

**Effectiveness of red propolis and *Mikania glomerata* in bitches's surgical analgesia**  
**Eficácia da própolis vermelha e *Mikania glomerata* na analgesia cirúrgica de cadelas**  
**Eficacia del propóleo rojo y *Mikania glomerata* en analgesia quirúrgica de perras**

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### **Abstract**

The aim of this research was to evaluate the analgesia provided by red propolis and *Mikania glomerata* oral administration in 40 female dogs submitted to ovariohysterectomy (OH) compared to standard treatment with non-steroidal anti-inflammatory ketoprofen. Through of a double-blind and randomized study, the animals were divided in four different groups with 10 animals of treatments: control treatment with ketoprofen (CT), propolis treatment (PT), *Mikania glomerata* treatment (MT), and propolis-*Mikania glomerata* treatment (PGT). All patients received one of these treatments two hours before OH and the pain evaluation was performed 1 (T1), 6 (T6), 12 (T12), and 24 hours (T24) after OH using University of Melbourne Pain Scale and the Glasgow Composite Measure Pain Scale. Glucose levels were also measured at the same times, except at 6 hours after OH (T6). All treatments achieved similar and satisfactory analgesia. During the experiment, only three animals were rescued, two belonging to the PT and another of the MT. Considering the lower rate of complications and no rescue necessity of in PGT and CT treatments, they were considered the most effective and safer. The combined treatment with propolis and *Mikania glomerata* could be a promising alternative method for OH surgery analgesia in bitches.

**Keywords:** Ovariohysterectomy; Ketoprofen; Pain; Natural products; Analgesia; Bitches; Anti-inflammatory alternative medicine.

### **Resumo**

O objetivo desta pesquisa foi avaliar a analgesia proporcionada pela própolis vermelha e pela administração oral de *Mikania glomerata* em 40 cadelas submetidas à ovariohisterectomia (OH) em comparação ao tratamento padrão com o anti-inflamatório não esteroide cetoprofeno. Por meio de um estudo duplo-cego e randomizado, os animais foram divididos em quatro grupos

com 10 animais de tratamentos diferentes: tratamento controle com cetofeno (CT), tratamento com própolis (PT), tratamento com Mikania glomerata (MT) e própolis-Mikania glomerata tratamento (PGT). Todos os pacientes receberam um desses tratamentos duas horas antes da OH e a avaliação da dor foi realizada 1 (T1), 6 (T6), 12 (T12) e 24 horas (T24) após a OH usando a Escala de Dor da Universidade de Melbourne e o Composto de Glasgow Medir a escala de dor. Os níveis de glicose também foram medidos nos mesmos momentos, exceto 6 horas após OH (T6). Todos os tratamentos obtiveram analgesia semelhante e satisfatória. Durante o experimento, apenas três animais foram resgatados, sendo dois do PT e outro do MT. Considerando o menor índice de complicações e a não necessidade de resgate nos tratamentos de PGT e TC, foram considerados os mais eficazes e seguros. O tratamento combinado com própolis e Mikania glomerata pode ser uma alternativa promissora para analgesia cirúrgica de OH em cadelas.

**Palavras-chave:** Ovariohisterectomia; Cetoprofeno; Dor; Produtos naturais; Analgesia; Cadelas; Medicina alternativa antiinflamatória.

### Resumen

El objetivo de esta investigación fue evaluar la analgesia proporcionada por el propóleo rojo y la administración oral de Mikania glomerata en 40 perras sometidas a ovariectomía (OH) en comparación con el tratamiento estándar con el antiinflamatorio no esteroideo ketoprofeno. A través de un estudio doble ciego y aleatorizado, los animales se dividieron en cuatro grupos con 10 animales de diferentes tratamientos: tratamiento control con ketófono (CT), tratamiento con propóleo (PT), tratamiento con Mikania glomerata (MT) y propóleo- Tratamiento con Mikania glomerata (PGT). Todos los pacientes recibieron uno de estos tratamientos dos horas antes de la OH y la evaluación del dolor se realizó 1 (T1), 6 (T6), 12 (T12) y 24 horas (T24) después de la OH utilizando la Escala de Dolor de la Universidad de Melbourne y el compuesto de Glasgow Miden la escala de dolor. Los niveles de glucosa también se midieron en los mismos momentos, excepto 6 horas después de la OH (T6). Todos los tratamientos obtuvieron una analgesia similar y satisfactoria. Durante el experimento, solo se rescataron tres animales, dos de PT y uno de MT. Considerando la menor tasa de complicaciones y la no necesidad de rescate en los tratamientos de PGT y TC, se consideraron los más efectivos y seguros. El tratamiento combinado con propóleo y Mikania glomerata puede ser una alternativa prometedora para la analgesia quirúrgica con OH en perras.

