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Clinical Validation of Ethno-Veterinary Preparation for Infectious Bovine Keratoconjuctivitis

Karthik Kumar[1], Sivaraman Subramanian[2], Yogeswari Ragavan[2*] and Dharmaceelan[2]

[1]Marutham Research Institute, Chembur, Sivagangai, INDIA
[2]Department of Clinics, Veterinary College and Research Institute, Namakkal, INDIA

**Corresponding author: Y Ragavan; E-mail: dryogavet@gmail.com*

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ABSTRACT

Infectious Bovine Keratoconjunctivitis (IBK) of cattle is a common ocular bacterial infection caused by *Moraxella bovis* and characterized by blepharospasm, conjunctivitis, lacrimation, and varying degrees of corneal opacity and ulceration. In severe cases, ocular rupture leads to blindness. The economic impact of the disease is due to its high contagious nature, cost and time required for the treatment. Ethno-veterinary medicine provides low-cost alternatives to allopathic drugs. Research in such field is often undertaken as part of a community-based approach that serves to improve animal health especially in rural areas. Hence a study was conducted to evaluate the clinical effectiveness of the flowers of *Tabernaemontana divaricate* and *Thespesia populnea*, in crossbreed cattle, affected by IBK. The incidence of IBK (n=15) is 73.3% in young animals and 26.6% in adult animals. Out of fifteen infected animals, two animals were affected bilaterally [13.3%] and thirteen animals were affected unilaterally [86.6%]. The occurrence of disease was found in eleven animals that were infected during the autumn season [73.3%], three animals infected during the monsoon season [20%] and one animal was infected in summer season [6.6%]. The fresh juices of the flowers were poured on the affected eyes two times daily until cure. The overall success rate was achieved in fourteen animals [93.3%] with a mean recovery period 14.6 days. Further the Pharmacognostic review of the medicinal properties and the phyto-constituents of the flowers substantiate the zero cost and safe therapy against IBK.

HIGHLIGHTS

- Infectious Bovine Keratoconjunctivitis (IBK) of cattle is a common ocular bacterial infection.
- Fresh juice extracts of *Tabernaemontana divaricate* and *Thespesia populnea* were found effective against IBK without any side effects.

Keywords: Infectious Bovine Keratoconjuctivitis, *Tabernaemontana divaricate, Thespesia populnea*

Infectious bovine keratoconjunctivitis (IBK), or commonly known as pinkeye is a highly contagious and infectious ocular disease of cattle characterized by conjunctivitis and ulcerative keratitis, which occur worldwide (Holzhauer *et al*., 2004). In India first reported in Uttar Pradesh state (Jeyabal *et al*., 2013). It is caused by *Moraxella bovis* bacteria. It is a Gram- Negative, beta-hemolytic, aerobic, rod-shaped bacterium. The bacterium adheres to the cells via its fimbriae and pili proteins and produces β–hemolysin toxins which lyse the corneal epithelial cells. Many researchers indicated that at least one among the following, environment, season, physical injury, vector transmission, dietary insufficiencies, and the host's immune system was a potential contributor to the occurrence and severity of an IBK infection (Ahmed Seid, 2019). The disease is most commonly transmitted by mechanical vector, the face fly (*Musca autumnalis*) also transmitted by direct contact, aerosols and fomites (Snowder *et al.,* 2005). Carrier animals can shed the organism for long period, so they

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are an important factor in the spread of the disease and its survival over winter. When the eyes of a carrier animal are irritated, its tear production increases which promotes the shedding of *M. bovis* (Whittier *et al*., 2009). Early treatment of cattle with pinkeye is important, not only for a successful outcome of the individual animal affected, but also to stop the shedding of the bacteria to decrease the risk of transmission to other cattle. Antimicrobial therapies were used for the treatment of IBK via intramuscular, sub- cutaneous, sub-conjunctivitis, intravenous and topical routes. The practical problem of treating several individuals within a herd 2 or 3 times daily has inspired several studies in search of alternate, more cost-effective therapies (Dima and Fikedu, 2021).

