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EFFECT OF SYSTEMIC ADMINISTRATION OF PHYTOSTEROL ON LACRIMAL QUALITY OF DOGS

EFEITO DA ADMINISTRAÇÃO SISTÊMICA DE FITOESTEROL NA QUALIDADE LACRIMAL DE CÃES

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ABSTRACT: The tear lipid layer (oily outer layer) reduces evaporation and prevents tear overflow. In dogs, reductions in the lipid components of this layer (cholesterol, triglycerides and phospholipids) can cause eye serious diseases. In this way, the tear crystallization test analyzes the lacrimal quality, however, it is less used in veterinary. As phytosterol reduces blood cholesterol, the objective of this study was to investigate, through the tear crystallization test, whether the systemic administration of this drug influences the lacrimal quality of healthy dogs and, in addition, to verify differences in the interpretation of the ophthalmic test between different evaluators. Eight beagles, healthy, of both sexes, young and adults, without clinical ophthalmic signs apparent were selected. Basal lacrimal samples (D0) were collected from the right and left eye of all animals with glass capillary tube and arranged on a glass slide for scanning the images and subsequent microscopic analysis. Subsequently, all were medicated with the phytosterol (Collestra® 650 mg: 1 capsule, orally, every 12 hours, for 15 days). After seven (D7) and fifteen (D15) days of this systemic administration, the tear crystallization test in both eyes of all dogs was again performed for statistical comparison with the baseline results. The photographs of the slides were classified by four evaluators (AV1 and AV2 with professional experience in ophthalmology and AV3 and AV4 without previous professional experience in ophthalmology), following standards established by Rolando (1984). The results were statistically verified by analysis of simple variance (ANOVA One-Way). There was no statistical difference in the tear crystallization test between the established periods and in relation to the different ophthalmic test evaluators ($p \leq 0.05$). Although phytosterols reduce blood cholesterol levels, it was observed in the present study that these drugs when administered systemically did not interfere in the tear lipid layer and, consequently, in the lacrimal quality of healthy dogs, and may be prescribed as lipid-lowering agents for patients with ocular diseases, especially the lacrimal ones.

KEYWORDS: Lipids. Veterinary ophthalmology. Lacrimal quality. Tear crystallization test.

INTRODUCTION

The lipid layer of the tear is composed of cholesterol, triglycerides and phospholipids (TORRICELLI; WILSON, 2014; HSU et al., 2015) which decrease evaporation, increase break time and prevent tear leakage (BRON et al., 2004; DAVIDSON; KUONEN, 2004). Thus, the lipid reduction at this layer contributes to the ocular disorders (OFRI et al., 2007).

In this sense, the test of tear crystallization (fern test) is based on the formation of crystals, classified microscopically in patterns, according to the presence and amount of branches in the shape of fern leaves (MURUBE, 2004; FELDBERG et al., 2008). In the pattern I, the crystallization originates multi-branched trees, with no empty spaces between the branches, characterizing better lacrimal quality when compared to the other standards. In pattern II, the branches are shorter and less arbours, with more spaces between them. In pattern III, the spaces between the branches are large and rare and the pattern IV is characterized by clumps of crystals that seldom form branches (ROLANDO, 1984).

Phytosterols are biologically active chemical groups found in vegetable oils, being the grains, vegetables and seeds the main sources (MARTINS et al., 2004). Among the phytosterols, the campesterol, stigmasterol and β-sitosterol are well knowed (PIIRONEN et al., 2000; BRUFAU; CANELA; RAFECAS, 2008). The use of phytosterols isolated or associated with lipid-lowering drugs reduces systemic LDL-cholesterol and, consequently, cardiovascular and endocrine

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diseases (EUSSEN et al., 2010; VENERO et al., 2010; RUDERMAN et al., 2013).

As phytosterol reduces blood cholesterol, the objective of this study was to evaluate, through the tear crystallization test, whether the systemic administration of this drug influences the lacrimal quality of healthy dogs and, in addition, to verify differences in the interpretation of the ophthalmic test between different evaluators.

MATERIAL AND METHODS

The study protocol was approved by the Animal Care and Use Committee of the University of Franca (Approval n° 4594210616).

Eight Beagle dogs (*Canis familiaris*), healthy, young and adult, male and female, from the Experimental Kennel of the University of Franca were used. As inclusion criteria in this study, animals with no apparent ophthalmic signs were selected. The animals received water and commercial feed *ad libitum* during the experiment.