**Palabras clave:** Ovariectomía; Ketoprofeno; Dolor; Productos naturales; Analgesia; Perras; Medicina alternativa antiinflamatoria.

## 1. Introduction

In the last decade, the pain measurement and control in pets has been the subject of increasing concern and more researches due the improvement of pain pathophysiology scientific understanding and the relationship between pets and their owners. Ovariohysterectomy (OH) is among the most frequently performed and under-monitored postoperative pain, although it is presumed to cause moderate to severe abdominal pain (Slingsby *et al*, 2011; Morgaz *et al*, 2013).

As a result of it being impossible to quantify pain intensity in nonhuman animals due to their inability to rationalize and express their perceptions verbally, pain scales based on the analysis of physiological and behavioral parameters are used and extremely important in veterinary medicine (Zanuzzo, 2014). In dogs, the pain scale of the University of Melbourne (Muir and Gaynor, 2009) and the Glasgow composite measure pain scale (Murrell *et al*, 2008) are the most commonly used.

The efficacy of analgesic drugs is frequently investigated for use in the pet veterinary market, and many of non-steroidal anti-inflammatory drugs have been indicated for use in bitches submitted to OH, including ketoprofen, vedaprofen, meloxicam, and dipyron (Selmi *et al*, 2009; Morgaz *et al*, 2013; Zanuzzo *et al*, 2015). However, the use of natural products as analgesics is still relatively little explored. This is especially surprising considering that many potential natural products are of national and regional importance to Brazil, such as *Mikania glomerata* and red propolis from Alagoas, a protected and endemic species of this region designated recently by the National Institute of Industrial Property (INPI) (under registration number IG201101) (INPI – Brazil, 2012).

Ketoprofen is a non-steroidal anti-inflammatory drug with anti-inflammatory, antipyretic, and strong analgesic properties, which is known to be an effective treatment for pain due OH (Alves *et al*, 2001; Fantoni *et al*, 2015) that minimizes hyperglycemia and cortisol release in response to surgical pain in the 24-hour period containing and following OH surgery (Lacerda and Nunes, 2008).

The leaves of *Mikania glomerata*, popularly known as guaco, have been used for centuries in traditional and popular medicine for their tonic, bronchodilatory, anti-flu, antipyretic, and appetite-stimulating actions. According to Castro *et al*. (2006), they are extremely beneficial in the treatment of asthma, which suggests that guaco also has anti-inflammatory and antiallergic action, in addition to its known ability to block calcium channels (Czelusniak *et al*, 2012).

Propolis is widely used in Brazilian medicine and is marketed in many ways. Its main components are isoflavonoids and flavonoids (Bueno-Silva *et al*, 2015), the latter of which interact with different physiological processes, such as the action and absorption of vitamins, healing processes, antioxidant pathways, and antimicrobial and modulating functions of the immune system (). Additionally, Batista *et al*. (2015) verified an antinociceptive (i.e. pain-relieving) action similar to dipirone in mice.

The aim of this study was to evaluate the analgesic action of *Mikania glomerata* and red propolis from Alagoas (Brazil), in single and combined applications, in bitches submitted to OH. The analgesic effects of these compounds were compared with ketoprofen, using the University of Melbourne pain scale (UMPS) and the Glasgow composite measure pain scale (GCMPS) to assess pain levels in patients.

## 2. Materials and Methods

The experiment has a quali-quantitative nature and was approved by Animals Ethics Committee on the use of animals/Federal University of Alagoas (CEUA/UFAL), under approval number 039/2016. Forty healthy bitches were studied, with a mean  $\pm$  standard deviation (SD) age of  $2.77 \pm 1.38$  years old and mean body mass of  $13.15 \pm 4.88$  kg. All were subjected to clinical examinations to evaluate their heart rates (HR), respiratory rates (RF), rectal temperatures (T, in °C), detailed cardiac and pulmonary auscultations, mucosa, appetite disturbance, weight, and complete blood cell counts. All animals used in this study had normal values for the species of all of these measured parameters (Muir *et al*, 2013), and were thus considered healthy.

