Original Research

Journal of Advanced Veterinary Research
(2024) Volume 14, Issue 4, 586-590

Therapeutic effects of propolis ethanolic extract on infectious bovine keratoconjunctivitis in cows

Tohid Bahmani[1], Vahid Najarnezhad[1*], Abdolghaffar Ownagh[2]

[1]*Department of Internal Medicine, Faculty of Veterinary Medicine, Urmia University, Urmia, Iran.*
[2]*Department of Microbiology, Faculty of Veterinary Medicine, Urmia University, Urmia, Iran.*

ARTICLE INFO

Recieved: 01 January 2024

Accepted: 09 March 2024

***Correspondence:**

Corresponding author: Vahid Najarnezhad
E-mail address: v.najarnezhad@urmia.ac.ir

Keywords:

Cow
Infectious Bovine Keratoconjunctivitis
Pinkeye
Propolis Ethanolic Extract

ABSTRACT

Infectious bovine keratoconjunctivitis (IBK), commonly referred to as 'pinkeye,' is a prevalent ocular disease affecting cattle and caused by *Moraxella bovis*. Propolis, a natural substance collected by honeybees, is recognized for its diverse therapeutic properties, including antibacterial, antifungal, antiviral, and anti-inflammatory effects, along with its potential to promote wound healing and protect various organs. Given the substantial economic losses associated with IBK and the growing antibiotic resistance problem, this study aimed to explore the therapeutic potential of propolis' ethanolic extract in managing IBK-induced eye lesions in cows under field conditions. One hundred infected cattle aged between 18 months to six years old, organized into four distinct groups (n=25). Group I was treated topically with propolis ethanolic extract dissolved in glycerin (1000 µg/mL) once daily for four days. Group II was treated topically with tetracycline ointment (1%) once daily over the same four days. Group III received glycerin treatment once daily for four days, serving as a solvent control substance. Group IV, designated as the untreated control group, received no therapeutic intervention. Propolis ethanol extract improved all cases within 18 days while tetracycline improved only 84% of cases within 21 days. Remarkably, no discernible indicators of improvement manifested in group III and IV by the twentieth day of the investigative period. Propolis ethanol extract was able to resolve IBK eye injuries more completely in a shorter period than tetracycline. Therefore, it can be considered a good alternative compared to other antibiotics.

Introduction

Infectious bovine keratoconjunctivitis (IBK), commonly referred to as 'pinkeye,' is a prevalent ocular disease affecting cattle (Fonseca *et al*., 2020). This condition is also known by various names including keratitis contagiosa, infectious keratitis, contagious ophthalmia, New Forest eye, and blight in different geographical regions (Kneipp, 2021). Over the past half-century, extensive epidemiological investigations and experimental challenge models have consistently implicated *Moraxella bovis* (*M. bovis*) as the primary causative agent of IBK (Zheng *et al*., 2019). Highly contagious among bovine populations, IBK spreads rapidly within herds through direct contact, nasal or ocular discharges, and insect vectors, particularly when the corneal integrity is compromised (Zheng *et al*., 2019). The prevalence of IBK is influenced by multiple factors, including season, mechanical irritation, host immune response, eyelid pigmentation, presence of concurrent pathogenic bacteria, and *M. bovis* strain (Seid, 2019). While IBK affects both dairy and beef cattle of all ages, its impact is most severe on calves and confined animals (Fonseca *et al*., 2020). Notably, older cattle without prior exposure to IBK are also susceptible to severe outbreaks (Seid, 2019). IBK leads to substantial economic losses worldwide due to its painful nature, resulting in reduced milk production, weight gain, and disruption of breeding programs, alongside treatment expenses (Seid, 2019; Kneipp *et al*., 2021a).

Historically, a range of antibiotics, such as ampicillin, cephalosporin, nitrofurans, penicillin G, sulfonamides, tilmicosin, and others, have been widely employed for IBK treatment (McConnel *et al*., 2007; Alexander, 2010; Villarino *et al*., 2013; Maboni *et al*., 2015; Parin *et al*., 2017; Seeger *et al*., 2022). Recently the use of multiple antibiotics in the treatment of IBK under field settings is not recommended for economic reasons as well as from the standpoint of good stewardship relative to antibiotic use and concerns over the development of antimicrobial resistance (Angelos, 2015; Maboni *et al*., 2015; Parin *et al*., 2017; Seeger *et al*., 2022). Furthermore, these antibiotics are associated with various side effects, including conjunctival chemosis, conjunctival necrosis, hyper-salivation, head shaking, pawing at the ground and more (Senturk *et al*., 2007; Villarino, *et al*., 2013; Kneipp *et al*., 2021b). Instances of IBK recurrence following penicillin G treatment have also been documented (Alexander, 2010).

Propolis, a natural substance collected by honeybees, is recognized for its diverse therapeutic properties, including antibacterial, antifungal, antiviral, and anti-inflammatory effects, along with its potential to promote wound healing and protect various organs (Lotfy, 2006; Fernandes *et al*., 2007; Elumalai *et al*., 2022; Salatino, 2022; Wieczorek *et al*., 2022). Studies have highlighted propolis' ability to enhance macrophage activity and boost natural killer cell degradation of tumor cells (Sforcin, 2007; Ghosh *et al*., 2022). It has also been employed in the treatment of conditions such as goiter and difficult-to-heal wounds and ulcerations (Hartwich *et al*., 2000; Salatino, 2022).

