ulcers and abscesses and presence of scar tissue; 2, severe hyperaemia and erythema, presence of haemorrhage and small ulcers (up to 1 cm in diameter), presence of scar tissue and absence of abscesses; and 3, severe hyperaemia and erythema, presence of haemorrhage, extensive ulcers and abscesses.

## Histopathological evaluation of the buccal mucosa

In order to perform the histopathological evaluation, surgical specimens were obtained from the central region of the buccal mucosa, fixed in 10% neutral buffered formalin for 24 h, submitted to standard laboratory processing, and embedded in paraffin. Sections of 5-μm thickness were obtained and stained with standard haematoxylin and eosin.

A blind analysis was performed twice, by a third examiner, with an interval of 2 weeks between each analysis to assess the agreement of the two evaluations.

The material was classified according to standard scores on a scale of 0 to 3 according to Lima et al. [21]: 0, epithelial and connective tissue without vasodilatation, absent or discrete inflammatory cellular infiltrate, absence of haemorrhage, oedema, ulcers or abscesses; 1, discrete vascular congestion, areas of re-epithelialization, discrete cell infiltration of mononuclear leukocytes, absence of haemorrhage, oedema, ulcers or abscesses; 2, moderate vascular congestion, epithelial hydropic degeneration (vacuolation), moderate cellular infiltrate with predominance of polymorphonuclear leukocytes, presence of haemorrhagic areas, oedema and occasional small ulcers and absence of abscesses; 3, acute vascular congestion, marked vasodilation, marked cellular infiltrate, predominantly polymorphonuclear, presence of haemorrhagic areas, oedema, abscesses and extensive ulcers.

### In vivo MN test

The test was performed on day 15. As the positive control of this test ($n = 5$), the substance cyclophosphamide was orally administered by gavage within a 24-h treatment interval. To remove the bone marrow, the legs of the animal were cleaned with 70% alcohol and the femur (from both legs) was removed using tweezers and dissection scissors. The bone marrow was removed at 18–24 h after treatment from the femur using a syringe (5 mL) and foetal bovine serum. The removed material was centrifuged at 1000 rpm for 5 min and then smeared on microscopic slides (three slides for each animal). After drying, the slides were stained with 2% Leishman stain.

The slides were analysed by optical microscopy; from 2000 cells, the number of monochromatic and polychromatic erythrocytes were counted in the bone marrow to evaluate the cytotoxicity, as well as to determine the mutagenic potential and genotoxicity (occurrence of MN) induced by the test substance.

### Statistical analysis

The sample size was determined through a pilot study using ANOVA, where a significance level of 0.05 and test power of 80% was adopted. Statistical analysis of variance (ANOVA) or Kruskal Wallis tests were performed respectively, according to whether the data presented a normal distribution. When a significant difference between the groups was identified, Tukey's multiple comparison test (for the clinical and histopathological evaluations) and Bonferroni test (for the MN test) were used.

The evaluation of the degree of intra-examiner agreement for the clinical and microscopic classification method of OM was performed using the Kappa coefficient (κ). The level of significance was set at 5% ($p < 0.05$) in all tests. The BioEstat Program (version 5.0) was used to perform the tests.

## Results

### GC-MS analysis of oils

Andiroba oil is a viscous liquid with a characteristic yellow-brown colour and can become cloudy at low temperatures. Table 1 shows the chemical components of refined andiroba oil and in natura andiroba oil their respective concentrations. Nine compounds of refined andiroba oil were identified and ten compounds of in natura andiroba oil.

A higher amount of FA was observed in the in natura andiroba oil compared to that in the refined andiroba oil. The lower percentage of FA in the refined oil was attributable to the higher glycerol content.

The GC-MS chromatogram of essential oils of andiroba is shown in Fig. 1. In this figure, all components are numbered according to Table 1.

**Table 1** Lipid composition of refined *C. guianensis* oil and in natura *C. guianensis* oil

| RT | Compounds | Composition (%) |
|---|---|---|
| Refined *C. guianensis* oil | | |
| 15.43 | Glycerol | 28.43 |
| 27.77 | Palmitoleic acid | 0.26 |
| 28 | Palmitic acid | 18.81 |
| 29.81 | Linoleic acid | 5.82 |
| 29.86 | Oleic acid | 38.88 |
| 30.08 | Stearic acid | 5.82 |
| 31.58 | Arachidic acid | 0.62 |
| 32.8 | 2-monolinolenic | 0.24 |
| 33.02 | Behenic acid | 0.15 |
| In natura C. guianensis oil | | |
| 15.43 | Glycerol | 0.94 |
| 21.71 | Lauric acid | 0.35 |
| 29.04 | Palmitoleic acid | 1.17 |
| 29.26 | Palmitic acid | 29.02 |
| 30.68 | Linoleic acid | 8.67 |
| 30.75 | Oleic acid | 52.22 |
| 32.99 | 2-monopalmitin | 1.73 |
| 33.69 | 1-monolinoleni | 0.68 |
| 33.89 | 1-monoolein | 4.11 |
| 34.01 | 1- monoestearin | 0.16 |