Adaptation of the animals to recovery conditions occurred over a minimum period of 72 hours, during which they were kept in individual cages 2 m<sup>2</sup> in floor area and fed with commercial dog food and provided with water *ad libitum*. Walks were conducted twice daily for 15 minutes each throughout the experiment. Complete blood cell counts and blood glucose tests were performed 24 hours prior to the experiment.

After blood sample through the cephalic vein, the animals were submitted to trichotomy in the surgical area. The experimental procedures were performed at the Laboratory of Innovation in Surgery, Hemotherapy, and Veterinary Cellular Therapies of the Federal University of Alagoas (LABINOVET- UFAL). After 12 and 4 hours of food and water fast, respectively, before surgery.

The bitches were divided in four different groups with 10 animals received one of four different types of analgesic medication two hours before surgery, which was administered orally in a double-blind and randomized way as follows: control treatment (CT): 2 mg/kg of ketoprofen in the form of drops, in a commercial presentation of 20 mg/mL; propolis treatment (PT): 50 mg/kg of red propolis from Alagoas, at a concentration of 20% in hydro-alcoholic extract; guaco treatment (GT): 50 mg/kg guaco, at a concentration of 20% in hydro-alcoholic extract; or propolis-guaco treatment (PGT): 25 mg/kg of red propolis from Alagoas + 25 mg/kg of guaco, at a concentration of 20% each in hydro-alcoholic extract. The doses of propolis and guaco were calculated by extrapolating those used in popular or traditional medicine and established as effective through experiments with mice (Batista *et al*, 2015), which were then prepared in the Laboratory of Natural Products of the School of Nursing and Pharmacy of the Federal University of Alagoas.

The anesthetic procedures used on all animals consisted of the subcutaneous administration of 0.2% acepromazine (0.1 mg/kg) as a preanesthetic medication, and then after 15 minutes the cephalic vein was accessed for the administration of lactated ringer's solution at a rate of 10 mL/kg/hour throughout the surgical procedure. Anesthetic induction was performed with intravenous midazolam (0.5 mg/kg) and ketamine (10 mg/kg) in the same syringe, seeking to mimic the protocol used in small clinics in Brazil. All procedures were performed by the same surgeon, using the same surgical technique. Throughout the surgical procedure, heart rate, respiratory rate, and rectal temperature were monitored. At the end of the procedure, all animals received a single dose of 30000 IU/kg benzathine penicillin intramuscularly.

Pain evaluation was performed 1 (T1), 6 (T6), 12 (T12), and 24 hours (T24) after OH using both the University of Melbourne Pain Scale (UMPS) (Muir and Gaynor, 2009) and the Glasgow Composite Measure Pain Scale (GCMPS) (Murrell *et al*, 2008). Glucose levels were also measured at the same times, except at 6 hours after OH (T6). After collection, blood samples for glucose measurement were processed within less than 20 minutes.

In the cases of pain positivity, analgesic rescues of bitches with tramadol were performed intramuscularly at the dose of 2 mg/kg, as this has been shown to be an effective analgesic for use after OH (Mastrocinque and Fantoni, 2003; Morgaz *et al*, 2013), in addition to oral administration of ketoprofen at a dose of 2 mg/kg for another 4 days. The animals were evaluated for 24 hours because this was the period over which ketoprofen has been previously shown to have effective analgesic action (Selmi *et al*, 2009); the ketoprofen-treated CT animals were thus used as the control group for pain evaluation.

Statistical analyses were performed using repeated measures ANOVAs in time and a significance level of 5% ( $p \leq 0.05$ ) of the clinical parameters used in the pain scales (heart rate, respiratory rate, and temperature) and glucose measurements, which were then followed by multiple comparisons using LSD test. The non-parametric Kruskal-wallis test was used for analyses of the pain scale scores. All statistical analyses were performed using SPSS® software 17.0. A minimum confidence level of 95% ( $p < 0.05$ ) was considered in all statistical analyses.

### 3. Results and Discussions

The surgical data collected shows that the mean  $\pm$  SD length of the incision was  $4 \pm 1.2$  cm, and the mean surgery time was  $20 \pm 4.62$  minutes, requiring no additional anesthetic doses in all experimental dogs.