Ethno veterinary medicine is a branch of science which deals with the study of Traditional knowledge, methods, skills, and practices used for treating various ailments of livestock. At least 80% of people in developing countries depend largely on these traditional medicines for the control and treatment of various diseases and parasites that affect both animals and humans. These traditional healing practices are called ethno-veterinary practice (WHO, 2002). Since many plants have been shown to contain powerful antimicrobial compounds that are alternative to antibiotics, the demand for herbal medicines has recently increased to treat microbes that are resistant to antibacterial agents. Nearly, 262 medicinal plants were used in various types of ocular diseases (Sandhu *et al*., 2011). Among them the commonly available flowers of *Tabernaemontana divaricate* (Reddy *et al.,* 2010) and *Thespesia populnea* (Acharya *et al.,* 2010) were used for sore eyes and conjunctivitis in humans. Hence in this study, the clinical efficacy of the fresh juice of *Tabernaemontana divaricate* and *Thespesia populnea* were tested against Infectious Bovine Keratoconjuctivitis.

MATERIALS AND METHODS

Study area

This study was carried out in door step veterinary health services in Melur Taluk, Madurai District, Tamil Nadu. This area is densely populated with animals of different species, especially cattle. Most of these people practice dairy, sheep and goat farming. This study was conducted from the year 2020 to 2021.

Study population

This study was carried out in fifteen cross breed cattle belonging to different owners, suffering from ocular disorders. Animals were from both sexes (male and female), different breeds (local and cross) and different age groups (young and adults).

Clinical Examination of animals

Clinical examination of the infected animals including history, visual (Menace reflex and Palpebral reflex tests) and physical examination were done as illustrated by Jackson and Cockcroft (2002). Whittier *et al*. (2009) characterized Infectious bovine keratoconjunctivitis eye lesion into four stages. Likewise, in the present study fifteen infected animals were classified in four stages.

Stage 1

Stage 1 is indicated when there is an excessive tearing and increased sensitivity to light which progresses to a small ulcer in the Centre of the cornea which appears as a small white spot.

Stage 2

Stage 2 is indicated when the ulcer spreads across the cornea with the cornea becoming increased opacity.

Table 1: Scientific classification of the Medicinal plants used for the treatment

	Tabernaemontana divaricate	***Thespesia populnea***
Kingdom	Plantae	Plantae
Order	Gentianales	Malvales
Family	Apocynaceae	Malvaceae
Genus	Tabernaemontana	Thespesia
Species	*T. divaricate*	*T. populnea*
Tamil name	Nathiyavattai	Puvarasa
Hindi name	Chandani	Paras papal
Kannadam	Nandibatlu	Adavibendemara
Malayalam	Kutampale	Puvarassu
Common name	Crape Jasmine, East India rose bay, Pinwheel flowers	Portia tree, Pacific rose wood, Indian tulip tree.

Stage 3

Stage 3 is indicated when the ulcer has covered almost the entire cornea and inflammation continues to spread into the inner parts of the eye. The inside of the eye is filled with fibrin, which is a Pus-like substance that gives the eye a yellow appearance.

Stage 4

Stage 4 becomes obvious when the ulcer extends completely through the cornea, and the iris may protrude through the ulcer. The iris will become stuck in the cornea even after healing process has occurred.

Herbal Formulae used for the treatment

Four flowers each from *Tabernaemontana divaricate* and *Thespesia populnea* were taken and its fresh juice was extracted and used for the treatment of IBK cases. The scientific classifications of the plants used are given in the Table 1 and the pictures of the flowers are given in Fig 1. Fifteen infected animals were treated with the juice of the above-mentioned flowers to be poured, two times daily until cure. The duration taken for healing and the overall success rate with mean recovery period was calculated.

Fig. 1: Herbal flowers of *Tabernaemontana divaricate* and *Thespesia populnea* used in the study

RESULTS AND DISCUSSION

The Clinical signs in the IBK infected animals were anorexia, overflow of tears onto the face, rapid blinking, affected eye became cloudy or opaque, some cases corneal ulceration observed, swelling of eyelids, inside of the eye is filled with pus-like substance. Further the animals were grouped according to the stages of infection form 1 to 4 Whittier *et al*. (2009). Among the treated animals, five animals were in stage 1, five animals were in stage 2, two animals were in stage 3 and three animals were in stage 4.

