ORIGINAL ARTICLE

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Postoperative analgesic effect of *Cordia verbenacea* topical aromatherapy in female dogs undergoing ovariohysterectomy

Efeito analgésico da aromaterapia tópica com Cordia verbenacea *em cadelas submetidas à ovariohisterectomia*

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ABSTRACT: This study evaluated the effects of topical aromatherapy with *Cordia verbenacea* D.C. (Boraginaceae) essential oil on postoperative pain in female dogs who underwent elective ovariohysterectomy (OVH). Twenty dogs were used and randomly distributed into the aromatherapy and placebo groups, with ten animals each. The animals in both groups received pre-anesthetic medication (PAM) with morphine, acepromazine and midazolam, anti-inflammatory drug and antibiotic therapy before being anesthetized with propofol, isoflurane, ketamine, lidocaine and fentanyl and subjected to OVH. Animals in the aromatherapy group received *Cordia verbenacea* essential oil mixed with a biphasic carrier (avocado and rosehip carrier oils and gel) immediately after extubation and 3, 5 and 8 hours after PAM, while dogs in the placebo group received the biphasic carrier at the same times. All animals in the experiment were evaluated for pain before the PAM (M0) and 3 (M3), 5 (M5), 8 (M8), 12 (M12), and 24 (M24) hours after the surgical procedure using the visual analogue scale (VAS) and the short form of the Glasgow Composite Measure Pain Scale (CMPS-SF). The aromatherapy group showed a significantly lower pain score compared to the placebo group at M8 (P=0,0088 and P=0,00930, M12 (P=0,0028 and P=0,0004), and M24 (P=0,0265 and P=0,0475) and also required fewer administrations of analgesics in this period (P=0,0367). Therefore, aromatherapy with *Cordia verbenacea* essential oil showed an analgesic effect in the postoperative period of female dogs undergoing OVH.

KEYWORDS: analgesia; pain; phytotherapy; surgery.

RESUMO: Este estudo avaliou os efeitos da aromaterapia tópica com óleo essencial de *Cordia verbenacea* D.C. (Boraginaceae) na dor pós-operatória em cadelas submetidas à ovariohisterectomia eletiva (OVH). Vinte cadelas foram utilizadas e distribuídas aleatoriamente nos grupos aromaterapia e placebo, com dez animais cada. Os animais de ambos os grupos receberam medicação pré-anestésica (MPA) com morfina, acepromazina e midazolam, anti-inflamatório e antibioticoterapia antes de serem anestesiados com propofol, isoflurano, cetamina, lidocaína e fentanil e submetidos à OVH. Os animais do grupo aromaterapia receberam a aplicação tópica de óleo essencial de *Cordia verbenacea* misturado com um carreador bifásico (óleos carreadores de abacate e rosa mosqueta e gel) imediatamente após a extubação e 3, 5 e 8 horas após a MPA, enquanto os cães no grupo placebo receberam topicamente apenas o carreador bifásico nos mesmos momentos. Todos os animais no experimento foram avaliados quanto à dor antes da MPA (M0) e 3 (M3), 5 (M5), 8 (M8), 12 (M12) e 24 (M24) horas após o procedimento cirúrgico usando a escala visual analógica (EVA) e a forma abreviada da Escala Composta de Dor de Glasgow (CMPS-SF). O grupo aromaterapia apresentou pontuações de dor significativamente menores em comparação ao grupo placebo em M8 (P=0,0088 e P=0,00930, M12 (P=0,0028 e P=0,0004) e M24 (P=0,0265 e P=0,0475) e também necessitou de menos administrações de analgésicos neste período (P=0,0367). Portanto, a aromaterapia com óleo essencial de *Cordia verbenacea* apresentou efeito analgésico no período pós-operatório de cadelas submetidas à OVH.

PALAVRAS-CHAVE: analgesia; cirurgia; dor; fitoterapia.

INTRODUCTION

Analgesia in the postoperative period is important to provide welfare to animals, decrease recovery time, reduce complications, and avoid comorbidities (Dimitriou *et al.*, 2017). Traditionally, pharmacological therapy, such as anti-inflammatory medications and opioids, is used to promote analgesia in this period, although the use of these drugs can cause adverse effects such as nausea, vomiting, apnea, and sedation (NENADOVIC *et al.,* 2017).

