Ophthalmological pre-clinical research about essential oil from the *Origanum vulgare L.*, Lamiaceae

Investigação oftalmológica pré-clínica do óleo essencial de Origanum vulgare L., *Lamiaceae*

Jana Luiza Toscano Mendes de Oliveira[1], Adriano Francisco Alves[3], Maria Carmem Toscano Tavares de Araujo[4] , Walter Mendes de Oliveira Junior[4], Luiza Toscano Dias Rodrigues[4], Margareth de Fátima Formiga Melo Diniz[2].

Abstract

***Objective**: To evaluate acute eye irritation in rabbits following topical administration of essential oil. **Methods**: animals were divided into three groups, each containing three rabbits, with a total of 6 eyes per group. The difference between them was the concentration used (1, 3 and 9%). A single dose of 0.1 ml of the product was applied into the conjunctival sac of one eye of the animal, and the contralateral eye was used as control. The effects caused by the essential oil in the conjunctiva, iris and cornea were analyzed after 1, 24, 48 and 72 hours and at the end of the seventh day after topical application. Ophthalmologic evaluations were performed with the aid of a binocular indirect ophthalmoscope fluorescein and with and without the observed responses, before being graded according to the Draize scale. Pathological examinations were performed on all eyes studied at the end of the experiment. **Results**: in the group of animals subjected to the ocular instillation of 1% essential oil, there was no change. For treatment with 3% oil, conjunctival changes were found to be decreasing during the examination after 1 hour. Administration of the 9%essential oil induced conjunctival injection, without any change in the other ophthalmologic evaluation times. **Conclusion**: the evaluation contributed to meet the clinical changes in the ocular surface. Thus, it was possible to classify the oil at 1% as non-irritating and the concentration of 3% and 9 as mildly irritating, making it possible for clinical studies to establish the oil as an alternative therapy in bacterial conjunctivitis.*

***Keywords**: Origanum vulgare; Oils, volatile; Irritants/toxicity; Conjunctiva; Conjunctivitis, bacterial*

Resumo

Objetivo: Avaliar a irritação ocular aguda em coelhos, após a administração tópica de óleo essencial. **Métodos**: Para tanto, os animais foram divididos em três grupos, cada um com três coelhos, totalizando 6 olhos por grupo, e a diferença entre eles foi a concentração utilizada (1, 3 e 9%). Aplicou-se no saco conjuntival, de um dos olhos do animal, uma dose única de 0,1 ml do produto e o olho contralateral foi usado como controle. Analisou-se os efeitos causados pelo óleo essencial na conjuntiva, íris e córnea após 1, 24, 48, 72 horas e no final do sétimo dia após a aplicação tópica. As avaliações oftalmológicas foram feitas com o auxílio de um oftalmoscópio binocular indireto com e sem fluoresceína. As reações observadas foram graduadas segundo a escala de *Draize*. Foram realizados exames anatomopatológicos em todos os olhos estudados no final do experimento. **Resultados**: No grupo de animais submetidos à instilação ocular do óleo essencial a 1%, não se observou alterações. O tratamento com o óleo a 3% provocou alteração conjuntival no exame feito em 1 hora, o que foi reduzindo. A administração do óleo essencial a 9% induziu hiperemia conjuntival, não havendo qualquer alteração nos outros tempos de avaliação oftalmológica. **Conclusão**: A avaliação contribuiu para conhecer as alterações clínicas na superfície ocular. Desta forma, foi possível classificar o óleo a 1% como não irritante e nas concentrações de 3 e 9% como pouco irritante, tornando possível estudos clínicos, a fim de estabelecer o óleo como alternativa terapêutica em conjuntivites bacterianas.

Descritores: Origanum vulgare; Óleos essenciais; Irritantes/toxicidade; Conjuntiva; Conjuntivite bacteriana

[1]Department of Phisiology and Pathology, Universidade Federal da Paraíba; João Pessoa, PB, Brazil.
[2]Department of Pharmaceutical Sciences, Universidade Federal da Paraíba; João Pessoa, PB, Brazil.
[3]Post-graduation Program on Bioactive Natural and Sinthetic Products, Universidade Federal da Paraíba; João Pessoa, PB, Brazil.
[4]Universidade Federal da Paraíba; João Pessoa, PB, Brazil.

The study was conducted at the Laboratory of pre-clinical Toxicology of the Program on Bioactive Natural and Sinthetic Products (PgPNSB) at the Center of Health Sciences (CCS), Universidade Federal da Paraíba, João Pessoa, PB, Brazil.

