

## **Analgesic Efficacy of Cordia Verbenacea-based Gel in the Reduction of Pain Associated with Use of Separator Elastics**

## **Eficácia Analgésica do Gel à base de Cordia Verbenacea na Redução de Dor Associada ao Uso de Elásticos Separadores**

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**ABSTRACT**

**Introduction:** In Orthodontics separator elastics are used to create space for inserting the bands. This causes pain. Phytotherapy has been promising as a therapy. **Objective.** This double blind randomized clinical trial evaluated the level of pain due to the use of elastic separator (SE) after intraoral topical administration of *Cordia verbenacea* gel (CV). **Methods.** 50 volunteers received the SE on the mesial and distal surfaces of the maxillary first molars. In a blinded manner, 0.5 g of the gel was used in one hemi-arch, and placebo in the other hemi-arch on the free gingival margin of the first molar, every 8 hours, for 3 days. Pain was measured on a visual analog scale (VAS), before insertion of the SE (t0), immediately after (t1) and every hour after the following applications. Sensory analysis of the was performed for effects of taste and burning. Wilcoxon (comparison between sides) and Friedman and Nemenyi tests were performed for comparisons between times ( $\alpha=0.05$ ). **Results.** The pain was lower on the side that received the phytotherapeutic gel, from the first application until before the removal of the elastic ( $p< 0.05$ ). As regards the burning sensation, the experimental side had higher scores and 20% of the subjects considered the CV gel had an unpleasant taste. It was concluded that the use of the intrabuccal topic of CV has an analgesic potential for pain resulting from the insertion of SE.

**Keywords:** Pain, Orthodontics, Therapeutic action, *Cordea verbenacea*.

**RESUMO**

**Introdução:** Em Ortodontia os elásticos separadores são usados para criar espaço para a inserção das faixas. Isto causa dor. A fitoterapia tem sido promissora como terapia. **Objetivo.** Este ensaio clínico duplo-cego randomizado avaliou o nível de dor devido ao uso do separador elástico (SE) após a administração tópica intraoral do gel de *Cordia verbenacea* (CV). **Métodos.** 50 voluntários receberam o SE nas superfícies mesial e distal dos primeiros molares superiores. De forma cega, 0,5 g do gel foi usado em um hemi-arch, e placebo no outro hemi-arch na margem gengival livre do primeiro molar, a cada 8 horas, durante 3 dias. A dor foi medida em uma escala visual analógica (VAS), antes da inserção do SE (t0), imediatamente após (t1) e a cada hora após as seguintes aplicações. Foi realizada uma análise sensorial do SE para os efeitos do sabor e da queima. Wilcoxon (comparação entre os lados) e testes Friedman e Nemenyi foram realizados para comparações entre os tempos ( $\alpha=0,05$ ). **Resultados.** A dor foi menor no lado que recebeu

o gel fitoterápico, desde a primeira aplicação até antes da remoção do elástico ( $p < 0,05$ ). Quanto à sensação de ardor, o lado experimental teve pontuações mais altas e 20% dos sujeitos considerados que o gel CV tinha um sabor desagradável. Concluiu-se que o uso do tópico intrabucal do CV tem um potencial analgésico para a dor resultante da inserção do SE.

**Palavras-chave:** Dor, Ortodontia, Ação terapêutica, *Cordea verbenacea*.

## 1 INTRODUCTION

In the Orthodontics, the purpose of placing separator elastics is to create space for inserting the bands<sup>1</sup>. This causes pain, being continuous in the first days after placement, and becoming intermittent until it ceases completely<sup>2</sup>. The onset of pain generally occurs within the first 4 hours, and attains a maximum peak at 24 hours, tending to remission up to the seventh day<sup>3</sup>.

Oral administration of nonsteroid anti-inflammatory drugs (NSA) is considered the main therapeutic resource for analgesia of orthodontic pain<sup>4</sup>. *However, local allopathic and anesthetic medications taken indiscriminately may cause adverse side effects, particularly related to cardiovascular changes*<sup>5</sup>.

Phytotherapy, using exclusively plant-derived active raw materials, such as extracts, oils and tinctures, have shown to be increasingly promising and safe therapeutic resources for use in the treatment of different conditions and pathologies of the oral cavity<sup>6</sup>. However, its clinical applicability in local analgesia is limited<sup>7</sup>.

*Cordia verbenacea* is a medicinal plant known as "Whaling herb". Its volatile oil is extracted into the aerial part of the plant and has been used in folk medicine<sup>8</sup>. It has constituents with important anti-inflammatory, antimicrobial, analgesic and anti-rheumatic action, such as flavenoids, trans-caryophyllene,  $\alpha$ -humulene and others<sup>9</sup>. ACHEFLAN®, a herbal medicine containing volatile *Cordia verbenacea* oil, has been tested in the healing of skin wounds and its effectiveness has been proven<sup>10</sup>.

