Journal of Advances in Medicine and Medical Research

32(20): 44-57, 2020; Article no.JAMMR.61584
ISSN: 2456-8899
(Past name: British Journal of Medicine and Medical Research, Past ISSN: 2231-0614, NLM ID: 101570965)

In vivo Antistaphylococcal Activity Evaluation of *Ocimum gratissimum* Linn. (*Lamiaceae*) Ophthalmic Ointment

Adiko N'dri Marcelline[1*], Okpekon Aboua Timothée[2], Say Venance Martial[2], Akoubet Ouayogode Aminata[1,3], Koffi Agnely Armand[4] and Kablan Ahmont Landry Claude[5]

[1]Laboratoire de Pharmacognosie, Botanique, Biologie Végétale et Cryptogamie, UFR des Sciences Pharmaceutiques et Biologiques, Université Félix Houphouët-Boigny, 22 BP 714 Abidjan 22, Côte d'Ivoire.
[2]Laboratoire de Chimie Organique et de Substances Naturelles (LCOSN), UFR Sciences des Structures de la Matière et Technologie, Université Félix Houphouët-Boigny, 22 BP 582 Abidjan 22, Côte d'Ivoire.
[3]Chimie des Substances Naturelles, CNRS UMR 8076 BioCIS, Faculté de Pharmacie, Université Paris-Sud 11, 92296 Châtenay-Malabry Cedex, France.
[4]Laboratoire de Pharmacie Galénique, Biopharmacie et Législation Pharmaceutique, UFR des Sciences Pharmaceutiques et Biologiques, Université Félix Houphouët-Boigny, 22 BP 582 Abidjan 22, Côte d'Ivoire.
[5]UFR des Sciences Biologiques, Université Peleforo Gon Coulibaly, BP 1328 Korhogo, Côte d'Ivoire.

Authors' contributions

This work was carried out in collaboration among all authors. Author ANM performed the toxicity tests (OECD 404) and wrote the article. Authors OAT and SVM performed the antibacterial tests. Authors AOA and KAA produced the galenic formulations. Author KALC carried out the OECD 405 toxicity tests and reread the article. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JAMMR/2020/v32i2030680
Editor(s):
(1) Dr. Chan-Min Liu, Xuzhou Normal University, China.
Reviewers:
(1) Ayokunnumi Funke Obajuluwa, , Kaduna State University, Nigeria.
(2) Rosa Lizbeth Aparicio Zambrano, University of Los Andes, Venezuela.
Complete Peer review History: http://www.sdiarticle4.com/review-history/61584

Original Research Article

Received 10 August 2020
Accepted 17 October 2020
Published 07 November 2020

**Corresponding author: E-mail: adikomarc@yahoo.fr;*

ABSTRACT

The goal of this study is to evaluate the antistaphylococcal potential of ophthalmic ointment obtained from the leaves of *Ocimum gratissimum*. Cutaneous and ocular tolerances of methanolic extract of *O. gratissimum* leaves were evaluated according to the Organisation for Economic Co-operation and Development (OECD) 404 and 405 Guidelines. Gentamicin ophthalmic ointment 0.3 % was used as antibiotic reference. *In vivo* antibacterial activity evaluation of the phytomedicine was carried out in rabbits infected by *S. aureus* CIP 4.83. During 15 days, three groups of 6 animals were treated as follows: the excipient (vaseline + liquid paraffin), gentamicin ointment and *Ocimum gratissimum ophthalmic ointment* (OGO). 0, 5% Good cutaneous and ocular tolerances of methanolic extract were observed. The microbial contamination control carried out on the 5th and 240th days showed that the prepared formulation is consistent with the preparation of cleanliness standards for topical application to the European Pharmacopoeia. The subconjunctival injection of *S. aureus* suspension caused mucopurulent acute keratoconjunctivitis with edema, and sometimes eyelid erythema. It is therefore on these ocular observations that therapeutic efficacy of the phytomedicine was evaluated. The treated rabbits without anti-infective agent have developed leucoma, eyelids swelling and conjunctiva hyperemia. Only one panophthalmitis case was observed. From the 1st to 5th days, we observed significant and gradual regression on ocular damages in animals treated with gentamicin. On the 15th day, the eye became normal; the rabbits showed no lesion on cornea. The batch treated with OGO is experiencing a similar trend. The in vivo therapeutic activity of the OGO was similar to that of gentamicin.

Keywords: Ocimum gratissimuml; gentamicin ointment; skin tolerance; conjunctivitis.

1. INTRODUCTION

In traditional ophthalmology, administration of traditional medicines in ocular infections is not always harmless. Many publications have reported eye accidents due to their use. Some authors consider ocular traditional care as harmful practices [1-3] and are among the leading causes of blindness in children. For others, there is an over - estimation of complications due to the lack of studies reporting positive results of traditional treatment of eye diseases [4-6]. According to Goetz [4], practically all essential oil plants having antimicrobial activity, particularly antibacterial activity, can be used as external eye disinfectant, in a suitable pharmaceutical formulation. The use of plant species is a knowledge that must be inventoried and validated by experiments for the well-being of people who use it. This will eliminate the non-medicinal plants and identify the most effective active ingredients for therapeutic purposes.

Ocimum gratissimum Linn (Lamiaceae) is a plant widely used in Ivorian traditional medicine and also serve as a condiment. It is a weed that is commonly found along roadsides but also in pastures, grasslands, agricultural areas. In Côte d'Ivoire, *O. gratissimum* is found in all regions. It is used in the treatment of many diseases such as migraine, stomach cramps, diarrhea, tonsillitis, dermatitis, sinusitis, colds, chicken pox, hypotension, respiratory and eye disorders [7-10]. The phytochemical study of alcoholic extracts of the *O. gratissimum* leaves revealed the presence of phenolic compounds and terpenoids [7-11]. Many authors have demonstrated the antibacterial properties of *O. gratissimum* against Gram-positive Cocci and Gram-negative Bacilli which are: Bacilli which are: *Staphylococcus aureus, Staphylococcus albus, Streptococcus faecalis, Staphylococcus pneumonia, Escherichia coli, Pseudomonas aeruginosa, Proteus mirabilis, Proteus vulgaris, Klebisiella pneumonia, Enterobacter, Morganella morganii, Salmonella typhi, Salmonella enteritidis, Shigella dysenteriae, Shigella flexneri, Shigella sonnei* and *Shigella boydii* [12-17]. Staphylococci, particularly *Staphylococcus aureus* and *Staphylococcus epidermidis*, are the main bacteria involved in eye infections. Infections caused by Gram negative, *Pseudomonas aeruginosa* germs is the most cited [18-19]. *In vitro* and *In vivo* pharmacological essays conducted from *O. gratissimum* concerned mainly antidiarrheal, anti-inflammatory, analgesic and healing properties [20-30]. In the field of the toxicity, we shall note that the aqueous extract is non-toxic and the essential oil used in moderate doses can be tolerated [31-34]. The aim of this study is to produce an ophthalmic phytomedicine (OGO) from the leaves of *Ocimum gratissimum* and evaluate *In vivo*, its antistaphylococcal efficacy on rabbits in which conjunctivitis is

induced by subconjunctival injection of a *Staphylococcus aureus* suspension.

