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Therapeutic Implication of *Terfezia claveryi* Extract on Corneal Ulcer of Rabbit's Eye

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Authors' contributions

This work was carried out in collaboration between all authors. Author SMA designed the study and wrote the first draft of the manuscript. Authors YHA, MAQ and MIA managed the literature search and ophthalmic diagnostic analyses. Author AAK prepared the extract and performed serum biochemical tests. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Aims: The aim of this study was to investigate the antimicrobial activity of *Terfezia claveryi* crude extract on induced corneal ulcer in rabbit's eye.
Study Design: Study was carried out in Rabbits eye.
Place and Duration of the Study: This study was conducted in the experimental laboratories at College of Applied Medical Sciences of Qassim University during the period from March to June 2013.
Methodology: Crude aqueous extract of this truffle, in different concentrations, was introduced through intraperitoneal injection to rabbits for their safety dose.

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Corneal epithelial wound was induced in different groups of rabbit's eye with sodium hydroxide and later this wound was contaminated with some selected bacteria like *Staphylococcus aureus* and *Escherchia coli* to produce iatrogenic infection. The healing power of different concentrations of *T. claveryi* crude extract was observed by different clinical findings.

Results: We observed that 1.5% crude extract of this desert truffle significantly healed the corneal ulcer almost within 9 days. Delayed response in healing was observed with 3% *T. claveryi*, while 5% extract developed some extra corneal complications. The healing response of corneal ulcer to topical application of different concentrations of *T. claveryi* extract was compared with a synthetic antibiotic (Vigamox 0.5%) as a reference standard drug.

Conclusion: It was concluded that aqueous extract of *T. claveryi* has no significant toxic effects against liver and kidney function parameters. The antibacterial activity of this desert truffle recommends as an alternative medicine for some corneal infections. However, the therapeutic role of some specific ingredients present in this truffle needs further investigations.

Keywords: Terfezia claveryi; antimicrobial activity; corneal ulcer; rabbits eye.

1. INTRODUCTION

Corneal ulcer accounts for a significant proportion of eye infection worldwide [1] and is a common ocular disease found both in humans and animals and can lead to loss of vision and eye [2]. Apart from other risk factors, corneal trauma by different materials and dry eyes is one of the major causes of corneal ulcer [3]. In developed countries, wide use of contact lenses is a major contributing factor of corneal ulcer [4]. Furthermore, there are many virulent organisms, such as *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Staphylococcus pyogenes*, *Streptococcus* sp. and *Staphylococcus epidermis*, claimed to infect corneal ulcer [5,6].

Corneal ulcer can cause serious complications if not managed properly. Such complications include corneal perforation and blindness [7]. Corneal scarring is listed second to cataract as an important cause of blindness and visual impairment in many developing countries [8]. Many antibiotic preparations are used to treat eye infections including, chloramphenicol, fluoroquinolone, neomycin, aminoglycosides [9] but these medicines have limited distribution, and are expensive. In addition to this, many bacteria develop resistance against antibiotics which may induce other side effects [10-12].

Hence, the search for natural, safer and more effective antibacterial preparation has been continued tremendously. Moreover, the guidelines of WHO recommend for search on the beneficial uses of medicinal plants for the treatment of various infections [13]. The compounds of truffle aqueous extract have an important therapeutic role of anti-inflammatory, anti-carcinogenic, anti-mutagenic, immune-suppressor and anti-microbial properties [14].

Desert Truffles are considered one of the oldest food and medicinal plants used by the Arabs [15]. Truffles grow naturally in many parts of the world especially in Arabian deserts [16]. *Terfezia claveryi* (Kamma) are round, tan to brown colored like small sandy potatoes, having unique flavor, nutritional value and medicinal properties for different ailments [17]. *T. claveryi* ascocarps contain a good quantity of proteins and carbohydrates along with saturated and unsaturated fatty acids and minerals [18]. Truffle aqueous extract is used as a folk medicine in many gulf countries to treateyeinfections [19]. The *T. claveryi* extract is used as a nourishing and invigorating preparation for convalescents in Mediterranean countries [20]. Furthermore, its aqueous extract has been recommended by The Prophet Mohammad (PBUH) for the treatment of eye problems [21].

