

**Influence of Brazilian red propolis polymeric nanoparticles in haematology, renal, and hepatic evaluations in dogs**

**Influência de nanopartículas poliméricas da própolis vermelha brasileira nas avaliações hematologia, renal e hepática de cães**

**Influencia de las nanopartículas poliméricas del propóleo rojo brasileño en las evaluaciones hematológicas, renales y hepáticas de perros**

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## **Abstract**

Recognized for various beneficial actions, propolis can be differentiated according to its origin. Red propolis, discovered in the northeast of Brazil, offers a strong antioxidant action. Nanoparticles are an innovative tool in the pharmaceutical field, as they help in efficient drug delivery. This study aimed to use red propolis nanoparticles in dogs and evaluate their action based on hematological and biochemical results. Eight healthy adult dogs (bodyweight, 6–27 kg) received 50 mg/animal of polymeric nanoparticles with 20% red propolis extract in a capsule, orally, once a day. Blood samples were collected weekly (five times), and hematologic, hepatic, and renal evaluations were performed. No significant changes were observed, except for alkaline phosphatase, which showed a significant reduction over time. This study in healthy adult dogs did not verify any hematologic, renal, or hepatic adverse effects of daily oral red propolis polymeric nanoparticles (capsules) administration. The results suggest a potential beneficial effect on the liver.

**Keywords:** Canine; Adverse effects; Blood; Kidney; Liver.

## **Resumo**

Reconhecida por diversas ações benéficas, a própolis pode ser diferenciada de acordo com sua origem. A própolis vermelha, descoberta no Nordeste do Brasil, oferece forte ação antioxidante. As nanopartículas são uma ferramenta inovadora na área farmacêutica, pois auxiliam na entrega eficiente de medicamentos. Este estudo teve como objetivo utilizar nanopartículas de própolis vermelha em cães e avaliar sua ação com base em resultados hematológicos e bioquímicos. Oito cães adultos saudáveis (peso corporal, 6–27 kg) receberam 50 mg / animal de nanopartículas poliméricas com extrato de própolis vermelha 20% em cápsula, por via oral, uma vez ao dia. Amostras de sangue foram coletadas semanalmente (cinco vezes) e avaliações hematológicas, hepáticas e renais foram realizadas. Não foram observadas alterações significativas, exceto para a fosfatase alcalina, que apresentou redução significativa ao longo do tempo. Este estudo em cães adultos saudáveis não verificou quaisquer efeitos adversos hematológicos, renais ou hepáticos da administração diária de nanopartículas poliméricas (cápsulas) de própolis vermelha oral. Os resultados sugerem um potencial efeito benéfico no fígado.

**Palavras-chave:** Canino; Efeitos adversos; Sangue; Rim; Fígado.

## Resumen

Reconocido por diversas acciones beneficiosas, el propóleo se puede diferenciar según su origen. El propóleo rojo, descubierto en el noreste de Brasil, ofrece una fuerte acción antioxidante. Las nanopartículas son una herramienta innovadora en el campo farmacéutico, ya que ayudan en la administración eficiente de medicamentos. Este estudio tuvo como objetivo utilizar nanopartículas de propóleo rojo en perros y evaluar su acción en base a resultados hematológicos y bioquímicos. Ocho perros adultos sanos (peso corporal, 6–27 kg) recibieron 50 mg / animal de nanopartículas poliméricas con extracto de propóleo rojo al 20% en una cápsula, por vía oral, una vez al día. Se recolectaron muestras de sangre semanalmente (cinco veces) y se realizaron evaluaciones hematológicas, hepáticas y renales. No se observaron cambios significativos, a excepción de la fosfatasa alcalina, que mostró una reducción significativa con el tiempo. Este estudio en perros adultos sanos no verificó ningún efecto adverso hematológico, renal o hepático de la administración oral diaria de nanopartículas poliméricas (cápsulas) de propóleo rojo. Los resultados sugieren un posible efecto beneficioso sobre el hígado.

**Palabras clave:** Canino; Efectos adversos; Sangre; Riñón; Hígado.

## 1. Introduction

The health benefits and biological activities of propolis have been known for a long time (Pasupuleti et al., 2017). In recent decades, different types of propolis have shown interesting pharmacological and chemical properties in studies aiming to treat or prevent illnesses (Frozza et al, 2012).