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To assist in postoperative pain management, in addition to allopathic drug therapy, some complementary therapies have proven effective in promoting analgesia in dogs and cats, including acupuncture (Groppetti *et al.,* 2011) and Reiki therapy (Pacheco *et al.,* 2021). Aromatherapy is a complementary therapy that is effective in decreasing pain in human patients undergoing surgical procedures such as cesarean section, episiotomy, tonsillectomy and knee replacement. (Lakhan; Sheafer; Tepper, 2016; Abbasijahromi *et al.*, 2019). Aromatherapy uses plant-derived essential oils that can be administered by oral, topical and inhalation routes to improve the physical and emotional well-being of the animal; essential oils are complex mixtures of volatile compounds obtained from aromatic plants (Lakhan; Sheafer; Tepper, 2016; Sousa, 2022).

C. verbenacea is a native plant in many parts of Brazil, with a preference for areas on the coast, particularly in the Atlantic Forest (De Carvalho *et al.*, 2004). It is a perennial shrub with a height of 1.5–2.5 meters, numerous branches with aromatic leaves, and flowers and fruits in certain seasons (Lorenzi; Matos, 2002). *C. verbenacea* is known by popular names such as erva baleeira, catinga de barão, erva-preta, maria-milagrosa, maria-preta, maria-rezadeira, salicilina and cordia; the botanical nomenclature includes *C. curassavica, C. salicilina, C cylindristacgya, Lithocardium salicinum, and L. verbenaceum* (Lorenzi; Matos, 2002). In folk medicine, their leaves have been used for their anti-inflammatory and cicatrizing effects (Dutra *et al.*, 2016). During the last 20 years, a few groups have investigated the potential effects of this plant and the related compounds, especially concerning its promising actions against inflammatory conditions (Dutra *et al.*, 2016; Martim *et al.*, 2021).

Toxicological pre-clinical studies (with both rodents and non-rodents) revealed a very satisfactory safety profile for the essential oil from *C. verbenacea,* containing both α-humulene and trans-caryophyllene, and the subsequent phase I, II and III clinical trials confirmed the efficacy of this product as anti-inflammatory and analgesic when used topically. This finally resulted in the approval of the phytotherapy product Acheflan® for human use by the Brazilian regulatory agency ANVISA in 2005 (Dutra *et al.*, 2016). Using C. verbenacea extract in rats Sertié *et al.* (1988) and Sertié *et al.* (2005) observed low toxicity.

Aromatherapy with *Cordia verbenacea* D.C. (Boraginaceae) essential oil can effectively promote postoperative analgesia and provide comfort and well-being in animals, as it has chemical components that inhibit inflammation (Fernandes *et al.,* 2007) and stimulate opioid (Katsuyama *et al.,* 2013) and cannabinoid (Gertsch *et al.,* 2008) receptors. Thus, aromatherapy can promote analgesia and reduce the amount of allopathic medications administered postoperatively and, therefore, be a complementary therapy in the postoperative treatment of veterinary patients. Furthermore, promising analgesic results may encourage the pharmaceutical industry to develop new herbal products that could be used by many animals.

Considering the absence of studies verifying the analgesic efficacy of aromatherapy in animals and the need to improve postoperative analgesia in female dogs and decrease the use of drugs during this period, this study evaluated the analgesic efficacy of topical aromatherapy with *C. verbenacea* essential oil in the postoperative period of female dogs that underwent ovariohysterectomy.

MATERIAL AND METHODS

Animals

This study included 20 female dogs weighing 2–12 kg and aged between 6 months and 7 years, who were clinically healthy, normally hydrated and non-pregnant. All animals underwent a screening process 1 week before the experiments, which involved clinical examinations (auscultation, palpation, checking mucous membranes) and laboratory tests such as blood count and levels of alanine serum aminotransferase (ALT), urea, and creatinine. No electrocardiogram or echocardiogram was performed on any animal; all animals were classified as ASA 1 patients. After these examinations, the female dogs were randomly separated into aromatherapy and placebo groups with ten animals in each group. The animals were randomized using simple random samples in Microsoft Excel software. The sample size was determined using the free version of the Action Stat software (Estatcamp- Consultoria Estatística e Qualidade, São Carlos, Brazil). A confidence interval of 0.95 and a maximum sampling error of 0.5 and a sample standard deviation of 0.8 was used, obtained from previous studies that used pain scores.

