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Non-Radioactive Therapeutic Effects of Molluscs in Non-Trachomatous Corneal Opacities (Case of Bivalves and Gastropods in the Province of Haut Katanga)

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ABSTRACT

***Aim**: To show the effectiveness of extracts of mollusc shell powder used in the current practice of traditional practitioners in the management of non-trachomatous corneal opacities in the urban-rural population of Haut Katanga due to a shortage of grafts.*

***Material and Method**: Randomized experimental study carried out on the urban-rural population of Haut Katanga from 2011 to 2016 in two corneal opacities therapy centers. The University Clinics of Lubumbashi (control group of patients treated with conventional drugs) and five structures of traditional practitioners (experimental group of patients treated with mollusc shells). The size of the corneal opacity was assessed at the slit lamp in the absence of OCT and the shell extracts were analyzed in the laboratory.*

***Results**: Out of 1244 patients with corneal lesions, 281 patients had corneal opacity: 22.59%. The control group: 183 or 65.1%, the experimental group: 98 or 34.9%. The mean age of the patients was 25.2±15.9 years. Dominant male sex (57.3%). Left eye (52.7%). Visual acuity ≥ 3/10° (38.1%). Corneal blindness (58.4%), trauma (36.3%) and leukoma (deep opacity: 39.9%) and superficial opacity (60.1%). Classical medical treatment (Corticosteroid therapy: 71%, Vitamin B12: 11.5%), treatment with shell extracts (34.9%). The association was non-significant in mean age, sex and affected eye (p>0.05). From 6 to 12 months of treatment, 95.9% with good progress noted for the treatment of shells (RR=4.281; CI: [3.259-5.623]) with regression of the size of corneal opacity from the second month of treatment (p<0.000).*

***Conclusion:** The presence of non-radioactive elements in the shells have an antibacterial, antiseptic, anticancer and antimycotic principle that allowed the regeneration of damaged corneal tissues quickly and optimally. Cyanogenic glycosides are absent in these shells.*

Keywords
Corneal opacities, Bivalve molluscs and gastropods, Classic medical treatment.

Introduction
Corneal opacity accounts for 3.46% of global blindness, 1.65% of global blindness and visual impairment [1]. It is the leading cause of blindness in children [2]. In developing countries, corneal blindness affects all age groups, approximately 80% of cases are preventable [3].

In the DRC, the epidemiology of corneal blindness is less reported. In Kinshasa, the authors showed that 5% of cases concerned corneal problems [4] and 4.9% corneal leukoma [5].

An observation was made on some patients with corneal opacity received in consultation in the ophthalmology department of the University Clinics of Lubumbashi and referred to specialized entities for a corneal transplant or for another adequate measure of adequate management of these opacities. Some of these patients being financially limited confided in traditional practitioners to be treated by them and returned to our institution with a remission of these opacities treated successfully by some traditional practitioners of the place with extracts of powder of bivalve shells (venerupis clam) and gastropods (alamy cyppraca tigris). Contact with these different traditional practitioners met in approximately five centers (Hewa bora in the commune of Kampemba, in the health area of Kipushi: Dilanda towards Kafubu, Kasumbalesa, Mokambo and Sakania). According to these different traditional practitioners encountered during this period, these treatments prove to be effective, but to this day have no scientific basis and are never documented in our circles. Following the information collected from them through patients examined and followed up from the treatment centers of traditional practitioners, which allowed us to discover the beneficial effects of powder extracts of bivalve and gastropod shells which remain uncertain in our circles and have no proof of its effectiveness. Molluscs are vertebrates with a soft body, with a common point a dorsal part at the level of their body called mantle which secretes shells of different shapes depending on the species. This mantle can fold on itself giving a cavity which contains an anus and genitals. Molluscs feed on the calcium and mineral rich paints for the manufacture of their shell (about 4.78 kg / year of consumption) and for the synthesis of tissues. Georges Cuvier 1795 categorized molluscs into 8 classes according to the different existing forms (approximately 50,000 to 120,000 species): solenographs (350), caudofoveates (100), polyplacophores (900), monoplacophores [15], gastropods (103,000), cephalopods (786), bivalves (12,000) and scaphopods (400 species): molluscs with shells live in fresh, salty, warm or cold water [6,7].

The shell is an external skeleton secreted by the mantle. Histologically we have 3 layers at the level of the shell: The Periostracum or cuticle (contains conchiolin, nitrogen). The ostracum or layer of prisms contains argonite), it is necessary for the decoration of the surface of the shell and its growth in length about 2-6mm, about 1mm per month is worth 10% increase in the weight of the shell. The Hypostracum or lamellar or pearly, this layer allows the growth in thickness of the shell, it is discontinuous. The shell has the capacity to decontaminate itself, selects its food and rejects the non-useful for it by filtering the water, it is an indicator of the environment [6]. From the 18th century to the present day, several studies: Figuier, 1840; Baron B., 1855; have proven that molluscs have given several beneficial effects in almost all systems of the body, including some pharmaceutical preparations that have proven their effectiveness (gastropod molluscs (Helix pomatia) since these authors to the present day their acute or chronic toxicity has not yet been demonstrated [6]. Meurnier S., 2007, moreover in his thesis affirmed that gastropod molluscs could be useful in parasitoses (anthelminths) and play a very important role in the environment (can be placed in sewers) [8]. No one is unaware of the benefits that we are the object of in our cultures by using natural sources to treat various pathologies [6].