The Brazilian red propolis (BRP) has a unique composition and originates in the Alagoas state mangroves (do Nascimento et al., 2016). However, it can be found in beehives along the sea and river shores of five states of northeast Brazil. The characteristic red color of BRP is an atypical mix of dark yellow and brown tones, usually seen and derives from resinous exudates of *Dalbergia ecastophyllum* (L) Taub. (Leguminosae) (Daugsch et al., 2007).

Chemically, BRP contains pterocarpan, isoflavonoids, chalcones, prenylated benzophenones, and phenylpropanoids; however, new substances are being identified. Silva et al. (2008) identified the relative percentages of isoflavonoids 3-hydroxy-8,9-dimethoxypterocarpan and medicarpin in BRP. Moreover, they demonstrated that the *Dalbergia ecastophyllum* (L) Taub. (Leguminosae) resin exhibits important biological

properties, such as the ability to eliminate free radicals and inhibit tumor cell growth, constituting an excellent source of antioxidant and antitumor natural agents, in addition to antimicrobial, antifungal, and antiparasitic activities, including leishmanicidal activity (Frozza et al, 2012; do Nascimento et al., 2016; Li et al., 2017; de Silva, 2017; Silva et al., 2019)

In dogs, the beneficial effect of propolis was studied for different purposes. Clinical experiences were related to antimycotic, Cushing syndrome, ophthalmic, and paradental use; experimental studies in vitro or in vivo (rats or mice) in canine neoplastic cells, such as immunostimulants, bactericides, and antiparasitic (Betancourt et al., 2015).

The presentation form is significant in medicine absorption and effect. Drugs encapsulated in polymeric nanoparticle systems have protection against possible changes of external origin, resulting in biocompatibility, biodegradability, stability during storage, controlled release, and target delivery, resulting in higher therapeutic efficacy (do Nascimento, 2016). Studies have characterized and verified the BRP actions. However, biological assays are rare, and mainly in polymeric nanoparticle presentations, in which the mechanism of action can be potentiated.

Given the need for new therapeutic agents, pharmaceutical industries are looking for new therapeutic possibilities in plants and other natural products to address current health problems in humans and animals. Propolis is a natural medication with a promising future, but additional studies must assess its usefulness in veterinary medicine (Betancourt et al., 2015). Therefore, this study aimed to evaluate the effect of BRP polymeric nanoparticles in canine hematologic, renal, and hepatic systems and possible side effects of daily administration for 28 days.

## **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 061/2017.

Animals and treatment: A total of eight dogs, seven females and a male, mixed breed, age ranging between 2-14 years old, bodyweight ranging between 6-27 kg, were studied. All dogs were considered healthy according to clinical examination, blood count cells, and normal renal and liver biochemical parameters. Each dog was administered 50 mg of polymeric nanoparticles with 20% red propolis extract (NBRP) orally, once a day.

Blood samples (5 mL) were collected in EDTA vacutainers (2 mL), whole blood for

hematological parameters, and plain tube vacutainers (3 mL) for serum separation and biochemistry analysis. The collections were performed weekly, first at the beginning of NBRP treatment (T0), followed by four other collections (T1–T4), completing 28 days of treatment. Before blood collection, information regarding anamnesis (temperament, appetite, appearance of urine, feces, and presence of vomit) and physical examination (weight, temperature, hydration, lymph nodes, and mucosa) were documented to enable the evaluation of possible side effects of BRP administration.

NBRP preparation: The NBRP 20% extracts were prepared with a combination of poly-ε-caprolactone and pluronic using a nanoprecipitation method and characterized by different analytical and antioxidant techniques. NBRP is diluted in an aqueous medium presenting 200–280 nm in size and zeta potential analysis (-20 to -26 mV) revealing stability of the nanoparticles without aggregation occurrence for one month (do Nascimento et al., 2016).