In terms of the clinical parameters evaluated (heart rate, respiratory rate, and temperature) and glucose, there were no statistically significant differences among treatments. The most significant differences ( $p < 0.05$ ) were only found among times in all treatments (Tab. 1)

**Table 1.** Mean  $\pm$  standard deviation (SD) values of the heart rate, respiratory rate, temperature, and glucose variables calculated and compared between all treatments at different times in relation to OH, where T0 is 24 hours before, T1 is 60 min after, T6 is 6 hours after, T12 is 12 hours after, and T24 is 24 hours after surgery.

Vari- able	T0	T1	T6	T12	T24
HR	112. 85 $\pm$ 30.55 <sup>a</sup>	144. 25 $\pm$ 37.35 <sup>b</sup>	129. 3 $\pm$ 32.08 <sup>c</sup>	126. 15 $\pm$ 26.41 <sup>c</sup>	116. 15 $\pm$ 20.77 <sup>a</sup>
RR	30.5 5 $\pm$ 12.66 <sup>a</sup>	29.8 7 $\pm$ 9.14 <sup>a</sup>	25.0 2 $\pm$ 9.00 <sup>b</sup>	23.4 7 $\pm$ 5.66 <sup>b</sup>	26.0 2 $\pm$ 10.00 <sup>b</sup>
T°C	38.4 4 $\pm$ 0.52 <sup>a</sup>	36.6 1 $\pm$ 0.74 <sup>b</sup>	37.8 2 $\pm$ 0.44 <sup>c</sup>	38.0 8 $\pm$ 0.51 <sup>c</sup>	37.9 1 $\pm$ 1.61 <sup>c</sup>
Glucose	91.2 2 $\pm$ 12.03 <sup>a</sup>	115. 62 $\pm$ 38.44 <sup>b</sup>	----- -	105. 47 $\pm$ 24.26 <sup>c</sup>	101. 00 $\pm$ 22.54 <sup>c</sup>

Different letters in the same row indicate statistically significant differences ( $p < 0.05$ ) between times, as determined by repeated measures ANOVAs followed by multiple comparisons with LSD test.  
 Source: Research data.

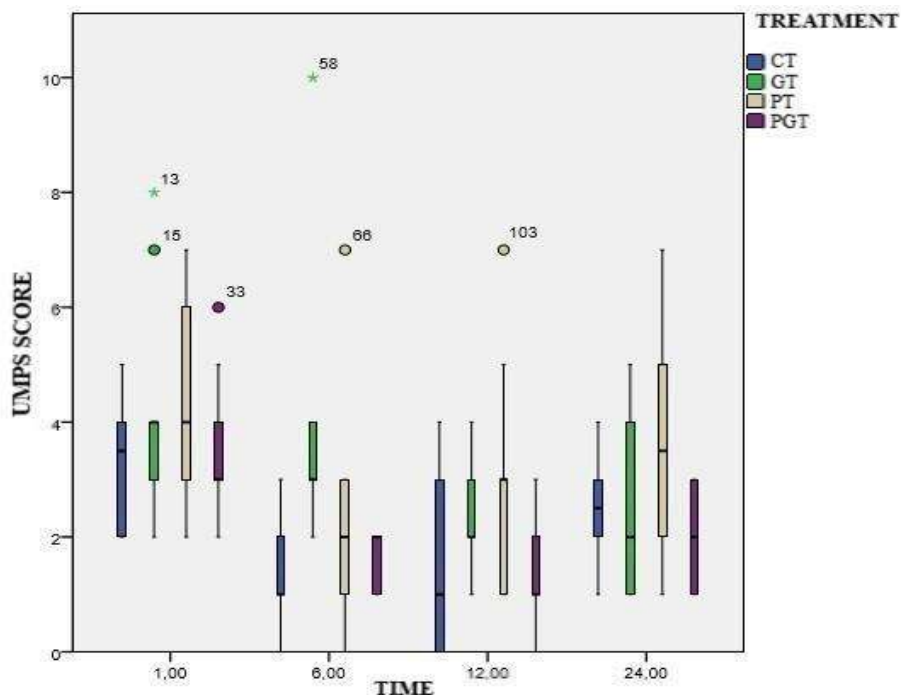
Table 1 shows the data before and after the surgery where was a visible increase in HR between T0 (where the animals had their normal parameters) and T1. Between T6 and T12, the

averages still differed significantly from baseline values, and baseline values were not restored until the T24 evaluation. It is also notable that regarding RR, it was noticed that there was no difference between T0 and T1, but there was a decrease at T6. There was a decrease in T°C by T1

At T0, glucose levels in all bitches were within the normal range for the species, which is from 77 mg/dL to 126 mg/dL (Muir *et al*, 2013). However, at T1 a significant increase was observed ( $p = 0.042$ ).