Pain, sedation and physiological parameters evaluations

The animals arrived at the hospital at least 4 hours before the surgery having fasted for 8 hours. During this period, 1 hour before the pre-anaesthetic medication (PAM), the first assessments of pain, sedation, and physiological parameters were performed (time point M0). These assessments used the sedation scale suggested by Wagner; Hecker; Pang (2017), which is the short form of the scale used by Grint; Burford; Dugdale (2009), two pain scales [visual analogue scale (VAS) and the short form of the Glasgow Composite Measure Pain Scale (CMPS-SF)]. Respiratory rate, heart rate, and rectal temperature were also measured, in that order, by a trained male evaluator (face to face evaluator).

This evaluator made 2-minute videos of the animals' behaviour in the cage, moving outside the cage, and during pain and sedation assessment. The videos were collected using a Canon 60D camera with a wide-angle lens.

The sequence of activities of the face-to-face evaluator was as follows: 1) turn on the video camera; 2) observe the animals

in their cages and complete the sedation scale and VAS; 3) put a leash on the animals and observe their movement outside the cage; 4) palpate the abdomen and flanks of the animals and complete the CMPS-SF; 5) turn off the video camera; 6) measure the respiratory rate; 7) measure the heart rate; 8) measure the rectal temperature; 9) rescue analgesic if necessary.

The video footage was sent to another trained female evaluator who first completed the VAS and then the CMPS-SF; This second evaluator was not aware of the grades given by the first evaluator and was not present on the day of the surgery and the recording of the videos. The scores given by this evaluator were not used to recommend rescues, as these assessments were carried out after the animals had finished their post-operative period. The pain scores of each animal were the average of the scores of the two evaluators. Two evaluators of different genders were used as there may be a difference in scores given to the same animals depending on the scales used (Tomio, 2019).

The evaluators were blinded to the treatment of the animals; that is, they did not know to which group each animal belonged. So that the on-site evaluator could not identify the animals in the aromatherapy group, the essential oil mixture was applied by another member of the research team who was not an evaluator after the evaluation and the characteristic aroma of C. verbenacea had disappeared by the time of the next evaluation.

Anesthetic and surgical protocol

After first evaluation, the dogs received intramuscular pre-anaesthetic medication (PAM) (Figure 1), which consisted of 0.3 mg/kg morphine (morphine sulfate, Hipolabor Farmacêutica Ltda, Brazil), 0.03 mg/kg acepromazine (Apromazin®, Syntec do Brasil, Brazil) and 0.2 mg/kg midazolam (Dormium®, União Química Farmacêutica Nacional S/A, Brazil).

Subsequently, each animal was prepared with venous access with a 22G catheter in the cephalic vein for infusion of 5 ml kg^{-1} per hour of lactated ringer's solution (ringer lactate; JP Indústria Farmacêutica AS, Brazil) and abdomen trichotomy. At this time, the animals were administered intravenous meloxicam (0.2 mg/kg, Flamavet®, União Química Farmacêutica Nacional S/A, Brazil) and the combination of benzylpenicillin benzathine (10,000 IU/kg), benzylpenicillin procaine (10,000 IU/kg) and dihydrostreptomycin (20 mg/kg) intramuscularly (Shotapen®, Virbac, France).

Twenty minutes after PAM, the animals received an anaesthetic induction (Figure 1) of 1 mg/kg ketamine (Cetamin®, Syntec do Brasil, Brazil), 1 mg/kg lidocaine (Anestt®, Syntec do Brasil, Brazil), and 2 mg/kg propofol (Propotil®, DongKook Pharm. Co., South Korea) administered intravenously. They were then intubated with an endotracheal tube. Anaesthesia was maintained by the continuous infusion of 1.2 mg/kg/h of ketamine and 4 mg/kg/h of lidocaine by infusion pump (SDAMed model SDA101, SDAMed, Brazil), 10µg/kg/h of fentanyl (Fentanest®, Cristália Produtos Químicos Farmacêuticos Ltda, Brazil) by continuous infusion syringe pump (SDAMed model SDA401, SDAMed, Brazil), and inhalational anaesthesia with isofluorane (Isoforine®, Cristália Produtos Químicos Farmacêuticos Ltda, Brazil) diluted in 100% oxygen and adjusted vaporization in a universal vaporizer.

Ten minutes after anaesthetic induction, the animals underwent the hook-and-loop ovariohysterectomy (Figure 1). All surgical procedures were performed by the same technique and by the same surgeon and all anesthetic procedures were performed by the same anesthesiologist.

Immediately after extubation, the animals were taken to an air-conditioned anesthetic and surgical recovery room, where they remained for 24 hours (Figure 1).