The topical use of *Cordia verbenacea* has been tested in animal models for evaluation of the anti-inflammatory effect. ACHEFLAN® was administered locally in a model of periodontitis induced in rats, and caused a rise in the levels of interleukin-10 (IL-10)<sup>11</sup>. that stimulate the endogenous production of anti-inflammatory cytokines and inhibit the production of pro-inflammatory cytokines<sup>12</sup>. This proved the effect of this herbal medicine on the inflammatory process. The aim of this study was to evaluate the

analgesic effect promoted by topical intraoral use of *Cordia verbenacea* in the control of pain associated with the use of elastic orthodontic spacers.

## 2 MATERIAL AND METHODS

This double-blinded, randomized clinical trial with the split-mouth experimental design was submitted to the Institutional research ethics committee, in accordance with the National Health Council resolution, and was approved under Protocol Number 01901818.0.0000.5385. All the study participants signed the Term of Free and Informed Consent.

### Sample

The sample was composed of fifty volunteers, balanced as regard gender (1:1), with a mean age of 24 years (standard deviation 5.8 years). The participants were selected from among students of the Dentistry Course at UNIRARAS. Dimensioning was performed by using the Gpower programs (G\*PowerTech Specs. Version 3.1.9.4.) and R (R Foundation for Statistical Computing, Vienna, Austria). The sample size of 50 participants provided a test power of 0.80, level of significance of 0.05 of a mean effect size (0.38).

The inclusion criteria used were: participants in good general health, without systemic diseases, without use of analgesics or anti-inflammatories, caries-free dentition with healthy periodontium, without previous endodontic treatment on posterior teeth, close contact of the first molar with the teeth adjacent teeth, presence of teeth antagonistic to the first molar, without posterior open bite, complete upper dentition, except for the third molars. The excluded patients were those who lost the elastic band or did not use the gel correctly, volunteers who had poorly adapted restorations in the proximal portion of the first and second molars and second premolars, those who used analgesics or anti-inflammatories during the experiment.<sup>13,14</sup> (Al-Melh & Andersson, 2017; Eslamian, Akbarian, Rahbani, & Mortazavi, 2018).

### Insertion of separator elastics

The volunteers received of separator elastics (size 5/32, blue; Morelli® Sorocaba, São Paulo, Brazil) on the mesial and distal surfaces of the maxillary first molars on both sides of the dental arch by a single operator<sup>13,15,16</sup>.

## Chemical analysis of the volatile oil of *Cordia verbenacea* by Gas Chromatography coupled to Mass Spectrometry (CG-EM).

Analysis by Gas Chromatography coupled to Mass Spectrometry (CG-EM) of volatile oil was performed in accordance with the modified method described by Proestos, Sereli & Komaiti (2006)<sup>17</sup>. 400 µL of the sample was exposed to 1 mL of a trimethylsilyl solution for silanization. The sample was analyzed in a gas chromatograph (QP 2010 Plus, Shimadzu Co., Japan) coupled to a mass spectrometer, equipped with a capillary column DB-5 (J&W Scientific, Palo Alto, California, US). The samples (0.5 µL) were injected, by using the *splitless* injection technique. The other chemical compounds were identified by comparison with the data of the mass spectrum from the library of the equipment (Willey-138 and Nist-98).

## Development and mode of application of the gel containing volatile oil of *Cordia verbenacea*

The formulation was developed with *Cordia verbenacea* volatile oil (Laszlo® Belo Horizonte, Minas Gerais, Brazil) according the formulation (Table 1).

Table 1. *Cordia verbenacea* gel Formulation

Components	%	Function
<i>Cordia verbenacea</i> oil	1,5	Active
carboxymethylcellulose	1	Polymer
propylene glycol	3	Humectant
methylparaben	0,15	Preservative

The gels were supplied in 5.0 mL syringes. The first application of the gel was made by the volunteer, after the insertion of the elastic separator. They were instructed to apply approximately 0.5 mL of gel (experimental and placebo) across the free gingival margin of the first molar. The randomization unit was the dental hemiarch with the application side being random<sup>14</sup>.

## Pain evaluation by the visual analog scale (VAS)

The volunteers were instructed to mark the pain they felt, on a visual analog scale (VAS), which was presented as a straight line ten centimeters long, with reference for absence of pain (0) at one extremity and maximum pain (10) at the other extremity<sup>18</sup>.