2. MATERIALS AND METHODS

2.1 Plant Material

Leaves of *O. gratissimum* were harvested in November 2008 at Grand-Bassam, Côte d'Ivoire. The plant was identified by Professor Ake-Assi Laurent of the National Floristic Center of Abidjan. A voucher specimen (ANM-OG) was deposited at the Pharmacognosy Laboratory of the Faculty of Pharmaceutical and Biological Sciences, Felix Houphouët Boigny University.

2.2 Drug and Excipients

Gentamicin ophthalmic ointment 0.3% (Chauvin) was used as a reference drug in the experimental study. The conventional excipients, Vaseline (Cooper, lot 08040059/D) and liquid Paraffin (Cooper, lot 08030177/A) were used as well as other local vegetable fats. These vegetable oils were obtained from almonds of *Ricinodendron heudolotii* (Euphorbiaceae) and *Irvingia robur* (Irvingiaceae) by hot extraction. Coconut oil (*Cocos nucifera*, Arecaceae) and shea butter (*Vitellaria paradoxa*, Sapotaceae) were sampled from the market. The palm oil (*Elaeis guineensis*, Arecaceae) was harvested from refinery (Unilever, Côte d'Ivoire). They have been clarified with bleaching earth (Qianhe-QH-070 #).

2.3 Microorganism

Staphylococcus aureus bacterial strain used was obtained from Pasteur Institute (CIP 4.83).

The bacterial suspension was prepared from a single colony on agar Chapman in 10 mL of Buffered Glucose Broth (BGT) and incubated for 4 h at 37°C.

2.4 Animals

The 24 animals used were domestic albino rabbits, male and female (1.62–2.53 kg), from the animal facility of the Faculty of Pharmaceutical and Biological Sciences of the University of Cocody. The rabbits were acclimated for two weeks before their infestation with the *Staphylococcus aureus* bacterial strain.

2.5 Preparation of Plant Extract

Leaves of *O. gratissimum* were dried at room temperature for one month and crushed. A 10 % (w/v) extraction by maceration of the powder in methanol under stirring for 24 hours at laboratory temperature was performed. The extract obtained after solvent evaporation to dryness was placed under vacuum for 24 hours in order to eliminate methanol traces.

2.6 Preparation of Ophthalmic Phytomedicament Ointment (OGO)

Pharmaceutical preformulations were carried out from various fats in order to select adequate excipients. The basic formula was the gentamicin ophthalmic ointment with Vaseline and liquid Paraffin as vehicles in 65:35 ratio. The concentration of active ingredient in the prepared ointment was 0.5 mg/mL according to the MIC of the methanollic extract leaves of *O. gratissimum* against *Staphylococcus aureus* obtained in preliminary essays.

Formula for 100 g ointment formula:

- Active ingredient: Extract of *Ocimum gratissimum*: 0.5 g
- Excipients: Vaseline / Paraffin (65:35): 99.5 g

The method of preparation was carried out as follows: *O. gratissimum* extract was mixed with liquid Paraffin in a turbine and centrifuged at 1200 Trs/min for 1 hour; Vaseline was then incorporated to the supernatant of the turbine for 5 minutes at a speed of 600 Trs/min.

2.7 Rheological Characterization Test

The rheological characteristics of excipients and ointments were determined using a texture analyzer TA-XT2i type monitoring by a computer. The test used was the BACK EXTRUSION RIG. The compression disk linked to the probe had 35 mm diameter. The probe descended into sample at a 2 mm/s speed, and the compression carried out at a speed of 1 mm/s up to 75% of the sample volume which was 20 mL. The compressive force was 0.09 Newton. Curve analysis (strengths/time) allowed to determine the sample texture parameters (firmness, consistency and viscosity).

2.8 Microbial Contamination Control Test

To assess the cleanliness of the ointments produced we referred to the control of microbial contamination of the European Pharmacopoeia entitled: "control of microbial contamination of a

preparation for local application" [Pharm. Eur.6.3].The cleanliness conditions must comply with the following values:

- Enumeration of Total Aerobic Germs <102 / g, in Trypticase Soy Agar (TSA).
- Enumeration of Total Molds and Yeasts <102 / g in medium composed of Sabouraud and Chloramphenicol.
- *Pseudomonas aeruginosa* absence in 1 g, in Cetrimide.
- *Staphylococcus aureus* absence in 1 g, in Mannitol-salt.

The experiment is conducted as follow: in a sterile Erlenmeyer flask, 2.5 g of ointment to be tested was introduced into 22.5 mL of neutralizing solution. After homogenization, a contact time of 30 minutes was observed. From this 1/10 dilution, two dilutions (10^{-1}, 10^{-2}) were made and then seeded in specific media. An incubation of 5 to 7 days at 25°C for yeasts and molds while that of bacteria lasts 18 to 24 hours at 35°C. [35].

2.9 Cutaneous and Ocular Tolerance Tests

The leaves methanol extract of *Ocimum gratissimum* was tested for cutaneous and ocular tolerances according to OECD 404 and 405 Guidelines [36,37]. To perform the skin test, healthy young adult rabbits were used. Approximately 24 hours before testing, the dorsal regions of the animal trunks were sheared flush. A single dose of 0.5 g of the test substance was applied to a portion of their skin (approximately 6 cm2) and covered with gauze, secured with a non-irritating tape, the untreated areas served as control (reference). After 4 hours exposure period, the test substance was removed with water or an appropriate solvent. The tested skin was observed respectively at 1 h, 24 h, 48 h and 72 h after applying the test substance.