A promising preliminary *in vitro* study showed antimicrobial effect of *Terfizia claveryi* aqueous extract against *Staphylococcus aureus* [22]. Recently, we have done a further studies for the *in vitro* antibacterial activity of *Terfezia claveryi* using the disc diffusion, well diffusion method, minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC), where *T. claveryi* exhibited excellent antibacterial activity against all clinical isolates of corneal ulcer tested, especially against *Pseudomonas aeruginosa, Staphylococcus aureus* and *Escherichia coli* [23] and this results recommended further research to examine its *in vivo* mechanism of action, toxicity, and therapeutic effect.

The present study was designed to check properly *in vivo* safety and therapeutic effects of *T. claveryi* aqueous extract on the induced corneal ulcer of rabbits via experimental work.

2. MATERIALS AND METHODS

Fifty four male rabbits were obtained from the farm at Qassim University. All animals were examined properly and were found to be free from any corneal disease. The rabbits were kept in animal house for one week before starting the experiment. All selected animals were healthy, weighing between 2-2.5 kg, and housed in standard aluminum cages (3rabbits/cage). The animal house was maintained at 18-22°C, 12 hr. dark/light condition. This experimental study was approved by the Institutional Animal Care Department and rabbits were fed with standard diet and normal tap water and handled as per the international rules of experimental laboratory animals, Qassim University.

2.1 Preparation of Crude *Terfezia claveryi* Extract

Freshly harvested *Terfezia claveryi* was washed with running tap water to remove the attached dust and micro sand particles. The *Terfezia* tubers were refrigerated for about 6 hr. at 4°C before peeling the soft skin with a surgical knife. 75 gram of peeled *Terfezia claveryi* was homogenized with 100 ml pre-cooled 50 mM sodium phosphate buffer pH 7.0 in a tissue homogenizer for 3 min. The extract was passed through four layers of cheese cloth to remove the major debris. The filtrate was centrifuged at 10,000 rpm for 15 min at 4°C. The supernatant was passed through micropore filter paper to make the solution sterile. Further, the sterility of this supernatant was checked on culture plate and no bacterial strain was observed. The supernatant was further diluted with the same homogenizing buffer when used. The supernatant was considered as crude aqueous extract of *T. claveryi* and stored at 4°C for experimental use. An *in vitro* antibacterial activity of *Terfezia claveryi* crude extract was done using MIC and MBC and proved excellent antibacterial activity against *Pseudomonas aeruginosa, Staphylococcus aureus* and *Escherichia coli* [23].

2.2 Test Microorganisms

Antibacterial activity tests of some selected bacteria like *S. aureaus* and *E. coli*, were performed. These bacterial strains were sub-cultured at 37°C on Mueller-Hinton agar (Oxoid, Hampshire, UK) slants every two weeks and stored at 4°C. In parallel to this study, the bacterial isolates were also obtained from clinical cases, suffering from corneal infections, from various hospitals of Qassim region. All microbiology related experiments were performed in Department of Medical Laboratories, Qassim University using the standard microbiology techniques [24].

2.3 Preparation of Bacterial Suspension

Bacterial inoculum were prepared by cultivating each bacterial species onto nutrient agar for 24 hours at 37°C, then 5-7 colonies were transferred to a tube containing 5 ml sterile normal saline solution. The tubes were vortexed to make a bacterial suspension with turbidity equal to 0.5 Mc Farlands' standard solution. Freshly prepared bacterial suspension containing 105 CFU/ml was used for contamination of induced corneal epithelial wound.

2.4 Experiment 1: Safety of *Terfezia claveryi* Aqueous Extract

Several concentrations of *T. claveryi* extract were prepared as 5%, 15% and 30%, and 1 ml of each concentration was inoculated in 6 rabbits (total 18 rabbits) through intra-peritoneal injection. The inoculated animals were observed for different clinical disease signs (with special reference to mortality, off food, diarrhea, nervous manifestation), for 2 weeks. Periodic blood samples were taken after sacrificed the animals on 3rd, 6th and 12th days of follow-up for evaluating liver and kidney function parameters.

2.5 Experiment 2: Therapeutic Effect of *T*erfezia and Vigamox for Treating Corneal Ulcer

For the induction of corneal epithelial wound, first the eye of 36 rabbit was anesthetized by application of topical alcaine (proparacain) eye drops for 5 minutes. Next, the eyelids were secured in open position by fingers, and 5 mm circular filter papers (Whatmann No. 3), immersed in 1 N NaOH were used for inducing central corneal epithelial wound. The filter paper was kept for 5 seconds in contact with central cornea and removed with forceps followed by a thorough irrigation with normal saline [25].

