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Effects of the *Ottonia martiana* Miq. (Piperaceae) extract on dog's ocular surface

[*Efeitos do extrato de* Ottonia martiana *Miq. (Piperaceae) sobre a superfície ocular de cães*]

C.B.S. *Lisbão*[1], M.A. *Corrêa*[2], J.P.D. *Ortiz*[1], B.C. *Martins*[1], J.L. *Laus*[1*]

[1]Faculdade de Ciências Agrárias e Veterinárias - UNESP - Jaboticabal, SP
[2]Universidade Braz Cubas - Mogi das Cruzes, SP

ABSTRACT

The anesthetics effects of aqueous extract of *Ottonia martiana* leaves were studied on the ocular surface of healthy beagle dogs. The dogs were divided in three groups (n=15): control group (CG), proxymetacaine group (PG) and Ottonia group (OG), which were treated with 0.9% saline, 0.5% proxymetacaine hydrochloride ophthalmic solution and *O. martiana* extract respectively. An oftalmic evaluation was performed before the treatments. Eye drops were instilled at time 0 (T0) and 3 minutes later (T3). Axial corneal sensitivity was evaluated by esthesiometry 5 and 10 minutes after T0. Tear production and intraocular pressure were evaluated 10 minutes after T0. Slit lamp biomicroscopy was performed 10 and 20 minutes after T0 and the eyes were stained with fluorescein 20 minutes after T0. The STT was reduced in PG. Conjunctival hyperemia was observed in 13 animals from PG and constituted the only ocular alteration observed during the study. Esthesiometry revealed a decreased corneal sensitivity for PG and OG. Those results show that the *O. martiana* extract acts reducing corneal sensitivity in dogs. Moreover, its use does not decrease the tear production and does not cause any clinical ophthalmic alteration.

Keyword: dog, *Ottonia martiana*, Piperaceae, corneal sensitivity, tear production

RESUMO

Estudaram-se os efeitos do extrato das folhas de Ottonia martiana sobre a superfície *ocular de cães hígidos da raça Beagle. Compuseram-se três grupos de tratamento (n=15): grupo controle (GC), grupo proximetacaína (GP) e grupo Ottonia (GO), tratados, respectivamente, com solução fisiológica, colírio de cloridrato de proximetacaína a 0,5% e extrato de* O. martiana. *Após avaliação oftálmica inicial, os tratamentos foram realizados no tempo 0 (T0) e decorridos 3min (T3). Avaliaram-se a sensibilidade axial da córnea por estesiometria (T5 e T10) e a produção lacrimal e a pressão ocular (T10). Realizaram-se a biomicroscopia com lâmpada em fenda (T10 e T20), e o teste do tingimento pela fluoresceína (T20). Relativamente ao teste de Schirmer, observou-se diminuição nos cães do GP. Houve alteração clínica somente nos do GP, em que 13 animais apresentaram hiperemia conjuntival. Relativamente à estesiometria, houve diminuição da sensibilidade corneal nos animais do GP e do GO. Admite-se que o extrato de* O. martiana *age diminuindo a sensibilidade corneal em cães e que sua utilização não diminui a produção lacrimal, tampouco causa alterações clínicas oftálmicas.*

Palavras-chave: cão, Ottonia martiana, *Piperaceae, sensibilidade corneal, produção lacrimal*

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*Autor para correspondência (*corresponding author*)
E-mail: jllaus@fcav.unesp.br

INTRODUCTION

The Piperaceae family can be found from north to south in Brazil and its species have been used for medical purposes since ancient ages. Therefore, it is an exhilarating source for phytochemistry and pharmacology research (Marques *et al*., 2008). There are about 1400 different species distributed around all tropical areas in the planet. In Brazil, there are about 460 species divided in 5 genus: *Pothomorphe* Miq., *Sarcorhachis* Trel., *Peperomia* Ruiz and Pav., *Piper* L. and *Ottonia* Spreng (Barroso *et al*., 2002). The genera *Ottonia* and *Piper* are among those used in the "Brazilian popular medicine" (Pessini *et al*., 2003).

The *Ottonia* genus is known for its diversity of bioactive secondary metabolites, including the amides, which are of great medicinal interest (Antunes *et al*., 2001). Phytochemistry studies show that its species are mainly constituted by the amide piperovatine (Cunico *et al*., 2005), which acts as sialagogue, piscicide and local anesthetic (McFerren and Rodriguez, 1998). The piperovatine mechanism of action as local anesthetics has not been totally elucidated but it has been shown that it increases significantly the neural intracellular calcium and acts as a sodium channel agonist. This characteristic may explain its use for fishing by the Indians, since fish are hypersensitive to the piperovatine, as with pyrethroids and DDT. Despite the similar action, the piperovatine is a sodium channel agonist while the other local anesthetics block those channels (McFerren *et al*., 2002).