Concerning the ocular test, both eyes of each experimental animal were examined 24 hours before starting the test. Animals that showed signs of eye irritation, ocular defects or corneal damage were excluded. An ocular instillation of 0.1 mL, in a single dose of the test substance, was performed into the conjunctival cul-de-sac of one eye of each animal; the untreated eye was used as a control. The eyes were examined respectively at 1 h, 24 h, 48 h and 72 h after applying the test substance. The test substance for ocular tolerance was 30% (w/v) suspension of the extract of *O. gratissimum* leaves and in liquid Paraffin. If the results of these tests indicated that the substance was corrosive or severely irritating the skin and eyes, the experiment was stopped.

2.10 Induced Conjunctivitis

Two infestation types were performed with *S. aureus* CIP 4.83. The first was done by ocular instillation and the second by subconjunctival injection of the bacterial suspension. Prior to injection, an instillation in the eye was made using anesthetic eye drops.

2.11 Statistical Analysis

Data were entered into Excel software and analysed using Graph Pad Prism® 7.0 software.

Dunnett's test allowed comparison of mean values ± standard deviation (SD) by analysis of variance (ANOVA) at risk $\alpha = 0.05$.

2.12 *In vivo* Antistaphylococcal Evaluation

After induction of the conjunctivitis, an amount of ointment, equivalent approximately corresponding to 50 mg, was put into the inferior conjunctival cul-de-sac of the treated eye. The treatment was continued during 15 days, on the basis of one application per day.

3. RESULTS

3.1 Cutaneous and Ocular Tolerances

Cutaneous contact during 4 h of the plant extract did not cause erythema or edema. Similarly, instillation of the suspension of the *Ocimum gratissimum* extract leaves did not cause eye damage (Fig. 1). Cutaneous and ocular applications of the active ingredient were used to record the non-irritant extract. Confirmatory tests performed with additional animals gave similar results. These results showed that there was a good cutaneous and ocular tolerance of the methanolic extract of *O. gratissimum* leaves.

3.2 *O. gratissimum* ophthalmic ointment (OGO)

For the choice of the excipients, *Irvingia robur* was not retained because of the strength of its ointments not in accordance with the reference ointment. The results obtained from shea butter had higher values. The characterization data of the ointments obtained from Vaseline were shown in Table 1.

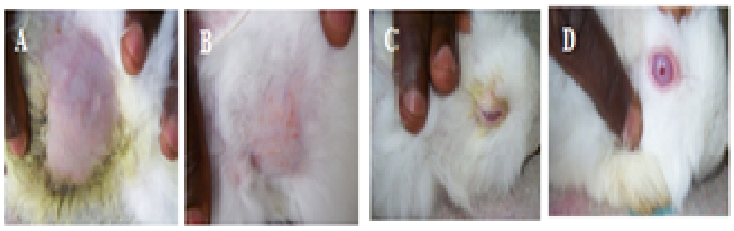

Fig. 1. Skin and eye tolerances test
A: Skin exposed to the treatment after 4h
B: Skin non test (positive test)
C: Eye treated with plant extract suspension
D: Eye aspect after 24 hours

Table 1. Preformulations of 65 % vaseline containing

Oils used	Macroscopic aspect			Texture analysis (in Newton)		
	Stability*	Texture	Color	Firmness	Consistency	Viscosity
Liquid Paraffin	Stable	Soft	White	0.72	- 0,55	- 1,20
Coconut oil	Stable	Soft	White	1.04	- 1,12	- 2.33
Palm oil	Stable	Soft	Light Yellow	0.70	- 0,72	- 1.45
Refinery palm oil	Unstable	Soft	Light Yellow	0.98	- 1,11	-1.87
R. heudolotii oil	Stable	Soft	Lemon	0.60	- 0.56	-1.20

** Stability after 24 hours*

Values of Vaseline/*R. heudolotii* oil and Vaseline/Palm oil ointments were similar to those of the basic formula Vaseline/Paraffin (underlined values). However, due to inability to completely get rid of the smell and color of the R. heudolotii and palm oil ointments as recommended by the European Pharmacopoeia [38,39], the Vaseline and liquid Paraffin were therefore used as excipients in the formulation of *Ocimum gratissimum* ophthalmic phytomedicine (OGO) (Fig. 2). The rheological characteristics of OGO were compared with those of gentamicin ointment. The results (Table 2) showed that the phytomedicine had rheological values close to those of ophthalmic gentamicin ointment.

3.3 Microbial Contamination

The microbial contamination controls of the phytomedicine (OGO) were carried out at D5 (day 5) and D240 (day 240). The number of aerobic bacteria, molds and yeasts were less than 10^2/g. The result of the microbial contamination is described in Table 3. Thus, on D5 and D240 we note an absence of Total Aerobic Germs as well as an absence of *Staphylococcus aureus* and *Pseudomonas aeruginosa.* Therefore, we can conclude that the ophthalmic ointment based on leaves of *Ocimum gratissimum*, complies with the standards of preparation cleanliness for local application of European Pharmacopoeia 6.3.

Fig. 2. *Ocimum gratissimum* ophthalmic phytomedicament (OGO)

Table 2. Rheological characterizations of ointments

Products	Macroscopic aspect			Texture analysis		
	Stability	Texture	Color	Firmness	Consistency	Viscosity
Gentamicin ointment	Stable	Soft	White	0.92	- 0.75	- 1.02
Phytomedicament (OGO)	Stable	Soft	Greenish	1.28	- 1.05	- 1.16

Table 3. Control of microbial contamination on D5 and D240 of OGO

Enumeration of Total Aerobic Germs (ETAG) <10²/g	**TSA**		
	10^{-1}	0	< 10 CFU */g
	10^{-1}	0	
	10^{-2}	0	
	10^{-2}	0	
Total Mold and Yeast Enumeration (TMYE) <10² / g	**Sabouraud + Chloramphenicol**		
	10^{-1}	0	< 10 CFU */g
	10^{-1}	0	
	10^{-2}	0	
	10^{-2}	0	
Search for *Staphylococcus aureus*	**Appearance of the bottle**		**Clear**
	Mannitol-salt	Lack of colonies	Absence of *Staphylococcus aureus* in 1 g.
Search for *Pseudomonas aeruginosa*	Cetrimide	Lack of colonies	Absence of *Pseudomonas aeruginosa* in 1 g.

