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RESEARCH ARTICLE

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Clinical assessment of surgery and cryotherapy in bovine ocular squamous cell carcinoma

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Bovine ocular squamous cell carcinoma (BOSCC) is an economically important ocular neoplasm in cattle that causes visual impairment and reduced productivity in affected animals. The present study was conducted to evaluate the efficacy of surgical excision, cryotherapy, and their combination in the management of BOSCC in cattle. Eighteen cattle diagnosed with BOSCC based on clinical and pathological examinations were randomly allocated into three equal groups (n=6): group I underwent surgical excision, group II received cryotherapy, and group III was treated with a combination of surgical excision and cryotherapy. The parameters evaluated included tumour regression, healing time, pain score, recurrence, disease-free interval, and overall survival. The combined treatment group demonstrated the highest tumour regression (89.3%), the shortest healing time (12.7 days), the lowest pain score (3.1), complete recovery in all animals (6/6), and no recurrence during the six-month follow-up period. Haematological and physiological parameters remained within normal physiological limits throughout the study, indicating the systemic safety of the treatment protocols. In conclusion, the combination of surgical excision and cryotherapy proved to be the most effective treatment modality for the management of bovine ocular squamous cell carcinoma in cattle.

Keywords: Bovine, Cryotherapy, Squamous cell carcinoma, Surgical excision

Bovine ocular squamous cell carcinoma (BOSCC) otherwise called as "cancer eye," is one of the most commonly encountered neoplasms affecting cattle worldwide and represents a significant clinical and economic concern in dairy production systems (Priyanka *et al.,* 2021; Vijay *et al*., 2024). The disease primarily involves periocular tissues such as the eyelids, conjunctiva, corneoscleral junction and often progresses from benign plaques and papillomas to invasive malignant lesions (Prasanna *et al*., 2024). Chronic exposure to ultraviolet radiation, periocular depigmentation, breed susceptibility and old age are recognized as major predisposing factors influencing disease incidence (Islam *et al*., 2017). Progressive tumour development results in ocular pain, visual impairment, excessive lacrimation, off feed, decreased body weight and decreased milk productivity thereby adversely affecting animal welfare and farm income (Vivek *et al*., 2025).

Medical management of BOSCC under field conditions remains challenging due to high recurrence rates and limitations in advanced surgical facilities. Surgical excision is widely practiced for physical removal of neoplastic tissue; however, incomplete tumour margin clearance frequently leads to regrowth (Fornazari *et al*., 2017). Cryotherapy has emerged as an effective minimally invasive modality that destroys neoplastic cells through rapid freezing, intracellular ice crystal formation, osmotic injury and vascular stasis, while preserving surrounding healthy tissues (Priyanka, 2021). Recent veterinary oncological approaches emphasize that combination therapy combining surgical excision with adjunct cryotherapy significantly enhance local tumour control and reduce recurrence compared to single-modality interventions (Vijay *et al*., 2024).

Combination therapy is particularly advantageous in large animal practice where radical procedures such as enucleation are economically undesirable and cosmetically limiting. Application of cryotherapy to surgical margins effectively eliminates residual microscopic tumour foci and improves long-term prognosis (Fornazari *et al*., 2017). Recent clinical investigations have demonstrated superior tumour regression, accelerated wound healing and improved cosmetic recovery following integrated treatment protocols (Islam *et al*., 2017). Despite increasing clinical application, comparative evidence evaluating the efficacy of surgical excision, cryotherapy and combined therapeutic approaches under standardized field conditions remains limited. A systematic evaluation of tumour response, recovery indices, recurrence patterns and systemic safety is essential to establish evidence-based therapeutic guidelines for veterinary practitioners. Therefore, the study was carried out to assess the efficacy of surgical, cryotherapeutic, and combined interventions in cattle affected with BOSCC using clinical, haematological, and biochemical outcome measures.