*RT* retention time

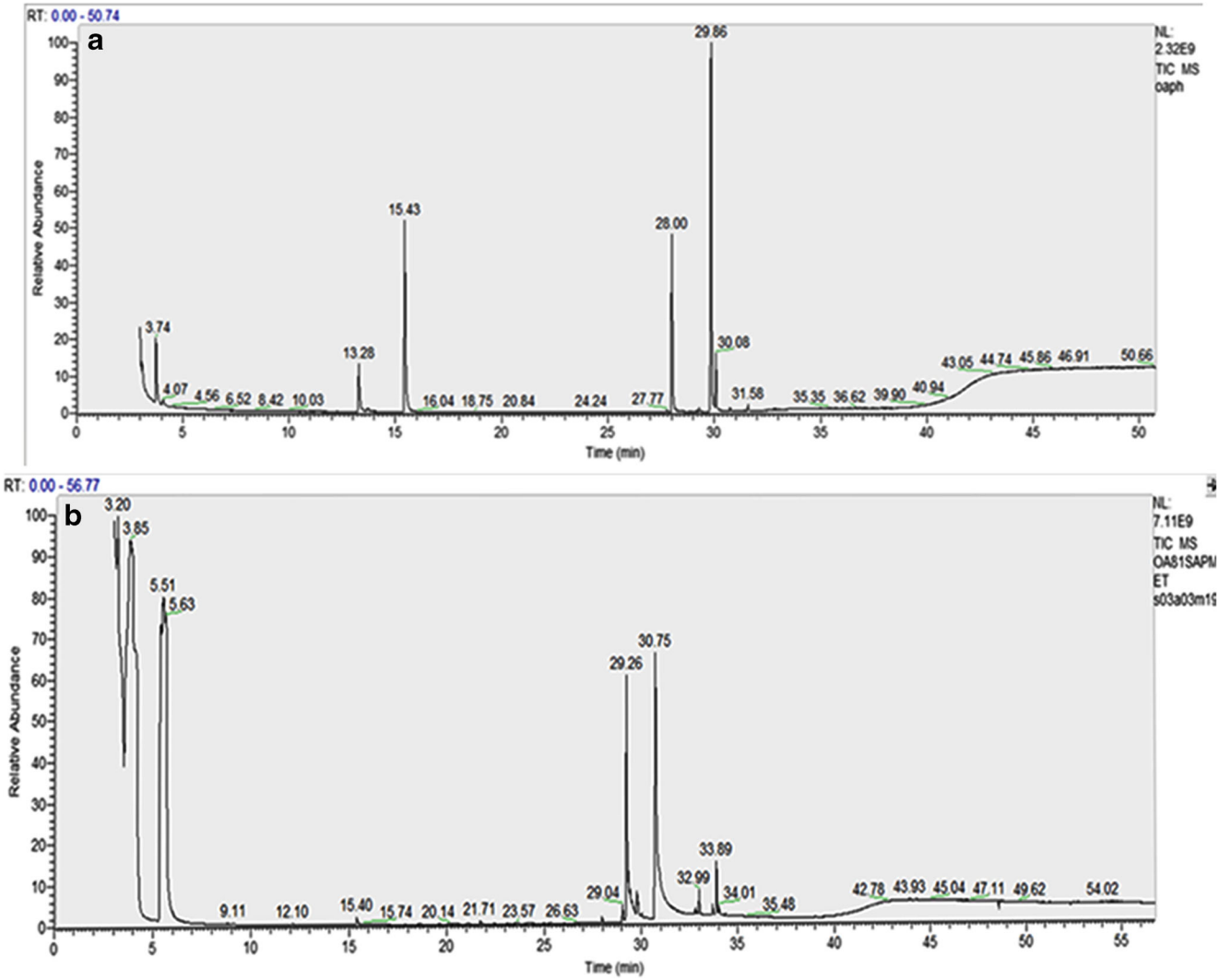



**Fig. 1** GC-MS chromatogram of refined *C. guianensis* oil and and in natura *C. guianensis* oil. Component number (RT) according to Table 1. **a** Chromatogram of refined *C. guianensis* oil and **b** in natura *C. guianensis* oil

## Clinical and histopathological analysis

Both clinical and histopathological analyses were performed at two distinct time periods by a trained examiner. Intra-examiner agreement was determined using the Kappa coefficient test, with $\kappa > 0.7$ and $p$ value $< 0.001$ for clinical analysis and $\kappa > 0.8$ and $p$ value $< 0.001$ for histopathological analysis. The arithmetic means of the clinical and histopathological scores of the studied groups and on distinct days is shown in (Fig. 2).