Postoperative procedures

The animals were evaluated 3 (M3), 5 (M5), 8 (M8), 12 (M12) and 24 (M24) hours after PAM (Figure 1) in the same way as in M0 by the same in-person evaluator, using the same scales, who filmed the evaluation and sent it to the other evaluator, who was the same for all videos including M0. Animal evaluation was carried out 3 hours after PAM to avoid a possible influence of post-anesthetic sedation on pain assessments.

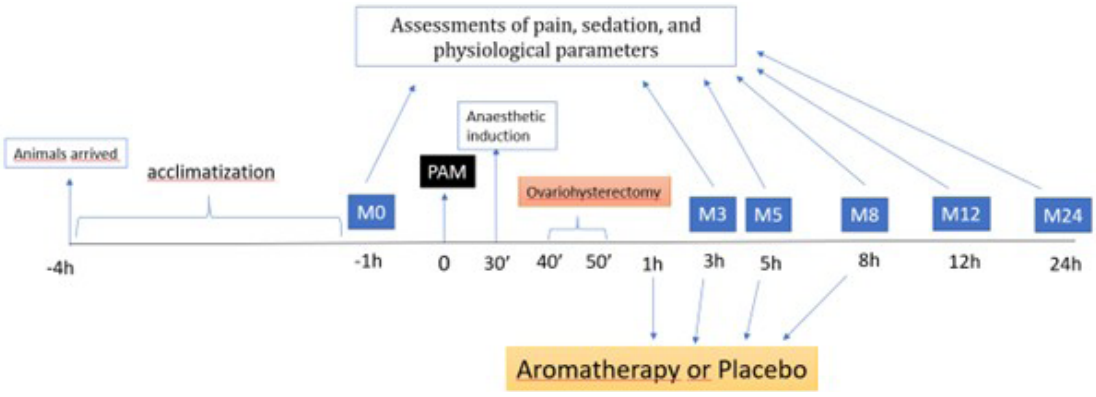


Figure 1. Experimental protocol

This time interval also mitigated the influence of analgesics used in PAM and anesthesia on pain assessments.

Animals with a CMPS-SF score ≥6 at any assessment received 0.2 mg/kg morphine intramuscularly. Animals that received additional analgesics were not removed from the experiment, i.e., all animals were evaluated at all times. The VAS was not used to recommend rescue in any evaluation of this experiment. Morphine was the analgesic of choice for analgesic rescues as it is the analgesic commonly used in studies to evaluate postoperative pain (Pacheco *et al.*, 2021) and its analgesic effect probably did not interfere with subsequent evaluations due to its half-life of action. Additional analgesic administrations were carried out by the face-to-face evaluator immediately after completing the CMPS-SF.

After the 24-hour evaluation, the animals were returned to their owners and received Dipyrone (25mg/kg every 8 hours) for 48 hours.

No laboratory tests were performed after the surgical and aromatherapy procedures. Aromatherapy toxicity assessments have also not been carried out.

Aromatherapy procedures

For topical aromatherapy applications, a moist and heated compress was placed on the application region for 2 minutes to cause local vasodilation and humidify the skin to improve the absorption of the essential oil. The mixture with essential oil was applied immediately after removing the compress in the largest area possible from the abdomen through delicate massage.

The animals in the aromatherapy group received 0.1 mL/kg applications of a topical (on the ventral skin of the abdomen) mixture of *C. verbenacea* essential oil (10%) (Yanih Produtos Cosm. E Farm. LTDA, Erva Baleeira, Brazil), avocado vegetable oil (40%) (Argila Ind. E Com.de Cosméticos Ltda, Óleo gorduroso extra-virgem de abacate, Brazil), Rosehip vegetable oil (20%) (Argila Ind. E Com. de Cosméticos Ltda, Óleo gorduroso extra-virgem de Rosa mosqueta, Brazil), and ultrasound gel (30%) after surgery, immediately after the dogs returned to their cages after extubation, and immediately after the M3, M5 and M8 evaluations, totalling 4 applications (Figure 1) and 0.4 mL/kg of the mixture and 0.04 mL/kg of essential oil. Avocado and rosehip vegetable oils and the gel formed the biphasic carrier vehicle for *C. verbenacea* essential oil. The placebo animals also received topical applications of 0.1 mL/kg of the biphasic carrier vehicle, a mixture of avocado vegetable oil, rosehip vegetable oil and ultrasound gel, in the same proportions and at the same times as the aromatherapy group (total 0 .4 mL/kg of the mixture). These animals received only the carrier vehicle so that it was possible to observe the effect of the C. verbenacea essential oil.