Materials and Methods

In the present study, cattle presented with periocular growths (Fig. 1A) were examined, and a total of 18 cattle exhibiting clinical signs suggestive of bovine ocular squamous cell carcinoma (BOSCC) were included in the study after the diagnosis was confirmed by histopathological examination. The representative tissue specimens were evaluated using histopathological procedures and characteristic neoplastic epithelial cells showing keratin granules

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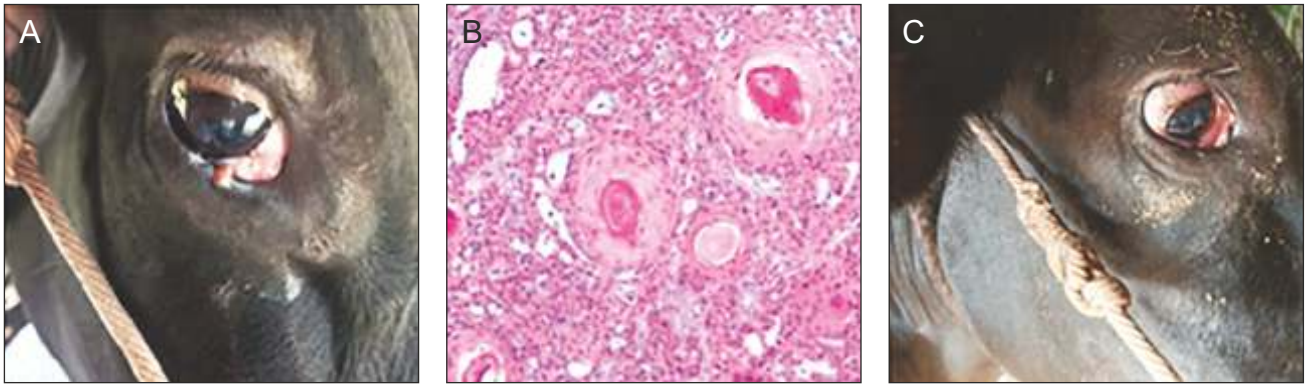

Fig. 1: (A) Ocular growth in cattle; (B) Histological section of BOSCC showing neoplastic epithelial cells with keratin granules; and (C) Clinical recovery.

was observed (Fig. 1B). Though metastasis was not explored further in the present study, there was no indication of any enlargement and other pathological conditions in the regional lymph nodes. Cattle were grouped randomly into three different treatment groups, each group including six animals. In group I, surgical excision was performed, in group II, cryotherapy was used and in group III, both surgical excision and cryotherapy were performed. These animals were grouped randomly regardless of their age, breed, sex, and eye sites because all of them showed characteristic clinical signs of BOSCC.

Before treatment, the animals underwent thorough physical examination with assessment of the tumour's location, size, corneal involvement, lacrimation and visual function (Table 1). Tumour size measurement was done using a measuring scale and the results were recorded in centimeters. Pain assessment was performed using a numerical pain scale (from 0 to 10). The animals were restrained in standing position and sedated with administration of xylazine HCl (0.05 mg/kg of body weight, i.m.). Local analgesia was achieved with auriculopalpebral and Peterson's retrobulbar nerve blocks using 2% lignocaine hydrochloride.

Table 1: Preoperative clinical findings in BOSCC affected cattle.

Parameters	Observation
Tumour location	Eyelid, conjunctiva, third eyelid and corneoscleral junction
Tumour size	0.5 to 1.5 cm
Surface morphology	Papillomatous, nodular, ulcerative, cauliflower-like
Corneal involvement	Mild
Lacrimation	Present in most cases
Ocular discharge	Serous to purulent
Vision status	Normal
Parotid lymph node involvement	No abnormal enlargement observed

In group I, excision of the tumour was carried out through surgery as a solitary treatment modality using routine methods of eye surgery with sufficient margins of safety. Haemostasis was accomplished by ligation, applying pressure and then suturing with sterile absorbable sutures. In group II, sterile cotton ear buds impregnated with liquid nitrogen (LN2) were used for applying cryotherapy on the superficial eye lesions as cryoguns could not be used under field conditions. LN2-impregnated cotton ear buds were applied over the surface of the tumour and its margins for about 30 seconds in each application for a total of four applications within one session. This treatment was performed for three consecutive weeks with one-week intervals between them. In group III, surgery was performed for removal of the tumour mass via traditional methods of ophthalmologic surgery with appropriate margins. Cotton ear buds saturated with liquid nitrogen (LN2) were then carefully placed on the margins of the surgical wound for about 30 seconds, and repeated four times at the same sitting in order to cause localized freezing and necrosis of remaining cancerous cells.

The clinical assessment was done on days 0, 7, 14, and 28 after treatment. The parameters assessed included tumour regression percentage, tumour regression in cm, healing time in days, pain intensity in a range of 0-10, time taken for appetite recovery and tumour recurrence percentage. Blood samples were collected in EDTA-coated vacutainer by aseptic venipuncture from the jugular vein for assessment of haemoglobin content, packed cell volume, total leukocytes count and differential leukocytes count. For biochemical analysis, serum was obtained by centrifugation and analyzed for AST, ALT, total proteins, albumin, globulin and cortisol levels using the Tulip biochemical analyser with Coral diagnostic kits. The data were analysed using appropriate statistical method.