The details on clinical and histopathological development of oral mucositis induced by 5-FU injection followed by mechanical trauma to the jugal mucosa of hamsters in both the untreated group and the andiroba-treated groups on the different days of the experiment are described in Table 2.

At D4, when comparing the groups, there was a statistically significant difference between the untreated group and Ol 100% with a $p$ value of 0.024 and 0.019 for clinical and histopathological analysis, respectively. The Ol 100% group presented less severe clinical and histopathological lesions, with score median 2, variation (0–3). On the contrary, the NT group showed for the clinical and histopathological analysis with score 3, variation (2–3). At D8, there was a partial regression of OM in all groups, but the scores did not show a statistically significant difference for both clinical ($p$ value 0.150) and histopathological (p value 0.163) analyses. However, the Ol 100% group presented lower mean of the clinical and histopathological scores with variation (1.2).

At D12, a statistically significant difference was observed in the clinical analysis between the Ol 100% group with the Ol 10% and NT groups, with a $p$ value of 0.007. Group Ol 100% presented smaller scores with variation (0–1). In the histopathological analysis, there was a statistical difference between the group Ol 100% and control with a $p$ value of 0.039. Histopathological analysis in the untreated group showed a mean score of 2, with a variation (2–1), containing moderate cellular infiltrate. The Ol 100% group presented a significant reduction in the OM severity, with a mean score < 1.

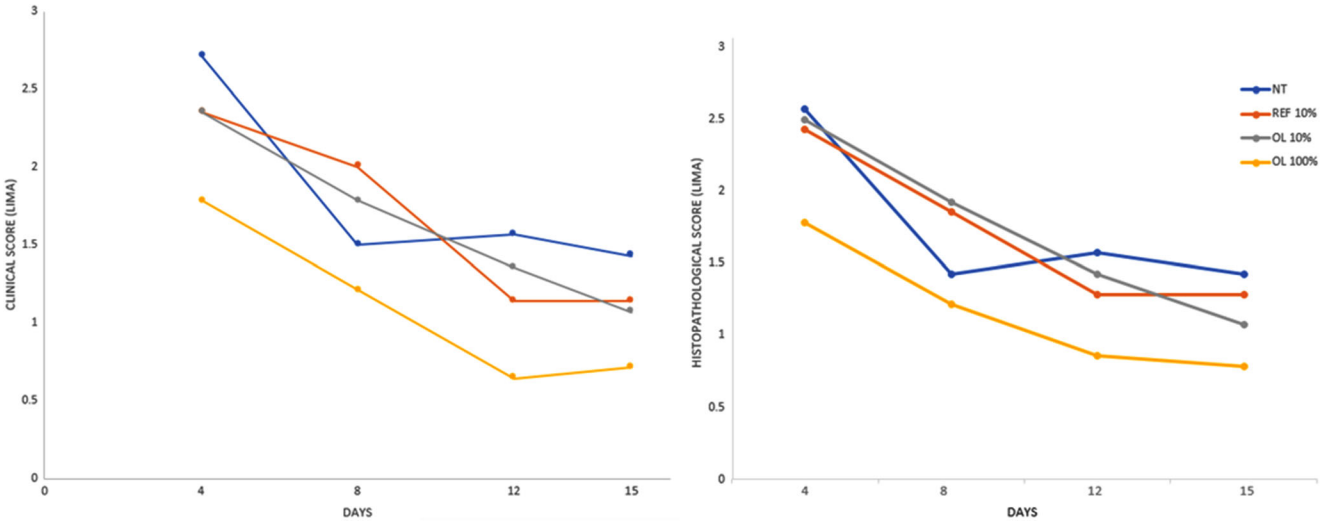



**Fig. 2** Evaluation of the means of clinical and histopathological scores on days 4, 8, 12 and 15 of the experiment. The mean score for the Ol 100% group was low on all days of the experiment. The highest score was reported for the Ol 10%, followed by the Ref 10% group and the no treatment group

On the last day of experiment, D15, when comparing the groups with each other, there was no statistical difference between the groups with a $p$ value of 0.051 and 0.075 in the clinical and histopathological analysis, respectively. Group Ol 100% showed a score < 1, with variation (0–1), which was observed in relation to the other groups. There were no spontaneous deaths during the experiment.