The baseline characteristics of the treated animals were given in the Table 2. The animals were kept under poor hygienic conditions in an open area exposed to wind and dust. The floor of the enclosures was not clean with plenty

Table 2: Base line characteristics of infected animals

Case No	Breed	Age (In years)	Unilateral/ Bilateral	Date attended	Stage of eye lesion	Response to treatment
1	HF cross	6 years	Bilateral	25-12-2021	Stage 3	5 days
2	HF cross	5 years	Bilateral	24-08-2021	Stage 1	6 days
3	Jersey cross	3 years	Unilateral	27-11-2020	Stage 1	7 days
4	Jersey cross	2 years	Unilateral	27-03-2021	Stage 1	7 days
5	Jersey cross	7 years	Unilateral	03-12-2020	Stage 1	7 days
6	Jersey cross	2 years	Unilateral	16-08-2021	Stage 1	2 days
7	ND	3 years	Unilateral	27-11-2020	Stage 2	6 days
8	Jersey cross	2 years	Unilateral	14-11-2020	Stage 4	57 days
9	HF cross	3 years	Unilateral	03-10-2021	Stage 2	8 days
10	Jersey cross	1 years	Unilateral	18-10-2020	Stage 3	14 days
11	Jersey cross	2 years	Unilateral	19-12-2020	Stage 2	11 days
12	Jersey cross	3 years	Unilateral	20-10-2020	Stage 2	9 days
13	Jersey cross	6 years	Unilateral	15-11-2021	Stage 4	15 days
14	HF cross	2.5 years	Unilateral	25-11-2021	Stage 1	5 days
15	HF cross	1.5 years	Unilateral	21-08-2021	Stage 4	Not cured

of dung and urine. Plenty of *Musca domestica* flies were seen around the face of the animals. In the present study, the incidence rate of IBK was significantly higher in young animal's aged from one to three years. Among the treated animals, 73.3% was young animals and 26.6% were adult animals of above 3 years of age. It appears that the younger age-group is more susceptible than the adult as interpreted by Takele and Zeribum (2000). Their recorded incidence was higher in the 2-3 years (3-54%) age group than above three years (1.8%) age group. Increased susceptibility of the younger animals may be due to the underdevelopment of immune factors and lack of ocular antibodies on the other hand, adult cattle have protective antibodies on the surface of the eye (Dima and Fikedu, 2021).

In the current study out of fifteen animals, two animals (13.3%) were bilaterally infected and thirteen animals (86.6%) were unilaterally infected. Previously Takele and Zeirbum (2000) reported similar findings. They documented 16 animals were bilateral (14.5%) and 94 animals were unilateral (85.5%). In many animals, at the onset of the infection, only one eye is involved, but as the disease progress, it is common that the infection from the first eye to migrate to the other eye resulting in both eye being infected (Bedford, 1992). In this study, the occurrence of disease was found in eleven animals that were infected during the autumn season (73.3%). Three animals were infected during the Monsoon season (20%) and one animal was infected in the summer season (6.6%). The occurrence of disease was more in autumn season because of the high population of flies. Radostits *et al.* (2006) reported that the IBK can occur in all seasons, predominately occurring in summer and autumn.

All the infected animals showed good responses to treatment in the first few days. Average healing duration of the infected animals in different stages was shown in the Table 3. Five animals affected with stage 1 IBK lesion were completely cured in 2 to 7 days (average 4.5 days). Likewise, five animals of stage 2 IBK lesion, two animals of stage 3 IBK lesion and two animals of stage 4 IBK lesion were completely cured in 6 to 11 days (average 8.5 days), 5 to 11 days (average 9.5 days) and 15 to 57 days (average 36 days) respectively.