2.6 Animal Grouping

Rabbits were divided randomly into four main groups and corneal wound was induced in all animals.

Group 1- Negative control (6 rabbits): Non-contaminated without treatment.

Group 2- Positive control (6 rabbits): Contaminated and without treatment.

Group 3- Test group: subdivided into 3 sub-groups all were contaminated (where 3 rabbits of each subgroup contaminated with *Staphylococcus aureus* and 3 other rabbits contaminated with *E. coli)* and treated with different concentrations of *T. claveryi*.

Sub-group A (6 rabbits): usage of *T. claveryi* 1.5%

Sub-group B (6 rabbits): usage of *T. claveryi* 3%

Sub-group C (6 rabbits): usage of *T. claveryi* 5%

Group 4- Test group (6 rabbits): contaminated and treated with vigamox (0.5%)

After a few minutes of inducing corneal epithelial wound, the rabbits eye of positive control (group 2) and test groups (groups 3 & 4) were contaminated with 0.5 ml (10^5 CFU/ml) bacterial suspensions from each of *Staphylococcus aureus* and *E. coli* (3 rabbits/bacteria/sub-group) with the help of a dropper, respectively. After 24 hours, the treatment was started in rabbits of group (3) with topical application of crude extract of *Terfezia claveryi* in 3 different concentrations (1.5%, 3% and 5%) to sub-groups (A-C). Besides this, synthetic antibiotic moxifloxacin hydrochloride (vigamox) 0.5% (Alcon Laboratories SA, Pvt. Ltd), was applied in test group (4) based on its anti-bacterial sensitivity. This treatment was carried out 4 times daily in respective groups for 2 weeks (Table1).

2.7 Ophthalmic Diagnostic Test

For the eye examination of different groups of rabbits, the cornea was first stained with flourescein paper strips soaked with a drop of alcaine. The detailed examination of corneal ulcer was performed by using portable Slit-Lamp Microscope (KJ5S2, Suzhou Kangjie Medical). The rabbit eyes of different groups were followed up every day for the signs of corneal inflammation and infection (Table 2).

2.8 Serum Biochemistry Techniques

The blood samples were collected from all rabbits of different experimented groups in tubes without anticoagulant and were allowed to clot at room temperature for 30 min. the serum was collected by centrifugation at 1600×*g* at 4°C for 15 min. The liver and kidney function test parameters were performed by using commercially available kits (Human GmbH, Wiesbaden, Germany), as per the instructions of manufacturer.

2.9 Statistical Analyses

A probability at level of 0.05 or less was considered significant. Means and standard errors were also estimated. All statistical analyses were run on the computer, using the SAS program (SAS, 2003).

Table 1. Groups and sub-groups of rabbits showing induced corneal wound and contamination with bacteria and its treatment

Group	Treatment	Induction of epithelial wound and corneal ulcer	Contamination *Staphylococcus aureus*	*E. coli*
1	Negative control	+	-	-
2	Positive control	+	+	+
3	Sub groups A-(1.5%) *T. Claveryi*	+	+	+
	B-(3%) *T. Claveryi*	+	+	+
	C-(5%) *T. Claveryi*	+	+	+
4	(0.5%) Vigamox	+	+	+

This table shows a correlation of different groups and subgroups of rabbits with induced corneal ulcer and contamination with two selected bacteria (S. aureus and E. coli) and different treatments with varying percentage of T. claveryi and vigamox. A total of 36 rabbits were used, 6 rabbits in each group (3rabbits/bacterial infection)

3. RESULTS

To check the safe dosage of *Terfezia claveryi* in rabbits (experiment 1), the three different concentrations showed no clinical signs of intoxication and no remarkable change in the parameters of liver and kidney function tests up to 3 days, 1 week or 2 weeks of intraperitoneal injection, except creatinine. There was a slight non-significant increase in creatinine level after 2 weeks of intra-peritoneal injection (Table 3).