Figure 3 shows the clinical and histopathological aspects of OM in the hamsters of the different groups, evaluated on D4, D8, D12 and D15.

## In vivo MN test

Cells were stained with 2% Leishman solution. Two thousand cells were analysed per animal; the number of micronucleated polychromatic erythrocytes (MNPCEs) was counted for the evaluation of genotoxicity and the relationship between the number of polychromatic and normochromatic erythrocytes was used for cytotoxicity evaluation.

Number of micronuclei (MN) observed from a total of 2000 cells analysed in the bone marrow of hamsters (*Mesocricetus auratus*) after induction of 5-FU oral mucositis and subsequent local treatment with andiroba oil (*Carapa guianensis* Aubl.). For statistical analysis, one-way ANOVA, with Tukey's post test (*** value $p < 0.0001$) was used. Data expressed are the mean of five animals per group. NC (negative control), CPA (cyclophosphamide, micronucleus positive control), NT (no treatment group), Ol 100% (100% pure oil), Ol 10% (*in natura* oil, 10%) and Ref 10% (refined oil at 10%) in Table 3.

We observed that the induction of oral mucositis by 5-FU did not induce an increase in the MN frequency compared to that reported for the negative control group. The number of micronucleated cells increased (34.76%) in the group of animals treated with cyclophosphamide (CPA) compared to that in the negative control and 5-FU groups; this difference was statistically significant ($p < 0.0001$). Treatments with 10% refined andiroba oil, oil in natura at 10% and oil at 100%, increased the frequency of MN (1.85, 10.51, 34.3%, respectively) compared to that in the above-mentioned groups. However, only the Ol 100% group showed a significant increase ($p < 0.0001$) compared to that reported for the NC and 5-FU groups, which was equivalent to that observed in the CPA treatment group.

Figure 4 shows the percentage of polychromatic (PCE) and normochromatic (NCE) erythrocytes in the different groups. Treatment of animals with 5-FU and with andiroba oils produced a significant increase in PCE compared to that in the NC groups (a* $p < 0.05$; a** $p < 0.01$) and CPA (b*** $p < 0.001$). However, there was no statistically significant difference between the NC and CPA groups, or between 5-FU, Ol 100%, Ol 10% and Ref 10%.

The relationship between PCE/NCE was used for statistical analysis. One-way ANOVA was used, with Tukey's post test, where a statistically significant increase of the PCE/NCE ratio was found between the groups treated with NT, Ol 100%, Ol 10% and Ref 10% with the NC groups (a** $p = 0.01$; a*** $p = 0.003$) and CPA (b** $p = 0.02$; b*** $p = 0.0008$). Table 3, all values for the evaluation of this parameter of cytotoxicity are summarized.

## Discussion

The anti-inflammatory and healing activities of two types of andiroba oil at different concentrations were studied after

**Table 2** Means of clinical and histopathological OM scores of the groups (NT, Ref 10%, Ol 10% and Ol 100%) on days 4, 8, 12 and 15 of the experiment

| | Clinical | | Histopathological | |
|---|---|---|---|---|
| Days/groups | Average | Median (MM) | Average | Median (MM) |
| Day 4 | | | | |
| NT | 2.714 | 3 (2–3)* | 2.571 | 3 (2–3)* |
| Ref 10% | 2.357 | 2.5 (1–3) | 2.428 | 2.5 (1–3) |
| Ol 10% | 2.357 | 3 (1–3) | 2.500 | 3 (1–3) |
| Ol 100% | 1.785 | 2 (0–3)* | 1.785 | 2 (0–3)* |
| Day 8 | | | | |
| NT | 1.5 | 1 (0–3) | 1.428 | 1 (0–3) |
| Ref 10% | 2 | 2 (0–3) | 1.857 | 2 (0–3) |
| Ol 10% | 1.785 | 2 (0–3) | 1.928 | 2 (0–3) |
| Ol 100% | 1.214 | 1 (0–2) | 1.214 | 1 (0–2) |
| Day 12 | | | | |
| NT | 1.571 | 2 (1–2)** | 1.571 | 2 (1–2)* |
| Ref 10% | 1.142 | 1 (0–2) | 1.285 | 1 (0–2) |
| Ol 10% | 1.357 | 1.5 (0–3)** | 1.428 | 1.5 (0–3) |
| Ol 100% | 0.642 | 1 (0–1)** | 0.857 | 1 (0–2)* |
| Day 15 | | | | |
| NT | 1.428 | 1 (1–3) | 1.428 | 1 (1–3) |
| Ref 10% | 1.142 | 1 (0–2) | 1.285 | 1.5 (0–2) |
| Ol 10% | 1.071 | 1 (0–2) | 1.071 | 1 (0–2) |
| Ol 100% | 0.714 | 1 (0–1) | 0.785 | 1 (0–1) |

