Ophthalmology Research: An International Journal

Volume 18, Issue 6, Page 41-58, 2023; Article no.OR.110471
ISSN: 2321-7227

Evaluation of Lycium Shawii Schult & Roem Eye Drops as a Novel Treatment for Old Corneal Scars in Rabbits

Nabeel Muhsin Ziad [a*], Khalid M. Faisel [b], Mohammed Mohsen Ziad [c], Abdul-Malik Abudunia [d] and Ali Al-Kaf [e]

[a] Department of Ophthalmic, Al-Dorra General Hospital's in Jahana, Sana'a Governorate, Yemen.
[b] Department of Ophthalmic, Al - Dofeah Hospital's in Behan, Shabwah, Yemen.
[c] Department of Research and Development, Yemeny Drug Company for Industry and Commerce, Sana'a, Yemen.
[d] Department of Biotechnology Laboratory (Med-Biotech), Faculty of Medicine and Pharmacy, University Mohamed V, Morocco.
[e] Faculty of Pharmacy, Sana'a University, Yemen.

Authors' contributions

This work was carried out in collaboration among all authors. The author NMZ was in charge of the first draft, research technique, data organization, formal analysis, writing, printing, supervision, and editing while the authors KMF and MMZ authors contributed to the research technique, data organization, writing, printing, supervision, and review. The Authors AMA and AAK contributed to the initial conceptualization and study protocol, as well as data collection and review. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/OR/2023/v18i6407

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/110471

Original Research Article

Received: 10/10/2023
Accepted: 14/12/2023
Published: 18/12/2023

ABSTRACT

Purpose: to evaluate the efficacy of eye drops extracted from Lycium Shawii Roem&Schult leaves in treating old corneal scars in rabbit corneas. A corneal scar is a condition in which the cornea becomes opaque. The cornea is a transparent, avascular tissue. Corneal opacity/scar is the fifth leading causes of blindness and vision impairment worldwide.

**Corresponding author: E-mail: dr.nabeelziad@gmail.com; dr.nabeelziad@yahoo.com;*

Methods: In a randomized controlled trial. twelve European pigmented rabbits with a twenty four eyes with a mean weight of 1.12 kg (Standared Deviation ± 0.16 kg), aged 5 to 6 months old, were subjected to a partial thickness (120–180 μm) sterile surgical excision on the anterior central part of the cornea with a 7 mm diameter. After the surgical procedures, we waited six weeks for stable old corneal scars to develop, after that rabbits received topical drops as one eye drops three times per day for two months, lycium shawii leaves extract for treatment group (10%; n=12), placebo (normal saline 9% ;n=10) for control group. Therapy effects on corneal scar and ocular safety were evaluated by slit-lamp microscope, Modified Fantes haze scale for grading.
Results: The treatment group showed a significant reduction in corneal scar (*P* <0.000), mean 2.75 score , standard deviation ± 0.45, and a 68.75% improvement in corneal clarity (*P* <0.000), mean 0.25 score, standard deviation ± 0.45.
Conclusion: The therapy is effective and safe in treating old corneal scar in rabbit and restores the transparency without ocular toxicity even after 2.5 years of follow up.

Keywords: Corneal scar; opacity; lycium shawii; awsaj; zarb.

1. INTRODUCTION

corneal scar is a condition in which the cornea becomes cloudy or opaque .The cornea is a transparent, avascular tissue that covers the outermost part of the eye ball and provides approximately two-thirds of the refractive power of the eye. The human cornea is composed of five layers: 1. Epithelium. 2.Bowman. 3. Stroma. 4.Descemet membrane. 5. Endothelium [1]. The most common causes of corneal opacity / scarring include trachoma, infectious keratitis, xerophthalmia and ocular trauma [2]. According to WHO corneal scar is the fifth cause of blindness and vision impairment. Approximately 2.2 billion people worldwide are visually impaired. Corneal opacity is recognized as a significant health concern in the realm of eye diseases, affecting people of all ages and posing the risk of profound blindness. The treatment of this condition can be difficult and occasionally unsuccessful. The annual global costs of productivity losses associated with vision impairment estimated to be US$ 411 billion (WHO).Corneal opacity is estimated to account for 3.2% of all causes of blindness. In general, infectious keratitis is the most common problem in low and middle income countries. The most popular treatments of corneal scar is corneal transplantation, but this is not always possible, practical, or successful, and thus the mission to eliminate corneal blindness requires more than simply increasing access to transplantation [3]. Following corneal transplantation, a visually significant complication that can occur even years after initial surgery known as post-penetrating keratoplasty, corneal ectasia. Endothelial failure caused long-term failure [4,5]. Drug delivery to the corneal tissues is difficult and challenge because the mechanical barriers that protect the cornea as multi layered epithelium, tight junctions that limit ocular drug delivery. Lipophilicity, partition coefficient, and molecule size are the primary factors that affect corneal medication absorption. Nano carriers may be a viable option for targeted ocular medication delivery [6]. The breakdown of these suspended particles contributes to ocular medication absorption. The biopharmaceutical influence and interplay of formulation parameters such as viscosity and particle size have not been studied or analyzed in depth. These factors may have a major impact on ocular medication absorption in the eye. Ocular bioavailability of drugs after eye drop administration to rabbits is limited to less than 5% of the instilled dose, often less than 1%. This is due to several limiting factors:

1- The permeation barrier of the corneal epithelium. 2- Rapid drainage of the instilled drug from the ocular surface. 3- Effective trans conjunctival drug absorption into the systemic blood circulation [7].

The aim of this study is to evaluate the efficacy of Lycium Shawii Roem & scult leaves extracts as eye drops on old corneal scars and to find a treatment in the form of eye drops that can be used anywhere, is easily accessible to patients in need, and is inexpensive for the treatment of corneal scars. The rabbit model was chosen in ophthalmic experimental studies because the eyes of rats, hamsters, guinea pigs, and mice are small that surgery is difficult to perform. Rabbit corneas, on the other hand, have a large diameter of about 15 mm. the arrangement of the corneal epithelium, stromal lamellae, and endothelium is also somewhat similar to that of human corneas [8]. There are some differences

between the human and rabbit corneas, which we will explain as follows:

1. Differences in drug delivery between rabbit and human, the fact that the conjunctival corneal surface area ratio in man is approximately twice that of rabbit shows that there is a substantially large surface area for drug adsorption via the conjunctiva in man vs. rabbit, as well as a smaller surface area for drug absorption via the cornea in man [9]. 2.The rabbit stroma fibrils are more organized than human stroma fibrils may be due to variances in the state of the final implanted tissue rather than an actual quantitative difference between species [10]. 3- In adults human , the cornea measures around 12 mm in the horizontal meridian and approximately 11 mm in the vertical meridian [11]. Rabbit corneas, on the other hand, have a large diameter of about 15 mm. 4. Rabbit corneas do not have a well developed Bowman's membrane, The descemet's membrane is also present, but the collagen arrangement is different from that of the human cornea [8]. 5.The pigmented rabbit's central corneal thickness is nearly 150 mm thinner than the human's. Similar to the human cornea distribution, the inferior temporal quadrant has the thinnest corneal thickness [12]. The central cornea in humans is 0.5 mm thinner than the peripheral cornea, which is 1.0 mm thicker, with an average radius of curvature of 7.8 mm [11]. The central corneal thickness in rabbits is 341+_14mm at one month, 391± 16mM at six months, and 404 ±12mm at twelve months. 6.The central corneal thickness (CCT), corneal curvature, and refractive state of the rabbit's cornea change dramatically during the first few months of life. Keratometry decreases with age, while CCT and spherical equivalent refraction increase. In the human newborn to 6-month old group, the mean keratometry in diopter (D) was 46.82 ± 2.11 D (range: 44.08 - 49.50 D). Keratometry gradually dropped and appeared to stabilize at about 54 months. The mean corneal keratometry in rabbits aged 1-6 months was 45.60 ±2.77 D (range:41.24 - 51.88 D) and appeared to settle at around 9 months (41.30 ± 1.37 D). In corneal experimental studies using rabbits as an animal model, it is critical to account for ocular parametric changes [8].

The old corneal scars formed in a rabbit model of a deep corneal wound between 6-8 weeks of healing, resulting in deep and dense scars. [13,14] and other studies discovered no significant change in measurements from day 28 to day 56, indicating that the opacity and its optical and morphological properties stabilized between these time-points [15]. Previous studies have shown that 4 weeks following a corneal wound, the stable scar time in mice is ideal [16].