In general, corneal transplantation remains a reference treatment for corneal opacity. Several institutions treat corneal opacities using this practice, but in the Democratic Republic of Congo (DRC) following the shortage of grafts, the high cost of this adequate care in specialized entities, the difficulty experienced by some patients in being able to access appropriate care (financial barrier for needy families) and the low technical platform to date, pose a problem of care for patients with corneal opacities who remain victims of these blinding and long-term incurable and irreversible pathologies in our environments. Several studies have been published on molluscs in Africa and elsewhere, but none of them to date have reported on a therapeutic approach in eye diseases. To supplement this relative rarity elsewhere, we conducted this study with the aim of showing the effectiveness of powdered extracts of bivalve and gastropod mollusc shells used in the current practice of traditional practitioners for the management of non-trachomatous corneal opacities by analyzing them in the laboratory to search for elements with therapeutic potential contained in these shells and which would be the basis of the regeneration of damaged tissues. We want to make these data available to allow the practitioner of the next day to be able to approach the management of patients with corneal opacities differently in the days to come. However, we believe that treatment with extracts from mollusc shells would be less expensive and beneficial for our population.

Material and Method
Our survey was carried out in two centers for the management of corneal opacities, we have the ophthalmology reference center of the University Clinics of Lubumbashi and five structures of traditional practitioners in the province of Haut-Katanga over a period of 6 years from 2011 to 2016. It should also be noted that the ophthalmology reference center of the University Clinics of Lubumbashi was the monitoring and evaluation center for all patients selected for corneal opacity in this study.

The study is experimental randomized. The sample size was calculated at 281 patients. We included in the study all patients with corneal opacity. Patients with trachoma and dystrophy, the use of extracts of shell powder diluted with palm oil or breast milk were excluded from the study. Two study groups were divided into control groups in the urban environment (ophthalmological reference center of the University Clinics of Lubumbashi where 183 patients received in consultation were treated with available conventional drugs (corticosteroids, vitamin B12 and vitamin A capsule or ointment). The second group is experimental in the rural environment which consisted of 98 patients from the five structures of traditional treatment of corneal blindness by traditional practitioners, this is the structure of Hewa bora in the commune of Kampemba, in the health area of Kipushi: Dilanda towards Kafubu, Kasumbalesa, Mokambo and Sakania) and having been treated with powder extracts of the shells of bivalve molluscs: clam venerupis and gastropods: alamy cyppraca tigris used in the current practice of traditional practitioners. The variables studied were age, sex, visual acuity, affected eye, causes and types of corneal opacities, layers of affected corneal lesions, types of treatment received, types of shells used, patient evolution in 3 treatment phases (≤ 1 month: 1st phase; 2nd month: 2nd phase; 6 months to 1 year of treatment: 3rd phase) and survey settings. The size of the corneal opacity was assessed at the slit lamp in the absence of OCT.

The shell extracts were analyzed in the laboratory in collaboration with laboratory technicians and pharmacists to identify the elements with therapeutic potential contained in these bivalve and gastropod mollusk shells. The data were collected from the records available at the ophthalmology department of the University Clinics of Lubumbashi and from those recorded from patients examined in the five traditional practitioner structures for the traditional management of corneal blindness. The medical team consisted of 2 doctors and 1 nurse. On examination, each patient underwent a complete ophthalmological examination. Based on the interview and clinical examination, an etiology (infectious, traumatic,) was determined, the presence of pain, changes in corneal transparency (edema, scars, new vessels), changes in corneal thickness (thickening, thinning, descemetocele, change in curvature), hypertonia, whether or not corticosteroid therapy was used and this is how all forms of corneal opacities were diagnosed and measured using the slit lamp due to the lack of OCT. The edges (regular, irregular, short-winded), corneal sensitivity were determined, the Seidel test was used (to test for perforation or not) the depth of the corneal lesion (epithelial, stromal, endothelial); the topography of the corneal lesion in relation to the visual axis (central: which occupies the entire pupil, paracentral: which spares the pupillary area, peripheral: which occupies the anterior surface of the limbus) , the number of lesions, the dimensions, the thickness, the size, the location of the opacity, a drop of fluorescein was instilled in search of a corneal lesion and if present, it was identified, measured and classified using the slit lamp. We noted for a corneal opacity superficial any corneal opacity limited to the corneal epithelium and deep any opacity in the subepithelial or in the deep layers of the cornea after the corneal epithelium. Corneal opacity is a corneal opacity ≤ 5 mm in corneal diameter, nephelion goes to ≥ 5 mm and leukoma goes to more than 1 mm reaching the stroma or corneal endothelium. Treatments for patients with corneal opacity had been described (conventional medical or powdered extracts of mollusc shells). The treatment was administered in the form of eye drops or drops, tablets, capsules, dressings or surgery as needed and constituted the classic medical treatment including vitamin A (ointment or capsule), vitamin B12 and corticosteroid therapy depending on the affected corneal layer. Other types of treatments were traditional used by financially limited patients to access better management of this blinding disease. The presence of an inflammatory reaction was also investigated.

The interview with the traditional practitioner(s) was done by interview. He specified how the treatment based on molluscs is done, how does he prepare the medicine? How does he administer it and for how long, how does he follow up? The treatment was administered in the form of powder extracts of mollusc shells mixed either with distilled water used as a solvent or the powder used in its pure state depending on the severity of the corneal lesion. The powder that the traditional practitioner made by pillaging the mollusc shells or scraping the inside or outside of the shell depending on the indication of the corneal lesion (2mg of powder diluted in 3 to 7 ml of distilled water, if the corneal lesion is minimal or not) and was put in the patient's eye 2 to 3 times a day depending on the severity of the corneal expectation. Treatment with mollusc shell powder extracts was used for 2-4 weeks for patients with epithelial opacity or 4 or ≥ 24 weeks if the lesions were extensive or deep to the level of the corneal endothelium. The patients were followed up weekly for at least 6 months. During the follow-up, the patient underwent a routine ophthalmologic examination: measurement of acuity, slit lamp examination to assess the size of the corneal opacity or to assess other corneal lesions. Patients with an active lesion were treated with conventional available drugs.