Before the insertion of the separator elastic, the volunteer first marked the VAS scale, in the intensity of the pain felt at the time (t0) and immediately after the insertion

of the separator elastic (SE) (t1). Together with the researcher, the volunteer made the first application of the gel, according to the established protocol.

After 60 minutes, the pain felt was again noted (t2). After the next meal and oral hygiene, the volunteers performed a new application of the gels, and recorded the pain on the visual analog scale, 60 minutes after the application (t3).

Thus, after the main meals, the volunteers performed applications and subsequent pain measurements, until the conclusion of the period of 72 hours (t11) after insertion of the SE. After these time intervals had passed, the volunteers continued with use of the separators in position, until they completed 07 days (t11). However, in this time interval they were instructed to apply the gels only if necessary, in the case of feeling pain, and report on the questionnaire, the number of applications and the times at which these were made.

### **Sensory Analysis of the Gels**

Patients received a questionnaire about the sensations of taste and burning produced by the tested gels, which were quantified on an ordinal scale, with scores ranging from 0 to 10<sup>19</sup>. The questionnaire was completed and delivered at the time of consultation to remove the separators. After assigning the scores, the values were categorized and plotted on a graph.

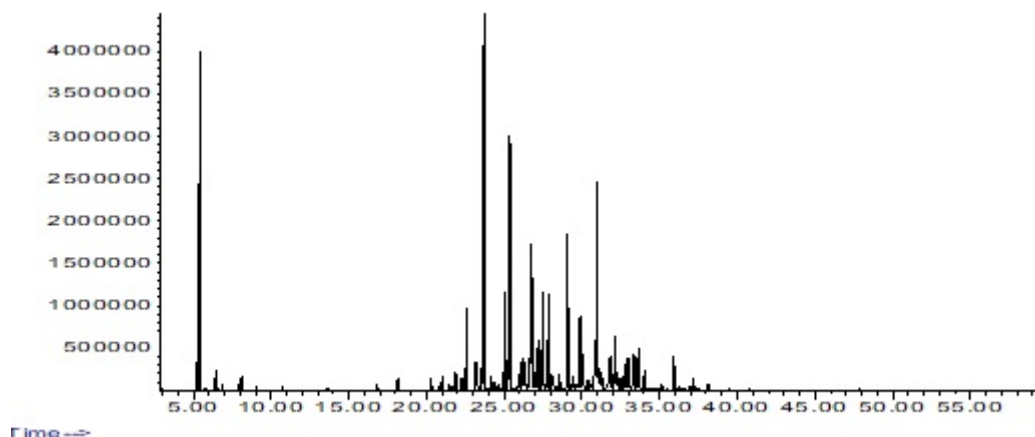
### **Statistical Methodology**

Descriptive and exploratory data analysis was performed. Wilcoxon and Friedman and Nemenyi tests were used. All analyzes were performed using the resources of the R program, considering the significance level of 5%.

## **3 RESULTS**

The chromatogram obtained, and its retention peaks are presented in Figure 1.

Figure 1: Chromatogram of EO of *Cordia verbenacea*.



In phytochemical analyzes, monoterpenes, sesquiterpenes, triterpenes, flavenoids and fatty acids were identified. Most chemical compounds have been described in retention times (TRs) between 5.06 and 38 minutes. 51 compounds were identified, the most representative of which are shown in chart 1. Of the analytes found, and those with anti-inflammatory and analgesic activity, those with the highest relative percentage were trans-karyophyllene, beta-PMD and alpha-pinene.

**Chart 1** - Main components identified in the volatile oil of *Cordia verbenacea* after chemical analysis by Gas Chromatography coupled to Mass Spectrometry (GC-MS). \*Retention time #Retention Index &Relative percentage

tr (min)*	IR#	Identification	% rel.
5.38	932	Alpha-pinene	6.15
22.57	1389	beta-elemene	2.84
23.7	1417	trans-caryophyllene	16.39
25.03	1450	alpha-humulene	3.18
25.35	1458	beta-PMD	9.65
26.76	1493	alpha-zingiberene	5.06
27.47	1511	Cubebol	3.53
27.85	1521	delta-cadinene	3.58
29.88	1574	Spathulenol	2.77

With regard to pain perception, the scores were significantly lower on the side that received the *Cordea verbenacea* gel than the placebo application side, right from the first application on the first day until the day of removing the elastic ( $p < 0.05$ ), as may be observed in Table 2. On the experimental side, as from 60 minutes after the third application on the second day, the pain level was significantly lower than the pain felt immediately after placement of the elastic ( $p < 0.05$ ). On the placebo side, the pain only

tended to return to initial levels ( $p < 0.05$ ) 60 minutes after the second application on the third day.