Mechanism of corneal scar formation: Corneal wound closure following a serious ocular trauma commences the process of corneal fibrosis, transforming a healthy cornea into a scared cornea. The migration and proliferation of corneal epithelial cells is followed by the differentiation of stromal keratocytes into myofibroblasts, the excess deposition of extracellular matrix (ECM) components, an increase in alphasmooth muscle actin (-SMA) expression, and ECM remodeling, resulting in corneal opacity. The corneal scarring process continues long after the wound has healed, exacerbating the problem. Keratocytes resume their dorman condition during wound healing. and the immune cells are no longer present. Due to its low regenerative capacity, the disrupted Bowman's membrane does not regenerate. This causes extracellular vesicles and persistent fibroblast cells to invade the stromal layer on a continuous basis, accumulating Transforming growth factor-β (TGF-β) and Platelet-derived growth factors (PDGF) and [17]. promote tissue repair [18]. Repetition of corneal ECM remodeling as a result, scarring of the cornea is a direct result of the multiplexed corneal wound-healing process [17]. The corneal epithelium is made up of 4-6 layers, including 1-2 layers of superficial squamous cells, 2-3 layers of broad wing cells, and a layer of columnar basal cells in the innermost layer. Tight junction between surface epithelial cells prevent tear fluid from penetrating the stroma. The replacement of damaged layers is caused by the constant proliferation of limbal stem cells. This process takes between 7 and 14 days, which results in a layer with a nearly uniform refractive index and minimal light scattering. Bowman layer is an acelluare membrane that lies anterior to corneal stroma layer that helps maintain the shape of the cornea. When there is a damage it will not regenerate. Corneal stroma accounts for roughly 90% of total corneal thickness. A clear cornea requires a regular arrangement of stromal cells (keratocytes), fibers, and extracellular matrix. They are fibroblasts that have been flattened and are found between the stromal collagen lamellae. The stroma is made up of an extracellular matrix made up of collagens and proteoglycans. Type I and type V fibrillar collagens are intertwined with type VI collagen filaments. Decorin (associated

with dermatan sulfate) and lumican (associated with keratan sulfate) are the two most important corneal proteoglycans. Corneal transparency is dependent on keeping the corneal stroma's water content at 78%. Corneal hydration is largely controlled by intact epithelial and endothelial barriers, as well as the endothelial pump's function. Descemet membrane is the corneal endothelial basement membrane. Corneal endothelial cells form a monolayer of closely interdigitated cells on the cornea's posterior surface. Human endothelial cells are thought to have limited proliferate , but they can divide in culture. Cell density decreases with age at an average rate of about 0.6% per year, starting at about 3400 cells/mm2.through its functions as a barrier to the aqueous humor and as a metabolic pump that moves ions and draws water somatically from the stroma into the aqueous humor, the endothelium maintains corneal transparency by controlling corneal hydration and maintaining stromal deturgescence [1]. The cornea endothelium When injured, it heals through migration and enlargement of the cells. existing cells. interleukin-1(IL-1) which causes keratocytes in the stroma to die, helps to increase cell migration during endothelial healing Endothelial damage causes to keratocyte apoptosis in the stroma posterior. It is unknown whether endothelial Myofibroblasts are formed in the stroma as a result of injury. Endothelial cells, on the other hand, acquire the myofibroblastic phenotype, also known as endothelial to the transition from the mesenchymal to the epidermis, this transition results in a potentially hazardous situation. endothelial fibrosis complication that forms a retrocorneal membrane [19].

To the best of our knowledge, the current study is the first to evaluate the efficacy of Lycium shawii Roem & Schult leaves extract in treating the old corneal scar in vivo.

Treatment options for corneal scar, when it comes to treating corneal opacity. 1. corneal transplantation is considered the the golden treatment. However, there are alternative techniques to penetrating keratoplasty (PK) include: Lamellar corneal transplants, which replace only the damaged layers,Both the anterior (deep anterior lamellar keratoplasty, (DALK) and posterior modalities, Descemet's membrane endothelial keratoplasty (DMEK) and its modifications, of these lamellar methods are now in use and are replacing PK in the majority of referral centers worldwide. To present, clinical studies and meta-analyses have shown that PK has similar results and prognoses to lamellar corneal transplantation [20]. Post penetrating keratoplasty corneal ectasia is a visually significant complication that can occur even years after initial surgery. Long-term failure was caused by endothelial failure [4,5]. The most common reasons for high failure rates are lax corneoscleral tissue, a more aggressive inflammatory response, persistent intraocular inflammation and glaucoma. The survival rates of grafts treated for acquired traumatic corneal opacities are poor (63% failure rate). Regraft procedures also have failure rates of up to 80%. Graft survival in pediatric patients is less promising than in adult patients and is heavily dependent on the patient's age [5]. With penetrating keratoplasty, the Australian Corneal Graft Registry reported 89% graft survival at 5 years, 49% at 15 years, and 17% at 23 years. There are approximately 12.7 million people on the global waiting list for corneal transplantation [21]. Corneal transplantation surgery treatments are limited due to postoperative complications such as infection and delayed healing, immune rejection and failure of the transplant, intraocular pressure elevation, and a general scarcity of donor corneas as a result of the increased popularity of laser refractive surgery [22,23]. Femtosecond lasers (FSLs) are a recent technological breakthrough in ophthalmic surgery. Theoretically, refractive surgery and corneal surgery have advantages over manual techniques that involve cutting corneal tissue. Femtosecond laser-assisted ophthalmic surgery. FS-LASIK: femtosecond laser-assisted laser in situ ,keratomileusis. SMILE: small incision lenticular extraction. FS-AK: femtosecond laser assisted astigmatic keratotomy. FSPKP: femtosecond laser-assisted penetrating keratoplasty. FSDALK: femtosecond laser-assisted deep anterior lamellar keratoplasty. FSDSEK: femtosecond laser-assisted Descemets stripping endothelial keratoplasty. FS-DMEK: femtosecond laser-assisted Descemet' s membrane endothelial keratoplasty [24].

2. Stem cell therapy: A variety of treatments have been attempted to improve scarless corneal regeneration. Stem cell therapy using various types of stem cells can rebuild the cornea by renewing and restoring damaged cells and breaking down the scar extracellular matrix. Stem cell secretions (secretomes, extracellular vesicles, and exosomes) have also recently been employed in corneal regeneration. Stem cell

therapy can stimulate corneal regeneration by differentiating into corneal epithelial cells, keratocytes, or endothelial cells to replenish the lost/damaged cells; and activating the resident stem cells at the site of injury to aid in damage repair. Secreting regenerative cytokines or growth factors that can reduce inflammation, remodel scar deposits, and boost regeneration by activation of various signaling pathways [21]. The use of the amniotic membrane as an ocular bandage because of its antiangiogenic and antifibrotic properties is a popular treatment method. However, they are use is limited because of ethical problems related to tissue donation, potential risks of the transmission of diseases, inter-amnion variability, and reproducibility [25]. The amnion's bandaging function dampens the cascade of fibro genic signaling during the various phases of repair. Alternative therapy options include the creation of amniotic membrane extract drops [18]. There was a study that discovered the group that only received amniotic stem cells to treat corneal opacity caused by chemical alkaline burn showed transient improvement, wile the other two groups that received limbal stem cells alone or in combination with amniotic stem cells improved their recovery time quickly [26]. Another study revealed that mesenchymal stem cells might restore corneal transparency by secreting hepatocyte growth factor. The ability of umbilical mesenchymal stem cell transplantation to cure corneal opacity in the congenital mice model but not in the acquired [22].

3. The most common available treatments are steroids and other medications that entail the danger of various side effects, necessitate repeated administrations, and are frequently inefficient in entirely recovering vision. Although mitomycin C (MMC) is common topical treatment for corneal fibrosis, its usage remains controversial due to long-term negative effects. MMC, toxicity, and DNA damage to endothelial cells may have long-term deleterious effects on ocular health [21,27]. Topical eye drops are a simple method of administration, but their limited bioavailability and fast ocular surface drainage limit their popularity [25].

4. New methods therapy or trail therapy incluede gene therapy (exosomes, micro RNAs), recombinant viral vectors, bioactive molecules, growth factors, histone deacetylase inhibitors, and nanotechnology have been targeted as therapeutic tools for treating scarred cornea [17]. Cholesterol-conjugated completely modified asymmetric small interfering RNAs (siRNAs) [self-deliverable siRNAs (sdRNAs)] are a unique method for in vivo gene knockdown that transfects cells and tissues without the need of any extra formulations [28]. Nonviral gene delivery techniques based on synthetic polycations, such as polyethylene amine (PEI), demonstrate potential as delivery systems in order to use gene therapy [29]. A gold-standard method for preventing corneal scarring is still being researched. Decorin, glucosamine, acetylcholine, and chitosan are examples of biomolecules [17].