Oral mucositis was induced in hamsters by 5-FU injection followed by mechanical trauma to the jugal mucosa. The groups treated with andiroba oil (*Carapa guianensis* Aubl.), Ref 10%, OL 10% and OL 100% and untreated (NT) group. The data represent average, median and variation of the scores (MM—minimum and maximum), attributed in 7 hamsters, considering the clinical parameters evaluated: presence and intensity of erythema, hyperaemia, haemorrhage and ulcers and for histopathological analysis, vasodilatation; intensity of cellular infiltrate; presence of hemorrhagic areas; edema and ulcers

*$p < 0.05$ statistically significant values compared with Ol 100% and NT groups

**$p < 0.05$ statistically significant values compared with (NT and Ol 100%) and (Ol 100% and Ol 10%)

(ANOVA and Tukey)

topical use in hamsters with oral mucositis. Clinical and histopathological evaluation, GC-MS analysis of the essential oils, and analysis of toxicity and genotoxicity using a MN test were performed.

The oil consists of saponifiable components, such as palmitic, oleic (approximately 50%), stearic and linoleic acids, and an unsaponifiable fraction (25%) composed mainly of bitter substances called meliacin or limonoids [22]. Limonoids are responsible for the antiseptic, anti-inflammatory, healing and insecticidal activity of this oil [23]. The in natura andiroba oil used in the study had a higher concentration of these FA compared with the usually commercialised refined oil, in which a higher amount of glycerol was detected.

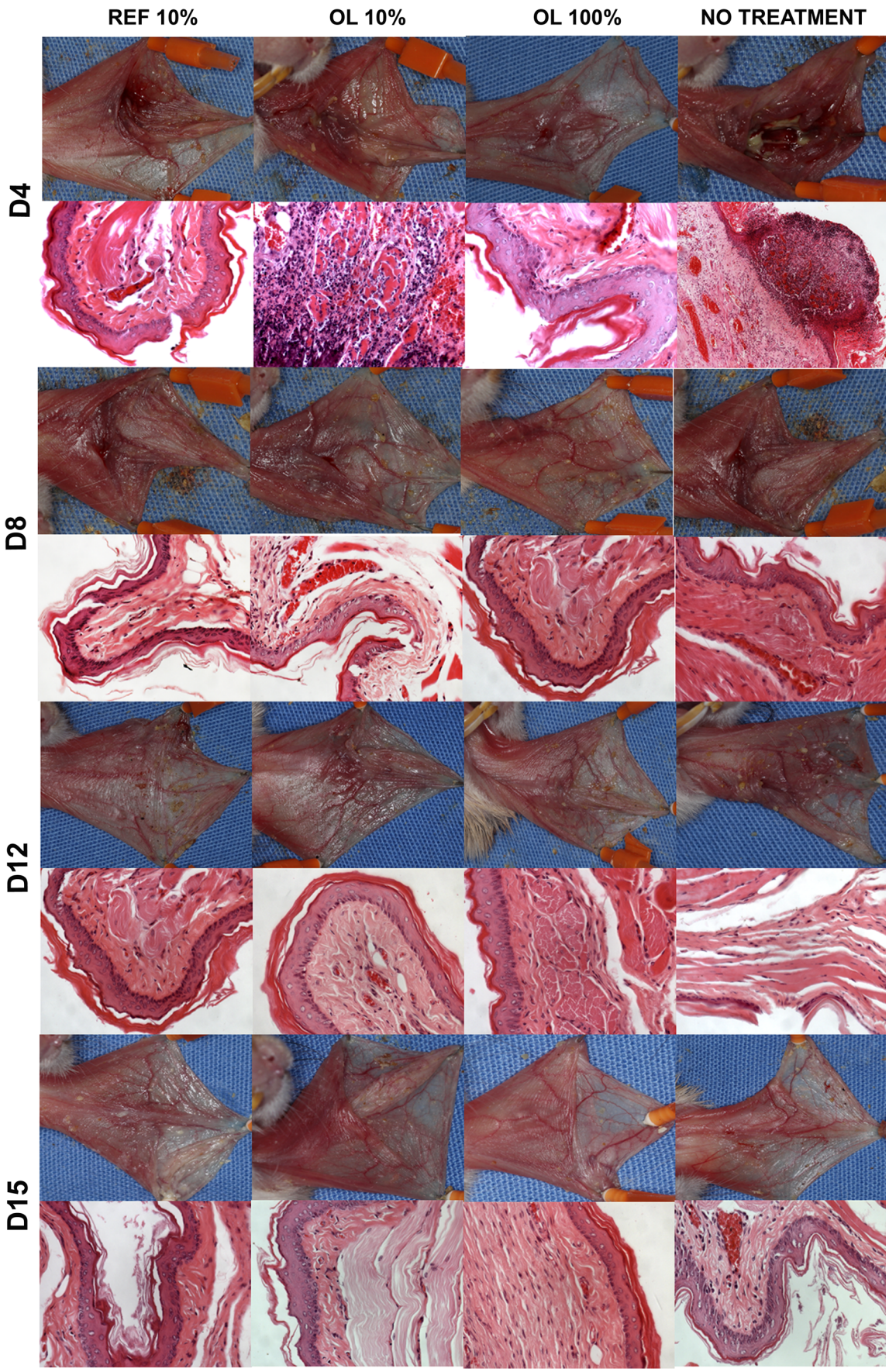
Although inflammatory diseases are treated with both steroids and non-steroidal anti-inflammatory drugs (NSAIDs), in vivo studies and clinical trials have reported the anti-inflammatory effects of medicinal plants in OM [13, 24]. Andiroba consists of bioactive agents that show great potential for the treatment of OM; some clinical and non-clinical trials have shown the healing activity of andiroba oil against open wounds when applied topically [15]. They also reported the anti-allergic, analgesic and anti-inflammatory activities of the oil following oral administration [15, 25]. However, only a few studies on these activities have been performed and no research related to OM can be found in the literature; this is the first study to evaluate the healing activity of andiroba oil against OM.