On clinical examinations, the central induced corneal epithelia wound of non-contaminated (negative control) group 1 non-treated rabbits eye, healed spontaneously within 7 days (Table 4). On the other hand, the rabbits eye of non-treated group 2 (positive control), contaminated with *Staphylococcus aureus* and *E. coli*, after induced corneal epithelial wound, produced mild stage of infection (Table 2), which gradually healed and developed dense corneal opacities (Fig. 2). One rabbit from the same group, developed severe corneal infection resulting in abscess with the formation of hypopyon which ultimately perforated within 9 days (Fig. 1, Table 4).

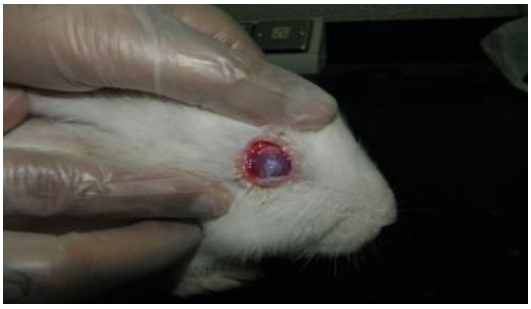

Fig. 1. Rabbit eye showing signs of infection. One week after contamination with S. aureus, showing red eye, chemosed conjunctiva with pussy discharge threatened to perforate corneal abscess

In treated group 3 (A) after the topical therapy of 1.5% *Terfezia* extract, the healing phase of induced corneal ulcer of rabbits eye, infected with *S. aureus* and *E. coli* showed improved clinical signs from moderate to mild stage within 9 days (Table 2, Fig. 3a). Later, the rabbit's corneal ulcer completely healed to form nebular type corneal opacities by 12 days of follow up (Fig. 3b). The treated group 3 (B), induced contaminated corneal ulcer (Fig. 4a) were treated by 3% extract of *T. claveryi* showed delayed healing until 14 days and formed macula type of corneal opacities (Fig. 4b). On the other hand, in treated group 3 (C), the contaminated induced corneal ulcer of rabbits eye were treated by 5% *T. claveryi* extract, developed severe complications (dry eyes, hypopyon and ultimately perforation) by one week (Table 4).

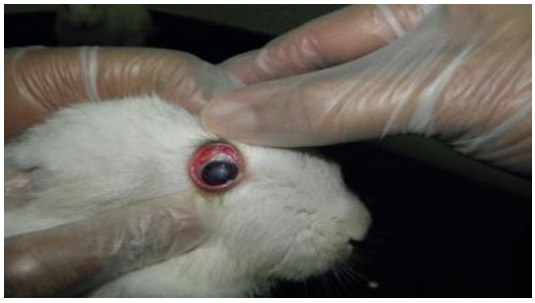

Fig. 2. Rabbit eye showing Leucomatous cornea, Second week after contamination of induced corneal ulcer by *E. coli*, showing quiet eye self-healed central corneal opacity

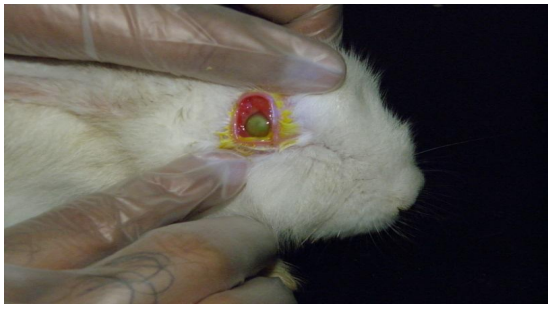

Fig. 3a. Rabbit eye cornea showing positive fluorescein staining with exudates and conjunctiva congestion, day after contamination of induced corneal ulcer by *S. aureus*

Fig. 3b. Rabbit eye showing healed nebula type corneal opacity formed within 12 days, after contamination of induced corneal ulcer by *S. aureus* and treated with 1.5% *Terfezia claveryi*

Table 2. Clinical rating scale of corneal epithelial wound and ulcer of rabbit's eye

S. No.	Stages of corneal ulcer	Clinical and portable slit-lamp examination
1	Only wound	Fluorescein stain +, photophobia and ciliary injection.
2	Mild stage	Fluorescein stain +, photophobia, conjunctiva congestion, lid edema, gray color ulcer with surrounding edema and mild discharge.
3	Moderate stage	Fluorescein stain +, photophobia, blephrospasm, lid edema, matted eye lashes, conjunctiva congestion and edema, gray white ulcer with surrounding edema and pussy discharge.
4	Severe stage	Fluorescein stain +, lid edema, blephrospasm, conjunctiva injection, corneal abscess, pussy discharge, hypopyon and dry eye.
5	Healing stage	Fluorescein stain -, slight ciliary injection, no discharge, no photophobia