Apart from traditional practitioners, the work was done in collaboration with local pharmacists and laboratory technicians (the laboratory of the Faculty of Sciences in 2013 and that of the University Clinics of Lubumbashi in 2014 and 2016). With the help of pharmacists and laboratory technicians, the laboratory analysis of extracts from bivalve and gastropod mollusc shells was done in 2 stages including basic bacteriological and physicochemical analysis (using the spectrophotometer) to know the elements with therapeutic potential contained in these mollusc shells.

The bacteriological analysis concerned:

1) First, the step of searching for the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC), which are the 2 concepts to know before any process of searching for an antibiogram in the laboratory for a given substance. The MIC or Minimum Inhibitory Concentration is the lowest concentration of the antibiotic for which bacterial growth is inhibited (no growth of the population; 100% survivors) and the MBC or Minimum Bactericidal Concentration is the lowest

concentration of the antibiotic allowing 99.99% of the bacteria present at the start to be destroyed (i.e. one surviving bacterium out of 10,000). Measuring the MIC makes it possible to determine whether a strain is sensitive or resistant to the antibiotic tested. For each antibiotic, the serum concentrations obtained in the patient (human) were measured in the context of a normal dosage. We then distinguish:

- The strain is said to be resistant: the MIC cannot be reached by treatment using this antibiotic without being toxic to the animal.
- The strain is said to be sensitive: the MIC can be reached by usual treatment carried out using this antibiotic.
- The strain is said to be intermediate: the MIC can only be reached by increasing the doses.

If CMB < 5 MIC, that is to say that the antibiotic is very effective. On the contrary, if CMB > 10 MIC, it is considered to be not very effective. Once the antibiotic power is tested in the shells of the mollusks, it was necessary to move on to the antibiogram to test on culture medium, the action of antibiotic molecules on a bacterial strain, it will therefore give indications on the in vitro effectiveness of these antibiotics.

2) Secondly, the search for antibiogram in the extracts of the shells of the mollusks proceed as follows:
The shells of bivalve molluscs more commonly used than gastropod shells were analyzed in depth in the laboratory for antibacterial activity as follows:
After weighing the powder of the extracts of the shells of bivalve molluscs and gastropods using a precision balance and then mixed with physiological serum at concentrations of 10%, 20%, 30%, 40% and 50% in each test tube (the solution obtained was mixed using the vortex). The antibiogram on Mueller Hinton agar medium had been carried out. Colonies of bacteria were inoculated into the antibiotic discs soaked in the solutions of extracts of these shells of molluscs used by traditional practitioners in their current practice.

The basic physicochemical analysis was carried out using the Ultraviolet-visible (UV-vis) spectrometer. Spectrometry is a device designed to measure the distribution of complex radiation as a function of the wavelength or frequency in the case of waves, the mass or energy of individual particles in the case of particles, it is a dispersion device that separates broadband radiation into wavelengths, it is an absorption spectrometry that allows the analysis of less complex samples in the liquid or gas phase. The spectrometer consists of a dispersive system (prism, grating,), a light source that generates a broad band of electromagnetic radiation from the UV-visible spectrum. The principle of the spectrometer is to measure the intensity of the light it receives, once it has passed through a transparent container (the material must be adapted to the wavelength), containing the solution [9].

The patient was informed and gave his consent to participate in the study (obtained before filling out the forms kept anonymous). The data were entered by Excel 2016 on the basis of the data, they were analyzed with IBM SPSS software version 23. The dispersion parameters calculated were the mean and the standard deviation, the association between qualitative variables was evaluated with the chi 2 test, the student test was used to compare the means regarding the association of a qualitative variable and a quantitative variable. The significance threshold was set at 5% (p-value < 0) with its 95% CI, the cohort test was used to determine the treatment efficacy.

Results

Relative frequency of corneal opacities and sociodemographic and clinical characteristics of patients

Frequency of Corneal Opacities

Of 31,038 subjects examined, 1244 patients had corneal involvement, i.e. 4% of all corneal blindness: 963 patients had an active corneal lesion, i.e. 77.41%: corneal ulcer was the leading cause in 29.39% followed by keratitis with 17.55% (Table 1) and 281 patients had corneal opacity, i.e. a frequency of 22.59% (the control group in the urban environment: n=183, i.e. 65.1% and the experimental group in the rural environment: n=98, i.e. 34.9%).

Table 1: Distribution of patients according to other corneal lesions.

Other corneal lesions	Frequency	%
Limbal thickening	120	12, 46
Epibulbar tumor operated Pterygium operated	30 62	3, 12 6, 44
Keratouveitis	56	5, 81
Corneal edema	105	10, 90
Keratitis (bacterial, fungal)	169	17, 55
Corneal abscess	71	7, 37
Corneal ulcer	283	29, 39
Viral keratitis (Herpes simplex)	38	3, 95
Wound	29	3, 01
Total	963	100

Sociodemographic and Clinical Characteristics

The mean age was 25.2±15.9 years, patients aged 21 to 30 years were in the majority with 26% (n=73/281 patients). The age extremes were 1 year and 70 years. The sex ratio M/F was 1.3. The right eye was more affected in 52.7% (n=148); Most patients in 38.1% of cases had VA ≥ 3/10°, poor vision was 25.3%; light perception: 17.1% and nil vision: 11%. Trauma was the cause of remarkable corneal opacity in 36.3% and leukoma was the leading cause of corneal opacity in 39.9% of cases. More than half of the patients or 60.1% (n=169) had superficial corneal lesions (Table 2).