After dogs mechanical restraint, tear was collected from both eyes in the conjunctival sac with a capillary glass tube for hematocrit (Biocap AS®, Argentina - Buenos Aires), in order to perform the tear crystallization test, according to Farias et al. (2013). The aliquots were then placed on glass microscope slides (Bioglass Ltda, Taubaté - SP), in the center of a circle, previously identified with a hydrographic pen (Faber-Castell®, São Carlos - SP) to facilitate the localization of the tear sample during microscopic examination. These steps were performed by the same professional and in the same conditions, without the prior use of ocular and systemic anesthesia (MURUBE, 2004).

Shortly after drying the slides at room temperature (approximately 10 minutes), they were placed under a light microscope (Leica Microsystems® DMLB, Wetzlar - Alemanha) and digitized with a specific camera attached to the microscope (Leica Microsystems® DFC 295, Wetzlar - Alemanha). The photographs were classified by four different evaluators (AV1 and AV2 - with previous professional experience in ophthalmology, AV3 and AV4 - without previous professional experience in ophthalmology), as well as the basal (D0) lacrimal crystallization standards, following the criteria proposed in medicine by Roland (1984).

Then, all dogs were medicated with the Collestra phytosterol (Collestra®, Aché Laboratórios Farmacêuticos, Guarulhos - SP), independent of weight: 1 capsule of 650 mg, orally, every 12 hours, with the usual meals, as recommended by the package insert, for 15 consecutive days. This phytosterol is extracted from soybean (60-70%), sunflower (5-10%), corn (1-5%) and canola (20-30%).

After 7 (D7) and 15 (D15) days of systemic administration of phytosterol, tear fractions were collected again from both eyes of all dogs to perform the tear crystallization test, following the previous criteria (Figure 1), aiming the statistical comparison of the four different evaluators with the baseline results (D0).

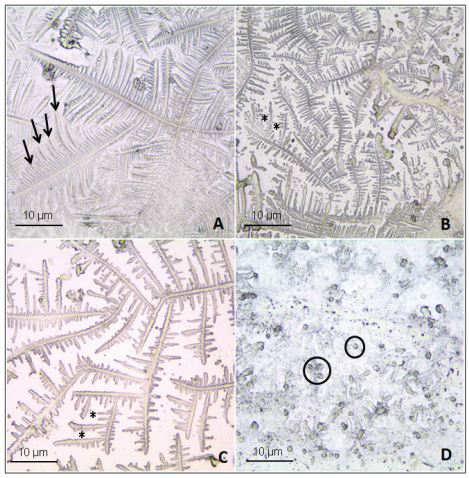

Figure 1. Photomicrography of the dog tear (10x), showing the patterns of the lacrimal cristalization test, according to Rolando (1984). In A: pattern I (multi-branched trees (arrow), with no empty spaces between the branches); B: pattern II (Short branches and less arbours, with spaces (*) between them; C: pattern III (large and rare spaces spaces between the branches and D: pattern IV (clumps of crystals (circles) that seldom form branches).

The results of the tear crystallization test, for the different moments of analysis (D0, D7 and D15) and evaluators (AV1, AV2, AV3 and AV4), were evaluated by single-variance analysis (ANOVA One-Way) with calculation of the F and its respective "P-value". In cases where $p \leq 0.05$, the means were compared by Tukey test, with the calculation of the minimum significant difference to $\alpha = 0.05$, using the Graphpad Prism Software 6.0® Program.

RESULTS

It was observed no statistical difference in the tear crystallization test for the different moments of analysis (D0, D7 and D15) (Figure 2).

Similar results were found in relation to the different evaluators (AV1, AV2, AV3 and AV4) of the tear crystallization test, independant of the degree of professional experience (Figure 3).