Results and Discussion

The results presented in table 2 showed that the highest tumour regression was observed in group III (89.3±3.9%), followed by group II (75.8±5.1%) and

Table 2: Tumour response, clinical recovery and recurrence.

Parameters	Group I	Group II	Group III
Tumour regression (%)	62.5± 4.8[a]	75.8±5.1[b]	89.3±3.9[c]
Tumour size reduction (cm)	2.8±0.3[a]	3.6±0.4[b]	4.5±0.3[c]
Healing period (days)	17.7±1.2[a]	15.0±0.9[b]	12.7±0.8[c]
Pain score (0-10)	6.2±0.6[a]	4.8±0.5[b]	3.1±0.4[c]
Return of appetite (days)	5.6±0.7[a]	4.2±0.6[b]	3.0±0.5[c]

Means bearing different superscripts (a–c) within rows differ significantly.

Table 3: Hematological parameters.

Parameters	Group I	Group II	Group III
Hemoglobin (g/dL)	9.6±0.6[a]	9.9±0.5[a]	10.1±0.4[a]
Packed Cell Volume (%)	31.2±1.7[a]	32.0±1.5[a]	32.5±1.3[a]
Total Leukocyte Count ($\times 10^3/\mu L$)	8.4±0.7[a]	8.7±0.6[a]	8.9±0.5[a]
Neutrophils (%)	34.5±2.1[a]	33.8±1.9[a]	32.6±1.8[a]
Lymphocytes (%)	58.2±2.8[a]	59.4±2.6[a]	60.8±2.4[a]
Monocytes (%)	4.6±0.4[a]	4.4±0.5[a]	4.3±0.3[a]

Means within rows do not differ significantly

Table 4: Serum biochemical and stress parameters.

Parameters	Group I	Group II	Group III
AST (IU/L)	76.5±4.2[a]	74.1±4.0[a]	72.3±3.7[a]
ALT (IU/L)	39.7±2.1[a]	38.5±2.0[a]	37.9±1.8[a]
Total Protein (g/dL)	6.8±0.3[a]	6.9±0.4[a]	7.1±0.3[a]
Albumin (g/dL)	3.2±0.2[a]	3.3±0.2[a]	3.4±0.2[a]
Globulin (g/dL)	3.6±0.3[a]	3.6±0.3[a]	3.7±0.2[a]

Means within rows do not differ significantly.

Table 5: Overall survival time and clinical outcome in BOSCC.

Parameters	Group I	Group II	Group III
Complete recovery (No.)	3/6	4/6	6/6
Partial regression (No.)	2/6	1/6	0/6
Recurrence observed (No.)	3/6	2/6	0/6
Disease-free interval	3.0 ± 0.2[a]	4.0 ± 0.3[b]	6.0 ± 0.0[c]
Overall survival time (OST)	4.8 ± 0.4[a]	5.3 ± 0.3[b]	6.0 ± 0.0[c]

Means bearing different superscripts within rows differ significantly among groups (P<0.05)

group I (62.5±4.8%). A significantly greater reduction in tumour size was recorded in group III (4.5±0.3 cm) compared with group II (3.6±0.4 cm) and group I (2.8±0.3 cm). The shortest healing time was also observed in group III (12.7±0.8 days), followed by group II (15.0±0.9 days) and group I (17.7±1.2 days). Similarly, animals in the combination therapy group exhibited lower pain scores (3.1±0.4) and a faster return of appetite (3.0±0.5 days) than those in the other treatment groups.

These findings indicate that the superior tumour regression (Fig. 3), shorter healing period, improved clinical outcome, lower pain scores, and reduced tumour recurrence observed in group III may be attributed to the enhanced therapeutic efficacy of combining surgical excision with cryotherapy for the treatment of bovine ocular squamous cell carcinoma. Surgical excision effectively reduced the primary tumour mass, while subsequent cryotherapy likely improved local tumour control by inducing intracellular ice crystal formation, vascular stasis, and destruction of residual neoplastic cells. Similar findings have been reported by Priyanka *et al.* (2021) and Vijay *et al*. (2024), who demonstrated that combination therapy was more effective than either treatment modality alone in the management of bovine ocular tumours. Although cryotherapy alone also produced better tumour regression and faster healing than surgical excision alone, its therapeutic advantage is likely attributable to its direct cytotoxic effect on neoplastic tissue, as reported by Sozmen *et al*. (2019). Furthermore, cattle treated with the combined approach showed earlier recovery of appetite and a lower incidence of tumour recurrence, highlighting the clinical superiority of combination therapy.