This table show different clinical stages with different clinical signs of corneal ulcer, staining, photophobia, blephrospasm, edema of conjunctive and cornea, discharge and response of healing

Table 3. Biochemical parameters of rabbits intraperitoneally injected with different concentrations of *Terfezia claveryi*

Time duration	Intraperitoneal injection of *Terfezia* (%)	Urea (mg/dL)	Uric acid (mg/dL)	Creatinine (mg/dL)	ALT (U/L)	AST (U/L)
3 days	Control	29.53±2.38	0.64±0.08	0.87±0.02	27.92±1.98	14.59±2.18
	5	29.53±3.24	0.42±0.76	0.94±0.01	30.78±2.75	5.71±1.19
	15	23.69±2.19	0.42±0.79	0.91±0.08	31.41±2.54	2.22±0.08
	30	20.06±1.98	0.30±0.03	0.91±0.06	28.66±2.53	11.42±1.75
1 week	Control	15.09±1.42	0.53±0.06	1.08±0.05	24.75±2.45	9.20±1.15
	5	23.61±2.98	0.24±0.65	1.01±0.08	31.41±3.45	17.13±1.76
	15	16.06±1.50	0.13±0.07	0.77±0.07	30.46±3.27	14.59±1.95
2 weeks	Control	31.96±2.43	0.42±0.01	0.77±0.09	31.48±2.12	12.32 ±1.2
	5	33.42±2.65	0.37±0.01	1.33±0.09	27.29±3.31	14.25±1.42
	15	25.34±3.12	0.46±0.05	1.00±0.08	25.70±2.16	12.85±0.06
	30	22.45±2.12	0.42±0.09	1.19±0.07	26.97±2.64	16.41±2.13

*Values are given as ±SD for a given group of rabbits; values are statistically significant; * p< 0.05 compared to control*

Comparatively in test group 4, treated with standard synthetic topical antibiotic Moxifloxacin hydrochloride (Vigamox 0.5%) dramatically improved the signs of contaminated induced corneal ulcer from moderate to healing phase within 5 days without any complications (Tables 2 and 4).

In experiment (2), liver and kidney function test parameters were also observed in all groups of rabbits and in comparison with the control (positive and negative) group, a marked significant decrease in the urea, uric acid and creatinine levels were observed in group 4 rabbits While, all the rabbits in group 3 showed non-significant increase in urea and non-significant decrease in uric acid and creatinine levels. No measurable changes were observed in alanine aminotransferase (ALT) and aspartate aminotransferase (AST) parameters in all groups of rabbits (Table 5).

Fig. 4a. Rabbit eye showing exudate, ciliary congestion and central corneal ulceration with chemosis of conjunctiva. First week after contamination of induced corneal ulcer by *E. coli* and treated with 3% *Terfezia claveryi*

Table 4. Clinical findings of rabbits eye of different groups on different days of treatment

Groups	Treatment	Bacteria	Day 1	Day 3	Day 5	Day 7	Day 9	Day 12	Day 14
Group 1.	(Negative control)Non-contaminated Corneal wound Without Treatment		Stain +ve, photophobia, moderate ciliary injection	Faint stain, photophobia mild ciliary injection	Stain –ve, very mild ciliary injection	Healed			
Group 2.	(Positive control) Contaminated Corneal Ulcer Without Treatment	*Staphylococcus aureus*. (3 Rabbit)	Mild stage	Mild stage	Mild stage (2 rabbit) Moderate stage (1 rabbit)	Mild stage (2 rabbit) severe stage (1 rabbit)	Healing phase (2 rabbit) perforated (1Rabbit)	Central corneal opacity (2 rabbit)	Leucoma type corneal opacity (2 rabbit)
		E. coli (3 rabbit)	Mild stage	Mild stage	Mild stage	Mild stage	Healing phase	Central corneal opacity	Leucoma type corneal opacity
Group 3. (Test group).Contaminated Corneal Ulcer Treated with Terfezia claveryi.	*Terfezia claveryi* 5%(subgroup C)	*Staphylococcus aureus* - *E. coli* (3 +3 (6) rabbit)	Mild to moderate stage	Moderate stage	Severe stage	Severe stage	Perforation		
	Terfezia claveryi 3%(subgroup B)	*Staphylococcus aureus* - *E. coli* (3 +3(6) rabbit)	Moderate stage	Moderate stage	Mild stage	Mild stage	Mild stage	Healing phase	Macula type corneal opacity
	Terfezia claveryi 1.5%(subgroup A)	*Staphylococcus aureus* - *E. coli* (3 +3(6) rabbit)	Moderate stage	Moderate stage	Mild stage	Mild stage	Healing phase	Nebula type corneal opacity	
Group 4. (Test group) Contaminated Corneal Ulcer Treated with Vigamox 0.5%	Vigamox 0.5%	*Staphylococcus aureus* - *E. coli* (3 +3(6) rabbit)	Moderate stage	Mild stage	Healing phase	Cornea almost transparent			