** CFU = Colony Forming Unit*

3.4 *In vivo* Antistaphylococcal Evaluation

3.4.1 Conjunctivitis experimental

Unlike the ocular instillation of *S. aureus* CIP 4.83 suspension, which gave no ocular reaction, subconjunctival injection of this suspension gave significant damage to the rabbit eyes. The following ocular lesions generated after 24 hours were observed: very abundant muco-purulent secretions (Fig. 3B), conjunctival hyperhemy, significant eyelid edema causing eye closing (Fig. 3C) and cornea edema. It is on these lesions that the activity of the ointments will be evaluated.

3.4.2 Antistaphylococcal activity of ointments

The application of 50 mg of the ointment was performed once per day in the affected eye of the rabbit for 15 days. The results are summarized in Fig. 4 and illustrated in Figs. 5, 6 and 7.

The table of lesion quotations is transcribed in the appendix (Table A).

We observe:

- *: Significant difference compared to control (Excipients) at 5% risk: gentamicin ointment 0.3% (p= 0.0197); *Ocimum gratissimum* ointment 0.5% (p = 0.0197)
- ***: Hight significant difference compared to control (Excipients) at 5% risk: gentamicin ointment 0.3% (p =0.0001); *Ocimum gratissimum* ointment 05% (p = 0.0001)

Indeed, animal batch 1 was treated with excipient underwent severe eye damages. At the cornea, the rabbits developed significant corneal lesions with the leucoma (white spot on the cornea) and neo vessels. A normal cornea had to be transparent and composed of three parts: an epithelium corresponding to the outer part directly exposed to the outside environment, a stroma forming the thickness of the cornea and an endothelium which was the inner layer. A leucoma (Fig. 5A) may be due to a destruction of the corneal epithelium which was caused by trauma of the cornea (wound or burn) or infectious keratitis (herpes or cornea abscess) (Fig. 5A). The appearance of neo vessels (Fig. 5B) in the corneal stroma, normally avascular, was a response to attacks against this organ and mainly after inflammations (blood vessels converge to the injured area).

In addition to corneal lesions, there were the persistence of secretions and conjunctival hyperhemy, edema of the eyelids sometimes accompanied by erythema. One of the rabbits developed the most important lesions, namely a panophthalmitis (Fig. 5C). It was a suppurative inflammation of the entire eye (due to intraocular penetration of bacteria). It caused severe eye pain. Because of the severity of the infection, the animal was killed.

Batch 2 received gentamicin ointment. There was no damage to the cornea (Fig. 6). From D1 to D5, we noted significant regression of conjunctival redness, the inflammation going from intensity 3 to 1, only redness persisted at the injection point. Moreover, we observed a resorption of secretions and a decreased of eyelid edema from 4 to 2 (Fig. 6B). At D15, the eye became normal (Fig. 6C). Concerning the bath 3 which was treated with the ophthalmic phytomedicine OGO, we noted a favorable

ocular manifestation (Fig. 7). At the cornea, from D1 to D15, no corneal damage was observed. The conjunctival hyperhemy, intensity level 3, became 0 after 5 day treatments. Secretions were disappeared at D5. Regression of the eyelid swelling was significant at D7. At D15 (Fig. 7B), the eye had a normal appearance compared to the control eye (Fig. 7C). At D15, we noted no corneal damage. Until D5, secretions, conjunctival hyperhemy and palpebral tumefaction were gradually regressed. From D6, conjunctival secretions were completely resorbed, there was a slight irritation at the injection point that disappeared completely at D15 just like eyelid edema; thus, the treated eye became normal again.

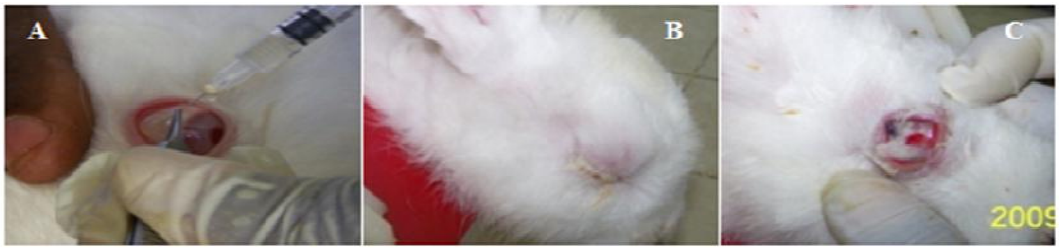

Fig. 3. Conjunctivitis experimental

A: Subconjunctival injection of 0.1 mL suspension of S. aureus
B: Important swelling of the eyelids
C: Abundant purulent secretions

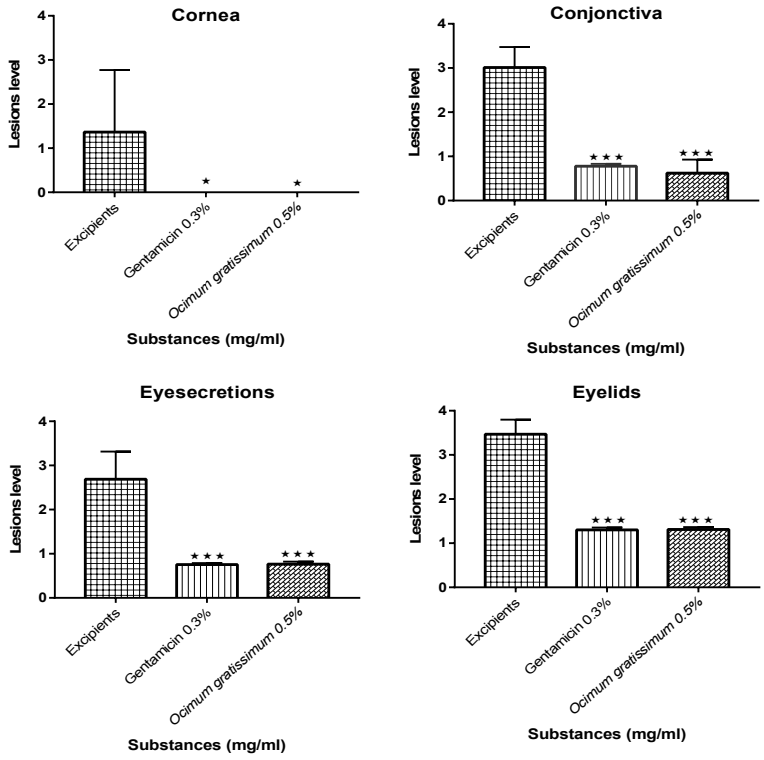

Fig. 4. Dunnett's test. Values expressed as mean ± SEM (standard error of the mean)