Evolution of Patients and Virtues of Treatment with Mollusc Shells

We noted a good evolution in 135 patients or 48% (135/281) of patients. There were 95.9% with good evolution in the experimental group (patients who had been treated with shells) from 6 to 12 months of treatment compared to those who had received the classic treatment (RR = 4.281; CI: [3.259-5.623]) against 22.4% in the control group (classic treatment). The association was not significant between the type of treatment and the evolution of patients remarkable at less than 1 month of treatment.

Table 2: Sociodemographic and clinical characteristics.

Features	Frequency	%
Age groups	average: 25.2±15.9 years	
≤ 10 years	53	18.9
11 to 20 years old	68	24.2
21 to 30 years old	73	26
31 to 40 years old	33	11.7
41 to 50 years old	30	10.7
> 50 years old	24	8.5
Sex	sex ratio: M/F 1.3.	
Female	120	42.7
Male	161	57.3
Eye affected		
Right eye	133	47.3
Left eye	148	52.7
Visual acuity before PEC		
≥ 3/10°	107	38.1
≤ 1/10 (MM, CD)	71	25.3
PL	48	17.1
No	31	11
Not determined	24	8.5
Causes of opacities		
Burn	6	2.1
Foreign body	5	1.8
Corticosteroids	40	14.2
Insects	4	1.4
LCET stage 4	79	28.1
Trauma	102	36.3
Other causes	37	13.2
Not determined	7	2.5
Types of corneal opacities		
Leukoma	112	39.9
Nephelion	58	20.6
Pillowcase	36	12.8
Affected layers		
Deep lesions (leukoma)	112	39.9
Superficial lesions	169	60.1

Types of Treatments

Table 3: Types of treatments received.

Treatments received	Effective	Percentage
Conventional medical treatment		
Corticosteroids	130	71
Bandage +ATB+ATV+ATF	16	8.7
LIVE A	16	8.7
VIT B12	21	11.5
Shell treatment		
Bivalve	87	88.8
Gastropod	11	12.1

Types of treatments and sociodemographic and clinical characteristics of patients

Table 4: Distribution of patients according to sociodemographic and clinical characteristics according to types of treatment.

Features	Conventional medical treatment	Treatment with shell extracts	p-value
Mean age ± standard deviation	26.4 ±15.9	23.1 ±15.9	0.099
Sex (%)	**SR M/F: 1.3**	**1.5**	
Female	81 (44.3)	39 (39.8)	0.471
Male	**102 (55.7)**	**59 (60.2)**	
Eye affected (%)			
Right eye	79 (43.2)	54 (55.1)	0.056
Left eye	104 (56.8)	44 (44.9)	
Visual acuity (%)			
AV ≥ 3/10°	79 (43.2)	28 (37.8)	
B AV ≤ 1/10 (MM, CD)	49 (26.8)	22 (29.7)	0.000
PL	35 (19.1)	13 (17.6)	
No	20 (10.9)	11 (14.9)	
Causes of opacities (%)			
Burn	4 (2.19)	2 (2.04)	
Foreign body	0	5 (5.10)	
Corticosteroids	23 (12.57)	17 (13.35)	
Insects	0	4 (4.08)	0.000
LCET stage 4	71 (38.79)	8 (8.16)	
Trauma	59 (32.24)	43 (43.88)	
Other causes	21 (11.48)	17 (17.35)	
Indeterminate	5 (2.73)	2 (2.04)	
Opacities types (%)			
Leukoma	57 (31.1)	**55 (56.1)**	0.000
Nephelion	32 (17.5)	26 (26.5)	
Pillowcase	23 (12.6)	13 (13.3)	
Layers affected (%)			
Deep lesions (subepithelial)	57 (31.1)	**55 (56.1)**	0.000
Superficial lesions (epithelium)	**126 (68.9)**	43 (43.9)	

Legend: *Other causes: in utero use of chloramphenicol, history of measles, dry eye due to facial paralysis (lagophthalmos), ophthalmic zoster, superficial punctate keratitis, use of medicinal plants.
*Trauma (tree hit, spoon hit, knife hit).

Comparing the regression of the size of the lesions according to the evolution in the different phases of treatment in the two groups (classic treatment and shell treatment), we found that at the first month of treatment, there was no significant difference ($p = 0.341$). At two months of treatment (2nd phase), the regression of the size of the corneal opacity compared to the type of treatment was remarkable ($p < 0.000$). The number of patients with corneal lesion size ≤ 0.5 mm increased from 36 (23 for conventional treatment and 13 for shell treatment) at the first month, to 91 (27 patients or 14.8% for conventional treatment and 64 patients or 65.3% for shell treatment) at the second month with a gain of 55 patients (among whom 4 patients or 2.2% had benefited from conventional treatment and 51 patients or 52% for shell treatment). From 6 to 12 months of treatment (3rd phase), the association was very significant ($p = 0.000$) between the type of treatment and the regression of lesion size. The number of patients with corneal lesion size ≤ 0.5 mm increased from 36 in the first month to 135

Table 5: Distribution of patients according to corneal lesion size and treatment evaluation phases.