This table shows the clinical stages of experimental rabbit's eye of different groups and follow-up the responses from day one to two weeks

Table 5. Effect of therapeutic treatment (using *Terfezia* and *Vigamox*) on liver and kidney function tests of rabbits with contaminated corneal ulcer

Group	Urea (mg/dL)	Uric acid (mg/dL)	Creatinine (mg/dL)	ALT (U/L)	AST (U/L)
Control	29.53±2.98	0.64±0.07	0.87±0.13	27.92±2.97	14.59±1.63
Corneal ulcer +E. coli + Terfezia5%	46.75±4.87	0.46±0.7	0.63±0.06	31.09±2.95	13.01±2.80
Corneal ulcer +Staphylococcus aureus + Terfezia 5%	43.00±2.90	0.46±0.08	0.63±0.08	26.65±2.56	21.26±1.59
Corneal ulcer +E. coli + Terfezia3%	42.31± 3.35	0.52± 0.06	0.75±0.24	28.17±2.65	15.31±2.23
Corneal ulcer +Staphylococcus aureus + Terfezia 3%	45.32±4.25	0.72±0.04	0.68±0.32	29.32±2.95	16.38±1.98
Corneal ulcer +E. coli + Terfezia1.5%	32.21±2.41	0.51±0.98	0.60±0.34	32.32±2.06	17.43±1.87
Corneal ulcer +Staphylococcus aureus + Terfezia 1.5%	30.63±2.87	0.48±0.65	0.65±0.43	30.64±1.65	18.32±0.43
Corneal ulcer +E. coli + Vigamox (0.5%)	18.01±2.67*	0.21±0.03*	0.47±0.02*	28.40±1.65	14.12±2.96
Corneal ulcer +Staphylococcus aureus+ Vigamox (0.5%)	16.30±1.79*	0.39±0.08*	0.52±0.01*	31.09±2.51	20.22±0.08

This table shows the correlation of liver and kidney function test parameters of induced corneal ulcer with E.Coli and Staphyloccus aureus and its treatment with Terfezia claveryi and standard antibiotic.
Values are given as ±SD for a given group of rabbits. Values are statistically significant.
** p< 0.05 compared to control*

4. DISCUSSION

Besides high production cost and limited availability, constant use of synthetic antibiotics results in bacterial resistance [12]. In addition to this, corneal ulcer treatment with many antibiotics leads to extra-ocular resistance [26]. So for this search of new alternative medicines, truffles represent best examples as they are rich sources of multiple therapeutic action compounds [14,23].

In our study, a slight non-significant rise in creatinine level was found and no other measurable changes were observed in liver and kidney function test parameters by using even 30% *T. claveryi* extract intraperitoneally (Tables 3 and 5). Similar observations have been observed by other researchers also [27]. In addition to this, the topical use of either *T. claveryi* extract (1.5-3.0 %) or vagomox (0.5%) for the treatment of induced corneal ulcer, showed no side effects in liver and kidney test function parameters.

Several studies have shown that alkali burn induced corneal damage occurs due to locally released corneal collagenase [28] therefore a wise approach for the prevention of corneal damage is to use the inhibitors of collagenase. Here in this regard, we hypothesize that there may be the presence of collagenase inhibitors in *T. claveryi* extract.