The authors declare no conflicts of interests.

Received for publication 10/10/2015 - Accepted for publication 25/01/2016

Introduction

Acute bacterial conjunctivitis is a common and highly contagious disease caused by direct contact with secretions from the infected eye[1]. It affects humans since antiquity[2], being characterized by a bacterial growth in the conjunctival surface which leads to an acute or chronic inflammatory process.

However, bacteria have a remarkable number of genetic mechanisms for the development of antimicrobial resistance, making indispensable the emergence of new drugs that can keep the therapy for infectious cases effective.

In this context, some medicinal plants and their essential oils, due to their antimicrobial properties, have aroused the interest of researchers around the world to research new antibiotics. Among these plants, we highlight the oregano (*Origanum vulgare*), which belongs to the genus Origanum (Lamiaceae) whose essential oil has antimicrobial activity against gram-positive and gram-negative bacteria (*Escherichia coli*, *Salmonella typhimurium*, *Staphylococcus aureus*, *Rhizobium leguminosarum*, *Bacillus subtilis*)[3]

OLIVEIRA et al[4] verified in their study that the essential oil of *Origanum vulgare L* presented, among the substances tested against the main bacterial strains of conjunctivitis, the highest antibacterial activity.

Given the stated antimicrobial activity of the essential oil of *Origanum vulgare L* on isolated bacterial strains from patients with conjunctivitis, the importance of the assessment of eye irritation was evident in order to determine whether the essential oil might be used in the topical treatment of eye disorders.

The aim of this study is to assess the acute eye irritation - single dose - in rabbits, and contribute to a new treatment option of conjunctivitis in humans.

Methods

The methodology used to evaluate the effect of the essential oil of *Origanum vulgare L.* in the eyes was based on the work developed by Draize et al. [5], with some modifications based on the protocol for testing eye irritation of the OECD Organization for Economic Cooperation and Development (OEDC)[6]

The albino rabbit was the model used due to allowing a better assessment of the eye irritation due to the absence of pigments in their ocular mucosa and presenting a broad and accessible ocular area, allowing the effects to be easily observed.

Nine healthy rabbits were selected, and about 24 hours before the test, the 18 eyes of the animals were carefully examined with a pen torch to check for the presence of any lesions, since if the animals had shown any signs of eye irritation or pre-existing corneal injury they would be excluded from the study.

The following doses of the essential oil of *Origanum vulgare* L. were used in the test: 1, 3 and 9%, corresponding to dose with bactericidal effect against bacterial agents collected from the conjunctival sac of patients with conjunctivitis, three and nine times this dose, respectively. With a syringe, 0.1 ml of the product was applied as a single dose directly into the conjunctival sac of the left eye of each animal. The contralateral untreated eye was used as control.

We analyzed the effects in the area of the cornea, such as opacity in the iris, as iritis, and in the conjunctival region, like hyperemia and chemosis. These reactions were observed after 1, 24, 48, 72 hours, and by the end of the seventh day after application of the product. The assessments were performed with the aid of an indirect binocular ophthalmoscope and the use of fluorescein eyedrops. All tests were performed by the same researcher.

Changes in the cornea, iris and conjunctiva were graded according to the Draize scale of grading of ocular reactions, and recorded in the appropriate forms.

The daily total for each of the parties analyzed is obtained according to Draize, as follows:

Cornea: (sum of the opacity values of rabbits 1... 3) x 5

Iris: (sum of the inflamation values of rabbits 1... 3) x 5

Conjunctiva: (sum of redness values + edema 1…3) x 2

The average daily rate (ADR) is obtained by summing up the values obtained for the cornea, iris and conjunctiva for each reading time, 1 h (ADR_1), 24 h (ADR_2), 48 h (ADR_3), 72 h (ADR_4) e 7 dias (ADR_7), and dividing the value thus calculated by the number of rabbits used in the test. According to the value obtained, the substances may be classified in the first approximation into one of the eight categories listed in Table 1. To this end, the ADR should be taken at the first four days of the test, and the higher value among the four is chosen. This value is the maximum average (MA), and the classification of first approximation is determined from it in Table 1.