Statistical analysis

The normality of the data obtained from the pain scales, sedation scale, and measurements of physiological parameters was evaluated using the d'Agostino & Pearson test. The results of the pain and sedation scales were compared between the two groups using Mann–Whitney tests, while the pain and sedation were compared within the same group at the various time points using Kruskal–Wallis test followed by Dunn's multiple comparison tests. The physiological parameters of the two groups were compared using unpaired T-tests. To compare the results within the same group among the various time points, one-way analysis of variance (ANOVA) tests were performed, followed by Dunnet's multiple comparison tests. To compare the number of additional analgesic administrations in the postoperative period in each group, chi-square and Fisher's exact tests were performed. The results obtained from the pain and sedation scales were presented as medians and maximum and minimum values. The physiological parameters were presented as means ± standard deviation. Differences were considered significant for $P<0.05$. All statistical tests were performed using the test version in GraphPad Prism 8 (GraphPad Software, San Diego, USA).

RESULTS AND DISCUSSIONS

Animals and surgical time

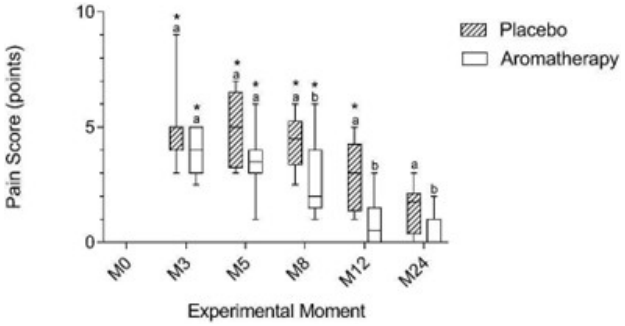
The dogs in the placebo group weighed 6.478 ± 3.902 kg and were 2.956 ± 2.592 years of age. The surgical time in this group was 14.56 ± 2.404 minutes. The dogs in the aromatherapy group weighed 6.567 ± 1.544 kg and were 2.089 ± 1.646 years of age. The surgical time in this group was 13.78 ± 2.386 minutes. There was no significant difference between groups in relation to weight (P=0,9501), age (P=0,4096) and surgical time (P=0,5008).

Pain scores

Regarding the pain scores obtained with the CMPS-SF (Figure 2), the aromatherapy and placebo groups showed significant differences at M8 (P= 0.0088), M12 (P=0.0028), and M24 (P= 0.0265), with the aromatherapy group showing lower pain scores compared to the placebo group. Comparisons of pain scores between M0 and those at M3, M5, M8, M12, and M24 showed higher scores at M3 (P<0.0001), M5 (P<0.0001), M8 (P<0.0001), and M12 (P=0.0108) compared to M0 in the placebo group. In the aromatherapy group, only the values at M3 (P<0.0001), M5 (P<0.0001), and M8 (P=0.0005) were higher than that at M0; thus, in this group, M12 and M24 did not differ significantly from M0, while in the placebo group, only M24 did not differ from M0, showing that in the aromatherapy group the pain score increased after surgery and returned to the M0 level already. in M12 and in the placebo group only in M24.

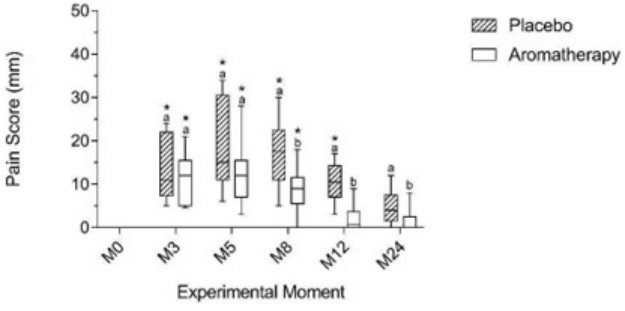
Using the VAS, pain scores obtained in the aromatherapy group were also significantly lower than those in the placebo group at M8 (P=0.0093), M12 (P=0.0004) and M24 (P=0.0475) (Figure 3). Comparisons of pain scores between M0 and those at M3, M5, M8, M12, and M24 showed higher scores at M3 (P=0.0008), M5 (P<0.0001), M8 (P<0.0001), and M12 (P=0.0040) compared to M0 in the placebo group. In the aromatherapy group, only the values at M3 (P<0.0001), M5 (P<0.0001), and M8 (P=0.0004) were higher than that at M0; thus, in this group, M12 and M24 did not differ significantly from M0, while in the placebo group, only M24 did not differ from M0. These results were similar to those obtained with CMPS-SF.