Lesion size	Healed		Not cured		Gain		P
1st month	shell	medical	shell	medical	Shell	medical	
≤0.5 mm	13(13.27)	23(12.57)	0	0	0	0	0.341
0.5 to 1 mm	0	0	28(28.57)	68(37.16)			
>1 mm	0	0	57(58,16)	92(50.27)			
2nd month					51(52.04)	4(2,19)	0.000
≤0.5 mm	64(65.31)	27(14.75)	0	0			
0.5 to 1 mm	0	0	5(5,10)	66(36.07)			
>1 mm	0	0	29(29.59)	90(49.18)			
6 to 12 months					81(82.65)	18(9.84)	0.000
≤0.5 mm	94(95.92)	41(22.40)	0	0			
0.5 to 1 mm	0	0	0	55(30.05)			
>1 mm	0	0	4(4.08)	87(47.54)			

(including 41 patients or 22.4% for conventional treatment and 94 patients or 95.9% for shell treatment) patients between 6 and 12 months, a gain of 99 patients (among whom 18 patients or 9.8% had received conventional treatment and 81 patients or 82.7% had received shell treatment) (Table 5).

Laboratory analysis of mollusc shell powder extracts from 2013 to 2016

Basic physicochemical analysis by UV-vis spectrometer of powder extracts from the shells of bivalve and gastropod molluscs from 2013

Table 6: Organic and inorganic substances found in mollusc shells after laboratory analysis.

	Organic substances	Non-organic substances
Present	Tannins	Carbon in the form of Calcium Carbonate, Cobalt, Copper, Manganese, Selenium, Zinc, Cadmium, Chromium, Lead, Sodium, Magnesium, Potassium, Calcium, Fe I, Fe II, Fe III (Iron in its different oxidation forms).
Absent	Saponin, Flavonoids, Anthocyanins, Steroids, Terpenoids, Alkaloids, Cyanogenic heterosides	

We noted that the powder extracts of bivalve and gastropod mollusc shells treated in the laboratory are rich in organic and inorganic substances, we have selected only the substances with therapeutic potential useful in ophthalmology represented in this table.

In vitro analysis of bacterial activity of powder extracts from mollusc shells bivalves and gastropods in 2014-2016

Table 7: Distribution of CMI and CMB significance in bivalve and gastropod shells.

Powder shells	Concentration in mg/ml	CMI	CMB
Bivalve	100	1/32	0
Gastropod	100	1/16	0

We proceeded to calculate the minimum inhibitory and bactericidal concentration in search of the antibiotic power existing or not in the powder extracts of the shells of the mollusks before making the antibiogram. The observation was that the MBC is 0.03125 for the bivalve shell and also 0.0625 for the gastropod shell from where the powder extracts of two shells of the mollusks have a very effective antibiotic power.

Table 8: Antibacterial activity spectrum of powder extracts from mollusc shells.

Mollusc shell powder extracts	Bacteria	
	Gram +	Gram -
Bivalve	+++	+++
Gastropod	+++	+++

After weighing the powder of the extracts of the shells of bivalve molluscs and gastropods using a precision balance and then mixed with physiological serum at concentrations of 10%, 20%, 30%, 40% and 50% in each test tube (the solution obtained was mixed using the vortex). The antibiogram on Mueller Hinton agar medium had been carried out. Colonies of bacteria were inoculated into the antibiotic discs soaked in the solutions of extracts of these mollusc shells. The observation was that the powder extracts of the mollusc shells show bacterial activity for Gram-positive (Staphylococci aureus) and Gram-negative (Escherichia coli) bacteria. The greater the dilution (at 10%), the broader the spectrum of antibiotic activity and if a high concentration of the product (50%), the more destruction of the ocular flora occurs.

Discussion and Comments

Frequency of corneal opacities

Corneal lesions in our series represented 4% of all patients examined, among which corneal opacities concerned 281 patients or 22.59%. In a study published in 1997 by Kaimbo et al. [4], corneal lesions had a number that varied between 4.5% to 5%, this percentage is close to ours, on the other hand Prabhasawat et al. [10] revealed in their study at Siriraj Hospital in Thailand that the prevalence of the causes of corneal blindness was 1.7%, this percentage is lower when compared to the figure reported in our series. In this same study women were more affected than men respectively 53.7% and 36.3%, Anas et al. [11] in their study on corneal blindness in Morocco also found a female predominance of 58% unlike our study where the sex. M/F ratio was 1.3 in the control group versus 1.5 in the experimental group in favor of men in all patients with corneal opacity. In the same study concerning Anas et al. [11], the mean age of the patients was 36 years [7-83], the origin was rural in 64% of cases and urban in 36%, bilateral blindness in 62%, the main causes of blindness were dominated by the sequelae of infectious keratitis considered as the first cause of corneal blindness in 22%, in our series, the urban environment had a population of 65.1% and the rural environment 34.9%, the patients had a mean age of 26.4 years in the control group of the urban environment and 23.1 years in the experimental group of the rural environment, there was no significant difference between the two study groups with regard to the mean age, sex and the affected eye (p=0.099). Trauma was the primary cause of corneal opacity in 36.3% followed by stage 4 LCET with 28.1%. The results of Chenge Borasisi et al. [12] in their 2003 study on limboconjunctivitis showed that out of 422 children examined, 139 children had LCET in its mixed form in 38% of cases and also its bulbar form in 3%, our results corroborate with those of Chenge et al. [12] in the mixed form and a little different in the bulbar form; in the same, keratitis had 1.9% and trauma 9.2. Among other causes of corneal blindness, bacterial and fungal keratitis had 17.6% and keratitis viral 3.95% (herpes simplex). Corneal leukoma was the most remarkable corneal opacity in 39.9% of cases, it represented 31.1% of cases in the control group against 56.1% in the experimental group (p = 0.000), nephelion came in second position with 20.6% of cases. Our results more or less agree with those of Anas et al. [11] in relation to the average age, sex and numbers found by considering the origin of the patients inversely. However, a difference in the proportions found was more noted in the causes of corneal opacity and blindness. According to the study of Kayembe L. [5], corneal leukomas represented 4.9%, there is a large gap in proportion between what the previous author found, this can be explained by the fact that a disease can evolve in time and space by the change of many demographic and clinical parameters.