Table 1

Classification values of the first approximation

Maximum Average (MA)	Classification of the first approximation
00	(N) non-irritanting
<2,5	(PN) pratically non-irritanting
2.5-15.0	(M_1) maximally annoying
15.1-25.1	(M_2) mildly irritanting
25.1-50.0	(M_3) mildly moderated
50.1-80.0	(S) severely irritanting
80.1-100.0	(E) extremely irritanting
100.1-110.0	(M_x) maximally irritanting

The final classification corrects the classification of the first approach, and takes into account the duration and intensity of the reaction observed. To this end, in addition to the average daily rate of the first four days of the test (ADL_1 to ADL_4) those obtained on the seventh day (ADL_7) and the total individual values (VTI) of the seventh day should be considered. If the ADL_7 is different from the value obtained in the classification of the first approximation, the final classification must be done according to Table 2.

The cornea, iris and conjunctiva of the eye treated and the control were subjected to histopathological examination. When this inflammation is rated as mild, moderate and severe according to the amount of polymorphonuclear neutrophils (PMNs) present. The inflammatory process was considered mild when few cells were visualized; moderate when an increased amount of cells was clearly visible, and severe when there was excessive amount of inflammatory cells, involving an extensive area.

The numerical results were expressed as mean ± standard error of the mean (SEM). Differences between groups were determined by the analysis of variance "*one-way*" (ANOVA) followed by Dunnett's test, in which the values of $p < 0.05$ were considered significant. The analyses were performed with the aid of *GraphPad Prism* version 4 (GraphPad Sofware Inc., USA).

Table 2

Determination of final classification

Classification of the 1st approximation	Cases	Condition	Final Classification
(N) non-irritating	1	Up to ADL2=0	(PN) practically non-irritating
	2	Up to ADL3=0	(M_1) minimally annoying
	3	Up to ADL4=0	(M_2) mildly irritating
	4	ADL7<20;VTI<10	(M_3) mildly moderated
	5	20<ADL7<40,VTI<30	(S) severely irritating
	6	40<ADL7<80;30<VTI<60	(E) extremely irritating
(M_1) minimally annoying	1	Up to ADL2=0	(M_1) minimally annoying
	2	Up to ADL3=0	(M_1) minimally annoying
	3	Up to ADL4=0	(M_2) mildly irritating
	4	ADL7<20;VTI<10	(M_3) mildly moderated
	5	20<ADL7<40,VTI<30	(S) severely irritating
	6	40<ADL7<80;30<VTI<60	(E) extremely irritating
(M_2) mildly irritating	1	Up to ADL2=0	(M_2) mildly irritating
	2	Up to ADL3=0	(M_2) mildly irritating
	3	Up to ADL4=0	(M_2) mildly irritating
	4	ADL7<20;VTI<10	(M_3) mildly moderated
	5	20<ADL7<40,VTI<30	(S) severely irritating
	6	40<ADL7<80;30<VTI<60	(E) extremely irritating
(M_3) mildly moderated	1	Up to ADL2=0	(M_3) mildly moderated
	2	Up to ADL3=0	(M_3) mildly moderated
	3	Up to ADL4=0	(M_3) mildly moderated
	4	ADL7<20;VTI<10	(M_3) mildly moderated
	5	20<ADL7<40,VTI<30	(S) severely irritating
	6	40<ADL7<80;30<VTI<60	(E) extremely irritating
(S) severely irritating	1	Up to ADL2=0	(S) severely irritating
	2	Up to ADL3=0	(S) severely irritating
	3	Up to ADL4=0	(S) severely irritating
	4	ADL7<20;VTI<10	(S) severely irritating
	5	20<ADL7<40,VTI<30	(S) severely irritating
	6	40<ADL7<80;30<VTI<60	(E) extremely irritating
(E) extremely irritating	7	40<ADL7<80;30<VTI<60	(E) extremely irritating
(Mx) maximally irritating	8	ADL<80, VTI<60	(E) extremely irritating
	9	ADL7<80;VTI>60	(Mx) maximally irritating

Results

The structures assessed in the study were the conjunctiva, cornea and iris, being respectively observed: corneal opacity; iritis; chemosis and conjunctival hyperemia. Macroscopic findings observed in the eyes of animals during treatment are presented in tables 3, 4 and 5.