Recent attention has been directed towards the antibacterial potential of ethanol extracts of propolis (Lisbona-González *et al*., 2021; Andre *et al*., 2022; Hossain *et al*., 2022; Yazdanian *et al*., 2022). Studies have demonstrated the antibacterial efficacy of propolis against a range of Gram-positive and Gram-negative bacteria (Eidi-Sheikhrobat *et al*., 2018; Pobiega *et al*., 2019; Salatino, 2022). Notably, an ethanolic extract of propolis from Taiwan exhibited effectiveness against various bacteria in vitro (Chen *et al*., 2018). Additionally, propolis ethanolic extract has shown successful outcomes in treating canine superficial pyoderma caused by Staphylococcus aureus (Dégi *et al*., 2022).

Given the substantial economic losses associated with IBK and the

ISSN: 2090-6277/2090-6269/

growing antibiotic resistance problem, this study aimed to explore the therapeutic potential of propolis ethanolic extract in managing IBK-induced eye lesions in cows under field conditions.

Materials and methods

Ethical approval

All procedures used in the present study were approved by the Scientific Research Ethics Committee on animal research, Faculty of Veterinary Medicine, Urmia University, Urmia, Iran.

Preparation of propolis ethanolic extract

To prepare the propolis ethanolic extract, 30 g of propolis obtained from the West Azerbaijan region's beehives underwent a meticulous process. Initially, the propolis was subjected to pulverization, yielding a finely small peace form. These propolis pieces were then subjected to dissolution in 300 mL of 96% ethanol under controlled conditions of 25°C, allowing for a comprehensive extraction period spanning 48 h. The resulting solution was subjected to a dual filtration process through Whatman filter paper (number 42), performed iteratively to ensure optimal purity and clarity of the extract. Subsequently, the solvent within the solution was meticulously eliminated through the employment of a rotary evaporator, leading to the concentration and isolation of the propolis extract. For formulation, the concentrated propolis extract was judiciously reconstituted in glycerin, achieving a precise concentration of 1000 µg/mL. This formulation was carefully prepared to harness the therapeutic potential of the propolis extract, optimizing its efficacy for subsequent applications. In adherence to stringent sterility standards, the finalized solution was subjected to meticulous sterilization via a 0.20 µ sterilized filter. This step was undertaken to ensure the elimination of any potential microbial contaminants, safeguarding the integrity of the prepared solution. The sterile propolis-glycerin formulation, thus prepared and validated, was methodically preserved under refrigeration conditions until its intended utilization, ensuring the stability and preservation of its therapeutic attributes.

Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

The chemical composition profiling of the propolis sample was meticulously executed through the utilization of Gas Chromatography-Mass Spectrometry (GC-MS) methodology. This analytical endeavor employed a Hewlett Packard HP 5890 Series II Gas Chromatograph, intricately interfaced with a mass spectrometer system, bolstered by an HP5-MS hair column (Agilent, USA). The Mass Spectrometer (MS) was judiciously operated under the electron ionization mode, harnessing its discriminatory capabilities. The carrier gas of choice was helium, adeptly adjusted to attain a controlled flow rate of 0.8 mL/min. The chromatographic partitioning and elucidation of the intricate chemical constituents were deftly conducted using a GC capillary column, specifically the HP-5MS, characterized by dimensions of 30×0.5 µm inner diameter (ID) and a film thickness of 0.25 µm. A meticulously designed temperature gradient regimen was meticulously executed, commencing with an initial column temperature of 60°C. This was adroitly augmented through an incremental temperature elevation of 5°C/min, culminating in a plateau at 300°C, effectively maintained for a duration of 10 min. A gap ratio of 1:10 was judiciously established to optimize chromatographic separation efficacy. The temperature of the injection port was meticulously set at 280 °C, ensuring optimal vaporization of the analytes. The ionization voltage within the mass spectrometer was adeptly calibrated to 70 V, thus ensuring the accurate generation of mass spectral data. This analytical configuration, comprising a meticulously orchestrated synergy of Gas Chromatography and Mass Spectrometry, engendered a comprehensive elucidation of the intricate chemical composition within the propolis sample, enabling the discernment of its diverse constituents with precision and fidelity.

Isolation of M. bovis

Referring to different infected cattle farms, cows with eye injuries suspected of IBK were identified. Their eyes were carefully examined, and samples were taken using a sterile cotton swab. The samples were then transferred in nutrient broth to the Department of Bacteriology of the Faculty of Veterinary Medicine, Urmia University and immediately cultured aerobically in a plate containing Columbia agar culture medium enriched with sterile defibrinated horse blood for 24 h at 37°C (Zbrun *et al*., 2011). The genus and species of *M. bovis* were then determined using the diagnostic keys methods (Bergey and Holt, 1994). The biochemical identification was confirmed by polymerase chain reaction (PCR((Angelos and Ball, 2007). The sequence of primers and temperature programs are given in the Table 1.