Decorin: Decorin consistently produces a surface coat on collagen fibrils on a regular basis, decorin expression is not elevated during mechanical and surgical damage in rabbits because of the surface coat. Decorin expression is adequate to sustain normal collagen fibrillogenesis in biglycan deficient mice and does not result in a compensatory increase. It is uncertain whether or if these compensatory interactions occur in humans during development and adulthood [19]. Glucosamine: This amino sugar has medicinal use in the treatment of osteoarthritis. However, it considerably enhances intraocular pressure. Park et al. discovered that glucosamine attenuated renal fibrosis by inhibiting SMAD2 phosphorylation. Acetylcholine: Cholinergic receptors release acetylcholine, a neurotransmitter. Acetylcholine binds to its nicotine receptor, generating an influx of Na+ or Ca2+ ions as well as an efflux of K+ ions. This affects several signaling pathways via protein kinases and phosphatases, and it can also activate many downstream signaling pathways via a murine receptor (mAchR) by activating protein kinase C. CECs include mAchR (M1, M3) and nAchR, and acetylcholine can induce reepithelialization in a damaged rat cornea [27].

1.1 Lycium Shawii Roem& Shult

Is a thorny shrub of the Solanaceae family, a genus of flowering plants in the nightshade family. There are around 90 different varieties of (Solanaceae).Because of the availability of diverse physiologically active components, they play key roles in nutritional and therapeutic applications [30]. Lycium Shawii Roem & Shult (L.S) has the Arabic name Awsaj and the Yemeni local name Awsaj or Zarb. It found throughout the Arabian Peninsula, Southwest Africa, and the Mediterranean basin. It has the potential to grow to a height of 4 meters. It is densely branched and has small thorns. The little dark green leaves are 7 mm broad, the blooms are star-like white or

lilac in hue, and the fruit is pea-size crimson berries [31]. L.S contains peptides, flavonoids, polysaccharides, alkaloids, ceramides, anthraquinones, coumarins, steroids, terpenoids, organic acids, carotenoids, cinnamic acid amides, lignans, neo-lignanamides, and lignanamides [32]. Plant leaves had a higher percentage of total flavonoids, tannins, saponins, and alkaloids than the stem. Argenine had the highest concentration of protein amino acids in plant leaves. The biologically active elements of L.S. leaves are: 2, 3 dihydroxy benzoic acid, quercetin, gallic acid, rutin, p-coumaric acid, ferulic acid, quercetin 3-methoxy glucoside, quercetin 3,7 diglucoside and quercetin 3-O-glucoside. L.S. leaves have a variable chemical composition depending on the season, total flavonoids increase gradually from autumn to spring, whereas total nitrogen, protein, and lipid are highest in winter, the percentage of (total and soluble) carbohydrates peaks in the summer. Flavonoids and phenolics perform a variety of functions, including antioxidants, superoxide radical scavengers, mineral uptake chelators, enzyme inhibitors, and regulators [33]. L.S. leaves are used to treat eye diseases and stomach tumors in Yemeni folk medicine [34]. The Lycium genus has anti-diabetic, anti-cancer, and antioxidant properties. In folk medicine, the aerial parts of the plant are used as a laxative and diuretic, while the fruit is used to heal jaundice and protect the liver from damage [35].

2. MATERIALS AND METHODS

2.1 Sample Collection and Authentication

The leaves of Lycium Shawii Roem & Schult (L.S) were collected in December 2016 from the city of Sana'a, Yemen, at an altitude of 2,200 meters (7,200ft), Coordinates: 15°20′54″N 44°12′23″E, with the agreement of the authorities and in accordance with the United Nations Convention on Biodiversity, and with the assistance of traditional medical practitioners. The plant material was authenticated by Professor Hasan Ibrahim (botanist from faculty of science- Sana'a University) and a voucher specimen was deposited in the herbarium of the botany department.

2.2 Samples Preparation And Extraction

Lycium shawii roem& schult leaves were washed with distilled water and dried in room temperature for one week in the semi dark room. The aqueous plant extract was prepared by adding 500ml distilled water to the 200 mg leaves powder ground in mortar and boiling for thirty minute decoctions were allowed to cool at ambient temperature before being filtered using Whatman Grade No.1 filter paper and evaporated to dryness using a rotary evaporator (Yamato Scientific Co., Ltd. USA) at 40 C, then freeze-dried and stored [36].

2.3 Study Design, Location, Duration and Approvals

A randomized controlled trial (RCT) was conducted from December 19, 2017 to July 31, 2023The experiment lasted eight months, beginning on February 19, 2017 and ending on October 17, 2017. However, due to the nature of the war and blockade that erupted in our country from 2015 to the present, the analysis of the results and publication of the study had to be postponed for five years. Over the course of five months, the results were analyzed, and the study was written and printed.

The study was conducted at the Ziad scientific research unit in Sana'a, Yemen. The ethics committees of the respective institutes approved the study, and the animals were handled inaccordance with the Association for Research in Vision and Ophthalmology (ARVO) statement on the use of animals in Ophthalmic and Vision Research (ARVO) statement on the use of animals in Ophthalmic and Vision Research. The IACUC Guidelines for Rodent andRabbit Anesthesia, Analgesia and Tranquilization, and Euthanasia were followed.

2.4 Experimental Animal Model of Corneal Scar

Twelve European pigmented rabbits weighing 1.00 to 1.200 kg (mean 1.12kg. SD ± 0.16) at 5 to 6 month old (young adult of both genders, seven female and five male), provided by a local contractor. As both eyes of each rabbit (n=24 eyes) were subjected to a partial thickness (120–180 μm) sterile surgical excision on the anterior central part of the cornea with a 7 mm diameter. All of the rabbits were healthy, and the general clinical and ophthalmic examinations revealed that they were within normal. They brought a week befor starting anesthesia and surgical procedures to allow rabbits to acclimate to the facility and reduce stress [37]. They were housed in a light-controlled environment at a predetermined room temperature, and they were fed on a regular basis. The cages were chosen with a single cage for each member, and the

cages were placed on the ground and identified by letters and numbers on a metal plate. The sample size was relatively small because of the scarcity of resources and the country's current state of war.

2.5 Surgical and Anesthesia Procedures

Rabbits were fasted for 2 hours before being anesthetized by diazepam 2.5 mg/kg (diazepam-Hamlin 5 mg/ml ampoules 10 mg/2 ml, Hamlin Pharma, Germany) with ketamine hydrochloride 70–100 mg/kg (Ketamine 50 mg/ml Rotexmedica, Bach: 20905, Trittan, Germany) as a subcutaneous injection into the dorsum of the neck using a 25-gauge needle of a 1 cc syringe (tuberculin syringe/1 cc needle). cube) 25ga.Household brand, USA). Topical lidocaine 2% (K.M. Lidocain HCL inj. 2%, Huons, Korea) was instilled to block corneal sensation, and supplemental heat was provided during anesthesia and recovery procedures, with monitoring for fetal signs.

Under aseptic condition the rabbit was placed on a surgical table, slightly tilted up word to elevate the head. The peri-ocular area was cleaned with a gauze dipped in 10% povidone iodine (Povicare extra 10%, pharmacareint. mfg. co, BNO: 21148, Yemen) and then covered with a sterile surgical towel.The eye was exposed using an eye speculum (Murdock, Eye speculum with locking screw, AA 1080 small# appasamy), one drop of povidone-iodine 5% was instilled in the conjunctival cul-de-sac, waited 5 minutes, then irrigated with 15 ml. of 0.9 normal saline.The surgical procedure was carried out with the aid of a surgical magnifying loupe (3.5X loupe Bp fram surgical, Nanchang Micare medical equipment's, China) by assisted of a head light, with the incision made as follows:The corneas were trephined by 7.0 mm single used vacuum trephine (Barron Radial vacuum trephine,Corzamedical ophthalmology, India) to induce corneal incision by rotated gently to create a circular incision about anterior one third to half of corneal thickness (120-180 µm) then the trephinated area dissected carefully by crescent 2.5 mm bevel up blade (Bioblade, Bio-Tech Vision Care Pvt. Ltd., India), the end of the incision was completed with a corneal scissor (micro corneal scissor curved, pointed tip, medium blade11mm, AA 4105, Appasamy, India) assisted by corneal forceps (Calibri forceps 0.4 mm,1x2 teeth with tie platform, AA 3035, Appasamy, India) to raise the corneal flap. Topical 1% tetracycline hydrochloride eye ointment (Zhejiang Jinwei pharmaceutical co., Ltd., China) was applied to the corneal woundbefore closing the eye lid. The wound is left to heal naturally. We had one case of corneal perforation as a surgical complication in the beginning of the operation, so we decided to stop the surgical procedure for this rabbit, moxifloxacin hydrochloride 0.5% preservative freeeye drops (vigamox 5% Alcon, USA) 3 drops were instilled, the eye lid was closed with aplaster eye pad, this rabbit was excluded and replaced by another, and the corneal perforated wound was closed in the next day by wound healing processes, and the moxifloxacin 0.5% eye drops were administered four times daily for two weeks. Each operation took about 20 minutes. The rabbits are closely monitored after surgery to ensure that there are no complications, and appropriate postoperative care is provided.To make therabbits more comfortable, they were placed in a quiet, semi-dark environment. The anesthetics and surgical procedures were both performed by the same trained ophthalmologist, who was also the only person who was aware of the treatment group allocation. Gentamicin sulphate 0.3% eye drops (gentamicin sulfate eye/ear drops 0.3% 10 ml, E.I.P.I.Co.Egypt) were instilled in the operated eyes one drop five times per day for two weeks. For a week after surgery, oral analgesic 0.2% meloxicam (meloxicam tablets 7.5 mg, Mobic — Boehringer Ingelheim, Germany) was mixed into the drinking water.