The haemato-biochemical parameters presented in table 3 revealed no significant differences among the treatment groups, with all values remaining within the normal physiological range throughout the study. The haemoglobin levels were high in group III (10.1±0.4 g/dL) compared to group II (9.9±0.5 g/dL) and group I (9.6±0.6 g/dL). The PCV ranged from 31.2±1.7% to 32.5±1.3%, while TLC ranged between 8.4±0.7 and 8.9±0.5 × $10^3/\mu L$. Differential leukocyte count including neutrophils, lymphocytes and monocytes were statistically similar between different groups. Therefore, the consistent pattern of haematological parameters obtained in the current study suggested that there were no adverse effects of surgical removal, cryotherapy and combined approach on the health status of the studied animals. Normal values for haemoglobin and packed cell volume indicated that there was no significant loss of blood postoperatively. Stable values for leukocytes indicated that there was no significant systemic inflammatory response postoperatively. In a similar observation, Yavuz *et al*. (2025) observed the maintenance of haematological balance after treatment of local ocular tumours in cattle. Leukocytosis and neutrophilia being absent indicated that the methods employed in the treatment of these local tumours were safe and effective under field conditions.

In the present study, serum biochemical and stress indicators did not deviate from the normal physiological range in all groups (Table 4). A slight decrease in the level of AST in group III (72.3±3.7 IU/L) was noted compared with that in group II (74.1±4.0 IU/L) and group I (76.5±4.2 IU/L). At the same time, ALT values in

all groups fluctuated between 37.9±1.8 and 39.7±2.1 IU/L. Values of total proteins, albumins and globulins also were preserved at normal levels. It should be noted that even if insignificant in group III, there were lower levels of cortisol, which can indicate the lack of physiological stress in the studied animals. This means that surgical removal, cryotherapy and combined therapy did not cause noticeable alterations in the functioning of the body. The maintenance of serum protein fractions also indicated proper nutrition and immunity of the animals during the recovery phase. As reported in other studies conducted by Prasanna *et al*. (2021) and Priyanka *et al*. (2021), there were insignificant changes in the biochemistry of ocular tumours in cattle after local management. The results of the current study confirmed the effectiveness and safety of the combination treatment method for BOSCC in cattle.

The overall survival time and clinical outcome of BOSCC-affected cattle following different therapeutic interventions are presented in table 5. In this study 100% of the animals (6/6) completely recovered when using combined surgery and cryotherapy compared with 50% (3/6) using surgery and 66.6% (4/6) of animals using cryotherapy. Lesion regression was partial in two animals from group I and one animal from group II. It means that lesions had not been completely healed when each type of therapy was administered individually. Disease recurrence was more frequent in animals where there was surgical excision (3/6) than in those receiving cryotherapy (2/6) alone. There were no instances of disease recurrence in the combined therapy group during the six months observation period (Fig. 1C). Disease-free time was relatively higher in combined therapy group (6.0±0.0 months) than group I (3.0±0.2 months) and group II (4.0±0.3 months). Likewise, the overall survival time was relatively higher in combined therapy group (6.0±0.0 months) than in group I (4.8±0.4 months) and group II (5.3±0.3 months).

This enhanced therapeutic outcome could have been due to the efficacy of cytoreduction combined with elimination of remaining cancerous cells using cryotherapy, which in turn resulted in lesser possibilities of tumour recurrence. This finding was supported by a study conducted by Priyanka *et al.* (2022) where they noted improvements in healing and lower chances of tumour recurrence after combining cryotherapy with other treatment regimens in ocular tumours. Similarly, according to another study conducted by Yavuz *et al*. (2025), there were better outcomes in terms of prognosis and disease-free survival period after employing a multimodal approach in patients diagnosed with BOSCC. The results obtained in the present study were in concurrence with the study performed by Prasanna *et al*. (2021), wherein they found longer survival and better clinical improvement after implementing combined therapeutic modalities in ocular tumours.

The present study demonstrated that the use of surgical excision along with cryotherapy would be more effective in managing BOSCC in cattle. It was also found that the use of surgical excision together with cryotherapy could be regarded as an efficient, economic, and practical approach to treat ocular tumours in cattle.

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