Determination of MIC and MBC of Propolis Ethanolic Extract on M. bovis

The broth micro dilution method was used to determine Minimal Inhibitory Concentration (MIC) and Minimal Bactericidal Concentration (MBC) of propolis ethanolic extract and tetracycline. MIC values were calculated according to the Clinical and Laboratory Standards Institute (CLSI) guidelines. Broth micro dilution method was used for testing in vitro the inhibitory concentration of the antimicrobial agent against specific bacterium (Cockerill *et al*., 2011). To determine the MBC, 10 µL of each well was transferred to Mueller- Hinton agar plates (Merck, Germany) and incubated at 37 ºC for 24 h. The MBC was considered as the lowest concentration of propolis associated with no visible growth of bacteria on the agar plates (Zeighampour *et al*., 2014).

Clinical study

The study cohort comprised 100 infected cattle from the cattle farms of West Azerbaijan province, during the summer and early fall of 2023, spanning an age range of 18 months to six years, organized into four distinct groups, each consisting of 25 individuals. In Group I, the cases were subjected to treatment involving the administration of propolis ethanolic extract dissolved in glycerin at a concentration of 1000 µg/mL (4 MIC). This treatment regimen was employed once daily over a span of four days. Group II was subjected to a comparative standard treatment, wherein tetracycline ointment 1% (Zisty-Kimia, Iran) was applied once daily over the same four-day period. Group III received glycerin treatment once daily for four days, serving as a solvent control substance. Group IV, designated as the untreated control group, received no therapeutic intervention. To gauge the progression of improvement, a meticulous daily examination of the afflicted eyes was conducted. The efficacy of the interventions was quantified based on the absence of lacrimation and the observed healing trajectory of corneal ulcers. Furthermore, the presence of *M. bovis*, the causative agent, was assessed through the methodical

Table 1. The amplification protocol name, thermal program for PCR and primer name and sequence and the size of PCR product (Dustin Loy and Brodersen 2014)

Protocol	Primer Name	Sequence 5--------3	PCR product size (bp)	PCR condition (Cycle)
Normal-PCR	Bovis-F Bovis-R	GTGAAGTCGTAACAAGGTAGCCGT ACCGACGCTTATCGCAGGCTATCA	671	95c for 5m, 95 c for 60s, 64c for 60s, 72c for 60s. 72c for 10m. (35)

collection of ocular secretions on days 0 (pre-treatment initiation), 4, and 20. This diligent surveillance facilitated a comprehensive assessment of the therapeutic impact and microbial dynamics throughout the investigative timeline. Healing time was defined as the time from enrollment (day 0) to the time of ulcer healing. For cattle in which treatment was not successful (persistent ulcers on day 20), the healing time was defined as 20 days (Angelos *et al*., 2000).

Results

Chemical Composition of Propolis

A total of nine distinct flavonoid compounds and 10 volatile organic compounds (VOCs) were meticulously identified through comprehensive gas chromatography analysis of the propolis sample. The quantitative assessment of the identified phenolic and flavonoid compounds, alongside the cataloged volatile organic compounds, is meticulously documented in Tables 2 and 3, respectively.

Table 2. The amounts of phenolic and flavonoid compounds in the propolis studied (ppm).

Flavonoid Acid and Phenolic compounds	ppm (µg/ml)
Cinnamic acid	27926.7
Apigenin	3282.6
Quercetin	1920.2
Rosemaric acid	1468.4
Coumaric acid	7209.3
Rutin	4285.7
Chlorogenic acid	432.1
Caffeic acid	2305.5
Gallic acid	1167.6

Table 3. The amounts of volatile organic compounds in the propolis studied (%).

Volatile Organic Compounds	Sensitivity (%)	Amount (%)
Aromadendrene	90	1.1
2-NaphtaleneMethanol	91	3.87
Alpha-Eudesmol	98	3.31
15-Tetracosanoic acid	64	1.79
Alpha-Atlantone	62	4.35
3,4-Dimethoxyphenylacetone	68	2.17
2-Methyl-7-oxo-4,7-Dihydro-1,2,4,T	59	1.34
2-Propen-1-one	98	7.24
1-Imidazole-1-yl-3-methylbut-2-en-1	52	11.18
Propanedinitrile	50	12.96

Culture, biochemical, and PCR tests

Suspected samples cultured on agar and the bacterium M. bovid identified by colony morphology and biochemical tests. To confirm the isolated *M. bovis* bacteria, PCR tests by amplification of Bovis gene with size 671 bp were used (Fig. 1.).

MIC and MBC Results

The MIC and MBC of propolis ethanolic extract tested on *M. bovis* were 250 µg/ml and 500 µg/ml respectively. The MIC and MBC values for tetracycline against *M. bovis* were 25 µg/ml and 50 µg/ml respectively. As seen propolis ethanolic extract and tetracycline exhibited comparable minimum inhibitory concentrations against *M. bovis*, suggesting potential antibacterial activity.

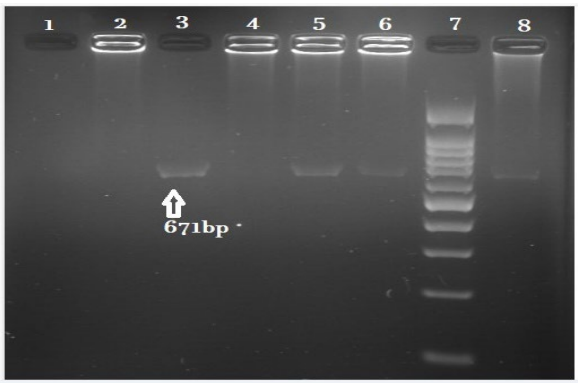

Fig. 1. Agarose gel image of amplified fragment of *M. bovis* (671 bp) using PCR. Lane 8, Positive control, Lane 7, 100- bp molecular ladder (Smobio Technology Inc., Taiwan); lanes 3, 5, 6 positive samples for *M. bovis*, Lane 2, 4, negative samples for *M. bovis*, lane 1, negative control.