In some parts of African countries like Nigeria, exudates from several herbal plants are mixed in a definite proportion and used for the treatment of corneal ulcer [29]. The topical application of supernatant crude extract of *T. claveryi* (1.5% and 3%) have found to be antimicrobial activity in contaminated induced corneal ulcer in rabbits eye. The corneal ulcer was healed within 9-14 days and left behind the opacity of central cornea (Fig. 3, Fig. 4). In a parallel study, Onwukaeme et al, 2007 have observed that the extract of *Pycanthusangolensis* showed healing activity within 10 days against the induced corneal ulcer of rabbit's eye [29].

A promising antimicrobial activity of *T. claveryi* was reported recently in our laboratory by *in vitro* studies, where it we observed that the truffle *T. claveryi* extract were bacteriostatic at lower

concentration but bactericidal at higher concentration against eight different strains of Gram positive and Gram negative bacteria [23]. In other study, it was found that, 5% aqueous extract of *Terfezia* inhibited the growth of *Staphylococcus aureus* by 66.4% [22]. Antibacterial activity against *S. aureus*, *S. pyogens*, *E. coli* and *P. auregenosa* has also been reported in the exudates of *P. angolensis* plant and healed the induced corneal ulcer in rabbits [29].

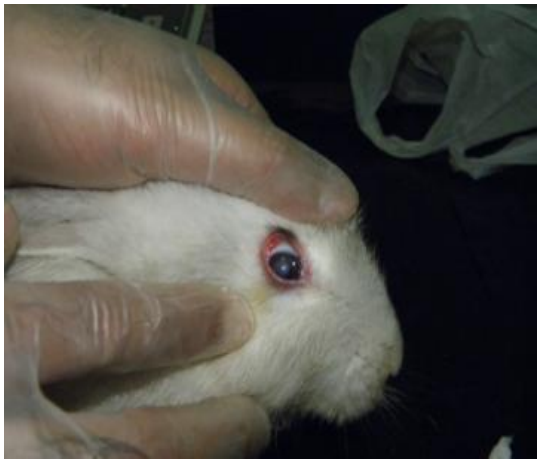

Fig. 4b. Rabbit eye showing healed central macula type corneal opacity, Second week after contamination of induced corneal ulcer by *E. coli* and treated with 3% *Terfezia claveryi*

In addition to above finding, our parallel published study evaluated the therapeutic effect of *Terfezia claveryi* in healing of corneal ulcer histopathologically in case of *Staphylococcus aureous* induced corneal ulcer through experimental inoculation of these bacteria in the corneal stroma of rabbit's eyes [30]. In another study, Propolis, a product collected from buds and produced from the honey bee has been used as a folk medicine possessing antimicrobial and anti-inflammatory activities for the treatment of corneal ulcer in rabbits. It was observed that induced corneal heals within ten days of treatment, confirmed by histopathological studies also [31].

Based on data, fortified topical antibiotic are as effective as or more effective than sub-conjunctival injection [32]. Topical treatment by some selected concentration of *T. claveryi* extract might have equal effect as topical fortified preparation. This was attempted by *in vivo* studies on therapeutic effect of *T. claveryi* extract used as an alternative choice for antibiotics for the treatment of corneal ulcer.

5. CONCLUSION

We conclude that aqueous extract of *T. claveryi* has no toxic effects on biological parameters; moreover, we obtained good antibacterial activity at 1.5% to 3% as observed through the healing of the induced corneal ulcer in rabbit's eye. These findings indicate that *T. claveryi* aqueous extract may be an alternative to others antibacterial treatments of corneal ulcer. The supernatant crude extract of *Terfezia* may work by different modes of action like antioxidant, antiradical and antimicrobial activities. However, the exact precise mode of action may need to be elucidated through further research and the effective compound from the crude extract of *T. claveryi* need to be explored further and tested on wide range of microorganisms to prove as alternative drug of choice.

CONSENT

It is not applicable.

ETHICAL APPROVAL

The Ethical Research Committee, College of Applied Medical Science has discussed the protocol and the methodology of the experiments of the research project entitled "Therapeutic Implication of *Terfezia claveryi* Extract on Cornial Ulcer of Rabbits Eye" to be performed on loboratory animals.

We approved this experimental study and the animals should be properly handled as per the International rules of experimental loboratory animals followed by Qassim University.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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