The *Ottonia martiana* species is an herbal bush with ribbed branches, a few short petiolated and oval-elliptical shaped leaves, spike flowers and drupaceous oblong to oval fruit (Figure 1). It can be found throughout the Atlantic Forest and has many popular names as "Jaguarandi" and "Taburutá" in Santa Catarina State (Guimarães *et al*., 1978) and "anestésica" in the shores of Paraná State, where the population use its root or leaves for the treatment of odontalgia by chewing or mouth washing (Lopes, 1989; Cunico *et al*., 2003).

Among the members of the Beneficent Spiritist Center União do Vegetal (UDV – www.udv.org.br), the use of medicinal plants is a popular knowledge, registered by its founder, Mr. José Gabriel da Costa (Master Gabriel) who worked as a rubber tapper in the Amazon region in the 60's and has the record of knowledge of various plants. Among these species, many species of the *Ottonia* and *Piper* genus, including *Ottonia martiana*, are known as "João brandim" or "João brandinho" and are used as antipyretic, anti-migraines, local anesthetics, muscle relaxing and as eyedrops for conjunctivitis and ocular irritation. The plant can be prepared by extraction in water or maceration in cereal alcohol (personal communication, Corrêa, 2009, Universidade Braz Cubas).

Studies with the ethanolic gross extract of total organs (EBEtOH) of *O. martiana* were performed by Cunico (2007). A cutaneous reflex inhibition was observed in guinea pigs after subcutaneous administration. The same author studied the extract anesthetic activity on the ocular surface of rabbits following the guidelines suggested by Vogel (2002) and no corneal reflex inhibition was observed.

The use of topical anesthetics in veterinary ophthalmology is not recent. Procedures like tonometry, corneal sutures and foreign bodies removal, conjunctival biopsies and intracameral injection are only feasible after anesthesia of the ocular surface (Herring *et al*., 2005). Those substances are used to reduce pain, but its short-term effect obligates repeated administrations. However, they acutely reduce the Schirmer's tear test (STT) values. Hamor *et al*. (2000) reported that the corneal desensibilization reduces the tear production by blocking its reflexive production. The authors also observed a significant decrease in the STT values after the instillation of 0.5% proparacaine ophthalmic solution (main from 20.3mm to 6.2mm).

The aim of this study was to verify the effects of *Ottonia martiana* extract as a local anesthetic on dog's ocular surface.

MATERIAL AND METHODS

The project was submitted to and approved by the Committee of Ethics and Animal Welfare of the College of Agricultural and Veterinarian Sciences – UNESP – Jaboticabal (Protocol 003292-08). Bioethics cares follow the guidelines from Association for Research in Vision and Ophthalmology (ARVO), and

National Institutes of Health Publications No 85-23: Revised 1985.

The extract was prepared from the *O. martiana* leaves obtained at one of the nuclei of the Beneficent Spiritist Center União do Vegetal, in Sao Paulo city, SP, Brazil. Before the preparation, the leaves were cleaned with sterile solution and dried with sterile gaze. As standard, 10g of fresh leaves were added to 100mL of cereal hydrated ethyl alcohol (EtOH 70%), in a 1:10 (m/v) ratio. The extract was kept under cold maceration for 30 days in a sterile amber bottle. The filtered liquid extract was evaporated under reduced pressure to remove the solvent so that 90mg of dry extract could be obtained (Sonaglio *et al*., 1999). The dry extract was resuspended in 5ml sterile saline solution, obtaining an 18mg/ml concentration. This final extract was kept in sterile dropper bottles under refrigeration up to two days.