After completion of the eye irritation test with the essential oil of *Origanum vulgare* 1%, there were no changes in the cornea, iris or conjunctiva in any of the standard observation time by the Draize method (Figure 1), resulting in an maximum average zero, classifying the product at this concentration as non-irritating (Table 3)

Upon primary eye irritation test with the essential oil of *Origanum vulgare* 3%, we observed a change in the conjunctiva in the ophthalmologic assessment performed to complete 1 instillation of the substance (diffuse purple color in one of the animals included in the study) and only a mild hyperemia in one of the study animals in the examination performed after 24 hours of topical use of the essential oil (Figure 2). There was no evidence of other ocular abnormalities in any of the other assessment times performed in this test. The maximum average was 1.33,

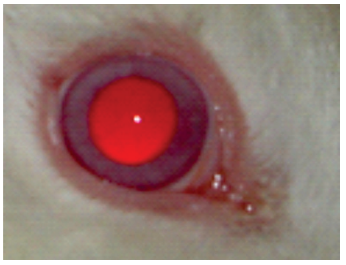

Figure 1: Eye treated with essential oil 1% showing normal aspect on the microscope

Table 3

Results of acute ophthalmic irritability induced by essential oil *Origanum vulgare L.* 1% in rabbits

Eye part assessed	Sum of the values obtained for each eye part assessed in rabbits belonging to the study in each observation time (hour)				
	1	24	48	72	168
Cornea	0	0	0	0	0
Conjunctiva	0	0	0	0	0
Iris	0	0	0	0	0
Average daily rate	0	0	0	0	0
Maximum mean ± SD	**0.00 ± 0.0**				

ranking the essential oil at this concentration as practically non-irritating (Table 4)

Table 5 shows the values obtained according to the parameters established by Draize after the eye irritation test, instilling the essential oil of *Origanum vulgare* 9% in the bottom of the conjunctival sac of rabbits. Changes were observed only in the conjunctiva, redness abundantly distributed in the first hour (Figure 3), and diffused purple color in the examination performed twenty-four hours after usage of the product. The tests carried out on 48, 72 and 168 hours after usage of the substance showed no change in any of the ocular structures

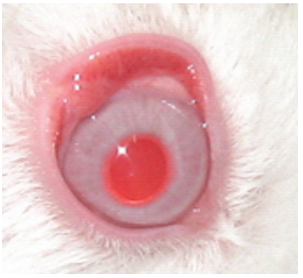

Figure 2: Eye after 24 hours of treatment with the essential oil of *Origanum vulgare* 3% showing mild conjunctival hyperemia

Table 4
Values obtained after the eye irritation test according to Draize scale for the essential oil of *Origanum vulgare* 3%

Ocular structure assessed	Sum of the values obtained for each eye part assessed in rabbits belonging to the study in each observation time (hour)				
	1	24	48	72	168
Cornea	0	0	0	0	0
Conjunctiva	2	1	0	0	0
Iris	0	0	0	0	0
Average daily rate	1.33	0.67	0	0	0
Maximum mean ± SD	**1.33 ± 0.64**				

Table 5

Values obtained after the eye irritation test according to Draize scale for the essential oil of *Origanum vulgare* 9%

Ocular structure assessed	Sum of the values obtained for each eye part assessed in rabbits belonging to the study in each observation time (hour)				
	1	24	48	72	168
Cornea	0	0	0	0	0
Conjunctiva	3	2	0	0	0
Iris	0	0	0	0	0
Average daily rate	2.00	1.33	0	0	0
Maximum mean ± SD	**2.00 ± 0.8**				

investigated. The maximum average was 2.00, ranking the essential oil at this concentration as practically non-irritating.

The histopathological examination showed no microscopic changes were observed in the eyes of the animals used in the study.

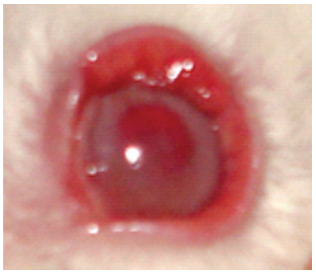

Figure 3: Conjunctiva of a rabbit eye after 1 hour in contact with essential oil of *Origanum vulgare* 9%, with generously distributed redness

Discussion

Bacterial conjunctivitis is a disease that affects humans since antiquity, but there is already an accurate description of the cardinal signs of disease made by the Egyptians 1550 years BC[2]. It is highly contagious, and typically the transmission occurs by hand-eye contact. However, there are other transmission forms, from exogenous and endogenous forms, through the blood contamination up to inoculation by the contra-lateral eye contamination[7].

The eye irritation test or Ocular Test of Draize is part of the toxicologic tests required by the regulatory agencies to register different kinds of substances. The results obtained from this test have great importance to decision of the toxicological classification of a product due to many times being the most critical data among the toxicological tests[8].