The overall success rate with a mean recovery period in 14 animals (93.3%) was 14.6 days. One animal in stage 4 showed no improvement beyond sixty days. No side effect was observed during treatment. The phtotgraphic documnetation of the IBK infected and cured animals of different stages are shown in the Fig. 1. This report was comparatively higher than the earlier reports of (Chaldi, 1992) who achieved a success rate of 92.5% recovery rate with a single injection of long-acting Oxytetracycline.

Table 3: Average Healing duration of the infected animals

IBK lesion	No. of cases cured	Duration of healed (In days)	Average days of healed
Stage 1	five cases	2- 7 days	4.5 days
Stage 2	five cases	6-11 days	8.5 days
Stage 3	two cases	5-14 days	9.5 days
Stage 4	two cases	15-57 days	36 days
The overall success rate with mean recovery period = 14(93.3%) with mean 14.6 days			

In Traditional medicine, *T. divaricate* is used to treat various eye infections (Ghani, 2003). *T. divaricate* possesses extensive series of useful actions like anti-bacterial, anti-inflammation, anti-oxidant and analgesic properties (Sandhya *et al*., 2006). The blossoms of *Tabernaemontana divaricate* can be used for ophthalmitis (Raut *et al.*, 2022), sore eye, conjunctivitis (Koppula *et al*., 2010) and cataract (Guha *et al*., 2010). *Thespesia populnea* is found to possess useful medicinal properties, such as antibacterial, antioxidant and anti-inflammatory (Vasudevan and Parle, 2006). *T. populnea* used in the treatment of conjunctivitis (Acharya and Acharya, 2010). The phytochemical compositions of the two plants are given in the Table 4.

Table 4: Phytochemical Composition

Tabernaemontana divaricate	***Thespesia populnea***
Flavonoids, Terpenoids, cardiac glycosides, Carbohydrates, Protein, Saponins, Tannins and Phenols (Kalaimgal and Umamaheswari, 2015)	Alkaloids, Tannins, Saponins, Terpenoids, Phenolics, Steroids, Gossypol and Kaempferol (Jayapriya *et al*., 2016)

Terpenoids act as regulators of metabolism and play a protective role as antioxidants along with it acquires anti-microbial, anti-allergic and anti-inflammatory activity (Wagner and Elmadfa, 2003). Phenols, Flavonoids and