Forty five healthy Beagle dogs (*Canis familiaris*, Linnaeus, 1758), adult, male or female were used. Schirmer's tear test (Schirmer Tear Test strips®, Ophthalmos Indústria Farmacêutica, Av. Brigadeiro Luiz Antônio 4790, São Paulo, SP, Brazil), slit lamp biomicroscopy (Slit Lamp SL – 14®, Kowa Optimed Inc., 20001 South Vermont Ave, Torrance, CA, USA), esthesiometry (Cochet-Bonnet esthesiometer, Luneau Ophthalmologie, Paris, França), applanation tonometry (TonoPen XL®, Medtronic Ophthalmics, 6743 Southpoint Drive North, Jacksonville, FL, USA) and fluorescein staining (Fluoresceína strips®, Ophthalmos Indústria Farmacêutica, Av. Brigadeiro Luiz Antônio 4790, São Paulo, SP, Brazil) were performed. Three groups of 15 animals each were composed as follows: control group (CG), proxymetacaine group (PG) and *Ottonia* group (OG).

Animals from CG, PG and OG were administered one drop of sterile saline, 0.5% proxymetacaine hydrochloride ophthalmic solution and *Ottonia martiana* extract, respectively, on the left eye at time 0 (T0) and 3 minutes after time 0 (T3). Evaluation of all treated animals was performed according to Table 1. Schirmer's tear test was performed immediately before treatments (STTa) and 10 minutes after each treatment (STTb), as proposed by Slatter (2005).

Slit lamp biomicroscopy was performed before treatments (SLBa), 10 minutes (SLBb) and 20 minutes (SLBc) after T0. Conjunctival hyperemia, corneal transparency and eventual inflammation signs were classified as absent, mild, moderate or severe.

Corneal sensitivity test was performed with a Cochet-Bonnet esthesiometer with the filament length adjustable between 0.5 and 6cm, where 0.5cm represented the smallest nylon filament and the most stimulant pressure on the cornea. Beginning with 4cm length, the nylon filament was put perpendicularly to the axial cornea (Figure 2). Measurements were performed 5 times using the same length of the nylon filament. The length of the nylon filament was decreased at 0.5cm increments until the dog respond with consistent corneal blink reflex.

Corneal sensitivity data was collected when the dog responded with a corneal blink reflex in at least three of five attempts to stimulate the cornea. A 0 value was admitted when no corneal reflex was observed at a 0.5cm nylon filament length. All tests were performed by the same veterinarian (Herring *et al*., 2005).

Intraocular pressure measurements were performed before treatments (IOPa) and 10 minutes after treatment (IOPb), as suggested by Slatter (2005). The eyes were stained with fluorescein 20 minutes after T0 to verify corneal integrity after the procedures, as suggested by Slatter (2005).

All collected data was analyzed by *Sigma Stat* software. Student's t-test was used to compare two groups and ANOVA (Kruskal-Wallis Analysis of Variance) was used when all three groups were compared. Results were considered significant when $P \leq 0.05$.

Figure 1. Photographic image of leaves and inflorescences of *Ottonia martiana*. Source: Maria Alice Corrêa.

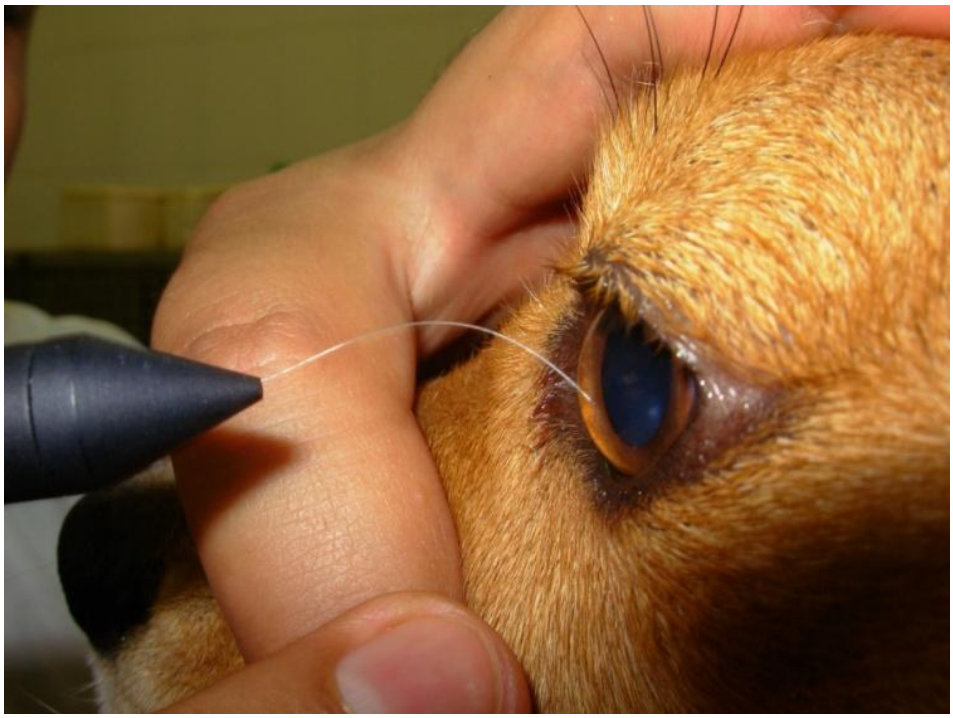

Figure 2. Photographic image of corneal esthesiometry in an adult male Beagle. The nylon filament is in contact with the axial cornea.