The present study showed that the andiroba oil in natura Ol 100% can reduce the healing time of OM compared to that reported for the no treatment group, 10% andiroba oil in natura groups, and refined 10% andiroba oil group (the latter two were applied in the form of orabase). When andiroba oil was applied topically three times daily on the oral mucosa of hamsters with 5-FU-induced oral mucositis and mechanical trauma, it showed significant inhibition of oral mucositis. This effect was characterized by lower OM severity scores in this group.

According to the literature, the peak phase of OM occurred between day 8 and day 12 [20]. In our results the maximum OM peak occurred in D4, an exacerbated inflammatory process was observed, with the presence of ulcers and abscesses. A comparison of the groups showed a statistically significant difference between the NT group and Ol 100% in the clinical and histopathological analysis with $p < 0.05$. The Ol 100% group presented less severe clinical lesions, with moderate to severe hyperaemia and erythema, and occasional small ulcers. In contrast, the NT group presented marked hyperaemia, erythema and the presence of haemorrhage, ulcers and abscesses. Clinical data were confirmed by histopathological evaluation, which showed moderate cellular inflammation, oedema, reduced haemorrhage and the presence of small ulcers in the Ol 100% group and a marked level of cellular inflammation, oedema and haemorrhage, and the presence of ulcers and abscesses in the NT group.

At D8, there was a partial regression of OM in all groups, but the analysis of the scores did not show a statistically significant difference for either clinical or histopathological evaluations. However, the Ol 100% group presented a lower mean of the clinical and histopathological scores.

At D12, a statistically significant difference was observed in the clinical analysis between the Ol 100% group with the 10% oil and NT groups with $p < 0.05$. The 100% Ol group presented a mean of the scores lower than one; consequently


REF 10%
OL 10%
OL 100%
NO TREATMENT
D4
D8
D12
D15

◀ **Fig. 3** Clinical and microscopic features (× 40 magnification, haematoxylin and eosin staining) of oral mucosa treated with andiroba (*Carapa guianensis* Aubl.). Groups Ref 10%, Ol 10% and Ol 100%, compared with the no treatment group, evaluated on days 4, 8, 12 and 15 of the experiment

there was a significant reduction in the severity of the clinical lesions. This group had erythema and absent or discrete hyperaemia, and absence of haemorrhage and ulcers, whereas the Ol 10% and NT groups had moderate to severe clinical lesions, with marked hyperaemia and erythema, haemorrhage and the presence of small ulcers.