The coding for significant statistic difference was as follows:
**: p ≥ 0.05: significant difference*
***: 0.001 ≤ p ≤ 0.01: very significant difference*
****: p <0.001: highly significant difference*

Fig. 5. Batch 1
A: Development of a Leucoma
B: Persistence of eye redness, neovascularization of the cornea
C: Panophthalmitis

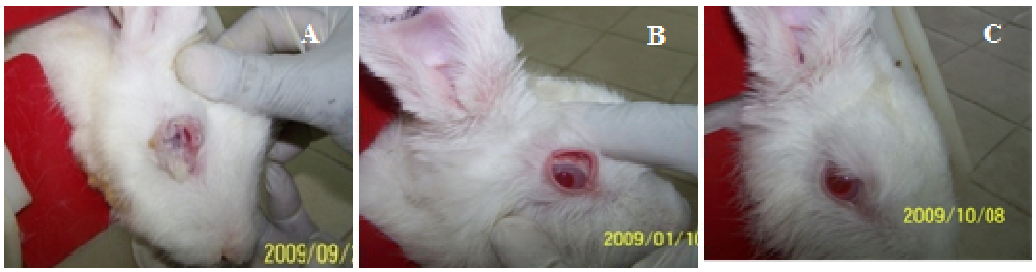

Fig. 6. Batch 2
A: Application of gentamicin ointment
B: Regression significant ocular
C: Appearance of the eye at D15

Fig. 7. Batch 3
A: Application of OGO
B: Appearance of the eye at D15
C: eye witness

These data carried out *in vivo* to infected rabbits by *Staphylococcus aureus* showed a therapeutic efficacy of the ophthalmic phytomedicine OGO similar to that of the gentamicin ointment, the reference drug used.

4. DISCUSSION

The ocular tolerance evaluation represented an essential step for this work. Generally, the assessment of local tolerance of product was based primarily on information on its cutaneous and ocular tolerances. These *in vivo* tests, carried out on rabbits, showed good skin and eye tolerances of the methanollic extract of the leaves of *O. gratissimum.* These data corroborated to those obtained by other authors [40,41,24]. Indeed, healing and anti-inflammatory properties of the plant were evaluated in rats and rabbits in which skin lesions were induced. The application of methanollic extract and essential oil of *O. gratissimum* leaves on the lesions for 10

days showed better healing compared to reference drugs used [27-30]. These encouraging data led to the use of methanolic extract of *O. gratissimum* leaves as active ingredient in this study to produce the eye ointment.

In order to establish an ophthalmic phytomedicine based on local products, galenical preliminary essays were conducted in order to search for excipients from local vegetable oils. These oils were derived from almonds of *Ricinodendron heudolotii*, *Irvingia robur*, *Vitellaria paradoxa*, *Cocos nucifera* and *Elaeis guineensis*. Characterization values of Vaseline/*R. heudolotii* oil and Vaseline/palm oil ointments were close to those of the basic formula Vaseline/Paraffin. These encouraging data require further studies. However, these formulas were not retained because of their poor drug stability and retention. The required quality was its inertia with respect to the active ingredient, the packaging material and the body. To date, we had no data on the inertia of these oils.

Moreover, according to the requirements of the European Pharmacopoeia, the excipients must be free of aromatic compounds and associated undesirable impurities to provide the highest degree of purity (absence of color, smell and taste) [37,42].

The Paraffin, a mixture of hydrocarbons with high resistance to oxidation and rancidity compared to vegetable oils (Oils of *Elaeis guineensis* and *R. heudolotii*), was selected.

Experimental conjunctivitis conducted by ocular instillation of *Staphylococcus aureus* suspension in rabbits did not produce the expected damage. These results corroborated to those of Behrens-Baumann et al. [43]. However, infection by subconjunctival injection of bacteria in creating trauma caused acute mucopurulent keratoconjunctivitis with edema and eyelids erythema occasionally. The obtained ocular staphylococcal blepharo-conjunctivitis and the panophthalmitis appearance were reported [44-49]. Siqueira et al. identified factors responsible for the virulence of *S. aureus* [45]. According to the authors, the leukotoxin with two components, LukD and LukE, can be considered as a major virulence factor of *Staphylococcus aureus*. A study was conducted by the latter on a corneal ulceration model with rabbit eyes that received leukotoxin by intra corneal injection after 24 and 48 hours. An intense ocular inflammation, severe corneal ulcers and a panophthalmitis array were observed. Thus, it was on these ocular manifestations caused by the subconjunctival injection of *S. aureus* that the evaluation of *O. gratissimum* ophthalmic ointment (OGO) in comparison with ophthalmic gentamicin ointment, the reference antibiotic was carried out. An antibiotic is defined by its spectrum activity which corresponds to all bacteria for which it is sensitive. A qualitative comparison of antibacterial spectrum activity can be established between gentamicin, from the pharmacological properties of Chauvin laboratories, and *O. gratissimum*, from antibacterial essays reported in literature [8,12-15,50-52]. Indeed, the antibacterial action of gentamicin, as well as that of *O. gratissimum*, encompassed Gram-positive and Gram-negative pathogen agents. It is important to note that most of the bacteria that caused infections of the anterior segment of the eye responded to local treatment with gentamicin [53-55]. It could be the same for *O. gratissimum* in relation to the *in vivo* evaluation of the antibacterial effect of the obtained ointment.

5. CONCLUSION

The results from this study demonstrated that *O. gratissimum* plant used in eye infections possessed an antibacterial potential. Ophthalmic ointment was prepared from the plant and its *in vivo* therapeutic efficacy was evaluated in rabbits. This experimental approach was used to validate the use in Ivorian traditional medicine of *O. gratissimum* for eye infection treatment. However, to have a close coloration to the conventional eye ointments, the fractionation and the isolation of active principles or the use of the essential oil of the plant could be used for new reformulations.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT

It is not applicable.