Table 1. Treatment and evaluation performed on the left eye of 45 adult Beagles, male or female, from all treatment groups

Time	Evaluation
Before treatment	Schirmer's Tear Test (STTa)
	Slit lamp biomicroscopy (SLBa)
	Esthesiometry (Ea)
	Intraocular pressure (IOPa)
T0	First administration
T3 (3 minutes after T0)	Second administration
T5 (5 minutes after T0)	Esthesiometry (Eb)
T10 (10 minutes after T0)	Schirmer's Tear Test (STTb)
	Slit lamp biomicroscopy (SLBb)
	Esthesiometry (Ec)
	Intraocular pressure (IOPb)
T20 (20 minutes after T0)	Slit lamp biomicroscopy (SLBc)
	Fluorescein staining

RESULTS

A significant decrease in the STT ($P \leq 0.01$) was observed for the PG when values before treatment (STTa) and after treatment (STTB) were compared. Also, STTb differs significantly between CG and PG, and between OG and PG. Tear production did not decrease significantly in CG and OG, when STTa and STTb were compared. The mean STT values obtained before and after treatment are shown in Table 2.

Slit lamp biomicroscopy observed no abnormalities in the CG and OG animals evaluated at any moment (SLBb and SLBc). Both evaluations performed after the treatment (SLBb and SLBc) revealed conjunctival hyperemia in 11 animals from PG.

Esthesiometry values significantly decreased in PG and OG groups, five minutes after treatment (Eb). After 10 minutes, esthesiometry values (Ec) did not differ from Eb. A statistical difference was observed when Ea, Eb and Ec were compared, in PG and OG groups ($P \leq 0.001$). Table 3 shows the mean esthesiometry values observed before and after treatment.

The intraocular pressure values obtained before (IOPa) treatment and 10 minutes later (IOPb) did not differ in any group (CG, PG, OG), and fluorescein staining test was negative in all animals.

Table 2. Mean Schirmer's tear test (STT) values obtained from left eyes of adults Beagles, males or females, from all treatment groups, before treatment (STTa) and after treatment (STTb)

	STTa (mm/min)	STTb (mm/min)
Control group (CG)	18.53	21.06
Proxymetacaine group (PG)	17.6	13.33
Ottonia group (OG)	20.3	21.3

Table 3. Mean esthesiometry values obtained from the central cornea of the left eye of 45 healthy Beagles, males or females, from all three treatment groups, using Cochet-Bonnet esthesiometer before treatment (Ea), after 5 minutes (Eb) and after 10 minutes (Ec)

	Ea	Eb	Ec
Control group (CG)	1.9	1.96	1.86
Proxymetacaine group (GP)	1.93	0	0
Ottonia group (GO)	1.26	0.46	0.46

DISCUSSION

There are few scientific studies regarding medicinal plants, especially considering the Brazilian biodiversity. The knowledge and use of those plants by the population are mainly promoted by oral and personal communication. In a study about the use of phytotherapy in veterinary medicine, Almeida *et al*. (2006) reported that the majority of veterinarian professionals believe that the use of medicinal plants would be more reliable if a larger number of scientific studies were conducted. The guidelines reported by Sonaglio *et al*. (1999) were followed during the preparation of *O. Martiana* extract. In addition, the *O. martiana* ethanolic crude extract (EbtOH) obtained was resuspended in 0.9% saline aiming the desired solution, since the EbtOH should not be directly applied, due to its viscous consistence and active components concentration that could be toxic to the corneal epithelium.

The topical anesthetic 0.5% proxymetacaine chloride was added to this study as a comparative variable, due to the frequent use in ophthalmology (Medeiros *et al*., 2000) and large number of reports regarding its effects (Medeiros *et al*., 2000; Hamor *et al*., 2000; Herring *et al*., 2005; Binder and Herring, 2006). Despite Herring *et al*. (2005) reporting that the proxymetacaine is the drug of choice for ocular surface anesthesia due to its minimal side effects, the manufacturer warns about possible ocular alterations following its administration, such as irritation, conjunctival hyperemia, tearing and corneal erosion.