Clinical Findings

Prior to the initiation of treatment, the afflicted bovine subjects exhibited characteristic ocular manifestations including photophobia, heightened lacrimation, corneal opacity, edema, ulceration, and neovascularization (Figs. 2a, 3a, 4a, and 5a.).

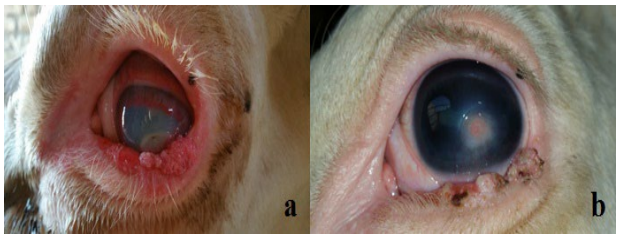

Fig. 2, a. Increased lacrimation, corneal opacity, edema, ulceration and neovascularization in a cattle with IBK. b. Improvement of corneal opacity, edema and neovascularization four days after the administration of propolis ethanolic extract

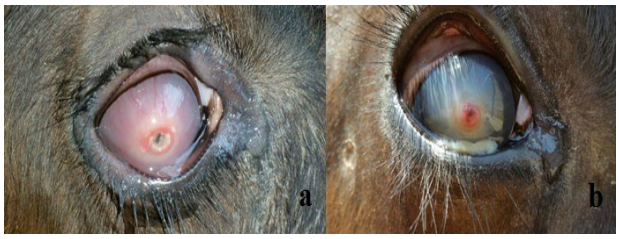

Fig. 3, a. Increased lacrimation, corneal opacity, edema, ulceration and neovascularization in a cattle with IBK. b. Improvement of corneal opacity, edema and neovascularization eight days after the administration of tetracycline

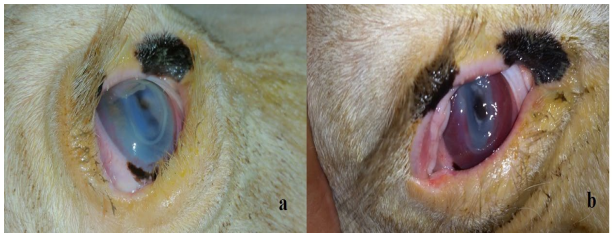

Fig. 4, a. Increased lacrimation, corneal opacity, edema, ulceration and neovascularization in a cattle with IBK. b. Observation of lacrimation, corneal opacity, edema, ulceration and neovascularization 20 days after the administration of glycerin

Upon the commencement of a daily regimen involving the administration of ethanol extract of propolis over a span of four days, a discernible amelioration in the active ocular lesions, attributed to *M. bovis*, was observed. Notably, this intervention achieved a comprehensive improve-

ment across all afflicted eyes (100% of the infected eyes) by the conclusion of the 14th day. Specifically, cessation of lacrimation was observed by the conclusion of the initial day of treatment, followed by notable enhancement in corneal opacity, edema, and neovascularization, culminating four days post-treatment (Fig. 2b.). Of significance, the outcome of bacterial culture assays yielded negative results at the culmination of the fourth day of therapeutic intervention.

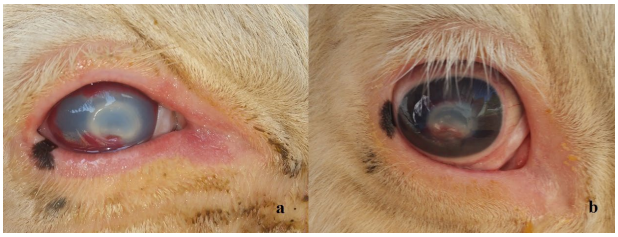

Fig. 5. a. Increased lacrimation, corneal opacity, edema, ulceration and neovascularization in a cattle with IBK. b. Observation of lacrimation, corneal opacity, edema, ulceration and neovascularization on the 20th day of the study.

A daily regimen involving the administration of tetracycline over a course of four days elicited a noteworthy amelioration in 21 active ocular lesions arising from *M. bovis* infection, thereby encompassing 84% of the initially afflicted eyes. This discernible therapeutic response culminated at the culmination of the 18th day. A notable observation was the persistence of profuse eye discharges for a span of two days post-initiation of tetracycline treatment. Subsequent to this period, a progressive mitigation of corneal opacity, edema, and neovascularization was discerned, achieving marked improvement within seven days post-treatment (Fig. 3b.). The investigative trajectory of bacterial culture assays, in parallel, yielded negative outcomes by the termination of the fourth day of the therapeutic protocol.