2.6 Post-processing, Photography and Grading

Fluorescein sodium ophthalmic strip (1mg per fluorescein strip, Madhu Instruments, India) used to detection corneal epithelia defects (corneal ulcer) assisted by portable slit lamp (Shin-Nippon XL-1 LED Slit Lamp, Ohira Co. Ltd. Japan) with cobalt blue filter every 2 days was started in third day then at ,5,7,9,11,13 day post operative day, until reach complete corneal epithelial healing. The corneal wounds completely reepithelialized in all corneas from 11- 13 day Post operatively. After the surgical procedure, the rabbits were randomly distributed and divided into two experimental groups: the treatment group and the control group, with six rabbits in each group. This was achieved by using the standard = RAND () function in Microsoft Excel to generate random numbers.There was no systemic difference between the two groups of rabbits. We waited six weeks after surgical procedures for stable, old

corneal scars to develop [13, 14, 15]. Then we started administering eye drops to both groups, which prepared as follows:

For the treatment group, the solid material extracted from L.S. leaves was diluted with distilled water at a concentration of 10% (L.S.D). For the control group, they received the placebo isotonic solution of 0.9% normal saline (NS).

The eye drops were then put inside a 5 cc syringe container to prevent the withdrawal of air from the surroundings, which could potentially be polluted during the instillation process. The needle was broken at the plastic junction. The instillation was performed vertically with a volume of approximately 25 µm per eye drop. Samples were discarded and replaced with fresh drops after 72 hours. The remaining drops were kept in a refrigerator at a temperature between 4 and 8 °C. For both groups, the instillation was administered as one eye drop threetimes a day for two months. The eye drops were coded by numbers and were administered by the pharmacist using a randomization table. The same ophthalmologist monitored all rabbits every day and examined the corneas with a surgical magnifying loupe every three days, as well as with a portable slit lamp every week. The examinations were done without anesthesia and used a diffused narrow slit light that was 1 to 1.5 mm wide,10.mm long, and angled at 45 degrees between the observation and illumination axes.

2.7 Digital Photography

Photography was taken according to IACUC protocol as much as possible by smart phone camera of Samsung galaxy S5 camera (Verizon G900V,16 mega pixel, F22, 145s, 48mm, iso 40, white balance, with auto flash) before installation of the first dose of the treatment and after at 3, and 7 days, then 2, 4, 8, and 16 weeks (± 1 day) until the end of the study. Funduscopy was performed with direct ophthalmoscope (haene, Germany) before the surgical procedure, before treatment instillation, and at the end of the study with of mydriasis pupils by instillation of tropicamide 1% eye drops (medriacyl 1%, Alcon,UK) to identify any abnormal findings that could appear as side effects of the eye drops extracted from (L.S.D). The following procedures were used in photography rabbits' eyes: hold rabbit gently against assistant torso with their head tucked under one of assistant arms and assistant opposite hand grasping the rabbit's scruff, eye opened by using examiner thumb and index fingers with gentle pressure against periocular bones especially in lower lid without anesthesia, phone camera placed vertically to corneal plan with auto flashing in quite light place, the same condition was used, the name of the group,the number of rabbits,the date, and the time were all recorded in the follow up chart.

At the end of the experiments stage, the rotating Scheimpflug camera corneal tomography (Pentacam, Oculus GmbH, Wetzlar, Germany) was used to capture corneal images. However, this imaging was only performed for half of the eyes in the treatment group. This limitation was due to the high cost of the imaging procedure and a shortage of Pentacam devices in our country due to the circumstances of the war in which the country passed for years. The procedure was performed under general anesthesia with ketamine hydrochloride 70mg/kg and diazepam 0.6 mg/kg injected subcutaneously in the dorsum of the neck, the outcome of the scheimpflug corneal tomography parameters were used to compare to previous references.

2.8 Euthanization

Were humanely euthanized with an intra-peritoneal injection of pentobarbitone (150 mg/kg) (pentobarsol injection 6 grains of pentobarbital sodium, 250ml. bottles. Dechra, USA) while under general anesthesia.

2.9 Evaluation of Test Results

Corneal scars were evaluated and graded using slit lamp examination, as described previously.The corneal scars were quantified using a modified Fantes haze scale, which was used to grade the severity of the corneal scar. Two external expert ophthalmologists who weren't aware of the treatment allocation in each experimental group were involved in the evaluation the process.

The following criteria were used to evaluate the outcomes of corneal scars:

a. Subtract the initial opacity reading from the final post-treatment opacity reading for each individual cornea, including the negative control. Then calculate the average change in opacity for the negative control corneas.
b. For each treated cornea, calculate a corrected opacity value by subtracting the average

change in opacity of the negative control corneas from the change in opacity of that treated cornea. c. Determine the mean opacity value of each treatment group by averaging the corrected opacity values of the treated corneas for each treatment group [38].

2.10 Exclusion Criteria

It will be excluded if the scar is less than one-third of the corneal thickness.

2.11 Statistical Analysis

Slit lamp examination data including grading of each cornea have been recorded using modified Fants scale scales.

To compare various characteristics suitable parametric tests were utilized. The paired T-test was used to compare changes in each outcome measure in rabbit corneal scar before and after eye drop instillation for treatment and control groups. The independent t-test was used to compare the percentage change in each parameter between treatment and negative control group. a P value < 0.05 was deemed statistically significant. Graph pad prism excel software was used for all statistical analyses. Commercial software (SPSS version 25.) was used for statistical analyses.

3. RESULTS

The treatment group has a statistically significant reduction in corneal scar ($P < 0.000$ independent samples t-test), mean 2.75 score and a standard deviation (SD) ± 0.45 compared with the control group. It also restored corneal transparency in treatment group by a mean of 68.75% ($P < 0.000$, paired t-test), mean = 0.25, SD. ± 0.45.

3.1 Descriptive Data

In the current randomized controlled trial, the outcomes included only 22 out of 24 eyes that underwent sterile excision of partial thickness (from one-third to half) of the anterior central part of the cornea with a diameter of 7 mm. One rabbit was excluded from the control group due to heart failure. We noticed Signs such as weight loss, decreased appetite, weakness, and irregular heart beat by auscultation for these criteria we decided to euthanize this rabbit humanely. Ketamine anesthesia may be the probable cause of heart failure [39, 40]. No rabbits were excluded from the treatment group. The number of rabbit corneas included in the results analysis for each group was as follows: Treatment group n=12; control group n=10 corneas. After the surgical procedures, we waited six weeks for stable, old corneal scars to develop. Afterward the rabbits corneas received topical drop as one drops three times per day for two months.

The treatment group received lycium shawii leaves extract (10%; n=12), while the controlgroup received a placebo (normal saline 9%; n=10). The only five rabbits from the treatment group were sent to the countryside and kept by our relatives for monitoring, allowing them tolive in a natural environment and mate. Out of the five rabbits, one was male and four were females, while the other male rabbit was excluded. Almost two and a half years after the experiment began a final check-up was performed as a follow-up. Only three rabbits were found, identified by signs of individuality such as the colors and unique pattern of a rabbit's fur, which were subjected to a general and ophthalmic examination.

3.2 Evaluation of the Corneal Scar

The effects of therapy on corneal scars and ocular safety were evaluated using a slit-lamp microscope, a modified Fantes haze scale for grading, and a direct ophthalmoscope. The corneal wounds in all rabbit eyes had completely reepithelialized within 11 to 13 days post surgery. The modified Fantes haze scale as depicted in Table 1. was used to grade the corneal opacification, ranging from 3 to 0. Grade 3 indicates severe opacity.