ETHICAL APPROVAL

The experimental procedures were carried out according to the ethical guidelines of the Félix Houphouët Boigny University relating to animal resources in Côte d'Ivoire. All procedures performed complied with European Union guidelines and statements regarding the handling and care of laboratory animals [42]. Animal Ethic committee approval has been collected and preserved by the author(s)

ACKNOWLEDGEMENTS

The authors acknowledge Dr Amélie Bochot, Professor at Pharmacy Faculty of Paris XI University, for its assistance in pharmaceutical formulations, Mr Assi Yapo Jean, Botanist technician at Centre National de Floristique, Félix Houphouët Boigny University, for its assistance in plant collection, and Institut Pasteur de Côte d'Ivoire for bacterial strains used.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. OMS. Prévention de la cécité et des déficiences visuelles évitables : Organisation Mondiale de la Santé EB124/7, Genève; 2008.
2. Mwanza JC, Kabasele PM. Complications cornéennes des traitements traditionnels locaux oculaires en République Démocratique du Congo. Méd. Trop. 2001; 61:500–502.
3. Foster A, Sommer A. Cécité infantile par ulcération cornéenne en Afrique: Causes, prévention et traitement. Bulletin de l'OMS. 1987;65:11–16.
4. Goetz P. Phytothérapie en ophtalmologie. Phytothérapie. 1987;4:145–147.
5. Gagnon L. Des remèdes traditionnels pour le traitement des maladies oculaires à Malawi. Ed. CRDI, Ottawa. 2000;3.
6. Géguénon J, Hinsa A. Œil et traitement traditionnel. Rapport scientifique de la Société Ouest Africaine d'Ophtalmologie, 2e congrès, Cotonou. 1995;13–16.
7. N'Guessan K, Kadja B, Zirihi GN, Traoré D, Aké-Assi L. Screening phytochimique de quelques plantes médicinales ivoiriennes utilisées en pays Krobou (Agboville, Côte-d'Ivoire). Sci. Nat. 2009;6:1–15.
8. Oussou KR, Yolou SF, Tue BB, Kanko C, Boti JB, Ahibo C. Étude chimique bio-guidée de l'huile essentielle d' *Ocimum gratissimum* (*Lamiaceae*). Eur. J. Sci. Res. 2010;40:50–59.
9. Ahoussou N, Koffi G, Sangaré A. Rapport de pays pour la Conférence Technique Internationale de la FAO sur les ressources phytogenetiques. Leipzig. 1996; 75.
10. Adjanohoun EJ, Aké-Assi L. Contribution au recensement des plantes médicinales de Côte d'Ivoire. Ed. Centre National de Floristique Abidjan, Côte d'Ivoire. 1979; 250.
11. N'Guessan HAO, Dago CED, Mamyrbékova-Békro JA, Békro YA. Teneurs en composés phénoliques de 10 plantes médicinales employées dans la tradithérapie de l'hypertension artérielle, une pathologie émergente en Côte d'Ivoire. Rev. Génie Ind. 2011;6:55–61.
12. Joshi RK. Chemical composition, *In vitro* antimicrobial and antioxidant activities of the essential oils of *Ocimum gratissimum*, *O. sanctum* and their major constituents. Indian J. Pharm. Sci. 2013;75(4):457–462.
13. Akujobi CO, Ogbulie JN, Njoku HO. The extract of *Ocimum gratissimum* on the dehydrogenase activities to clinical isolates of *Escherichia coli* and *Staphylococcus aureus*. J. Agric. Technol. 2010;6:57–65.
14. Matias EF, Santos KKA, Costa JGM, Coutinho HDM. Light-enhanced antibiotic activity of Brazilian medical plants (*Croton campestris* A*, Ocimum gratissimum* L and *Cordia* Verbenaceae DC). Asian Biomed. 2010;4:183–186.
15. Ahonkhai I, Ba A, Edogun O, Mu U. Antimicrobial activities of the volatile oils of *Ocimum bacilicum* L. and *Ocimum gratissimum* L. (*Lamiaceae*) against some aerobic dental isolates. Pak. J. Pharm. Sci. 2009;22:405–409.
16. Nweze EI, Eze EE. Justification for the use of *Ocimum gratissimum* L in herbal medicine and its interaction with disc antibiotics. J. Altern. Complement. Med. 2009;28(9):37;1–6.
17. Nwinyi OC, Chinedu NS, Ajani OO, Ikpo CO, Ogunniran KO. Antibacterial effects of extracts of *Ocimum gratissimum* and *Piper guineensis* on *Escherichia coli* and *Staphylococcus aureus.* Afr. J. Food Sci. 2009;3:77–81.
18. Sherwal BL, Verma AK. Epidemiology of ocular infection due to bacteria and fungus:

A prospective study. Journal of Medical Education and Research. 2008;10:127–131.