**Table 2** - Median (minimum and maximum value) of pain scores by the visual analog scale considering side and time

Day	Time	Gel applied		p-value
		<i>Cordea verbenacea</i>	Placebo	
First	Before elastic	0.0 (0.0; 0.0) Af	0.0 (-1000.0) Ad	-
	Immediately after	4.9 (0.0; 10.0) Aa	4.7 (0.0; 8.5) Aab	0.3946
	After 1st application	3.0 (0.0; 8.4) Bbcd	4.4 (0.0; 8.4) Aabc	0.0002
	After 2nd application	4.1 (0.0; 7.7) Bab	5.0 (0.0; 9.0) Aa	<.0.0001
	After 3rd application	3.2 (0.0; 8.4) Babc	5.1 (0.0; 8.4) Aa	<.0.0001
Second	After 1st application	4.2 (0.0; 8.4) Bab	5.0 (0.0; 10.0) Aa	<.0.0001
	After 2nd application	3.7 (0.0; 8.2) Bab	5.0 (0.0; 8.8) Aab	<.0.0001
	After 3rd application	2.9 (0.0; 7.5) Bbcd	4.6 (0.0; 8.1) Aabc	<.0.0001
Third	After 1st application	2.7 (0.0; 7.9) Bbcd	3.6 (0.0; 10.0) Aabc	0.0002
	After 2nd application	2.2 (0.0; 5.9) Bcd	3.5 (0.0; 7.1) Abc	<.0.0001
	After 3rd application	2.0 0.0 5.7	3.1 (0.0-6.8) Ac	<.0.0001
Seventh	Before removal	0.0 (0.0; 4.2) Bef	0.0 (0.0; 4.0) Ad	0.0403
p-value		<.0.0001	<.0.0001	

Medians followed by different letters, (capitals in the horizontal and lower case in the vertical) differ among them ( $p \leq 0.05$ ).

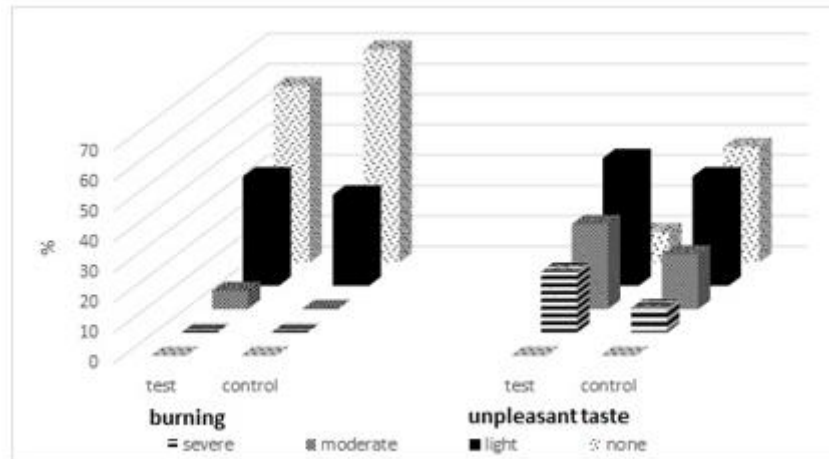
No patient used additional painkillers and one patient reported the need to use CV gel, having applied it once on the 5th day.

The results of the sensory evaluation are shown in Figure 2. The data revealed that no patient reported an intense or very intense burning sensation due to the gel based on *Cordia verbenacea*. 3 patients (6.0%) experienced moderate burning with a statistically significant difference ( $p = 0.0023$ ) between the Experimental and Control sides. In general, the burn scores were significantly higher on the experimental side ( $p < 0.05$ ) than on the placebo side.

When the unpleasant taste was analyzed, it was found that 80% classified it between light and intense, being statistically more significant than that of the placebo gel ( $p = 0.0038$ ).



Figure 2: Histograms classifying burning and unpleasant taste, considering side.



#### 4 DISCUSSION

The pain felt was owing to the constant pressure, ischemia, inflammation and edema in the periodontal ligament of the teeth involved in the treatment<sup>20</sup>. Dental movement involves compression of the periodontal ligament, preventing vascular circulation, cell differentiation and leads to the processes of cell and vascular degradation in a few hours. Slight inflammation occurs at the site of stimulus. The nerve endings sensitive to pain release substance *P* in the periodontal membrane and prostaglandins are synthesized by the local cells<sup>21</sup>.