The daily administration of glycerin over a span of four days did not yield any discernible amelioration in the ocular lesions associated with IBK. Notably, *M. bovis* was consistently isolated from the ocular samples obtained on both the fourth and 20th days of the investigative period. The ocular morbidities, characterized by heightened lacrimation, corneal opacity, edema, ulceration, and neovascularization, persisted unabated throughout the course of study (Fig. 4b.).

Within the cohort designated as the untreated group, a meticulous daily evaluation of the afflicted ocular structures was conducted over a duration of 20 days. Remarkably, no discernible indicators of amelioration or improvement manifested by the 20th day of the investigative period. Concomitantly, the process of ocular sampling led to the consistent isolation of *M. bovis* from specimens obtained on both the fourth and 20th days. Of salient note, the ocular sequelae characteristic of augmented lacrimation, corneal opacity, edema, ulceration, and neovascularization persisted unabated until the 20th day of the study, as elucidated by the ocular findings at that juncture (Fig. 5b.).

Discussion

Infectious bovine keratoconjunctivitis (IBK) known as 'pinkeye' is a contagious disease confined to the eyes of cattle. *M. bovis*, is commonly considered the cause of IBK (Prieto *et al*., 2013; Parin *et al*., 2017; Zheng *et al*., 2019). O'Connor *et al*. (2012), Angelos (2010), and Comin *et al*. (2020) also attributes a role to *M. bovoculi* in causing the IBK. In this study, only *M. bovis* was isolated from all animals with eye damages suspected of infectious bovine keratoconjunctivitis.

The pathogenesis of IBK, elucidated through comprehensive scrutiny, delineates the intricate interplay of *M. bovis* and its associated toxins in orchestrating a multifaceted cascade culminating in corneal ulceration. This complex sequence involves the insidious disruption of corneal epithelial integrity through hemolysins and proteases, subsequently leading to ulcerative lesions (Smith *et al*., 2020). Concomitant with this pathological tableau, the host's immune response engenders pronounced inflammation, characterized by ocular manifestations encompassing erythema, edema, augmented lacrimation (epiphora), conjunctival edema (chemosis), and corneal edema. Notably, the pivotal corneal endothelial cells that regulate stromal hydration equilibrium emerge as prominent actors in this intricate symphony, their perturbation contributing to edematous sequelae and attendant corneal opacification (Angelos, 2015; Smith *et al*., 2020; Kneipp, 2021).

Almost all researchers believe that the clinical symptoms of infectious bovine keratoconjunctivitis are limited to the eyes and include increased lacrimation, epiphora, serous or mucopurulent conjunctivitis, and, most significantly, keratitis, seen initially as focal corneal edema (blue) developing corneal ulceration, which would typically when mature be deep (hazy), circumscribed, oval-shaped, and paracentral (Kneipp, 2021). In this study, animals with IBK also showed signs of photophobia, increased lacrimation, corneal opacity, corneal edema, corneal ulcers and corneal neovascularization to varying degrees.

So far various drugs such as ampicillin, cephalosporin, nitrofurans, penicillin G, sulfonamides, tilmicosin, trimethoprim-sulfonamide, cloxacillin, erythromycin, gentamicin, tetracycline, streptomycin, clarithromycin, florfenicol and tulathromycin have been used in the treatment of infectious bovine keratoconjunctivitis in cattle (McConnel *et al*., 2007; Alexander, 2010; Villarino *et al*., 2013; Maboni *et al*., 2015; Parin *et al*., 2017; Kneipp *et al*., 2021b; Seeger *et al*., 2022). The high cost of treatment, certain side effects on the body, and development of antibiotic resistance against some of these antibiotics (Senturk *et al*., 2007; Villarino *et al*., 2013; Kneipp *et al*., 2021b), prompted us to investigate a new drug for the treatment of infectious bovine keratoconjunctivitis.

Propolis is a natural resinous substance collected by honeybees from various plant sources. It has been widely studied for its potential therapeutic properties, including its antimicrobial, anti-inflammatory, and wound-healing effects (Movaffagh *et al*., 2019; Sokeng *et al*., 2020; Zulhendri *et al*., 2021; Rashid *et al*., 2022). While there is limited research specifically on the use of propolis for treating IBK caused by *M. bovis*, its properties suggest potential therapeutic effect on IBK.

In this study, the empirical examination of propolis as a therapeutic agent for Infectious Bovine Keratoconjunctivitis (IBK), induced by *M. bovis*, yielded substantive insights into its potential superiority over the tetracycline treatment.

Propolis, enriched with an array of bioactive compounds, emerges as a promising therapeutic modality. The multifaceted constituents of propolis, including flavonoids, phenolic acids, terpenoids, essential oils, and resins collectively converge to confer a spectrum of pharmacological actions (Šuran *et al*., 2021). Flavonoids, noted for their anti-inflammatory and antioxidant attributes, hold potential in mitigating inflammation-induced conjunctival hyperemia and chemosis (Xu *et al*., 2022). Phenolic acids, by virtue of their antimicrobial potency, hold the capacity to suppress *M. bovis* propagation and colonization, thereby facilitating corneal ulcer healing (Altuntas *et al*., 2023). Concurrently, terpenoids exhibit dual facets of anti-inflammatory modulation and wound-healing, accentuating their utility in curtailing ocular inflammation and fostering tissue repair (Oryana *et al*., 2018). The antimicrobial actions of essential oils hold promise in limiting bacterial proliferation, ensuring a conducive milieu for the healing process, while resins contribute to tissue repair, epithelialization, and protective occlusion (Yang *et al*., 2022).