19. Denis F, Ploy MC, Rogez S, Martin C, Mounier M, Gambarotto K. Infections oculaires bactériennes: Méthodes de diagnostic actuelles et prospectives. Ann. Biol. Clin. 1999;57:401–408.
20. Pereira SL, Oliveira JW, Angelo KK, Costa M, Costa F. Clinical effect of a mouth rinse containing *Ocimum gratissimum* on plaque and gingivitis control. J. Contemp. Dent. Pract. 2011;12:350–355.
21. Ashidi JS, Houghton PJ, Hylands PJ, Efferth T. Ethnobotanical survey and cytotoxicity testing of plants of South-western Nigeria used to treat cancer, with isolation of cytotoxic constituents from *Cajan uscajan* Millsp. leaves. J. Ethnopharmacol. 2010;128:501–512.
22. Okoli CO, Ezike AC, Agwagah OC, Akah PA. Anticonvulsant and anxiolytic evaluation of leaf extracts of *Ocimum gratissimum*, a culinary herb. Pharmacognosy Res. 2010;2:36–40.
23. Je-Chiuan Y, Meen-Woon H, Ching-Hung H, Wei-Chien W, Yao-Ching H, Wei-Chun C. Analysis of caffeic acid extraction from *Ocimum gratissimum* Linn. by high performance liquid chromatography and its effects on a cervical cancer celline. Taiwan. J. Obstet. Gynecol. 2010;49(3):266–271.
24. Tanko Y, Magaji GM, Yerima M, Magaji RA, Mohammed A. Anti-nociceptive and anti-inflammatory activities of aqueous leaves extract of *Ocimum gratissimum* (Labiate) in rodents. Afr. J. Tradit. Complement. Altern. Med. 2008;5:141–146.
25. Nangia-Makker P, Tait L, Shekhar MP, Palomino E, Hogan V, Piechocki MP. Inhibition of breast tumor growth and angiogenesis by a medicinal herb: *Ocimum gratissimum*. Int. J. Cancer. 2007;121: 884–894.
26. Okiemy-Andissa N, Miguel ML, Etou AW, Ouamba JM, Gbeassor M, Abena A. Analgesic effect of aqueous and hydroalcoholic extracts of three Congolese medicinal plants: *Hyptis suavolens*, *Nauclea latifolia* and *Ocimum gratissimum*. Pak. J. Biol. Sci. 2004;7:1613–1615.
27. Osuagwu FC, Oladejo OW, Imosemi IO, Adewoyin BA, Aiku A, Ekpo OE. Wound healing activities of methanolic extracts of *Ocimum gratissimum* leaf in Wistar rats a preliminary study. Afr. J. Med. Med. Sci. 2004;33:23–26.
28. Orafidiya LO, Agbani EO, Abereoje OA, Awe T, Abudu A, Fakoya FA. An investigation into the wound-healing properties of essential oil of *Ocimum gratissimum* Linn. J. Wound Care. 2003; 12:331–334.
29. Orafidiya LO, Agbani EO, Iwalewa EO, Adelusola KA, Oyedapo OO. Studies on the acute and sub-chronic toxicity of the essential oil of *Ocimum gratissimum* L. leaf. Phytomedicine. 2004;11:71–76.
30. Rabelo M, Souza EP, Soares PM, Miranda AV, Criddle DN. Antinociceptive properties of the essential oil of *Ocimum gratissimum* L. (Labiatae) in mice. Braz. J. Med. Biol. Res. 2003;36:521–524.
31. George S, Chaturvedi P. A comparative study of the antioxidant properties of two different species of *Ocimum* of southern Africa on alcohol-induced oxidative stress. J. Med. Food. 2009;12:1154–1158.
32. Egesie UG, Adelaiye AB, Ibu JO, Egesie OJ. Safety and hypoglycaemic properties of aqueous leaf extract of *Ocimum gratissimum* in streptozotocin induced diabetic rats. Niger. J. Physiol. Sci. 2006; 21:31–35.
33. Logarto PA, Silva YR, Guerra SI, Iglesias BL. Comparative study of the assay of *Artemia salina* L. and the estimate of the medium lethal dose (LD50 value) in mice, to determine oral acute toxicity of plant extracts. Phytomedicine. 2001;8: 395–400.
34. Aziba PI, Bass D, Elegbe Y. Pharmacological investigation of *Ocimum gratissimum* in rodents. Phytother. Res. 1999;5:427–429.
35. Pharm. Eur. 6.3. Contrôle de la contamination microbienne d'une préparation pour application locale. Pharmacopée Européenne; 2009.
36. OECD. Guidelines for the testing of chemicals. Acute Dermal Irritation/Corrosion. 2002;404.
37. OECD. Guidelines for the testing of chemicals. Acute Eye Irritation/Corrosion. 2002;405
38. AFSSSAPS (Agence Française de Sécurité Sanitaire des Produits de Santé). Recommandations aux fabricants ou aux responsables de la mise sur le marché relatives à l'évaluation de la sécurité pour la santé humaine d'un ingrédient ou d'une combinaison d'ingrédients à usage cosmétique. Ed. AFSSSAPS, France ; 2006.

39. Le Hir A. Pharmacie Galénique: Bonnes pratiques de fabrication des médicaments. Abrégés, 8ème éd., Éd. Masson. 2001;402.
40. Chah KF, Eze CA, Emuelosi CE, Esimone CO. Antibacterial and wound healing properties of methanolic extracts of some Nigerian medicinal plants. J. Ethnopharmacol. 2006;104:164–167.
41. Mahesh SP, Patil MB, Kumar R, Sachin R. Evaluation of aqueous extract of leaves of *Ocimum gratissimum* on wound healing activity in albino wistar rats. International Journal of PharmTech Research. 2009;1(3): 544-550.
42. Louhimies S. Directive 86/609/EEC on the protection of animals used for experimental and other scientific purposes. Altern Laboratory Animals: Atla 2002;30(2):217-219.
43. Behrens-Baumann W, Begall T. Antiseptics versus antibiotics in the treatment of the experimental conjunctivitis caused by *Staphylococcus aureus*. Ger. J. Ophthalmol. 1993;2:409–411.
44. McCormick CC, Caballero AR, Balzli CL, Tang A, Weeks A, O'Callaghan RJ. Diverse virulence of *Staphylococcus aureus* Strains for the Conjunctiva. Curr. Eye Res. 2011; 36:14–20.
45. Siqueira JA, Boteon JE, Do Carmo LS, Prevost G. Étude expérimentale sur le rôle des leucotoxines à deux composés LukE-LukD de *Staphylococcus aureus* dans la genèse des ulcères de la cornée. J. Fr. Ophtalmol. 2009;32(1):126–394.
46. Vancraeynest D, Hermans K, Haesebrouck F. Screening of high and low virulence *Staphylococcus aureus* isolates from rabbits for MSCRAMM. Proceedings of the 8th World Rabbit Congress, Puebla, Mexico. 2004;646–651.
47. Floranceau P. L'œil et les pathologies oculaires chez le lapin domestique. Thèse Université Paul-Sabatier, N° TOU 3 – 4073, Toulouse, France; 2002.
48. Barthelemy P, Monnereau L. Anatomie et pathologie de la tunique fibreuse de l'œil du lapin domestique. Rev. Méd. Vét. 2001; 152:5–28.
49. Floret D. Les toxines du staphylocoque. Méd. Thér Pédiatr. 2000;3:394–399.
50. Baba-Moussa F, Noumavo PA, Adéoti K, Akpagana K. Composition chimique et influence de differents tweens sur le pouvoir antimicrobien des huiles essentielles de *Ocimum gratissimum*, *Ocimum basilicum*, *Laurus nobilis* et *Melaleuca quinquenervia*. European Scientific Journal September. 2016;12(27). ISSN: 1857 – 7881 (Print) e - ISSN 1857-7431.
51. Talabi JY, Makanjuola AS. Proximate, phytochemical, and *In vitro* antimicrobial properties of dried leaves from *Ocimum gratissimum*. Prev Nutr Food Sci. 2017; 22(3):191–194. DOI: 10.3746/pnf.2017.22.3.191
52. Genta Chauvin ;2016. Available :http://documentrcp.vidal.fr/34/e04d97d76aae457cb072c759fe5bb634.pdf
53. Gan IM, Van Dissel JT, Beekhuis WH, Swart W, van Meurs JC. Intravitreal vancomycin and gentamycin concentration in patients with postoperative endophthalmitis. Br J Ophthalmol Nov. 2001;85(11):1289-1293.
54. Agence Française de Sécurité Sanitaire des Produits de Santé. Prophylaxie des infections conjonctivales du nouveau-né; 2010. Available:http://ansm.sante.fr/var/ansm_site/storage/original/application/8d7b81471c088327d5343c5c102feafa.pdf
55. Nehme S, Trouvé O, Renard G. La gentamicine en ophtalmologie. J Fr Ophtalmol. 1980;3:595-605.