In the histopathological evaluation, there was a significant difference between the Ol 100% and NT groups with $p < 0.05$. The histopathological analysis in the NT group demonstrated moderate cellular infiltrate and the presence of haemorrhagic areas, oedema and ulcers. The 100% Ol group presented a significant reduction in the severity of OM, obtaining scores close to zero, which may mean that the scarring of this group occurred early, that is, patients may experience a faster improvement in oral mucositis.

Data reported in the literature state that the healing of open wounds that were topically treated with andiroba increased the rate of epithelialization and reduction of the wound area compared with the normal course of the inflammatory process and tissue repair [19, 25].

Despite the literature indication that OM lesions induced in animals treated with 5-FU tend to regress spontaneously after day 15 owing to the wound-healing process [24], the NT group animals had scores varying between maximum and minimum values in the clinical and histopathological score scale presented with clinical hyperemia and erythema ranging from moderate to severe, scar tissue, and ulcers and abscesses, which was different from the results for the groups treated with andiroba that presented a gradual healing of OM lesions approaching complete healing at the end of the experiment: Groups Ref 10% and Ol 10% clinically presented with hyperaemia and moderate erythema, presence of scar tissue, and absence of ulcers and abscesses; the group Ol 100% presented with absent or discrete erythema and the absence of bleeding ulcers and abscesses.

It is worth noting that recovery in the in the Ol 100% group was faster than in the other two groups of the andiroba group (Ol 10% and Ref 10%). One possible interpretation of the healing process with reference to the Ref 10% group is that during the refinement process for commercial use, the glycerol content of the oil increased and the refinement altered the concentrations of FA that decreased in the sample as confirmed by GC-MS chromatography analysis; this decreased the therapeutic effects of the test substance. With regard to andiroba in natura at a concentration of 10% in orabase, it was concluded this concentration was not as effective in tissue repair. Literature suggested that the daily dose providing a healing effect until complete epithelization in rats was 250 mg/kg/day [19, 29].

In the studies on the acute toxicity of andiroba, the oral administration of various concentrations ranging from 0.375–5.0 g/kg/day resulted in no signs of toxicity in animals or their offspring [16, 17, 19].

A genotoxicity study for the oil of *C. guianensis* Aubl. seeds through the MN test has been reported, which showed no significant differences ($p < 0.05$) between the treatment and control groups with regard to the evaluated parameters, indicating the absence of genotoxic and cytotoxic effects [26].

The MN test is the most widely used in vivo assay for clastogenic (chromosome-breaking) and aneugenic agents (which induce aneuploidy or abnormal chromosomal segregation). As an internationally accepted testing assay, evaluation

**Table 3** Results of the micronucleus test and relationship between polychromatic erythrocytes (PCE) and normochromatic erythrocytes (NCE) in the bone marrow of hamsters (*Mesocricetus auratus*), obtained after induction of 5-FU oral mucositis and mechanical trauma for subsequent local treatment with andiroba oil (*Carapa guianensis* Aubl.)

| Groups | *n* | Average of MNPCE | Standard deviation | % H | PCE/NCE | Standard deviation |
|---|---|---|---|---|---|---|
| NC | 5 | 264.4 | 31.46 | – | 2215 | 0.1182 |
| CPA | 5 | 356.3 | 21.12 | 34.76*** | 1990 | 0.1476 |
| NT | 5 | 261.9 | 22.27 | – | 2835a**,b** | 0.3576 |
| Ol 100% | 5 | 355.1 | 19.39 | 34.30*** | 2870a**,b** | 0.3324 |
| Ol 10% | 5 | 292.2 | 35.08 | 10.51 | 3170a***, b*** | 0.4990 |
| Ref 10% | 5 | 269.3 | 22.68 | 1.85 | 2940a***, b*** | 0.6285 |

*MNPCE*, micronucleated polychromatic erythrocyte; *NCE*, normochromatic erythrocyte; *PCE*, polychromatic erythrocyte; *% H*, percentage of damage increase; *NC*, untreated control, *CPA* cyclophosphamide; *5-FU*, 5-fluorouracil; *Ol 100%*, pure oil; *Ol 10%*, 10% in natura oil and *Ref 10%* refined 10% oil

***Statistically significant values in relation to the groups ($p < 0.0001$)

a** and a*** Statistically significant values compared with NC animals ($p = 0.01$ and $p = 0.003$, respectively)

b** and b*** Statistically significant values compared with CPA animals ($p = 0.02$ and $p = 0.0008$, respectively)