For pain scores evaluated using the UMPS, a intense decrease in the score in all 4 treatments between T1 and the remaining times (T6, T12, and T24) was observed (Figure 1).

**Figure 1.** Boxplot of the University of Melbourne Pain Scale (UMPS) scores assessed in each of the 4 treatments at different evaluation times.



Source: Research data.

Figure 1 contain blue boxes that indicate the control treatment (CT), green indicates the guaco treatment (GT), beige indicates the propolis treatment (PT), and purple indicates the propolis-guaco treatment (PGT). The lines inside the boxes shows the values of the medians, the upper whiskers shows the maximum values and the lower whiskers shows the lowest values punctuated in the pain scale. The symbols \* and ° represent extreme values (outliers).

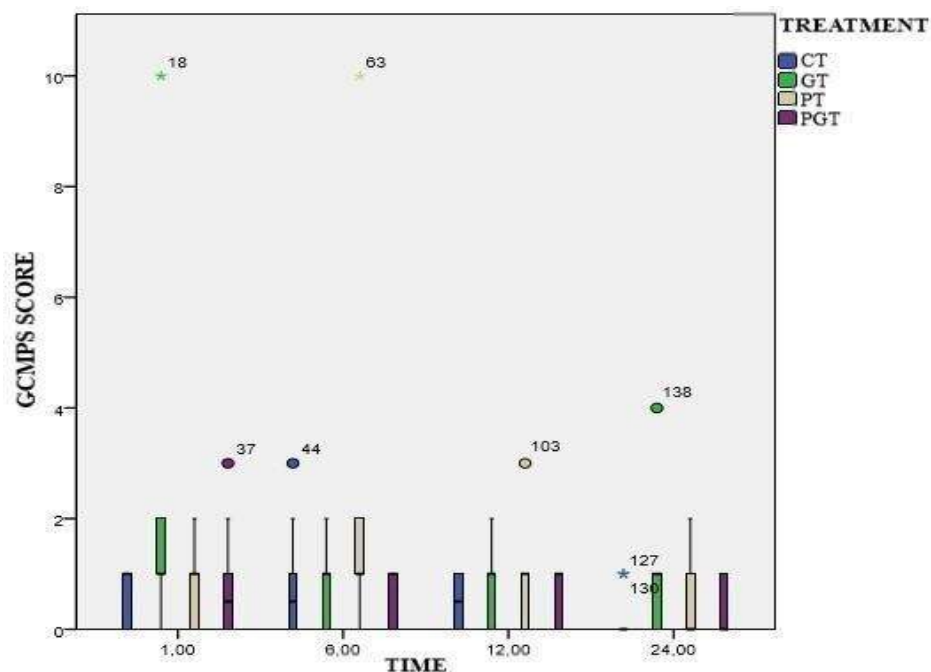


There was a significant difference in the magnitude of the decrease in the UMPS score at T6 between the GT and PGT ( $p = 0.001$ ). The decrease in the UMPS score at T6 was also significantly less for the GT than for either CT ( $p = 0.007$ ) or PT ( $p = 0.051$ ) in T6.

During the experiment, only three animals were rescued, two belonging to the PT and one to the GT. The UMPS scores for the two animals rescued in the PT were 7 and 8 at the moment of the rescue at T2, and for the animal rescued in the GT the score was 10 at T6.

Regarding the GCMPS, there were no significant differences in the pain scores recorded among any treatments or evaluation times based on this scale (Figure 2).

**Figure 2.** Boxplot of the Glasgow Composite Measure Pain Scale (GCMPS) scores assessed in each of the 4 treatments at different evaluation times.



Source: Research data.

Figure 2 shows blue boxes that indicate the control treatment (CT), green indicates the guaco treatment (GT), beige indicates the propolis treatment (PT), and purple indicates the propolis-guaco treatment (PGT). The lines inside the boxes shows the values of the medians, the upper whiskers shows the maximum values and the lower whiskers shows the lowest values punctuated in the pain scale. The symbols \* and ° represent extreme values (outliers).