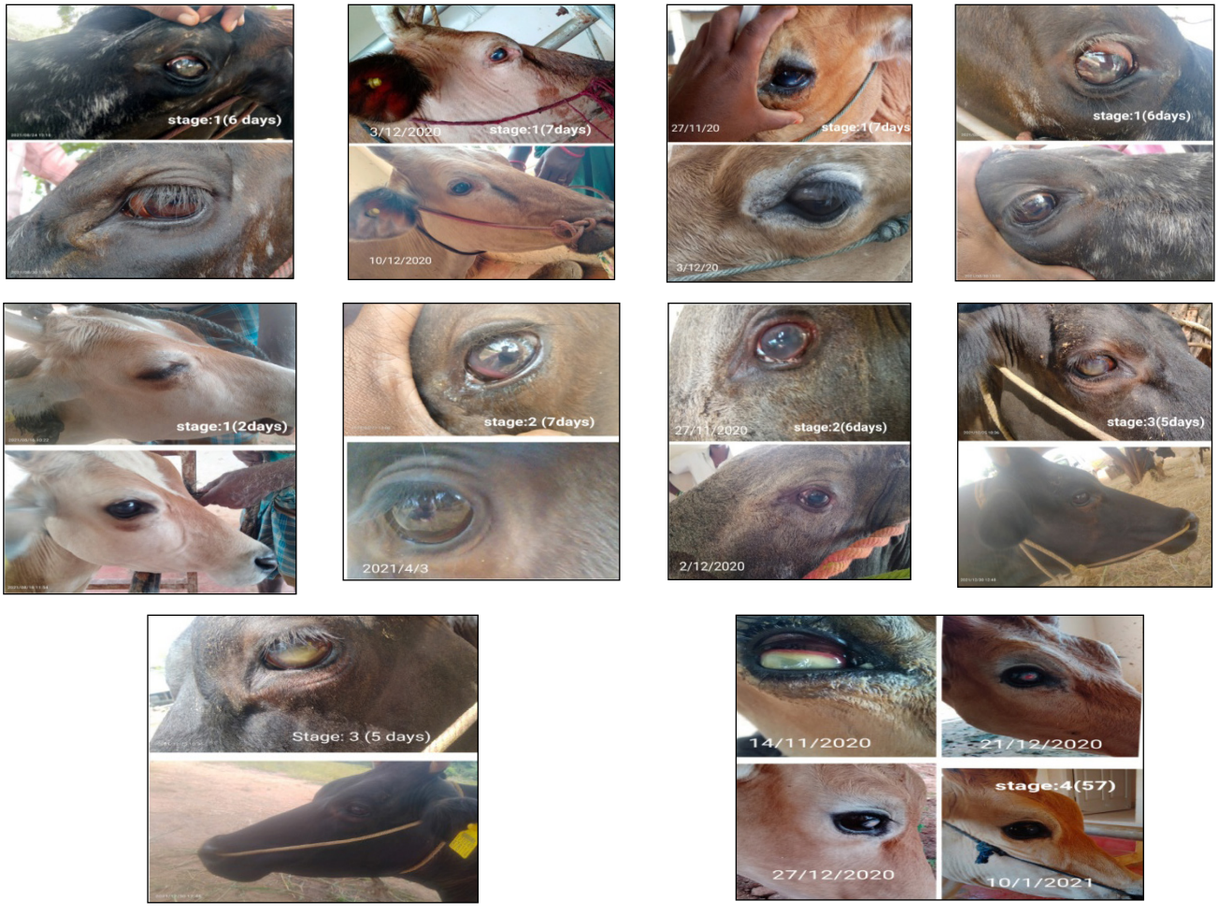

Fig. 2: Photographic documentation of the IBK infected and cured animals of different stages

Tannins act as antioxidant compounds which play a role as free radical scavengers (Tungmunnithum *et al.,* 2018). Tannins are astringent and are known bactericides because they react with proteins irreversibly, thus complexing within bacterial membranes, neutralizing their activity (Pizzi, 2021). Glycosides have analgesic, anti-inflammatory, antibacterial, antifungal and antiviral effects (Benito, 2022). Alkaloids are useful for curing burning sensation, ophathalmitis (Warrier *et al*., 1996). The above mentioned properties of the phytochemicals present in the herbal juice may be responsible for the healing the IBK in the infected animals.

Various scientists suggested different medicinal treatment for IBK like Ciprofloxacin and Chloramphenicol eye drops (Mukhtar *et al*., 2016), topical application of Cloxacillin (Fonseca *et al*., 2020), parental administrated Oxytetracyline (Ahmed, 2019). For past sixty years lot of antibacterial drugs were administered via intra muscular, subcutaneous, sub conjunctiva and topical routes to treat the infectious keratoconjunctivitis. Furthermore, antimicrobial therapy is the drug of choice for IBK, no regimen will ensure 100% success and also antimicrobial therapy may not eradicate the carrier state or improve the clinical disease. Now a day's a lot of antimicrobial drugs are available to treat the IBK, but the availability of veterinary services is not there in remote rural areas so in these areas traditional medicinal practices are a suitable way for the treatment of diseases (Chaudhary, 1994). Ethnoveterinary practices like this will reduce the problem of antibacterial resistance which is a great public health menace nowadays.

Infectious bovine keratoconjunctivitis cause a significant economic loss throughout the world, due to decreased weight gain, low calf growth rate, decreased milk production, drug cost for treatment, and decreased market value of animal due to corneal scarring (Arnold and Lebmkubler, 2012). It has been estimated that annual losses associated with only decreased weight gain from infected animals exceed 150 million dollars (Lane *et al*., 2006). Hence in this study, the fresh juices of the flowers of *T. divaricate and T. populnea* were found to be effective in the treatment of Infectious Bovine Keratoconjunctivitis. The results showed, the flowers of both the plants contain active compounds to cure the IBK without milk withdrawal period, side effects and it is cost effective.

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