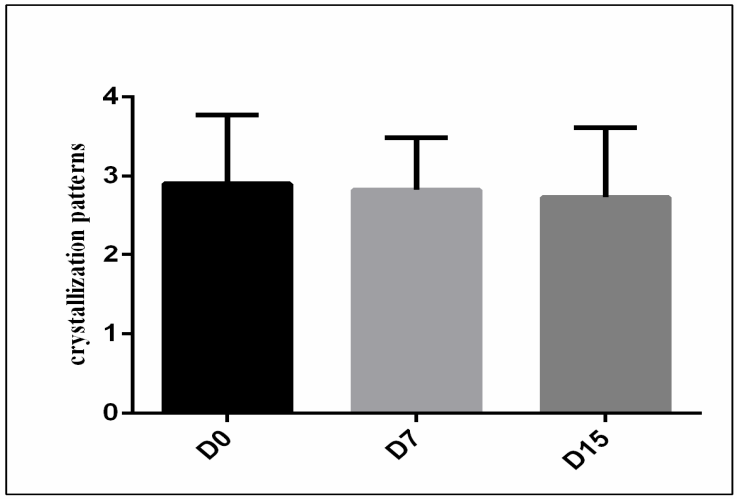

Figure 2. Graphic representation of tear crystallization patterns of Beagles dogs, before and after 7 and 15 days of phytosterol administration. D0 (basal) - without phytosterol systemic treatment. D7 - 7 days after phytosterol systemic treatment; D15 - 15 days phytosterol systemic treatment. No statistical difference between the different days of evaluation (P<0,05).

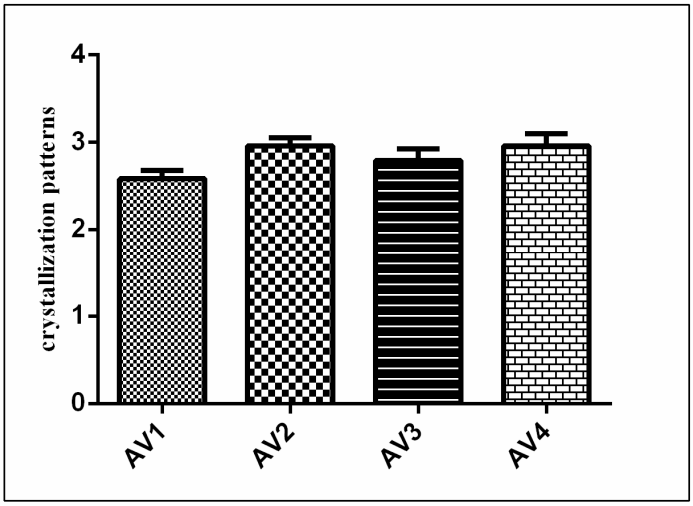

Figure 3. Graphic representation of tear crystallization patterns of Beagles dogs (performed by four different evaluators: AV1, AV2, AV3 e AV4), before and after the phytosterol administration. AV1 - First evaluator (experience in ophthalmology); AV2 - Second evaluator (experience in ophthalmology); AV3 - Third evaluator (without experience in ophthalmology); AV4 - Fourth evaluator (without experience in ophthalmology). No statistical difference between the different days of evaluation (P<0,05).

DISCUSSION

According to Masmali, Murphy and Purslow (2014), the chemical analysis of the components of the tear film is hard due to the small volume of the samples and the transparency of the tear. In the present study, lacrimal samples were collected in the conjunctival sac of each eye using a glass capillary tube, following the recommendations of Farias et al. (2013) and Willians and Hewitt (2017). According to Feldberg et al. (2008) and Silva et al. (2015), there are no descriptions in the scientific literature related to accidents or other complications during this collection technique in various animal species, making it feasible and reliable in veterinary practice (WILLIAMS; HEWITT, 2017).

The sampling condition was standardized, with doors and windows closed, so the tear crystallization did not suffer any changes due to oscillations in the air (MURUBE, 2004; FELDBERG et al., 2008).

Aiming to record the crystallization of the tear for subsequent analysis, chosed to photograph the glass slides after drying, since according to Norn (1987), the lacrimal aliquot is rapidly lost (about one hour after the blade is made). In this context, Murube (2004) discussed the need to stain the slides in eosin-hematoxylin prior to microscopic analysis; on the other hand, other researchers (FARIAS et al., 2013; SILVA et al., 2015) did not indicate this procedure because it did not compromise the reliability of the results.

As described by Murube (2004) and Feldberg et al. (2008), the tear when it dries, demonstrates crystallization of some of its components, which assume varied aspects, similar to the fern leaves. In this context, the standards established by Rolando (1984) are based on the presence and exuberance of these leaves. As the tear crystallization test involves a certain subjectivity, the present study was intended to verify, independently of the previous experience of each examiner, the ability to similarly classify the degrees of lacrimal crystallization in dogs, similar to that proposed by Rolando (1984) and Feldberg et al. (2008) in humans, because in the veterinary these standards are not well defined.

Thus, the data obtained in this research demonstrated that it is possible the reproducibility of human standards for dogs, allowing the diagnosis and early treatment of lacrimal ophthalmic disorders in this species, which can provide better quality of life and longer survival to the affected individuals.