According to Whitcher et al. [13], trauma and ulcerations were the important causes of corneal blindness that are often underreported but may account for 1.5-2.0 million new cases of unilateral blindness each year. Ukety TO [14] in 1991 in his study reported 2 cases of corneal ulcer associated with ulcero-erosive blepharitis and 6 patients who had a dendritic ulcer; Ngoie Maloba V et al. [15], proved in 2018 on 380 patients with ulcer a frequency of 0.85% of cases, these authors add that the average age of the patients was 38.67 years, patients aged over 40 were in the majority with 19.2%, children aged 0-5 years represented 8.5% and those aged 6-10 years had 10.3%. Our series reported a predominance in the age group of 21 to 30 years with 26% of cases and those under 10 years had 18.9% and corneal ulcers had 29.39%. Furthermore, Ezegwui [16] reported in his study on ulcers that apart from trauma, indigenous treatment was incriminated in 19.5%. The same author further states that 71.4% had visual acuity of the affected eye of 3/60 and 4 eyes or 6.2% had deteriorated visual acuity and that women were more affected than men respectively in 53.7% and 36.3%. Our study showed that the right eye was more affected with 55.1% in the experimental group and the left eye in the control group with 56.8% (p = 0.000). Most patients in 38.1% of cases had visual acuity ≥ 3/10°, corneal blindness was noted in (58.4%). The category of other causes in our study: in utero use of chloramphenicol, history of measles, dry eye due to facial paralysis (lagophthalmos), ophthalmic zoster, superficial punctate keratitis, use of medicinal plants represented 28.83%.

Ocular surface burns, often of chemical origin, have a high prevalence among ophthalmology emergencies, representing 7.7% to 18% of ocular trauma and causing conjunctival, corneal and limbal damage that varies depending on the nature of the agent involved, its concentration in the eye and the speed and quality of care. The most severe sequelae include limbic insufficiency syndrome, which includes extensive limbic ischemia affecting between one third and one half of the circumference following anterior segment surgery (pterygium, cataract and others). In industrialized countries, they mainly affect young men 66.7 to 86% [19]. Similarly, Sridhar et al. [17] reported 3 cases of ICSL in patients who underwent iterative ocular surface surgeries for the following pathologies: recurrent pterygium, perforated ulcer, and grafted keratomycosis. The main complication of pterygium surgery is the high risk of recurrence during the first postoperative year since 97% of pterygium that recur do so during this period [18]. Our series reported that burns represented 2.19% in the control group and 2.04% in the experimental group and that in other corneal blindness, limbal thickening was represented in 12.46%, operated pterygium 6.44%, and operated epibulbar tumor 3.12%.

Types of Treatments for Corneal Opacity

In most low- and middle-income patients, the most common acute and blinding corneal condition requiring intervention is microbial keratitis. This is often preceded by mild ocular trauma. If antibiotic prophylaxis is not given promptly after ocular trauma, infection may develop. In temperate climates, most infections are bacterial in origin. In contrast, in tropical regions, fungal keratitis is more common, accounting for about half of all cases of infectious keratitis [19]. Jihad S et al. [20], in their study of severe corneal ulcers in 74 patients hospitalized in the ophthalmology department, treatment was with broad -spectrum antibiotics or topical and systemic antibiotics combined with symptomatic treatment. The outcome was favorable in only 40 patients.

The management of corneal opacities includes several current methods according to one author to another [3,21]: human transplantation from a deceased donor practiced since 1887, this method has the disadvantage of a high risk of rejection and HIV infection, hence there is a shortage of grafts. The graft can be transfixing if it is total and lamellar if it is partial (corneal substitutes, regeneration is carried out from the collagen of the stroma or stem cells of the limbus, have an unlimited tissue renewal power by stimuli during a corneal lesion). Synthetic grafting is used from biological supports especially in severe lacrimal dysfunctions, ocular surface diseases. Autograft and amniotic membrane grafting, manual epitheliectomy and therapeutic laser photoablation. Other antioxidant drugs: vitamin A and vitamin C [25], our study however reported other antioxidants contained in mollusc shells in the form of mineral substances: Zinc, Copper and Selenium.

Laboratory Analysis of Mollusc Shell Powder Extracts

In view of all the above, we note that the MIC is 0.03125 for the bivalve shell and also 0.0625 for the gastropod shell proving that both shells have a very effective antibiotic power. The antibiogram on Mueller Hinton agar medium had been carried out and showed that the powder extracts of the shells of bivalve and gastropod molluscs showed bacterial activity for Gram positive (Staphylococci aureus) and Gram negative (Escherichia coli) bacteria and that these substances were endowed with a broad spectrum antibacterial principle at a high dilution of the powder extracts of the shells of bivalve and gastropod molluscs. Jihad S et al. [20], in their study on severe corneal ulcers, carried out a bacteriological study by corneal scraping before treatment and they demonstrated in the laboratory pneumococcus in 7 patients, klebsiella in 4 cases and alcaligermes xylosolidens in a single patient. Also noted in our series that the powder extracts of bivalve and gastropod mollusc shells analyzed in the laboratory were rich in non-radioactive organic and inorganic substances, however we selected the elements with therapeutic potential useful in ophthalmology.