3.3 The Results Were as Follows

The quantitative corneal scar scores based on the modified Fantes haze scale were obtained as follows: The average of the initial, final, and change in corneal scar score were obtained by subtracting the mean change in corneal scar of the control group's from the change in scar of each cornea of the treatment group after using the treatment as well as the average corrected scar value obtained by subtracting the mean change in corneal scar of the control group's from the mean change in corneal scar of the treatment group. These grades were assigned by two independent ophthalmologists who were masked to the treatment group after two months of instillation. Table 2. shows the average degree

Table 1. Modified Fantes haze scale

Grade	Description
Grade 0	Trace haze seen with careful oblique illumination.
Grade 1	Noticeable haze not interfering with visibility of fine iris details, haze smaller .than corneal area No neovessels ¼
Grade 2	Mild obscuration of iris details and haze larger than ¼ corneal area with or without neovascularization.
Grade 3	Obscuration of the iris and lens with or without neovascularizatio [16].
Grade	Description

Table 2. Shows the quantification of corneal scar (opacity) in both groups of rabbits using the modified Fantes haze scale, as well as the average of the initial, final, and changed corneal scar score

Group	Mean of Intial scar grade	Mean of Final scar grade	Mean of Change in scar grade	Average of corrected scar value	Percentage of scar reduction
Treatment group	3	0.25	2.75	2.75	68.75%
Control group	3	3	0	0	0

of scarring of the rabbits corneas in the treatment group, which before instillation was at the grade three, which significantly improved to 0.25 following the administration of treatment. The average change observed was 2.75 degrees, with a corresponding average correction of 2.75 degrees, resulting in a remarkable percentage improvement of 68.75%. While the average degree of scarring in the rabbit corneas of the control group rabbits was at the third degree before and after instillation, there were no change or correction in the average scarring.

Fig. 1 show the digital photography of corneal scars of control group before and after instillation of the placebo for a duration of two months. No significant change in corneal scar was observed.

The fig. 2. shows the digital photography of treatment group's corneas before and after topical instillation of L.S.D. 10% over a period of two months. Shows Significant reduction in corneal scar with improvement in corneal transparency.

(A) Rabbit cornea 40 days after surgical corneal ablation, and before instillation of treatment, when the old stable scar (opacity) had formed.Shows the scar is dense, severe and was at a grade 3 on the modified Fantes haze scale. (B) Rabbit cornea two months after administering of aqueous extracts derived from Lycium Shawii leaves as eye drops (10% concentration, one drop three times per day for two months) shows significant improvement in scar severity were it reduced to grade 0.

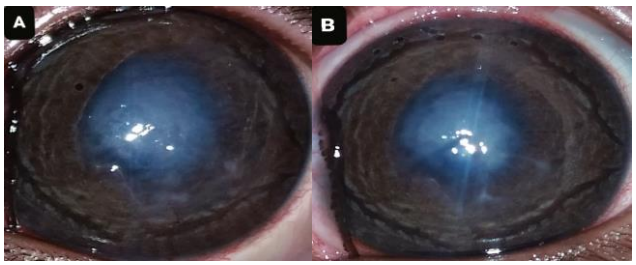

Fig. 1. presents the digital photography of the old corneal scar of rabbits in the control group. (A) Rabbit cornea 40 days after surgical corneal ablation, and before instillation, when the old stable scar (opacity) had formed. Shows the scar is dense, severe and was at a grade 3 on the modified Fantes haze scale. (B) Rabbit cornea two months after instillation of the placebo (0.9% normal salin, one drop three times per day for two months) it shows no improvement or decrease in the degree of the corneal scar. The degree of scar remained the same at grade 3 on the modified Fantes haze scale

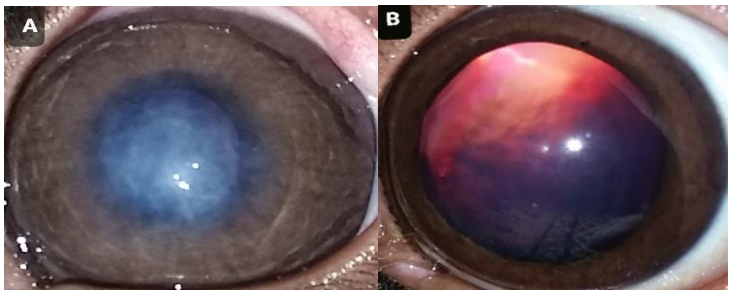

Fig. 2. presents the reduction in corneal scar in treatment group

(A) *Rabbit cornea 40 days after surgical corneal ablation, and before instillation of treatment, when the old stable scar (opacity) had formed.Shows the scar is dense, severe and was at a grade 3 on the modified Fantes haze scale.* ***(B)*** *Rabbit cornea two months after administering of aqueous extracts derived from Lycium Shawii leaves as eye drops (10% concentration, one drop three times per day for two months) shows significant improvement in scar severity were it reduced to grade 0.*

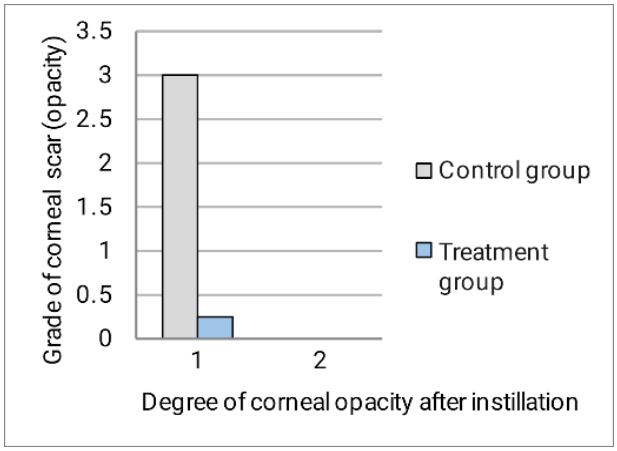

Fig. 3. displays a bar graph that compares the treatment groups with the control groups in terms of corneal scarring.

The corneal scars (opacity) in rabbits was evaluated using a modified Fantes haze scale. The results demonstrate a significant reduction in corneal scarring in the treatment group compared to the control group (P < 0.000), SD ± 0.45 in the treatment group, the degree of scar reduced to grade 0.25, which closely resembles a normal cornea. On the other hand, the control group maintained a degree of scar at grade three, similar to the state before the instillation

These images vividly show the treatment's efficacy in reducing the size and severity of corneal scars in rabbits, eventually leading to near complete recovery in the treated corneas with a statistically significant (P < 0.000) and SD of ± 0.45.

The Fig. 3. displays a bar graph comparing the treatment and control groups two months after instillation, which shows a significant reduction in the degree of corneal scarring in the treatment group compared to the control group.

The corneal scars (opacity) in rabbits was evaluated using a modified Fantes haze scale. The results demonstrate a significant reduction in corneal scarring in the treatment group compared to the control group (P < 0.000), SD ± 0.45 in the treatment group, the degree of scar reduced to grade 0.25, which closely resembles a normal cornea. On the other hand, the control group maintained a degree of scar at grade three, similar to the state before the instillation.

Fig. 4 displays the corneal parameters in the treatment group. In Table 3, the corneal parameters of the treatment group in the current study are compared to the corneal parameters reported in previous references, allowing for a comparative study.