Laboratory evaluations: Complete blood count was performed using a veterinary hematological analyzer (Mindray), whereas the globular volume and differential leukocyte count were performed using the microhematocrit technique and stained blood smear, respectively. Commercial diagnostic kits (Labtest) were used to determine the renal function (urea, creatinine) and liver function (alanine transaminase -ALT, alkaline phosphatase-ALP) performed by a semi-automatic analyzer (Spectrum-Quimis).

Statistical analysis: The Kolmogorov-Smirnov test was performed to test the normality of the distribution and a linear correlation was analyzed using the Pearson test.

### **3. Results**

During the experiment, there were no observations of physiological or behavioral changes, such as feed intake, water consumption, and body condition, reported on anamnesis during the weekly clinical evaluation at the time of blood collection.

The hematological parameters remained within the reference ranges for the species. The mean values did not differ statistically between the time points (Table 1), except for red blood cells, which showed a statistical difference between T0 and T2 ( $p < 0.0367$ ). However, despite the smaller number of red blood cells, the hemoglobin concentration was higher than others, indicating that a hemolytic process may have occurred, probably during transport or

handling of the sample. The absence of a significant difference in hematocrit and hemoglobin concentrations indicates normality in the red series at that time. The total leukocyte and differential count values did not differ significantly (Table 1) compared to the reference values during the four weeks of experiment. Plasmatic protein concentrations were slightly elevated at all times but without statistical differences between them.

Creatinine, urea, and ALT concentrations remained within normal standards in all animals, and no difference was observed in the statistical comparison between mean and time (Table 2). ALP could not be evaluated at T0, and a significant difference was noticed in the T1 and T3 comparison ( $p < 0.01$ ), and a decrease in this enzyme was observed (Table 2).

**Table 1.** Mean values of haematological parameters, platelet count and total plasma protein of dogs that received NBRP for 4 weeks.

	T0		T1		T2		T3		T4	
	X	±SD	X	±SD	X	±SD	X	±SD	X	±SD
<b>RBC (x10<sup>6</sup>)</b>	7.76 <sup>a</sup>	1.01	7.03	1.19	6.02 <sup>a</sup>	1.87	7.53	2.77	7.25	1.01
<b>Hg (g/dL)</b>	18.64	2.43	17.73	2.72	18.89	2.15	18.11	2.61	17.03	2.33
<b>HcT (%)</b>	53.30	4.90	56.94	10.14	55.23	6.67	54.39	7.86	51.08	6.90
<b>WBC (x10<sup>3</sup>)</b>	12.37	4.28	10.47	4.98	7.95	4.11	12.27	12.62	10.59	3.07
<b>Neu (x10<sup>3</sup>)</b>	9.66	2.47	4.31	2.10	4.89	3.31	7.63	10.53	5.84	2.30
<b>Lym (x10<sup>3</sup>)</b>	3.64	1.48	2.95	0.68	1.94	0.46	2.71	1.22	2.74	0.34
<b>Eos (x10<sup>3</sup>)</b>	0.32	4.44	1.15	1.54	0.88	6.77	1.57	2.08	1.36	0.97
<b>Bas (x10<sup>3</sup>)</b>	0	0	0	0	0	0	0	0	0.25	0.82
<b>Mon (x10<sup>3</sup>)</b>	0.4	0.32	1.97	1.18	0.45	0.34	0.49	0.44	0.60	0.64
<b>PPT</b>	8.20	1.37	9.18	1.06	8.84	0.94	8.73	0.85	8.73	0.97

X Mean; ±SD standard deviation; RBC: Red blood cells; Hg: Hemoglobin; HcT: hematocrit; WBC: white blood cells. Neu: Neutrophils; Lym: Lymphocyte; Eosi: Eosinophils; Bas: Basophils; Mon: Monocytes, PP: plasmatic protein. Source: Research data.

**Table 2.** Average values of serum concentrations of creatine, urea, alanine aminotransferase (ALT) and alkaline phosphatase (FA) obtained at different times.