The experimental outcomes underscore the superior efficacy of propolis vis-à-vis the conventional tetracycline intervention. Notably, the administration of propolis yielded expedited and comprehensive amelioration of active eye lesions, encompassing cessation of lacrimation, mitigation of corneal opacity, edema, and neovascularization. In contrast, the tetracycline regimen displayed a delayed and comparatively less efficacious profile. These observations signify the potency of propolis in accelerating the resolution of IBK-related ocular pathology, with implications for reduced discomfort, enhanced visual recovery, and potentially minimized sequelae such as corneal scarring and perforation.

Conclusion

The present investigation illuminates the therapeutic prowess of propolis as a viable and potent alternative for managing Infectious Bovine Keratoconjunctivitis (IBK) induced by *M. bovis*. This assertion is rooted in the multifaceted actions of propolis, leveraging its diverse bioactive constituents to effectively counter the complex cascade of events underpinning IBK pathogenesis. The observed superior therapeutic outcomes of propolis in comparison to the conventional tetracycline treatment substantiate its potential as a more efficacious intervention. The accelerated and comprehensive resolution of active eye lesions facilitated by propolis underscores its capacity to expedite recovery, reduce discomfort, and potentially mitigate long-term ocular sequelae. This study not only contributes to the advancement of veterinary ocular health but also underscores the rich therapeutic potential of propolis in addressing multifaceted pathophysiological processes.

Acknowledgments

The authors wish to express their gratitude to the Research Council of Urmia University, for financial support.

Conflict of interest

The authors declare that they have no conflict of interest.

References

Alexander, D., 2010. Infectious bovine keratoconjunctivitis: a review of cases in clinical practice. Vet. Clin. North Am. Food Anim. 26, 487-503.

Altuntas, U., Güzel, I., Özçelik, B., 2023. Phenolic Constituents, Antioxidant and Antimicrobial Activity and Clustering Analysis of Propolis Samples Based on PCA from Different Regions of Anatolia. Molecules. 28, 1-19.

Andre, I., Arief, I., Apriantini, A., Jayanegara, A., Budiman, C., 2022. Antimicrobial Activity of Propolis Extract and Their Application as a Natural Preservative in Livestock Products: A Meta-Analysis. Food Sci. Anim. Resour. 42, 280-294.

Angelos, J.A., 2010. Moraxella bovoculi and Infectious Bovine Keratoconjunctivitis: Cause or Coincidence? Vet. Clin. North Am. Food Anim. 26, 73-78.

Angelos, J.A., 2015. Infectious bovine keratoconjunctivitis (pinkeye). Vet. Clin. North Am. Food Anim. 31, 61-79.

Angelos, J.A., Ball, L.M., 2007. Differentiation of Moraxella Bovoculi sp. nov. from other Coccoid Moraxellae by the Use of Polymerase Chain Reaction and Restriction Endonuclease Analysis of Amplified DNA. J. Vet. Diagn. Invest. 19, 532-534.

Angelos, J.A., Dueger, E.L., George, L.W., Carrier, T.K., Mihalyi, J.E., Cosgrove, S.B., Johnson, J.C., 2000. Efficacy of florfenicol for treatment of naturally occurring infectious bovine keratoconjunctivitis. JAVMA. 216, 62-64.

Bergey, D.H., Holt, J.G., 1994. Bergey's Manual of Determinative Bacteriology, Ninth Ed. Williams & Wilkins. Baltimore, Maryland.

Chen, Y.W., Ye, S.R., Ting, C., Yu, Y.H., 2018. Antibacterial activity of propolins from Taiwanese green propolis. JFDA. 26, 761-768.

Cockerill, F.R., Wikler, M.A., Bush, K., Dudley, M.N., Eliopoulos, G.M., Hardy, D.J., Hecht, D.W., Hindler, J.A., Patel, J.B., Powell, M., Thomson, R.B., Turnidge, J.D., Weinstein, M.P., Zimmer, B.L., 2011. Performance standards for antimicrobial susceptibility testing: twenty-first informational supplement clinical and laboratory standards institute. Clinical and Laboratory Standards Institute. 31.

Comin, H.B., Domingues, R., Gaspar, E.B., Santos, J.R., Cardoso, F.F., 2020. Genetic differences among *Moraxella bovis* and Moraxella bovoculi isolates from infectious bovine keratoconjunctivitis (IBK) outbreaks in southern Brazil. Genet. Mol. Biol. 43, 1-7.

Dégi, J., Herman, V., Igna, V., Dégi, D.M., Hulea, A., Muselin, F., Cristina, R.T., 2022. Antibacterial Activity of Romanian Propolis against Staphylococcus aureus Isolated from Dogs with Superficial Pyoderma: In Vitro Test. Vet. Sci. 9, 299.

Dustin Loy, J., Brodersen, B.W., 2014. Moraxella spp. isolated from field outbreaks of infectious bovine keratoconjunctivitis: a retrospective study of case submissions from 2010 to 2013. J. Vet. Diagn. Invest. 26, 761 –768.