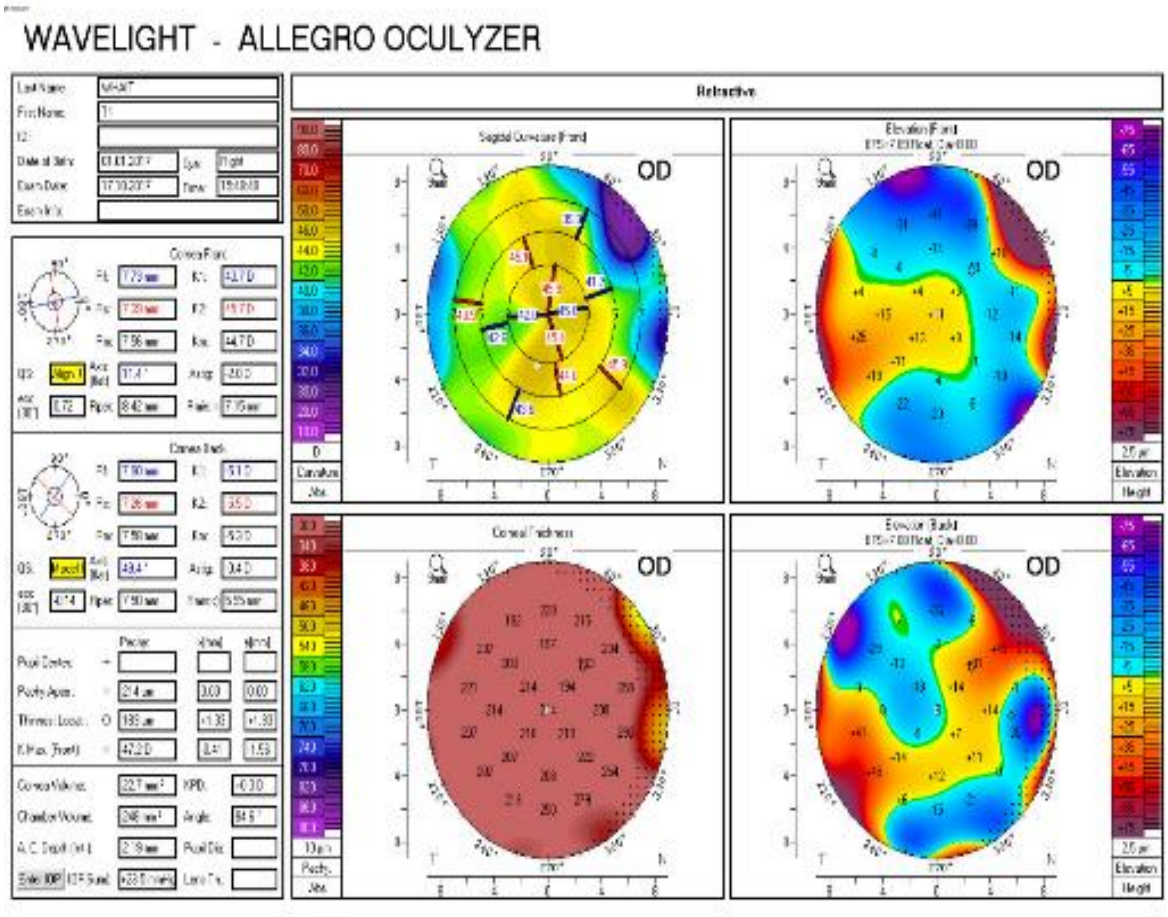

Fig. 4. presents a scheimpflug corneal tomography (pentacam) image of treatment group rabbit corneas after two months of therapy, displays corneal parameters that were close to normal value

Table 3. Comparison of corneal parameters in the current study's treatment group with previously established reference values for normal and scarred corneas

Parameter	Densitometry (mm^3)		Keratometry (D)		Thickness (µm)		Intraocular pressure(mmhg)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Rabbit Normal cornea (Reference)	18.7	±3.8	45.8	±0.9	407.3	±10.25	16	±4
Rabbit Normal cornea After treatment (Our study)	23.92	±1.1	43.14	±1.81	199	±85.48	24.55	±0.91
Rabbit scarred cornea (Reference)	54.5	±11.7	40.4	±2.25	273	±51.3		

D = Keratometric Diopters

Table 3. Displays the comparison of the outcome of corneal parameters in the treatment group of the current study with previously established reference values for normal and scarred corneas. Data from a previous study on densitometry, keratometry, and thickness were used as a reference point [15]. In addition, parameters for the normal range of intraocular pressure (IOP) in rabbits were obtained from another reference [41]. The parameters in our study were obtained from corneal tomography schiempflug (pentacam) image measurements performed

under general anesthesia with ketamine 70 mg/kg and diazepam 2 mg/kg, that lead to a temporary rise in intraocular pressure during anesthesia [41].

The treatment group (L.S.D. 10% therapy) showed a significant reduction in corneal scar scores compared to the corresponding control corneas (0.9% normal saline).

The treatment group has a statistically significant reduction in corneal scar ($P < 0.000$) mean 2.75 degree, SD ± 0.45, and with 68.75% mean improvement in corneal transparency ($P < 0.000$), mean 0.25 degree, Std. De ±0.45.

In the treatment group, nine corneal scars improved from grade three to grade zero, while three corneal scars improved from grade three to grade one. This variation in improvement may be attributed to differences in the depth of keratectomy or other factors.

On the other hand, the control group showed no significant change in corneal scar. The degree of scar remained the same at grade 3, with no signs of improvement.

Two and a half years later, during the follow-up period, there were no abnormal findings detected other than a slight degree of opacification noticed in the corneas under evaluation. The opacification was only noticeable in side illumination. The opacification scored as grade 0 on modified fantes haze scale, as seen in Fig. 5 with only slight variations observed among corneas.

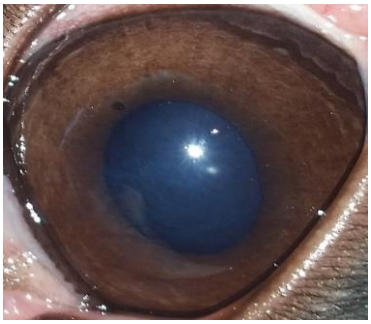

Fig. 5. Represents the digital photography of the treatment group's rabbit cornea after about two and a half years of follow-up, which shows the degree of corneal clarity, which was grade zero according to the modified Fantes haze scalethe, as no potential side effects or complications were observed

4. DISCUSSION

In our study, we demonstrated and approved the effectiveness of this topical drug extracted from leaves of L.S. in treating old corneal scars in rabbits. Corneal scar remains one of the main causes of blindness worldwide, which poses a challenge and an intractable dilemma for conventional medical treatment. Surgery is the only currently available and efficient method of treating corneal scars, but surgery has many limitations and complications.

To the best of our knowledge, our study is the first to evaluate the efficacy of LS. leaves in treating corneal scars in vivo, with the results indicating that it has a high efficacy in treating corneal scars.

Based on the findings of our preliminary study and our review of many of the manuscripts of Yemeni folk medicine, ethno botany textbooks, peer-reviewed academic publications, and research repositories that were used to gather the extensive review of literature, we have reached the conclusion that an extract derived from L.S. leaves has the potential to effectively treat corneal scar at all degrees ,layers and stages of corneal opacity formation. This conclusion is supported by interviews conducted with many traditional medicine practitioners in Yemen as well as our own observation which included ophthalmic examination of many patients with varying degrees of corneal opacity before and after their use of drops extracted from the L.S. by manual-squeezing methods.

Before conducting the preliminary study we met with traditional therapists and obtained participants consent to conduct these examinations and document. observations lasted for years before conducting this current study, as we did not notice any adverse potential effects, but rather On the contrary, we observed some benefits of this treatment on some other eye diseases that will require extensive scientific study in the future, and we will not discuss them in this topic.

When assessing the safety of the treatment approach, we observed no potential adverse effects during the experiment periods. We conducted thorough examinations of the cornea, conjunctiva, sclera, and retina using slit lamp and funduscopy. Our observations revealed no abnormalities except for a slight corneal haze that was noticeable under side illumination.

Importantly, this confirmed that the eye dropsextracted from LS. leaves (source) exhibited no side effects or toxicity.

There has been previous research on the toxicity of L.S leaves in animals (rats), there were no abnormal clinical signs and no deaths following oral administration of the methanol extract at the highest concentration of 4000 mg/kg body weight, indicating that oral use was safe. There are no toxicological studies on human L.S. leaves extracts. L.S. leaves could be used safely in pharmaceutical and natural therapies [42].

Based on the results of current study and our observations, we conclude that this treatment is effective and safe for treating corneal scars, and it is a promising treatment that is simple to prepare, manufacture, and inexpensive. It also foresees a bright future in the treatment of corneal scars in human, and the restoration of vision for many blind people.

As a result of our country's ongoing war from 2015 to the present, which has had an impact on the study, particularly since the air craft attacks by warplanes were very intense at the beginning of the experiment at the time of giving treatments to the experimental groups, as it led to us stopping giving treatments to groups of rabbits for intermittent periods that sometimes led to interruption for a period of almost two weeks while continuing to care for them, due to the noises of severe shelling and the vibration of the building, which may have an effect on the overall state of the animals and the results, we were afraid of being targeted at the location of the trials, which we were we decided to keep secret for safety and security considerations. For all experimental groups, the treatment time was two months.

We were unable to complete the study and analyze the results after we completed the experimental part (giving the treatments to all of the rabbits in the groups, evaluating the degree of opacity, photographing the rabbits' eyes, and documenting the outcome) due to the previously mentioned reasons, as well as other factors that caused the study to be suspended for several years, and when the war subside in 2023, we found an opportunity to complete the statistics.

The suggested mechanism of action in treatment of the scar are:

Although the aim of the current study is to evaluate the degree of effectiveness of L.S. leaves extracts in the treatment of corneal scars, particularly old ones, there have been previous studies on the role of polysaccharide extracted from Lycium barbarum in preventing the formation of Fibrosis in the corneas to evaluated the effectiveness of a pretreatment agent for corneal scarring made of polysaccharide from Lycium Barbarum (LBP) solution. To obtain significant physiological responses, fibroblasts were treated with LBP for 24 hours before matrix destruction and after that incubated with transforming growth factor 1 (TGF-1).LBP treatment reduced the expression of -smooth muscle actin, myofibroblast marker, vimentin, and type II and type III collagen extracellular matrix proteins. LBP therapy. Fibroblasts pretreated with LBP showed a reduction in angiogenic factor expression and inhibited unwanted proliferation [43].