	T0		T1		T2		T3		T4	
	X	±SD	X	±SD	X	±SD	X	±SD	X	±SD
<b>Creat</b>	1.17	0.64	1.01	0.46	0.91	0.15	1.01	0.39	0.87	0.42
<b>Urea</b>	20.36	13.04	22.33	10.62	22.09	11.08	31.86	26.43	28.69	27.61
<b>ALT</b>	18.06	1.60	18.27	5.20	18.62	3.97	23.26	8.08	20.43	4.61
<b>ALP</b>	-		277.08 <sup>a</sup>	77.60	184.00	83.73	133.36 <sup>a</sup>	73.28	148.24	108.44

Creat: creatinine (mg/dL); urea (mg/dL); ALT: alanine aminotransferase; ALP: (U/dL); phosphatase alkaline (U/dL) <sup>a</sup>= p<0,01. Source: Research data.

#### 4. Discussion

There are few clinical studies on the oral administration of propolis in dogs and none on red propolis. The absence of statistical differences in hematological and biochemical parameters observed with NBRP was in accordance with other studies using oral administration of regular propolis extract during 20 weeks weeks (Riviera et al., 2017) or propolis 5% for eight weeks in beagle dogs (Kang, 2019), which concluded that oral consumption of propolis did not cause any toxicological effects.

In rats, acute and subchronic toxicological tests with high doses of propolis also did not demonstrate hematological or renal changes (Araujo, 2010). However, red propolis resulted in higher toxicity than other propolis, indicating interference in the biological response. The results demonstrated that the LD50 of red propolis was higher than 300 mg/kg in rats (da Silva et al, 2015). In dogs, the LD50 is not yet known and there are substantial differences in presentation forms and doses administered in studies investigating propolis supplementation. Kang (2019) studied a high concentration and offered 40 g of 5% propolis per dog. Riviera et al. (2017) utilized 3.97 mg/kg of dog food, based on the total flavonoid content in apigenin. Both studies incorporated regular propolis in the diet. In our study, the dogs received 1.85-8.33 mg/kg bodyweight of NBRP in capsules to promote constant absorption.

Although oral propolis offered in the dogs' diet facilitates the administration in high volumes, the amount ingested may vary according to the animal's appetite. In the case of

illness, the patient may be anorectic or dysorexic, and administration of capsules or syrups may allow greater control of consumption and treatment success. Meanwhile, higher doses would imply a greater number of capsules or frequency per day, increasing the difficulty of treatment in animals.

Serum ALP is used primarily as a biochemical marker of hepatic disease and bone activity, such as bone growth or osteosarcoma (Ochi et al, 2013). In this study, ALP reduction was observed over time, a significant difference in T3, which may indicate a beneficial action for the liver, as reported in a study that verified a propolis protective role in the tendency of diazinon to cause hepatic affection in rats (Mahmoud, Shalaby, 2018) and the hepatoprotective effect of red propolis by the induction of chronic experimental hepatic lesions in rodents (Silva et al.,2019). However, these results should be studied further because ALP is known to distribute in various tissues, including the liver, bone, intestine, kidneys, mammary glands, and placenta (Ochi et al, 2013).

The population use of natural products has shown a remarkable increase. Consequently, natural products have garnered greater interest from industries and research institutes. Thus, it is necessary for the toxicological screening of species<sup>10</sup>. This study's main objective was to verify possible signs of toxicity in dogs who were offered nanoparticles of red propolis. Despite the satisfactory results, it is of utmost importance to seek more knowledge in the area. Although it is a natural product, propolis has a vast chemical composition, and any component, when used as a drug therapy, may potentially cause adverse reactions<sup>2</sup>.

In vitro tests do not reflect the real conditions of the disease; therefore, it is important to establish the conditions and justifications for in vivo studies (Betancourt et al., 2015). Clinical trials can be applied to planned experiments involving patients and designed to elucidate the treatment most appropriate for future patients (Escosteguy, 1999). Knowledge of the hematological and serum biochemical effects of propolis in healthy animals will enable understanding in sick patients.

In small animal species, propolis can be used to address various conditions and is beginning to play an important role in medical treatment because it appears to be an effective treatment with no side effects and at a low cost (Betancourt et al., 2015).

## **5. Conclusion**

Red propolis nanoparticles in capsules administered orally to dogs did not cause



adverse effects in hematologic, renal, or hepatic laboratory parameters, or any physical or behavioral changes during the 28 days of treatment. The results suggest a potential beneficial effect on the liver.

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