Eidi-Sheikhrobat, A., Sadat Naghavi, N., Karbasizadeh, V., 2018. Detection of the Effect of Bee Propolis Ethanol Extract against Molecularly Identified Multiple-Drug Resistant Bacterial Isolates in Wounds of Hospitalized Patients in Ahvaz City, Iran. J. Isfahan Med. Sch. 35, 1941-1948.

Elumalai, P., Muninathan, N., Megalatha, S.T., Suresh, A., Kumar, K.S., Jhansi, N., Kalaivani, K., Krishnamoorthy, G., 2022. An Insight into Anticancer Effect of Propolis and Its Constituents: A Review of Molecular Mechanisms. eCAM. doi: 10.1155/2022/5901191.

Fernandes, F.F., Dias, A.L., Ramos, C.L., Ikegaki, M., Siqueira, A.M., Franco, M.C., 2007. in vitro'' antifungal activity evaluation of propolis G12 ethanol extract on Cryptococcus neoformans. Rev. Inst. Med. Trop. 49, 93-95.

Fonseca, M.D.M., Maia, J.M.S., Varago, F.C., Gern, J.C., Carvalho, W.A., Silva, S.R., Mosqueira, V.C.F., Brandão, H.M., Guimarães, A.S., 2020. Cloxacillin nanostructured formulation for the treatment of bovine keratoconjunctivitis. Vet. Anim. Sci. 9, 100089.

Ghosh, S., Z. Al-Sharify, Z.T., Maleka, M.F., Onyeaka, H., Maleke, M., Maolloum, A., Godoy, L., Meskini, M., Rami, M.R., Ahmadi, S., Al-Najjar, S.Z., Al-Sharify, N.T., Ahmed, S.M., Dehghani, M.H., 2022. Propolis efficacy on SARS-COV viruses: a review on antimicrobial activities and molecular simulations. Environ. Sci. Pollut. Res. 39, 58628-58647.

Hartwich, A., Legutko, J., Wszolek, J., 2000. Propolis: its properties and administration to patients treated for some surgical diseases. Przegląd Lekarski. 57, 191-194.

Hossain, S., Yousaf, M., Liu, Y., Chang, D., Zhou, X., 2022. An Overview of the Evidence and Mechanism of Drug-Herb Interactions Between Propolis and Pharmaceutical Drugs. Front. Pharmacol. 13, 876183.

Kneipp, M., 2021. Defining and Diagnosing Infectious Bovine Keratoconjunctivitis. Vet. Clin. North Am. Food Anim. 37, 237–252.

Kneipp, M., Govendir, M., Laurence, M., Dhand, N.K., 2021a. Current incidence, treatment costs and seasonality of pinkeye in Australian cattle estimated from sales of three popular medications. Prev. Vet. Med. 187.

Kneipp, M., Green, A.C., Govendir, M., Laurence, M., Dhand, N.K., 2021b. Perceptions and practices of Australian cattle farmers for the treatment of pinkeye (infectious bovine keratoconjunctivitis). Prev. Vet. Med. 197, 105504.

Lisbona-González, M. J., Muñoz-Soto, E., Reyes-Botella, C., Olmedo-Gaya, M.V., Diaz-Castro, J., Moreno-Fernandez, J., 2021. Study of the Antimicrobial Effect of an Ethanolic Extract of Propolis in Periodontal Disease. Appl. Sci. 11, 1-10.

Lotfy, M., 2006. Biological Activity of Bee Propolis in Health and Diseas. APJCP. 7, 22-31.

Maboni, G., Gressler, L.T., Espindola, J.P., Schwab, M., Tasca, C., Potter, L., Vargas, A.C., 2015. Differences in the antimicrobial susceptibility profiles of *Moraxella bovis*, *M. bovoculi* and M. ovis. Braz. J. Microbiol. 46, 545-549.

McConnel, C.S., Shum, L., House, J.K., 2007. Infectious bovine keratoconjunctivitis antimicrobial therapy. Aust. Vet. J. 85, 65-69.

Movaffagh, J., Bazzaz, B.S.F., Yazdi, A.T., Sajadi-Tabassi, A., Azizzadeh, M.,Najafi, E., Amiri, N., Taghanaki, H.B., Ebrahimzadeh, M.H., Moradi, A., 2019. Wound Healing and Antimicrobial Effects of Chitosan-hydrogel/Honey Compounds in a Rat Full-thickness Wound Model. Wounds. 31, 228-235.

O'Connor, A.M., Shen, H.G., Wanga, C., Opriessnig, T., 2012. Descriptive epidemiology of *Moraxella bovis*, Moraxella bovoculi and Moraxella ovis in beef calves with naturally occurring infectious bovine keratoconjunctivitis (Pinkeye). Vet. Microbiol. 155, 374-380.

Oryana, A., Alemzadehb, E., Moshiric, A., 2018. Potential role of propolis in wound healing: Biological properties and therapeutic activities. Biomed. Pharmacother. 98, 469-483.

Parin, U., Kirkan, S., Gumus, S., 2017. Isolation of *Moraxella bovis* in Cattle and Detection of Antibiotic Susceptibilities. Int. J. Vet. Sci. 6, 228-231.

Pobiega, K., Kraśniewska, K., Derewiaka, D., Gniewosz, M., 2019. Comparison of the antimicrobial activity of propolis extracts obtained by means of various extraction methods. JFST. 56, 5386–5395.