In the current study, the results regarding the fern test did not differ among the evaluators with different professional experiences in the area, even though it was considered subjective (FELDBERG et al., 2008). Thus, this qualitative ophthalmic test can be routinely used in Veterinary Medicine as a complementary ophthalmic examination, mainly due to its low cost, fast and easy execution and interpretation, thus avoiding the chronicity of ocular symptoms (NORN, 1987; WILLIAMS; HEWITT, 2017). Similar results were found in the study by Feldberg et al. (2008), who verified the reproducibility of the classification of tear crystallization test patterns using five different examiners and compared these patterns among human patients with Sjögren's syndrome with those of individuals without ocular surface diseases.

In the experiment by Willians and Hewitt (2017), dogs with dry keratoconjunctivitis demonstrated abnormalities in tear crystallization patterns and the researchers attributed these findings to changes in the ratio of electrolytes and lacrimal macromolecules. In humans, Tabbara and Okumoto (1982) have demonstrated that tear crystallization is impaired in patients with lacrimal layer deficiency. In this theme, the objective was to verify if the phytosterols would influence the lacrimal quality of healthy dogs.

Phytosterols contribute to blood cholesterol lowering (MARTINS et al., 2004; BRUFAU; CANELA; RAFECAS, 2008; RUDERMAN et al., 2013); on the other hand, the results found in the present study demonstrated that the systemic administration of Collestra®, in the admitted periods, did not interfere in the tear lipid layer and, consequently, in the lacrimal quality of healthy dogs. Thus, such a natural drug can be prescribed for lipid-lowering (EUSSEN et al., 2010; VENERO et al., 2010) in dogs with ophthalmic conditions, especially lacrimal ones.

CONCLUSION

The systemic administration of phytosterol did not interfere in the tear quality of healthy dogs. In addition, independent of experience in the field of Veterinary Ophthalmology, there was no statistical difference between the different testers of the tear crystallization test.

RESUMO: A camada lipídica lacrimal (camada externa oleosa) reduz a evaporação e previne o transbordamento lacrimal. Em cães, reduções nos componentes lipídicos desta camada (colesterol, triglicérides e fosfolipídios) podem causar doenças graves nos olhos. Desta forma, o teste de cristalização lacrimal analisa a qualidade lacrimal, no entanto, é menos utilizado em veterinária. Como o fitoesterol reduz o colesterol sanguíneo, o objetivo deste estudo foi investigar, através do teste de cristalização lacrimal, se a administração sistêmica deste fármaco influencia na qualidade lacrimal de cães hígidos e, além disso, verificar diferenças na interpretação do teste oftalmológico entre diferentes avaliadores. Oito beagles, saudáveis, de ambos os sexos, jovens e adultos, sem sinais oftalmológicos clínicos aparentes foram selecionados. Amostras lacrimais basais (D0) foram coletadas do olho direito e esquerdo de todos os animais, com tubo capilar de vidro e, dispostas em lâmina de vidro para escaneamento das imagens e posterior análise microscópica. Ato contínuo todos foram medicados com o fitoesterol (Collestra® 650 mg: 1 cápsula, por via oral, a cada 12 horas, durante 15 dias). Após sete (D7) e quinze (D15) dias desta administração sistêmica, o teste de cristalização lacrimal em ambos os olhos de todos os cães foi novamente realizado para comparação estatística com os resultados basais. As fotografias das lâminas foram classificadas por quatro avaliadores (AV1 e AV2 com experiência profissional em oftalmologia e AV3 e AV4 sem experiência profissional prévia em oftalmologia), seguindo padrões estabelecidos por Rolando (1984). Os resultados foram estatisticamente verificados pela análise de variância simples (ANOVA One-Way). Não houve diferença estatística no teste de cristalização lacrimal entre os períodos estabelecidos e em relação aos diferentes avaliadores de teste oftalmológico ($p \leq 0,05$). Embora os fitoesteróis reduzam os níveis de colesterol no sangue, observou-se no presente estudo que esses fármacos quando administrados sistemicamente não interferiram na camada lipídica da lágrima e, consequentemente, na qualidade lacrimal de cães hígidos, podendo ser prescritos como agentes hipolipemiantes para pacientes com doenças oculares, especialmente as lacrimais.

PALAVRAS-CHAVE: Lipídios. Oftalmologia veterinária. Qualidade lacrimal. Teste da cristalização lacrimal.

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