The organic substances found by chemical screening were tannins which are plant molecules or amorphous substances very widespread in the wood, bark, leaves and/or roots of many plants having antimycotic properties with a bactericidal effect on staphylococci according to the NF standard in 1040 and Escherichia coli according to the NF standard in 1276. They delay the aging process, eliminate dead cells, have a natural capacity to precipitate proteins, take the place of starch by preventing the catalytic action of amylase in herbivores by interacting with digestive enzymes to inhibit them and are also used in the tanning of fabrics. Tannins are part of the phenol family (hydroxybenzene or carbolic acid, it is an aromatic hydrocarbon composed of a phenyl nucleus and a hydroxyl nucleus) which are the first antiseptic and a solvent used for the treatment of nails, in allergology, in the biochemical defense against microorganisms and fungi in certain species, useful for the manufacture of many plastic products, paint, synthetic fabrics, pesticides, and certain pharmaceutical products, present in disinfectants in general, anesthetic and antiseptic, superficial burns, the manufacture of salicylic acid, chlorphenols , picric acid and the preservation of meat [23]. According to a study by Ngom M [24] during clinical trials, noted that by administering the decoction of nep nep as a mouthwash three times a day to HIV-positive patients with oral-esophageal candidiasis, an 86.4% reduction in candida albicans colonies. However, mouth swabs were always positive for culture. On the other hand, Atefeibu Essodina Solim I [25] noted in his work that with the solid medium dilution method, the decoction inhibited the growth of Gram + and Gram - strains, but had no action on the candida albicans strain. The liquid medium dilution method, which is a method for searching for substances with antibiotic properties, the author noted that the decoction had no action on Gram - strains and also on candida albicans. Only Gram + bacteria were sensitive.

The inorganic substances found were Carbon in the form of Calcium Carbonate, Cobalt, Copper, Manganese, Selenium, Zinc, Cadmium, Chromium, Lead, Sodium, Magnesium, Potassium, Calcium, Fe I, Fe II, Fe III (Iron in its different oxidation forms). The ophthalmological role of minerals existing in mollusc shells [26]: Low dose Zinc is an antioxidant and maintains normal ocular function by stabilizing the photoreceptor membrane of the retina by interacting with taurine and vit A; protection against the occurrence of cataracts by its antioxidant effect and against corneal opacities (healing and antiseptic).

Manganese: repairs the structure and function of the eye, especially retinal cells, using mitochondrial superoxide dismutase.

Copper: anti-inflammatory, antioxidant and anti-infectious; it regulates circulation, IOP and fights against eye disorders and cell aging, promotes immune defense.

Calcium Carbonate: heals the eye. Cobalt and selenium: fight against increased IOP, free radicals and treat cancers.

Other studies [27] have shown that the chemical contents of copper in mussels (Mytilus californianus) collected in port areas are 10 times higher than those found in samples taken outside ports, and for other metals Nickel = < 0.8, Silver = 1, Cadmium = >1.6 to > 3.2, Zinc = 2.7 to 4.1, Chromium = 1 to 6.7, Lead = 3.5 to > 14, Tin = 2.6 to > 18, the difference in content in mollusc shells can be explained by the fact that the influence of organotins used in antifouling paints in aquatic environments can contaminate marine organisms, this contamination is higher in ports than in external areas according to Young et al. [28], hence it is important to note that when using indicator species of molluscs, the absence of toxicity in some indicator species is known to be more than in other species in the shellfish growing area. It is then appropriate to verify this correlation for each species of molluscs and for each group of toxins before retaining a species of mollusc as an indicator for a shellfish growing area. The composition of the shell generally includes and depending on the species: minerals or macroelements, microelements or mineral salts, calcium carbonate represents 89 to 95%. Magnesium, Selenium, Copper, Zinc, Aluminum, Iron, Lead, Cadmium, Chromium, Nickel, Cobalt, Sodium are also present in the shell, Vitamins: A, C, E, fatty acids: omega 3, proteins: chitin and conchiolin (argonite, calcite, etc.). It has been described that the shell is rich in minerals or macroelements, in microelements or mineral salts these are calcium carbonate (89 to 95%), Magnesium, Selenium, Copper, Zinc, Aluminum, Iron, Lead, Cadmium, Chromium, Nickel, Cobalt, Sodium, Vitamins A, C, E, fatty acids: omega 3 and proteins: chitin and conchiolin (argonite, calcite ...). According to the standards or guidelines set by the WHO) for metals in the body are for Aluminum 0.2 mg / l, Arsenic: 0.01 mg / l, Cadmium: 0.003 mg / l, Chromium: 0.05 mg / l, Copper: 2 mg / l, Lead: 0.01 mg / l, Nickel: 0.07 mg / l and Selenium 0, 01 mg / l.

Conclusion

Corneal opacities are one of the causes of visual impairment and blindness in our environment. Due to the shortage of grafts in the Democratic Republic of Congo, another treatment alternative to extracts of bivalve and gastropod mollusc shells used successfully in our environment by traditional practitioners has been very beneficial for needy families with limited financial resources for adequate care for corneal transplants in specialized entities.