Prieto, C., Serra, D.O., Martina, P., Jacobs, M., Bosch, A., Yantorno, O.M., 2013. Evaluation of biofilm-forming capacity of *Moraxella bovis*, the primary causative agent of infectious bovine keratoconjunctivitis. Vet. Microbiol. 166, 504-515.

Rashid, N.A., Mohammed, S.N.F., Halim, S.A.S.A., Ghafar, N.A., Jalil, N.A.A., 2022. Therapeutic Potential of Honey and Propolis on Ocular Disease. Pharm. 15, 1-26.

Salatino, A., 2022. Perspectives for Uses of Propolis in Therapy against Infectious Diseases. Molecules. 27, 1-26.

Seeger, M.G., Machado, C.S., Iglesias, B.A., Vogel, F.S.F., Cargnelutti, J.F., 2022. Antimicrobial efficacy of in vitro and ex vivo photodynamic therapy using porphyrins against Moraxella spp. isolated from bovine keratoconjunctivitis. World J. Microbiol. Biotechnol. 38, 103.

Seid, A., 2019. Review on Infectious Bovine Keratoconjunctivitis and its Economic Impacts in Cattle. JDVS. 9, 1-8.

Senturk, S., Cetin, C., Temizel, M., Ozel, E., 2007. Evaluation of the clinical efficacy of subconjunctival injection of clindamycin in the treatment of naturally occurring infectious bovine keratoconjunctivitis. Vet. Ophthalmol. 10, 186-189.

Sforcin, J. M., 2007. Propolis and the immune system: a review. J. Ethnopharmacol. 113, 1-14.

Smith, B., Van Metre, D., Pusterla, N., 2020. Large Animal Internal Medicine, Sixth Ed. Elsevier Mosby, United States, pp.1282-1283.

Sokeng, S.D., Talla, E., Sakava, P., Tagne, M.A.F., Henoumont, C., Sophie, L., Mbafor, J.T., Fohouo, F.N.T., 2020. Anti-Inflammatory and Analgesic Effect of Arachic Acid Ethyl Ester Isolated from Propolis. Biomed Res. Int. doi: 10.1155/2020/8797284.

Šuran, J., Cepanec, I., Mašek, T., Radi´c, B., Radi´c, S., Gajger, I.T., Vlaini´c, J., 2021. Propolis extract and its bioactive compounds—From traditional to modern extraction technologies. Molecules. 26, 1-21.

Villarino, N., Brown, S.A., Martín-Jiménez, T., 2013. The role of the macrolide tulathromycin in veterinary medicine. Vet. J. 198, 352-357.

Wieczorek, P.P., Hudz, N., Yezerska, O., Horčinová-Sedláčková, V., Shanaida, M., Korytniuk, O., Jasicka-Misiak, I., 2022. Chemical Variability and Pharmacological Potential of Propolis as a Source for the Development of New Pharmaceutical Products. Molecules. 28, 1600.

Xu, W., Lu, H., Yuan, Y., Deng, Z., Zheng, L., Li, H., 2022. The Antioxidant and Anti-Inflammatory Effects of Flavonoids from Propolis via Nrf2 and NF-κB Pathways. Foods. 11, 1-8.

Yang, J., Pi, A., Yan, L., Li, J., Nan, S., Zhang, J., Hao, Y., 2022. Research Progress on Therapeutic Effect and Mechanism of Propolis on Wound Healing. eCAM. doi: 10.1155/2022/5798941.

Yazdanian, M., Motallaei, M.N., Tahmasebi, E., Tebyaniyan, H., Alam, M., Abbasi, K., Seifalian, A., Ranjbar, R., Yazdanian, A., Mahmoodzadeh Hosseini, H., Moghaddam, M.M., 2022. Chemical Characterization and Cytotoxic/Antibacterial Effects of Nine Iranian Propolis Extracts on Human Fibroblast Cells and Oral Bacteria. Biomed Res. Int. 2022, 6574997. doi: 10.1155/2022/6574997.

Zbrun, M. V., Zielinski, G.C., Piscitelli, H.C., Descarga, C., Urbani, L.A., 2011. Dynamics of *Moraxella bovis* infection and humoral immune response to bovine herpes virus type 1 during a natural outbreak of infectious bovine keratoconjunctivitis in beef calves. J. Vet. Sci. 12, 347-352.

Zeighampour, F., Mohammadi, S.M., Shams, E., Naghavi, N.S., 2014. Antibacterial activity of propolis ethanol extract against antibiotic resistance bacteria isolated from burn wound infections. ZJRMS. 16, 25-30.

Zheng, W., Porter, E., Noll, L., Stoy, C., Lu, N., Wang, Y., Liu, X., Purvis, T., Peddireddi, L., Lubbers, B., Hanzlicek, G., Henningson, J., Liu, Z., Bai, J., 2019. A multiplex real-time PCR assay for the detection and differentiation of five bovine pinkeye pathogens. J. Microbiol. Methods. 160, 87-92.

Zulhendri, F., Chandrasekaran, K., Kowacz, M., Ravalia, M., Kripal, K., Fearnley, J., Perera, C., 2021. Antiviral, Antibacterial, Antifungal, and Antiparasitic Properties of Propolis: A Review. Foods. 10, 1360.