During the postoperative period, six administrations of additional analgesia were performed in 3 animals in the Placebo group (one in M3, 3 in M5 and two in M8), compared to only one administration in an animal in the Aromatherapy group (in M5), with significant difference between groups (P= 0.0367) (Table 1).



The letters indicate significant differences (P<0.05) between groups, with b<a. * represents values different from M0. The maximum score for this scale is 24. The results are presented as medians and maximum and minimum values.

Figure 2. Scores in the short form of the Glasgow Composite Measure Pain Scale in female dogs subjected to OVH and treated with *C. verbenacea* essential oil diluted in carrier oils and gel (Aromatherapy group) or treated with the carrier mixture alone (Placebo group).



The letters indicate significant differences (P<0.05) between groups, with b< a. * represents values different from M0. The maximum score on this scale is 100. The results are presented as medians and maximum and minimum values.

Figure 3. Visual analogue scale (VAS) scores in female dogs subjected to OVH and treated with *C. verbenacea* essential oil diluted in carrier oils and gel (Aromatherapy group) or treated with the carrier mixture alone (Placebo group).

No additional analgesic administrations were performed during the transoperative period.

Aromatherapy has been used until M8 to cover the period of greatest pain in the ovariohisterectomy postoperative period. Studies have shown that the periods of greatest pain for dogs undergoing ovariohysterectomy are experienced one to four hours after the surgical procedure (Srithunyarat *et al.*, 2016; Nenadovic *et al.*, 2017; Watanabe *et al.*, 2018).

We might have observed a difference in pain scores between the two groups at M3 and M5 if aromatherapy had been applied for a few hours before the beginning of surgery, as the topical route of aromatherapy application has a long absorption time, especially compared to the inhalation and oral routes, since the corneal extract forms a barrier for the absorption of the essential oil by the animals' skin (Moser *et al.*, 2001), although the absorption time and distribution of the active components of the essential oil were not measured in this experiment. The topical route was chosen for its safety and practicality in dogs.

The lower pain scores and lower number of additional analgesic administrations in the postoperative period in the aromatherapy group may be a result of the pharmacological action mechanisms of some constituents of the *C. verbenacea* essential oil. The *C. verbenacea* essential oil used has three major constituents, according to chromatography (Figure 4); namely, pinene (40.1%), caryophyllene (22%), and alpha-humulene (4%).

Table 1. Number of additional analgesic administrations in female dogs undergoing elective ovariohysterectomy treated with sham aromatherapy (placebo) or topical aromatherapy with Cordia verbenacea and biphasic carrier immediately after surgery, before pre-anesthetic medication (M0) and 3 (M3), 5 (M5), 8 (M8), 12 (M12) and 24 (M24) hours after pre-anesthetic medication.

Time points	M3	M5	M8	M12	M24	Total
Placebo group	1	3	2	0	0	6[a]
Aromatherapy group	0	1	0	0	0	1[b]

The letters indicate significant differences between the groups (P<0.05). Placebo> aromatherapy (P=0.0367).

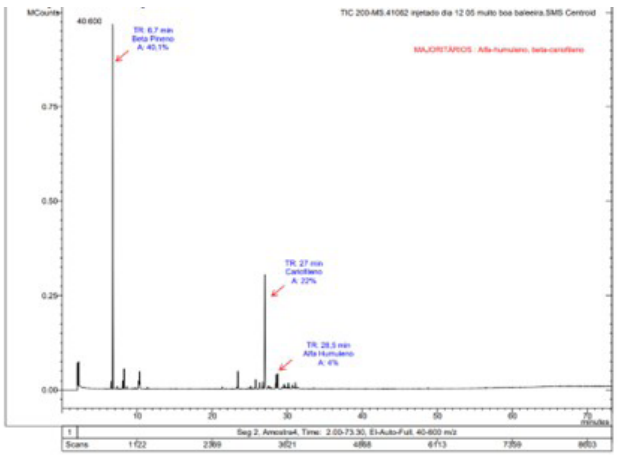


Figure 4. Chromatography of the *Cordia verbenacea* essential oil used in the experiment showing its main components.

The analgesic and anti-inflammatory effects of these *C. verbenacea* components have been studied extensively over the past few years.

Rahbar *et al.* (2018) reported that α-pinene decreased inflammation by decreasing cyclooxygenase-2 (COX-2) expression and nociception via γ-Aminobutyric acid type A (GABA-A) and opioid receptors. Li *et al.* (2016) found that α-pinene had anti-inflammatory and analgesic effects by decreasing nociceptive inflammatory infiltrates and COX-2.