.

APPENDIX

Table A. Quotation level of observed ocular lesions

Rabbits	Observation period														
	D1	D1	D 3	D 4	D 5	D 6	D 7	D 8	D 9	D 10	D 11	D 12	D 13	D 14	D 15
Batch 1 : Treatment with excipients															
Rabbit 1															
Cornea	2	2	2	2	2	2	2	2	3	3	3	3	3	3	3
Conjunctiva	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
Eye secretions	3	3	3	3	3	3	3	2	2	2	2	1	1	1	1
Eyelids	4	4	4	4	4	4	4	4	4	4	4	3	3	3	3
Rabbit 2															
Cornea	2	2	Panophthalmitis												
Conjunctiva	3	3													
Eye secretions	3	3													
Eyelids	4	4													
Rabbit 3															
Cornea	2	2	2	2	3	3	3	3	3	3	3	3	3	3	3
Conjunctiva	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
Eye secretions	3	3	3	3	3	3	3	3	3	3	3	2	2	2	2
Eyelids	4	4	4	4	4	4	4	3	3	3	3	3	2	2	2
Rabbit 4															
Cornea	2	2	2	2	3	3	3	3	3	3	3	3	3	3	3
Conjunctiva	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
Eye secretions	3	3	3	3	3	3	3	3	3	3	3	2	2	2	2
Eyelids	4	4	4	4	4	4	4	3	3	3	3	3	2	2	2
Rabbit 5															
Cornea	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Conjunctiva	3	3	3	3	3	3	3	3	3	2	2	2	2	2	2
Eye secretions	3	3	3	3	3	3	2	2	2	2	2	2	1	1	1
Eyelids	4	4	4	4	4	4	3	3	3	3	3	3	3	3	3
Rabbit 6															
Cornea	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Conjunctiva	3	3	3	3	3	3	3	3	3	2	2	2	2	2	2
Eye secretions	3	3	3	3	3	3	2	2	2	2	2	2	2	2	2
Eyelids	4	4	4	4	4	4	3	3	3	3	3	2	2	2	2
Batch 2. Treatment with gentamicin															
Rabbit 7															
Cornea	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Conjunctiva	3	3	2	2	2	1	0	0	0	0	0	0	0	0	0
Eye secretions	3	3	2	2	1	1	0	0	0	0	0	0	0	0	0
Eyelids	4	4	3	3	2	2	1	0	0	0	0	0	0	0	0
Rabbit 8															
Cornea	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Conjunctiva	3	3	2	2	1	0	0	0	0	0	0	0	0	0	0
Eye secretions	3	3	2	2	1	1	0	0	0	0	0	0	0	0	0
Eyelids	4	3	3	2	2	2	1	1	0	0	0	0	0	0	0
Rabbit 9															
Cornea	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Conjunctiva	3	3	2	2	1	1	0	0	0	0	0	0	0	0	0
Eye secretions	3	3	2	2	1	0	0	0	0	0	0	0	0	0	0
Eyelids	4	4	3	3	2	2	1	1	0	0	0	0	0	0	0
Rabbit 10															
Cornea	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Conjunctiva	3	3	2	2	1	1	0	0	0	0	0	0	0	0	0
Eye secretions	3	3	2	2	1	0	0	0	0	0	0	0	0	0	0

Rabbits	Observation period														
	D1	D1	D 3	D 4	D 5	D 6	D 7	D 8	D 9	D 10	D 11	D 12	D 13	D 14	D 15
Eyelids	4	4	3	3	2	2	1	1	0	0	0	0	0	0	0
Rabbit 11															
Cornea	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Conjunctiva	3	3	2	2	1	0	0	0	0	0	0	0	0	0	0
Eye secretions	3	3	2	2	1	0	0	0	0	0	0	0	0	0	0
Eyelids	4	4	3	3	2	2	1	1	0	0	0	0	0	0	0
Rabbit 12															
Cornea	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Conjunctiva	3	3	2	2	1	0	0	0	0	0	0	0	0	0	0
Eye secretions	3	3	2	2	1	0	0	0	0	0	0	0	0	0	0
Eyelids	4	4	3	3	2	2	1	1	0	0	0	0	0	0	0
Batch 3: treatment with OGO															
Rabbit 13															
Cornea	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Conjunctiva	3	3	2	2	1	0	0	0	0	0	0	0	0	0	0
Eye secretions	3	3	2	2	1	1	0	0	0	0	0	0	0	0	0
Eyelids	4	4	3	3	2	2	1	1	0	0	0	0	0	0	0
Rabbit 14															
Cornea	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Conjunctiva	3	3	2	2	1	0	0	0	0	0	0	0	0	0	0
Eye secretions	3	3	2	2	1	0	0	0	0	0	0	0	0	0	0
Eyelids	4	4	3	3	2	2	1	1	0	0	0	0	0	0	0
Rabbit 15															
Cornea	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Conjunctiva	3	3	2	2	1	0	0	0	0	0	0	0	0	0	0
Eye secretions	3	3	2	2	2	1	0	0	0	0	0	0	0	0	0
Eyelids	4	3	3	2	2	2	1	1	0	0	0	0	0	0	0
Rabbit 16															
Cornea	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Conjunctiva	3	3	2	2	1	1	0	0	0	0	0	0	0	0	0
Eye secretions	3	3	2	2	1	0	0	0	0	0	0	0	0	0	0
Eyelids	4	4	3	3	2	2	1	1	0	0	0	0	0	0	0
Rabbit 17															
Cornea	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Conjunctiva	3	3	2	2	1	0	0	0	0	0	0	0	0	0	0
Eye secretions	3	3	2	2	1	0	0	0	0	0	0	0	0	0	0
Eyelids	4	4	3	3	2	2	1	1	0	0	0	0	0	0	0
Rabbit 18															
Cornea	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Conjunctiva	3	3	2	2	1	0	0	0	0	0	0	0	0	0	0
Eye secretions	3	3	2	2	1	0	0	0	0	0	0	0	0	0	0
Eyelids	4	4	3	3	2	2	1	1	0	0	0	0	0	0	0

0: No lesion; 1: mild lesions; 2: moderate lesions; 3: lesions intense; 4: very intense lesions

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sdiarticle4.com/review-history/61584