The reason to study the effects of the oil of *Origanum vulgare* L.on the ocular surface lies on the fact that it has promising antibacterial activity on strains from patients with bacterial conjunctivitis, as well as on the use expectation of this product as a source of antibacterial compounds which can be used in the treatment of ocular infections[4,9].

In the original test of Draize three types of tissue are examined: Cornea, iris and conjunctiva. Each tissue has different characteristics to be assessed[5]. The test of Draize adapted by the OECD 405/2002 examines the density of opacity in the cornea, the irritation in the iris, and the hyperemia and edema in the conjunctiva.

After the eye irritation test with the essential oil of *Origanum vulgare* 1%, which is the therapeutic dose against bacteria causing conjunctivitis, no changes were identified in any of ocular structures examined in any of the observation times, when compared to the control. According to the test used, an average daily rate obtained by summing up the values obtained for the cornea, iris and conjunctiva for each reading time, and dividing the value thus calculated by the number of rabbits used in the test[10]. According to the value obtained, *Origanum vulgare* 1% was classified as non-irritating, suggesting that this component can be used on the ocular surface without causing toxic effects.

The same test performed with essential oil 3% generated conjunctival hyperemia after 1 hour of instillation of the product in the conjunctival sac, and such effect was well reduced after 24 hours, and absent in all other times of assessment. Hyperemia appears in the eye due to local vasodilatation, being a common manifestation in an organism in response to an irritating agent, aiming at releasing chemical mediators to deactivate the aggressor agent[11]. As the conjunctiva is widely vascularized and, from the anatomical point of view, is the first contact layer of the eye with the external environment, it is an important defense barrier of the ocular system. Consequently, conjunctival hyperemia is a very common clinical finding, even facing the weakly irritating agents[12]. In our study, only one animal presented said signal, which quickly disappeared, not being considered an important factor to consider the substance aggressive to the eye in this concentration. No other parameter was changed in any of the standard exam times.

The maximum mean is the highest value of average daily rate obtained, considering as the basis the average daily rates of the first four days of observation[5,6,10].The maximum mean with the essential oil 3% was 1.33, which rates the product as almost non-irritating, which suggests safe use as an ocular inoculant even in this concentration, which is three times higher than the therapeutic dose.

As stated by ANVISA in the guidelines for pre-clinical toxicity studies of phitotherapeutics, the assessment of ocular toxicity was also made with essential oil of *Origanum vulgare* 9%, a dose that is nine times higher than the one suggested for a possible treatment.

After 1 hour of inoculation of the essencial oil 9% in the conjunctival sac of the rabbit, a redness abundantly distributed was seen in the conjunctiva. This effect is decreased, being presented as a diffuse purple color in the ophthalmologic assessment carried out after 24 hours. In the other assessment times, there were no other conjunctival changes. Although the conjunctival inflammatory reaction was somehow more pronounced with the increase of the essential oil concentration, it is important to mention that it disappeared completely after 24 hours. None of the other ocular structures is changed in any of the standard times of ophthalmologic assessment. The maximum average calculated was 2.00, being the product rated as practically non-irritating, suggesting that even an accidental inoculation of the product in the eye will not result in ocular sequelae, demonstrating safety for use of the product.

Therefore, for the conjunctiva, the treatment with the essential oil in all doses studied induced changes which were mild and reversible in a short period after the use of the product.

Cornea is the most important structure to be assessed in the ocular irritation test due to the role it plays in the process of sight. Any problem in the cornea is the entrance for infections or the cause of functional problems[13]. The animals treated with all the doses assessed in the present study of *Origanum vulgare* L. did not have changes in the cornea in any of the assessment times.

The same was observed for the iris, since the treatment with *Origanum vulgare* L. did not induce iris irritation with any of the concentrations tested.

According to the literature, the rabbit's eye is more sensible to irritating agents than the human eye. The epithelial surface of the rabbit's eye is ten times more permeable to hydrophilic solutes than the human eye. The rabbit's cornea is thinner than the human one, with an average of 0.37mm thickness, whereas the human is 0.51mm. The Bowman's membrane of the cornea of these animals is six times thinner. The pain threshold of the rabbit is much higher than the human's, making the irritating substances to be slowly removed, which is enhanced by a less

efficient lacrimal system, delaying even more the removal of irritating substances from its surface. Besides, the cornea represents 25% of the surface area of the rabbit's eye, and only 7% of the human ocular surface area. Finally, the pH of the aqueous humor is 8.2, compared to 7.1 to 7.3 for men, making rabbits more susceptible to the damage caused by alkaline material.