In the GT, it was also worth noting that ten bitches presented nystagmus and tremors at T1, and six (60%) presented greater bleeding of the surgical wound than others.

All phases of the experiments were performed in a fluid and efficient way, without any complications in the pre-, trans-, and postoperative periods, except for the 3 rescues performed. In terms of the variables of the clinical parameters evaluated all treatments behaved in a similar way.

The increase in HR between T0 and T1 may have been associated with the effects of ketamine because according to Souza *et al.* (2002) it elevates heart rate and blood pressure as a result of stimulation of the central nervous system. The decrease in RR at T6 may be related to increased manipulation stress by this point. Acepromazine, on the other hand, can cause hypotension and hypothermia (Almeida *et al.*, 2000), which explains the T decrease observed at T1.

The glucose increase observed at T1 was associated with the manipulation of the animals and anaesthetic recovery, as corroborated by observations by Guimarães *et al.* (2007). According to Lacerda and Nunes (2008), a neuroendocrine and metabolic response to the painful stimulus during surgery begins immediately and lasts for 24 to 48 hours, which increases the release of cortisol, adrenocorticotrophic hormone, and glucagon, in addition to causing an inhibition of insulin secretion that may cause hyperglycemia.

The difference in magnitude in the UMPS score at T6 between GT & PGT, GT & CT and GT & PT indicated that better analgesia was achieved with PGT than in GT. The results also indicates that among the 4 treatments tested, the least effective at this time point was the GT one.

Although none of the 3 rescued animals scored 13, the minimum score that the literature defines are requiring rescue, in this study the surgical team decided to perform rescues if the UMPS score reached levels considered to represent moderate pain by Coutinho (2012), which were those between 6 and 17. Pohl *et al.* (2011) and Comassetto *et al.* (2017) correlated the UMPS with the Visual Analog Scale in bitches submitted to OH, and agreed that a UMPS score between 6 and 7 requires the administration of analgesic medication, which corroborates what was done in the present study. It should also be noted that the UMPS may have a somewhat subjective score in the period of anesthetic recovery, as seen at T1 (Figure 1), since the parameters evaluated are behavioral and physiological but such parameters can be altered by the use of anesthetics.

The GCMPS considers moderate pain to occur at scores between 6 and 24, but it assesses pain scores based only on behavioral evaluations (Coutinho, 2012); therefore, the analgesic recovery period may mask the actual pain signals from being detected by the observer. All mean values for the animals evaluated in this study were thus very low according to this scale.

However, we agree with this scale for the combined scores of two of the three rescued animals, one of which (in PT) had a score of 6 at T1 and another (in GT) of which had a score of 10 at T6 according to this scale; these rescued animals were thus in moderate pain based on the GCMPS as well as the UMPS.

The presence of nystagmus and tremors at T1 and greater bleeding of the surgical wound are a fact that can be explained by the presence of coumarins in guaco, which inhibit the formation of thromboxane in platelets (Czelusniak *et al*, 2012). In addition to these signs, the rescued GT animal presented: vocalization, reaction to palpation, and presence of edema in the surgical wound. It is noteworthy that the dose of guaco used was experimental, based only on the dose of propolis that was previously shown to be able to cause analgesia in mice (Batista *et al*, 2015). Such a dose of guaco appears to have caused adverse effects in the present study, or may even have interacted with another drug used during the anesthetic procedure or for antimicrobial prophylaxis. This demonstrated that further studies are needed to elucidate the function, drug interactions, and optimal dosing of this herbal medicine.

Comparing the analgesic action of the groups tested in relation to CT, and considering that ketoprofen decreases the action of prostaglandins and provides analgesic effects for a period of 24 hours, it was possible to affirm in the present study that PT and GT were effective analgesics in 80% and 90% of the bitches treated, respectively. However, PGT presented similar results to CT, with effectiveness in 100% of the bitches submitted to OH.

#### **4. Conclusion**

It was concluded that the four treatments had similarly satisfactory results in the postoperative analgesia of bitches submitted to OH. However, considering the number of complications and analgesic rescues that occurred when using GT, this was concluded to be the least Satisfactory treatment, while PGT and CT were both found to be more effective and safer.

To former researchers the treatment could be tested in another type of surgeries, using the Guaco and the Red propolis treatment with the same objectives as the pain control. The treatment could be tested in non-invasive clinical trials comparing the effectiveness of the red propolis and the guaco with another AINE's as the control group.

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