The analgesic effects of β-caryophyllene have already been tested extensively. Katsuyama *et al.* (2013) found that β-caryophyllene decreased the nociceptive response in rats by a mechanism mediated by cannabinoid CB2 receptors, which stimulated the release of β-endorphins by local keratinocytes, an effect that was blocked by a μ opioid receptor antagonist. Paula-Freire *et al.* (2014) also reported the analgesic effect in rats of β-caryophyllene mediated by opioid and cannabinoid receptors. In addition to the activation of these receptors, β-caryophyllene was also effective in preventing the increased expression of interleukin 1-β, tumour necrosis factor (TNF)-α, and interferon-gamma (Gertsch *et al.*, 2008; Aly; Khajah; Masocha, 2020), in addition to substance P and interleukin-6 (AVILA *et al.*, 2019), which had analgesic and allodynia-lowering effects.

Fernandes *et al.* (2007) observed that α-humulene and β-caryophyllene decreased acute and chronic edema and decreased prostaglandin-E2, COX-2, and inducible nitric oxide synthase (iNOS) production and that α-humulene further decreased interleukin-1β and TNF-α production. Medeiros *et al.* (2007) also observed that α-humulene decreased edema and the production of pro-inflammatory cytokines and prevented the up-regulation of bradykinin B1 receptors.

The intramuscular antibiotics may have increased the patients' pain scores, but all patients in both groups received this medication, therefore the use of this drug should not have influenced the difference in pain scores observed between the two groups, as that the only product used only in the aromatherapy group was essential oil.

Sedation and physiological parameters evaluations

The sedation scale scores did not differ significantly between the groups, suggesting that the chemical components in the essential oil used did not alter the level of sedation of the animals that received it topically (Figure 5).

No significant difference in respiratory frequency and rectal temperature was observed at any time point (table 2).

The heart rate was higher in the aromatherapy group compared to the placebo group at M3 (P= 0.0061) and M12 (P= 0.0461) (table 2 and Figure 6). Comparisons of M3, M5, M8, M12, and M24 in the placebo group to M0, showed a significantly lower value at M3 (P=0.05), which was not observed in the aromatherapy group. The aromatherapy group was expected to present lower heart rates compared to the placebo group at some time points due to an indirect effect by presenting lower pain scores. The heart rate values of the aromatherapy group may have been higher at some time points due to a possible activation of the opioid or cannabinoid receptors by some component of the essential oil used (PAULA-FREIRE *et al.*, 2014), but this hypothesis needs to be investigated further in subsequent experiments.

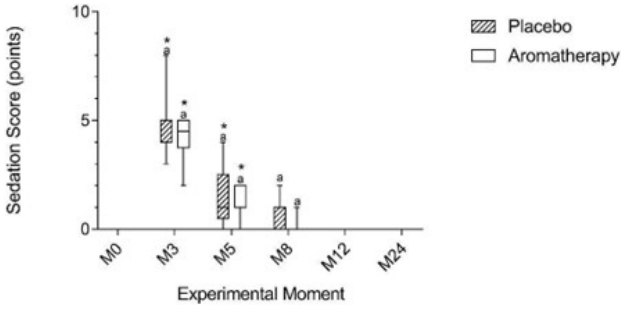
Aromatherapy procedures

Avocado and rosehip vegetable oils and gel were used as carriers and diluters of the essential oil with lipophilic and hydrophilic properties to favour the cutaneous absorption of the essential oil constituents through the lipophilic intercellular space and the hydrophilic transcellular pathway (Moser *et al.*, 2001).

The topical route of substance administration has several advantages, including minimizing the metabolic breakdown of drugs, eliminating gastric irritation, and possibly controlling the drug release profile to the skin (Choudhury *et al.*, 2017).

Although the skin is a barrier for chemicals to reach the blood circulation, mainly due to the presence of the stratum corneum, essential oils and their constituents are among the few molecules with specific physicochemical properties that can cross the skin barriers, so they are used as skin penetration enhancers to drugs with low permeability (Herman; Herman, 2014). Sesquiterpene (e.g. β-caryophyllene) and α-pinene, the main components of *C. verbenacea* essencial oil may increase the rate of absorption of the hydrophilic permeant. (Cornwell; Barry, 1994; Herman; Herman, 2014). This information shows that the active components of the essential oil may have been well absorbed by the animals' skin.