Most of cases, albino rabbits are more sensible than men to irritating agents. The results of the tests made in other animal species may reinforce those obtained with rabbits. Then, it is possible to consider the data for human beings[5,6,10].

Our study showed that the essential oil of *Origanum vulgare* 1% was not irritating, and almost non-irritating in the concentrations of 3 and 9%. As described above, the eyes of the rabbis are more sensible to toxic agents, suggesting that the effects found are probably even less significant in human eyes. Associated to the macroscopic findings, the microscopic assessment of the eyes after exposure to all concentrations of essential oil did not show any significant change when compared to the control eyes.

The ocular irradiation test showed that contact between the eyes studied and all concentrations assessed of the oil of *Origanum vulgare* L. produces minimal ocular lesions, which are generally reversible after 24 hours of exposure to the product.

Conclusion

Based on the data obtained, we can conclude that the assessment of ophthalmologic irritability contributed to the knowledge of macroscopic and microscopic clinical changes on the ocular surface of the rabbits' eyes exposed to the essential oil of *Origanum vulgare* L., in each test interval of ocular irritability. This way, it was possible to rate the essential oil of *Origanum vulgare* L 1%, which is the therapeutic dose, as non-irritating, and in concentrations as high as 3 and 9% as almost non-irritating, suggesting the need to deepen the study of this substance with clinical studies, and considering this product a possible therapeutic agent in the treatment o bacterial conjunctivitis.

References

1. Martínez BO, Ruiz RM, Pérez RM. Conjuntivitis bacteriana: patógenos más prevalentes y sensibilidad antibiótica. Anales Pediatr. 2004;61(1):32-6.
2. Hirschberg J. The history of ophthalmology, vol 1, the history of ophthalmology in antiquity. Bonn: JP Wayenborgh; 1982.
3. Sivropoulou A, Papanikolaou E, Nikolaou C, Kokkini S, Lanaras T, Arsenakis M. Antimicrobial and cytotoxic activities of Origanum essential oils. J Agric Food Chem. 1996; 44(5):1202-5.
4. Oliveira JL. Estudo da atividade antibacteriana de óleos essenciais contra agentes etiológicos da conjuntivite bacteriana simples [tese]. João Pessoa: Universidade Federal da Paraíba; 2006.
5. Draize JH, Woodard G, Calvery HO. Methods for the study to irritation and toxicity of substances applied topically to the skin and mucous membrane. J Pharmacol Experiment Ther. 1944; 82:377-90.
6. Organisation for Economic Cooperation and Development (OECD). Guidelines for the testing of chemicals, OECD 405. Acute eye irritation/corrosion. Paris: OECD; 2002.
7. Hwang DG, Schanzlin DJ, Rotberg MH, Foulks G, Raizman MB; Levofloxacin Bacterial Conjunctivitis Place-controlled Study Group. A phase III, placebo controlled clinical trial of 0.5% levofloxacin ophthalmic solution for the treatment of bacterial conjunctivitis. Br J Ophthalmol. 2003;87(8):1004-9.
8. Wilhelmus KR. The Draize eye test. Surv Ophthalmol. 2001;45(6):493-515.
9. Oliveira JL, Diniz MF, Lima EO, Souza EL, Trajano VN, Santos BH. Effectiveness of Origanum vulgare L. and Origanum majorana L. essential oils in inhibiting the growth of bacterial strains isolated from the patients with conjunctivitis. Braz Arch Biol Technol. 2009; 52(1):45-50.
10. Brito AS. Manual de ensaios toxicológicos in vivo. Campinas: Editora da UNICAMP;1994. 122p.
11. Gigliotti F, Williams WT, Hayden FG, Hendley JO, Benjamin J, Dickens M, Gleason C, Perriello VA, Wood J. Etiology of acute conjunctivitis in children. J Pediatr. 1981;98(4):531-6.
12. Huhtala A. Corneal epithelial and retinal pigment epithelial cell culture assays as potencial alternatives to animal experiments for the evaluation of ocular toxicity. Helsinki: Helsinki University of Technology; 2003.
13. Estévez, R. A. Efeito do tacrolino na reepitelização da córnea em coelhos [tese]. Belo Horizonte: Universidade Federal de Minas Gerais; 2004.

Corresponding author:
Adriano Francisco Alves.
Rua: Maria das Neves Coutinho, n 45, Bancários, João Pessoa, Paraíba, Brasil.
Phone/Fax: (83) 99675.9944.
Email: adrianofalves@gmail.com