The use of the elastic separator is associated with pain, but other authors have also used the elastomeric separator in their studies<sup>13</sup>, so that the main advantage of its use for induction of pain is to facilitate the correspondence between the samples and eliminate confounding factors that induce unequal forces and generate a systematic methodological error<sup>14</sup>.

Pain is a subjective perception, so the study had a split mouth design to lessen the effect of confounding factors.<sup>22,15</sup> The experimental model allowed the case and control groups and the effect of confounding factors to be compared on the same patient<sup>15</sup>. The medicinal properties of *Cordia verbenacea* have been attributed to its volatile oil extracted from its leaves.

These have anti-inflammatory, healing and anti-ulcerogenic action, due to the presence of mono and sesquiterpenes, with the outstanding agents being alpha humulene and trans-caryophyllene<sup>23</sup>. *Cordia verbenacea* has a potent anti-inflammatory activity and can be compared to well-established NSAIDs<sup>24</sup>. This activity can be attributed to the

negative regulation of pro-inflammatory mediators such as TNF- $\alpha$  and (Interleukin) -1 $\beta$  <sup>9</sup>.

Through GC-MS, alpha humulene was identified as one of the main active constituents isolated in the volatile CV oil, corroborating the findings of a study <sup>24</sup>. The study highlighted important activity of alpha humulene, which showed a rapid onset of action and good topical absorption, probably being the factor responsible for the anti-inflammatory and, consequently, analgesic actions of the volatile oil <sup>23</sup>.

Trans-caryophyllene is also a sesquiterpene found in the volatile oil, which was probably one of the compounds responsible for the control of orthodontic pain (%rel. 16.39). In the literature,  $\alpha$  pinene has also been reported as being a compound that has antinociceptive activity <sup>26</sup>.

The topical action of this component has been evaluated against an inflammatory process induced by carrageenan in rats. The results suggested that there was a reduction in cytokines such as tumor necrosis factor alpha (TNF- $\alpha$ ) <sup>27</sup>. Similarly, the oral administration of CV in rats exhibited the same anti-inflammatory effect, which was related to the presence of alpha humulene and trans caryophyllene <sup>9</sup>.

No studies were found to demonstrate the analgesic potential of the other active components, such as beta PMD. Components such as alpha-pinene have effects on pain control, such as its muscle relaxant action <sup>28</sup>. The analgesic activity of the CV has not been widely explored and, when evaluated, was administered orally. In a study evaluated the analgesic activity of a 70% ethanolic extract obtained from lyophilized aerial parts of CV, at doses of 1.24 and 2.48 mg / kg. The study concluded that there were mild analgesic effects when the highest dosage was administered <sup>24</sup>.

The onset pain caused by elastomeric separation, usually occurs in the first 04 hours after insertion of the separators, and tended to increase in a 24-hour time interval, when it reached its maximum peak. After this period, there is a continuous reduction of pain in 07 days until it completely ceases <sup>2</sup>. In this study it was observed that the experimental side had its peak of pain at approximately the same time of 24 hours, but with a lower intensity when compared to the control side. From that peak of pain, the intensity began to decrease until it ceased in 7 days, corroborating the findings in the literature <sup>3</sup>; in fact, on the experimental side, the pain has always been less when compared to the side with the placebo. The CV gel can therefore be used topically, however, the pharmacological properties of the oil components need to be evaluated in the future. The present study made it possible to determine the presence of chemical compounds with

proven anti-inflammatory activity in the volatile oil of *Cordia verbenacea*. The presence of significant differences in pain scores between the sides in the time intervals should be considered.

The gels were prepared with sufficient viscosity to adhere to the free gingival margin and, therefore, did not mix. After analysis and evaluation, the polymer selected was carboxymethylcellulose, as it is free of sugar, and of natural origin. In addition, it made it possible to obtain stable formulations<sup>29</sup>.

Burning and unpleasant taste were significantly greater on the side of the experimental gel, but no patient reported strong or extremely strong sensations. According to Simões, Schenkel, Gosmann, Mello, Mentz & Petrovic (2007)<sup>30</sup>, essential oils have an intense aroma, which may have influenced the perception of taste. The burning analysis revealed discomfort with the use of the gel, however, it was not considerably severe. 70% of the volunteers considered the palatability unpleasant, pointing to the need to change the formulation's organoleptic characteristics, such as the addition of flavoring and sweetening substances, or other components that mask the flavor.

Therefore, the gel used was considered acceptable for its intraoral use, being subject to adequate palatability.

#### 4 CONCLUSION

It could be concluded that *Cordia verbenacea* gel could be considered a therapeutic alternative to be used in a topical manner intraorally for the relief of orthodontic pain associated with the placement of separator elastics.

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