There was a non-significant increase in the tear production of the control group and *Ottonia* group, when STTa and STTb were compared. It is thought that it may have been the result of all the different stimulations (drug administration, tonometry and esthesiometry) applied to the corneal surface (Roberts and Erickson, 1962; Harker, 1970). As for the *Ottonia* group, there was no decrease in STT values after treatment.

The axial cornea was the chosen area for performing the esthesiometry since several studies, such as the one reported by Barret *et al*. (1991), showed it is the area with most nociceptor terminations. Moreover, this information is similar to those reported by Blocker and Van Der Woerdt (2001) in cats and by Brooks *et al*. (2000) in horses.

Barret *et al*. (1991) and Herring *et al*. (2005) observed that dolichocephalic dogs exhibit esthesiometry values of 1.75cm, similar to the results obtained in this study (1.7cm). Klaumann (2007) reported values varying from 1.5 to 2.5cm.

The administration of 0.9% saline in the control group did not alter the esthesiometry values at any moment. On the other hand, the 0.5% proxymetacaine administered in PG reduced the esthesiometry value from 1.93cm (Ea) to 0 (zero) (Eb and Ec), similar to previous literature report (Herring *et al*., 2005).

The administration of the *O. martiana* extract decreased the esthesiometry value from 1.26cm (Ea) to 0.46cm (Eb and Ec). It is postulated that this significant reduction of the esthesiometry values is due to the *O. martiana* analgesic or anesthetics properties, although its action mechanism is still unknown.

The anesthetic properties of the studied plant are attributed to one of its active principles, the piperovatine amide (Makapugay *et al*., 1983; McFerren and Rodriguez, 1998). The same active principle can be isolated from other species of *Piper* and *Ottonia* genera. The piscicide and sialagogue actions are also attributed to piperovatine. McFerren *et al*. (2002) studied its action mechanism and verified that the piperovatine is a sodium channel agonist, therefore a piscicide. Fish are sensitive to other sodium channel agonists such as DDT and pyrethroids. The authors report that the substance induces a tingling sensation on the oral mucosa and not an anesthetic sensation, different from the local anesthetics that are sodium channel blockers.

The popular use of *O. martiana* for the treatment of odontalgia is due to its analgesic or anesthetics activities, as observed in this study by the corneal sensitivity reduction after topical administration of the substance extract.

The reduction of corneal sensitivity differs from the reports of Cunico (2007). The author studied the corneal sensitivity in rabbits after topical administration of the ethanolic crude extract

using all parts of the same plant. It is important to observe that the Cochet-Bonnet esthesiometer was not used in that study. Also, the author followed the model proposed by Vogel (2002) that uses a non-adjustable nylon filament that produces an initial pressure on the cornea higher than the Cochet-Bonnet esthesiometer.

Moreover, different protocols were used for the extract production. In the present study only the plant leaves were used, whereas Cunico (2007) used all parts of the plant (root, stem, leaves and fruits), therefore inducing not only a higher concentration of amides in the cornea, but also other irritants and toxic substances. Although Cunico (2007) found no result for corneal anesthesia when the substance was topically applied, a decrease of skin sensitivity was observed when *O. martiana* was administered subcutaneously in guinea pigs. A similar result was obtained when lidocaine was administered subcutaneously in a comparative group (Cunico, 2007).

Slit lamp microscopy showed mild conjunctival hyperemia in 11 animals from the proxymetacaine group, in accordance to the manufacturer's warning (Anestalcon®) (www.medicinanet.com.br). No ocular alterations were observed in the animals from control group and *Ottonia* group, in contrast to the reports of Cunico (2007) that found severe ocular irritation after topical administration of *O. martiana* ethanolic crude extract using all parts of the plant in rabbits. It is postulated that the absence of ocular irritation in the present study is a result of several factors on the extract preparation such as only the use of fresh leaves, hydrated ethyl alcohol from cereals as a solvent and 0.9% saline for resuspension, which differ from the protocol used by Cunico (2007). Moreover, the concentration of active principles is different for each part of the plant (Gobbo-Neto and Lopes, 2007).

CONCLUSIONS

O. martiana extract acts reducing corneal sensitivity in dogs. Moreover, its use does not decrease the tear production and does not cause any clinical ophthalmic alteration.

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