The animals accepted the applications well and apparently had no adverse effects, but Pharmacokinetic tests such as speed of absorption and distribution, half-life of active components,

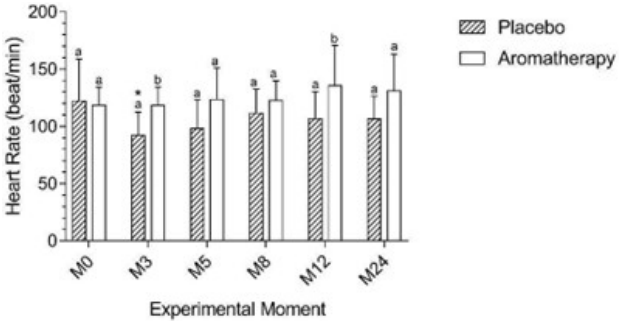


The same letters show that there was no significant difference between groups (P>0.05). * represents values different from M0. The maximum score on this scale is 12. The results are presented as medians and maximum and minimum values.

Figure 5. Sedation scores in female dogs subjected to OVH and treated with *C. verbenacea* essential oil diluted in carrier oils and gel (Aromatherapy group) or treated with the carrier mixture alone (Placebo group).

Table 2. Physiological parameters in female dogs undergoing elective ovariohysterectomy treated with sham aromatherapy [placebo (PLA)] or topical aromatherapy with Cordia verbenacea and biphasic carrier [Aromatherapy (AROMA)] immediately after surgery, before pre-anesthetic medication (M0) and 3 (M3), 5 (M5), 8 (M8), 12 (M12) and 24 (M24) hours after pre-anesthetic medication. Values are presented as mean ± standard deviation. Different letters show statistically significant differences between groups, where b>a.

Physiological Parameters	M0		M3		M5		M8		D12		D24	
	PLA	AROMA	PLA	AROMA	PLA	AROMA	PLA	AROMA	PLA	AROMA	PLA	AROMA
Heart Rate	122,2 ±36,5	118,6 ±15,9	92 ±20,5	118,3[b] ±16	98,2 ±24,9	123,4 ±27,8	111,5 ±21	122,8 ±17,1	106,8 ±23,8	135,6[b] ±35,1	106,3 ±20,1	131 ±32,1
Respiratory Frequency	35,5 ±15,8	31,1 ±9,1	18,2 ±5,7	21,2 ±3,8	21,1 ±3,3	22,4 ±6,4	24,3 ±6,4	21,8 ±4,2	30 ±8,9	24,8 ±8,4	30,8 ±12,1	23,9 ±5,8
Rectal Temperature	38,6 ±0,3	38,7 ±0,4	36,7 ±0,5	37 ±1	37,4 ±0,3	37,8 ±0,7	38 ±0,2	38,3 ±0,3	38,2 ±0,3	38,4 ±0,3	38 ±0,3	38 ±0,2



The letters represent differences between groups, b>a, while * represents different values with respect to M0. The results are presented as means and standard deviations.

Figure 6. Heart rate in female dogs subjected to OVH and treated with *C. verbenacea* essential oil diluted in carrier oils and gel (Aromatherapy group) or treated with the carrier mixture alone (Placebo group).

bioavailability and toxic effects must be developed. Using C. verbenacea extract in rats Sertié *et al.* (1988) and Sertié *et al.* (2005) observed low toxicity. Toxicological pre-clinical studies (with both rodents and non-rodents) revealed a very satisfactory safety profile for the essential oil from *C. verbenacea,* containing both α-humulene and trans-caryophyllene, and the subsequent phase I, II and III clinical trials confirmed the efficacy of this product as anti-inflammatory and analgesic when used topically (Dutra *et al.*, 2016).

Aromatherapy can promote analgesia and reduce the amount of allopathic medications administered postoperatively and, therefore, be a complementary therapy in the postoperative treatment of veterinary patients. In addition, promising analgesic results may encourage the pharmaceutical industry to develop new herbal products that can be used by many animals, as occurred in human medicine with the approval of the herbal product Acheflan® by the Brazilian regulatory agency ANVISA in 2005 (Dutra *et al.*, 2016).

In addition to the lack of information on the pharmacokinetics and pharmacodynamics of the components of *C. verbenacea* in dogs, other limitations of this study are the use of an empirical dose of essential oil, the use of only one dose of essential oil and the lack of measurement of other adverse effects of the essential oil.

CONCLUSIONS

Topical aromatherapy with *C. verbenacea* essential oil decreased pain scores and the amount of analgesic administered in the postoperative period of female dogs who underwent ovariohysterectomy.

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