The length of the course of treatment may have been affected by a number of factors, which may have increased the amount of time needed for corneal clarity. These elements are listed below

Drug ocular bioavailability in rabbits is restricted to less than 5% of the dose administered, and frequently less than 1%. This is because of a variety of limitations: 1. the corneal epithelium's permeability barrier. 2. quick ocular surface drainage of the administered medication [7]. 3. Protein binding is metabolized or destroyed by protease and esterase enzymes.4. The cornea's presence of static barriers. Therefore the logical design of the ocular therapeutic system necessitated increasing the drug holding duration and deeper penetration of the medication, which improved the drug's total bioavailability in the ocular tissue. Nano micelles, nanoparticles, Nano suspensions, liposomes, in situ gel, dendrimers, prodrugs, and contact lenses are examples of novel drug delivery methods [44]. One of the other factors that contributed to the treatment period being extended was that the rabbits used to shake their heads after receiving the drops, which caused the treatment to leak out of their eyes and they wiped their eyes with their hands before licking it. We did not notice this until about a month after they began administering the treatment, and in an attempt to prevent this, we placed masks over their eyes for ten to fifteen minutes after receiving the drops. When we used this method, the treatment effect was increased, resulting in a shorter treatment period. The cornea is a transparent organ, making it a perfect model for evaluating fibrolysis and antiscar treatments.We believe that using

inexpensive, effective and safe eye drops and herbal remedies can help to treat this issue.

Following the completion of the experiment's data collection we decided that the remaining rabbits in the control group should be rehabilitated and integrated into nature. So the rabbits of the control group were treated with drops extracted from L.S. one drop three times a day, and their corneal scar improved between five and eight weeks after treatment. They were then sent to the countryside to live and mate in the wild [45].

5. CONCLUSION

The results of the study showed that the L.S leaves extract was effective in treating scars on rabbit corneas and restoring their transparency in vivo. This innovative drug is expected to lead to the development of the first non-surgical treatment to treat old corneal scar. No significant adverse effects were observed, even after 2.5 years of follow-up.

The therapy group exhibits a statistically significant decrease in corneal scarring ($P > 0.000$), mean 2.75 score, SD. ± 0.45, as well as a mean improvement in corneal clarity of 68.75% ($P > 0.000$), mean = 0.25 score, and SD. ± 0.45. Since this treatment significantly reduced the degree of corneal scarring in treatment group, which improved from grade three (the highest degree of corneal scar severity on the modified Fantes haze scale) to grade zero (the closest to normal cornea clarity) in 75% of all corneas and from grade 3 to grade 1 in the remaining corneas.

The eye drops extracted from S.L. provide a non-surgical alternative treatment option for corneal scars, particularly old stable type. That is likely safe, simple to manufacture, and inexpensive. Further studies are needed to evaluate the safety and efficacy of L.S. eye drops in humans.

ETHICAL APPROVAL

Animal Ethic committee approval has been collected and preserved by the author(s)

CONSENT

As per international standards or university standards, Participants' written consent has been collected and preserved by the author(s).

FUNDING

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

ACKNOWLEDGEMENTS

We are grateful to Mr. Bashir Al-Fadhli, Director of the Ibn Al-Haytham Center for Ophthalmology in Sana'a, for his cooperation and for allowing us to perform schiempflug corneal topographic images (pentacam) for rabbits' corneas for free of charge at a time when other eye centers refused to cooperate at all. and grateful to dr. Ahmed Al-Asbahi, an ophthalmologist consultant, also provided us with surgical corneal trephines, Mr. Ahmed Mohsen Ziad, Director of the Ebsar Health Foundation for Ophthalmology, and dr. Ahmed Al-Jabbary for providing ophthalmic examination equipment.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Feder RS. External disease and cornea. American Academy of Ophthalmology. 2021;7.
2. World Health Organization. (n.d.). World Report on vision. World Health Organization. Available:https://www.who.int/publications-detail-redirect/9789241516570 -
3. Jeng BH, Ahmad S. In pursuit of the elimination of corneal blindness: Is Establishing Eye Banks and training surgeons enough? Ophthalmology. 2021;128(6):813–815. Available:https://doi.org/10.1016/j.ophtha.2020.06.042
4. Wang EY, Kong X, Wolle M, Gasquet N, Ssekasanvu J, Mariotti SP, Bourne R, Taylor H, Resnikoff S, West S. Global trends in blindness and vision impairment resulting from corneal opacity 1984–2020. Ophthalmology; 2023.Available:https://doi.org/10.1016/j.ophtha.2023.03.012 .
5. Wong YL, Liu S, Walkden A. Current perspectives on corneal transplantation (Part 2). Clinical Ophthalmology. 2022;16:647–659. Available:https://doi.org/10.2147/OPTH.S349582.

6. Sriram S, Gibson D, Robinson P, Tuli S, Lewin AS, Schultz G. Reduction of corneal scarring in rabbits by targeting the TGFB1 pathway with a triple siRNA combination. Advances in Bioscience and Biotechnology. 2013;04(10):47–55. Available:https://doi.org/10.4236/abb.2013.410A4005.
7. Toropainen E, Fraser-Miller SJ, Novakovic D, Del Amo EM, Vellonen K-S, Ruponen M, Viitala T, Korhonen O, Auriola S, Hellinen L, Reinisalo M, Tengvall U, Choi S, Absar M, Strachan C, Urtti A. Biopharmaceutics of topical ophthalmic suspensions: Importance of viscosity and particle size in ocular absorption of indomethacin. Pharmaceutics. 2021;13(4):452. Available:https://doi.org/10.3390/pharmaceutics13040452.
8. Riau AK, Tan NY, Angunawela RI, Htoon HM, Chaurasia SS, Mehta JS. Reproducibility and age-related changes of ocular parametric measurements in rabbits. BMC Veterinary Research. 2012;8(1):138. Available:https://doi.org/10.1186/1746-6148-8-138.
9. Watsky MA, Jablonski MM, Edelhauser HF. Comparison of conjunctival and corneal surface areas in rabbit and human. Current Eye Research. 1988;7(5):483–486. Available:https://doi.org/10.3109/02713688809031801.
10. Freund DE, McCally RL, Farrell RA, Cristol SM, L'Hernault NL, Edelhauser HF (n.d.). Ultrastructure in anterior and posterior stroma of perfused human and rabbit corneas. relation to transparency. Investigative ophthalmology; visual science; 1988. Available:https://pubmed.ncbi.nlm.nih.gov/7601631/
11. 2019-2020 basic and clinical science course, section 02: Fundamentals and principles of ophthalmology. American Academy of Ophthalmology. 2019;78.
12. Wang X, Wu Q. Normal corneal thickness measurements in pigmented rabbits using spectral-domain anterior segment optical coherence tomography: Thickness Measurement of Pigmented Rabbit Cornea By Sd-Oct. Veterinary Ophthalmology. 2013;16(2):130–134. Available:https://doi.org/10.1111/j.1463-5224.2012.01041.x.
13. Ke L, Wu QYS, Zhang N, Liu HW, Teo EPW, Mehta JS, Liu Y-C. Ex vivo sensing and imaging of corneal scar tissues using terahertz time domain spectroscopy. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy. 2021;255:119667. Available:https://doi.org/10.1016/j.saa.2021.119667.
14. Rawe IM, Zabel RW, Tuft SJ, Chen V, Meek KM. A morphological study of rabbit corneas after laser keratectomy. Eye. 1992;6(6):637–642. Available:https://doi.org/10.1038/eye.1992.137.
15. Joshi VP, Vaishnavi KS, Ojha SK, Singh V, Basu S. A reliable animal model of corneal stromal opacity: Development and validation using in vivo imaging. The Ocular Surface, 2020;18(4):681–688. Available:https://doi.org/10.1016/j.jtos.2020.07.017.
16. Cogswell D, Sun M, Greenberg E, Margo CE, Espana EM. Creation and grading of experimental corneal scars in mice models. The Ocular Surface. 2021;19:53–62. Available:https://doi.org/10.1016/j.jtos.2020.11.008.
17. Ghosh A, Singh VK, Singh V, Basu S, Pati F. Recent advancements in molecular therapeutics for corneal scar treatment. Cells. 2022;11(20):3310. Available:https://doi.org/10.3390/cells11203310.
18. Chouhan G, Moakes, RJA, Esmaeili M, Hill LJ, deCogan F, Hardwicke J, Rauz S, Logan A, Grover LM. A self-healing hydrogel eye drop for the sustained delivery of decorin to prevent corneal scarring. Biomaterials. 2019;210:41–50. Available:https://doi.org/10.1016/j.biomaterials.2019.04.013.
19. Kamil S, Mohan RR. Corneal stromal wound healing: Major regulators and therapeutic targets. The Ocular Surface. 2021;19, 290–306. Available:https://doi.org/10.1016/j.jtos.2020.10.006
20. Antunes-Foschini R, Adriano L, Murashima, ADAB, Barbosa AP, Nominato LF, Dias LC, Fantucci MZ, Garcia DM, Alves M, Rocha EM. Limitations and advances in new treatments and future perspectives of corneal blindness. Arquivos Brasileiros de Oftalmologia. 2021;84(3).