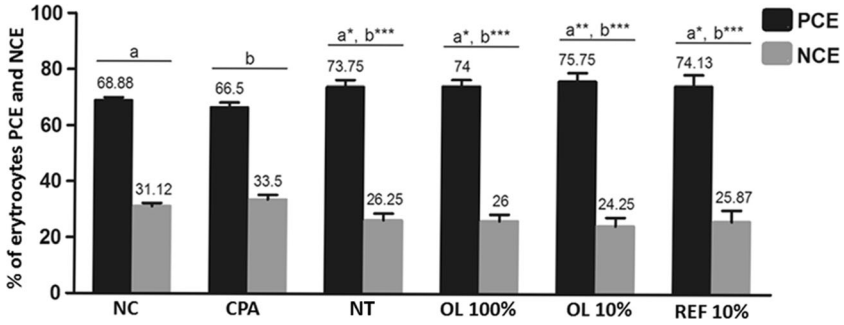



**Fig. 4** Mean percentage of polychromatic erythrocytes (PCE) and normochromatic erythrocytes (NCE) obtained from hamster bone marrow (*Mesocricetus auratus*) after induction of OM through 5-FU and mechanical trauma for subsequent local treatment with andiroba oil (*Carapa guianensis* Aubl.). For statistical analysis, two-way ANOVA was used, with Bonferroni post-hoc test. The values a* and a** were statistically significant when compared with NC animals ($p < 0.05$ and $p < 0.01$, respectively). The values b*** were statistically significant when compared to CPA animals ($p < 0.001$). Data are expressed as mean of five animals per group, NC (negative control), CPA (cyclophosphamide, micronucleus positive control), 5-FU (5-fluorouracil, oral mucositis-inducing positive control, Ol 100% (in natura oil, 100% pure), Ol 10% (in natura oil, 10%) and Ref 10% (refined oil)

of mutagenic potential and the registration of new chemicals entering the world market annually are recommended [27].

In this study, it was possible to observe that the induction of oral mucositis by 5-FU and mechanical trauma did not induce an increase in the MN frequency when compared to the NC group. The number of micronucleated cells increased in the group of animals treated with cyclophosphamide and in the 100% Ol group than in the CN and NT groups, this difference being statistically significant ($p < 0.001$). In the conditions studied, the results show that the frequency of MNs in the erythrocytes may be dependent on the dose, concentration and time of exposure. The data showed that a higher concentration resulted in a higher frequency of MN. We concluded that the percentage of MN was concentration-dependent, i.e. an increased concentration of essential oil resulted in a higher the percentage of MN, which indicated that this extract, at this concentration (Ol 100%), had a genotoxic potential on the cells of male golden Syrian hamsters.

However, it is important to highlight the need to test the clastogenicity of andiroba in other cell systems and in other conditions owing to the high consumption of this substance in the Amazon region.

The percentage of PCE of the total erythrocytes (PCE + NCE), i.e. the PCE/NCE ratio was determined in order to evaluate cytotoxicity to bone marrow. This data can also be used to document the bioavailability of a test chemical substance in the target tissue. Cytotoxicity is observed when there is a significant reduction in the percentage PCE, which indicated inhibition of division and maturation of erythropoietic cells [28].

The PCE/NCE ratio in the present study significantly increased the levels of PCE in the 5-FU and andiroba groups (Ol 10%, Ol 100% and Ref 10%) compared with the NC and CPA groups ($p < 0.05$), which indicated that this treatment induced cell proliferation in the bone marrow of these animals. This finding shows that the doses of andiroba oil tested did not generate cytotoxicity in the bone marrow of the golden Syrian hamsters; the absence of deaths or clinical signs of toxicity during the study reinforced this result.

We therefore concluded that andiroba accelerated the healing process in an experimental model of OM induced by 5-FU. The in natura oil at 100% concentration obtained better results compared with the no treatment and Ol 10% and Ref 10% groups. However, the use of this oil at a concentration of 100% for 15 consecutive days demonstrated genotoxic potential, although the frequency of PCE/NCE showed no cytotoxicity in all andiroba groups. Further studies are being conducted on the mechanisms that modulate the inflammatory and healing responses in oral mucositis.

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## Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interest.

**Ethical approval** This article does not contain any studies involving human participants performed by any of the authors. All procedures performed in studies with animals were in accordance with the ethical standards of the institutional and/or national research committee and all the care and use of animals were followed.

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