Mollusc shell powder extracts give satisfactory results after at least two months of treatment if the lesions are superficial and 6 to 12 months of treatment if the lesions are deep. Laboratory analysis of mollusc shell powder extracts sufficiently proves to us that the shells of bivalve and gastropod molluscs have antibiotic, anti-inflammatory, antiseptic, antifungal, anticancer, antioxidant, antiallergic and healing properties. The symbiosis of all the non-radioactive substances existing in these shells would be responsible for the regeneration of damaged corneal tissues quickly and optimally. Cyanogenic glycosides are absent in these shells; hence the use of these treatments would be less expensive and beneficial for our population. The mollusk and its shell always keep a part of mystery, this perhaps conceals other useful properties for future medicine, and even if this were not the case, the recent discoveries about this small animal prove at least that it was not useless to be interested in it.

References

1. Courtright P, Lewallen S. Ocular manifestations of leprosy. In: Johnson G, Minassian D, Weale R, West S (eds) The Epidemiology of Eye Disease Arnold: London. 2003; 306-317.
2. Solomon A. The State of the World's Sight. Vision 2020: The Right to Sight 1999-2005, Geneva: World Health Organization. WHO Press. 2005.
3. Gain P, Rémy Jullienne, Zhiguo He, et al. Global survey of corneal transplantation and eye banks. JAMA Ophthalmol. 2015.
4. Kaimbo wa Kaimbo D, Missotten L. Eye diseases and the causes of blindness in the southwestern Equator (equatorial ferest) in Zaire, data from an eye camp in three rural centers. Bull Soc Belgian Ophthalmol. 1997; 265: 59-65.
5. Kayembe L. Common causes of blindness in Zaire. Br Ophthalmol. 1985; 69: 389-391.
6. Pongombo C. Introduction to Negro-African medicine, lecture notes for 3rd year medical doctoral students, academic year. 2011-2012; 3-20.
7. http://hujtresandco.over-blog.com/categorie-988232.html
8. Meunier S. Molluscs of veterinary interest, state of the question in 2006, Thesis no. 3328/1, University of Limoges, Faculty of Pharmacy, medical pharmacy section, Vienna. 2007; 1-98.
9. Di Benedetto D, Breuil P. Principles of absorption spectrophotometry in the ultraviolet and visible, Techniques de l'ingénieur. 1987.
10. Prabhasawat P, Trethipwanit KO, Prakairungthong N, et al. Causes of corneal blindness, a multicenter retrospective analysis. Thai J Med Assc. 2007; 90: 2651-2657.
11. Anas Oulmodi, Mohamed Baali, Yassine Maslik, et al. Rayad: Blindness of corneal origin in Morocco from 2012-2017, Media Library l SFO-online - French Society of Ophthalmology. 2017.
12. Chenge B, Makumyaviri AM, Kaimbo wa Kaimbo D. Endemic limbo-conjunctivitis of the tropics in Lubumbashi, Democratic Republic of Congo, Bull. Soc. Belge Ophtalmol. 2003.
13. Whitcher P, Srinivasan M, Upadhyay MP. Corneal blindness: a global perspective. Bulletin of the World Health Organization. 2001; 79: 214-221.
14. Ukety TO, Maertens K. Ocular ulcerative herpes following measkes in Kinshasa, Zaire. Curr Eye Res.1991; 10: 131-137.
15. Ngoie Maloba V, Ngayuwa Nkiene J, Tunku Kabamba G, et al. Frequency of corneal ulcer in about 380 cases: retrospective study carried out in two hospital centers in DR Congo. Journal Français d'Opthalmologie. 2018; 41: 57-61.
16. Ezegwui IR. Corneal ulcers in a tertiary care hospital in Africa. J Natl Med Assoc. 2010; 102: 644-646.
17. Shridar MS, Bansal AK, Sangwan VS, et al. Amniotic membrane transplantation in acute chemical and thermal injury. Am J Ophthalmol. 2000; 130: 134-137.
18. Hirst LW. The treatment of pterygium. Surv ophthalmol. 2003; 48: 145-180.
19. Matthew J. Burton. Corneal blindness. Community Eye Health. 2009; 22: 33-35.
20. Jihad S, Ouarach N, Madaidou N, et al. 371 Epidemiological profile of severe corneal ulcers. About 74 cases, LISSA; Scientific Literature in Health. 2015.
21. Gatinel D, Nghiem M, Chaine G. Early limbus autograft after alkaline burn of the ocular surface. J Fr Ophthalmol. 2004; 88: 1280-1284.
22. Nakamura T, Inatomi T, Sotozono C, et al. Transplantation of cultivated autologous oral mucosal epithelial cells in patients with severe ocular surface disorders. Br J Ophthalmol. 2004; 88: 1280-1284.
23. Mamadou B. Pharmacological actions of tannins, cited 2, Doctoral thesis in pharmacy. 2002; 2-57.
24. Ngom NM. Randomized clinical trial of Acacia niloticaversus fluconazole in the treatment of oral-oesophageal candidiasis in people living with HIV/AIDS in Dakar Thes. Pharm. 2009.
25. Atefeibu Essodina Solim I. Contribution to the study of tannins and antibacterial activity of Acasia nilotica var adansonii, Doctoral thesis. Pharm. 2002.
26. Kanku mvita. Identification, dosage and isolation of some isoprene compounds in the flowers of Ceiba Pentan-Tandra Gaertn, TFC ISTM/Kinshasa, Nutrition Section. 1998-1999.
27. Olsen SM, Pedersen LT, Hermann MH, et al. Inorganic precursor peroxides for antifouling coatings. Journal of Coatings Technology and Research. 2009; 6: 187-199.
28. Young D, Alexander G, Mc Dermott Ehrlich D. Related contamination of southern California harbors by copper and other metals. Mar, Poll, Bull. 1979; 10: 50-56.