Available:https://doi.org/10.5935/0004-2749.20210042.

21. Kumar A, Yun H, Funderburgh ML, Du Y. Regenerative therapy for the Cornea. Progress in Retinal and Eye Research. 2022;87:101011. Available:https://doi.org/10.1016/j.preteyeres.2021.101011
22. Giva S, Abdelrahim A, Ojinna BT, Putrevu VP, Bornemann EA, Farhat H, Amaravadi K, Ben Abdallah M, Gutlapalli SD, Penumetcha SS. Safety and efficacy of mesenchymal stem cells for the treatment of evolving and established bronchopulmonary dysplasia: A systematic literature review. Cureus; 2022. Available:https://doi.org/10.7759/cureus.32598.
23. Escudero Bodenlle L, Domingo EO. Sclero-DALK: New approach for treatment of superficial corneal leukoma. American Journal of Ophthalmology Case Reports. 2019;16:100561. Available:https://doi.org/10.1016/j.ajoc.2019.100561.
24. Yan Q, Han B, Ma Z-C. Femtosecond laser-assisted ophthalmic surgery: From laser fundamentals to clinical applications. Micromachines. 2022;13(10):1653. Available:https://doi.org/10.3390/mi13101653.
25. Ghosh A, Singh VK, Singh V, Basu S, Pati F. Recent advancements in molecular therapeutics for corneal scar treatment. Cells. 2022;11(20):3310.Available:https://doi.org/10.3390/cells11203310
26. Salama A, Abudunia A-M, Al-kaf AG, Ziad NM, Ibrahimi A, Chabraoui L. Comparative study of the efficacy of stem cells in corneal regeneration in a chemical burn in rabbits. Universal Journal of Pharmaceutical Research; 2020.Available:https://doi.org/10.22270/ujpr.v5i4.445.
27. Talpan D, Salla S, Seidelmann N, Walter P, Fuest M. Antifibrotic effects of caffeine, curcumin and pirfenidone in primary human keratocytes. International Journal of Molecular Sciences, 2023;24(2): 1461. Available:https://doi.org/10.3390/ijms24021461
28. Boumil EF, Castro N, Phillips AT, Chatterton JE, McCauley SM, Wolfson AD, Shmushkovich, T, Ridilla M, Bernstein AM. Usp10 targeted self-deliverable sirna to prevent scarring in the cornea. Molecular Therapy - Nucleic Acids. 2020;21:1029–1043. Available:https://doi.org/10.1016/j.omtn.2020.07.032.
29. Gupta S, Fink MK, Ghosh A, Tripathi R, Sinha PR, Sharma A, Hesemann N. P., Chaurasia SS, Giuliano EA, Mohan RR. Novel combination bmp7 and hgf gene therapy instigates selective myofibroblast apoptosis and reduces corneal haze in vivo. Investigative Opthalmology & Visual Science. 2018;59(2);1045. Available:https://doi.org/10.1167/iovs.17-23308.
30. M Albarrak S. Supplementation with the methanolic extract of lycium shawii or rhanterium epapposum enhances immune responses of broilers. Advances in Animal and Veterinary Sciences. 2021;9(11). Available:https://doi.org/10.17582/journal.aavs/2021/9.11.1816.1828.
31. Rehman NU, Hussain H, Al-Riyami SA, Green Ivan R, Al-Harrasi A. Chemical constituents isolated from lycium shawii and their chemotaxonomic significance. Records of Natural Products. 2018;12(4):380–384. Available:https://doi.org/10.25135/rnp.34.17.09.160.
32. Ahmed FA, Abdallah NM, Ezz MK, El-Azab MM. Seasonal variations and identification of biologically active constituents of lycium shawii plant roem. & shult. (family solanaceae). International Journal of Innovative Science, Engineering & Technology. 2017;4(11):2348-7968
33. Frtwot N. Popular medicinal plants in Yemen medicinal plants used popularly for therapeutic purposes in some areas of Yemen.j.yemen. 2016;36:241. Available:https://www.researchgate.net/publication/336285528_alnbatat_altbyt_almstkhdmt_shbya_laghrad_lajyt_fy_bd_mnatq_alymn.)-
34. Nihad M. Fartout. Medicinal plants popularly used for therapeutic purposes in some regions of Yemen. Yemen journal. 2016;36:214-246
35. Gaweesh A, Sengab AENB, El-Hefnawy HM, Osman SM, Abdou AM. Phytoconstituents, Cytotoxic, Antioxidant and Hepatoprotective Activities ofthe Aerial Parts of L ycium shawii R. Growing in Egypt. Med Aromat Plants. 2015;4: 180. DOI:10.4172/2167-0412.1000180

36. Mikropoulou E, Vougogiannopoulou K, Kalpoutzakis E, Sklirou A, Skaperda Z, Houriet J, Wolfender J-L, Trougakos I, Kouretas D, Halabalaki M, Mitakou S. Phytochemical composition of the decoctions of greek edible greens (Chórta) and evaluation of antioxidant and cytotoxic properties. Molecules. 2018;23(7):1541. Available:https://doi.org/10.3390/molecules 23071541.
37. Harry Ransom Humanities Research Center, Kallendorf C, Wells MXZ37 ق- Aldine press books at the harry ransom humanities research center, the university of texas at austin: A descriptive catalog. Harry Ransom Humanities Research Center : Distributed by The University of Texas Press; 1998.
38. Cater KC, Harbell JW Prediction of eye irritation potential of liquid and granular laundry detergent formulas using the bovine corneal opacity and permeability (Bcop) assay. Cutaneous and Ocular Toxicology. 2013;32(3):210–221. Available:https://doi.org/10.3109/15569527 .2012.754459.
39. Rabbit_Anesthesia_guidance_ARC.the university of texas austin, animal resources center. (n.d.).
40. Li Y, Shi J, Yang B, Liu L, Han C, Li W, Dong D, Pan Z, Liu G, Geng J, Sheng L, Tan X, Sun D, Gong Z, Gong Y. Ketamine-induced ventricular structural, sympathetic and electrophysiological remodelling: Pathological consequences and protective effects of metoprolol: Metoprolol's effects on ketamine-induced remodelling. British Journal of Pharmacology. 2012;165(6): 1748–1756. Available:https://doi.org/10.1111/j.1476-5381.2011.01635.x.
41. Ghaffari MS, Moghaddassi AP. Effects of ketamine-diazepam and ketamine-acepromazine combinations on intraocular pressure in rabbits. Veterinary Anaesthesia and Analgesia. 2010;37(3):269–272. Available:https://doi.org/10.1111/j.1467-2995.2010.00531.x.
42. Ali SS, El-Zawawy, NA, Al-Tohamy R, El-Sapagh S, Mustafa AM, Sun J. Lycium shawii Roem. & Schult.: A new bioactive antimicrobial and antioxidant agent to combat multi-drug/pan-drug resistant pathogens of wound burn infections. Journal of Traditional and Complementary Medicine. 2020;10(1):13–25. Available:https://doi.org/10.1016/j.jtcme.20 19.01.004
43. Wang B, Han L, Liu J-M, Zhang J, Wang W, Li B-G, Dong C-X, Bai C-C. Lycium genus polysaccharide: An overview of its extraction, structures, pharmacological activities and biological applications. Separations. 2022;9(8):197. Available:https://doi.org/10.3390/separatio ns9080197.
44. Akhter MH, Ahmad I, Alshahrani MY, Al-Harbi AI, Khalilullah H, Afzal O, Altamimi ASA, Najib Ullah SNM, Ojha A, Karim S. Drug delivery challenges and current progress in nanocarrier-based ocular therapeutic system. Gels. 2022;8(2):82. Available:https://doi.org/10.3390/gels8020 082
45. Wang B, Han L, Liu J-M, Zhang J, Wang W, Li B-G, Dong C-X, Bai C-C. Lycium genus polysaccharide: An overview of its extraction, structures, pharmacological activities and biological applications. Separations. 2022;9(8):197. Available:https://doi.org/10.3390/separatio ns9080197.

Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle5.com/review-history/110471