

Comparative evaluation of continuous and superpulsed CO₂ laser in bovine ocular tumours

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ABSTRACT

The present study was undertaken to compare the efficacy of continuous and super pulsed modes of carbon dioxide (CO₂) laser for resection of bovine ocular tumours and to evaluate associated thermal tissue damage and surgical outcomes. Seventeen bovines with ocular tumours having a maximum diameter of ≤65 mm were randomly allocated into two groups. Tumour resection was performed using CO₂ laser in continuous mode in Group I and SuperPulsed mode in Group II under general anaesthesia. Clinical characteristics, laser parameters, postoperative recovery, thermal damage, and histopathological findings were evaluated. A total of 34 ocular tumours were studied, of which most were well demarcated and histopathologically diagnosed as dermoids. Ocular squamous cell carcinoma represented the major malignant tumour type encountered. The third eyelid and medial canthus were the most frequently affected sites. Mean power output, excision time, and thermal tissue damage were significantly higher in the continuous mode than in the Super Pulsed mode. Physiological parameters and Schirmer tear test-I values remained within normal limits throughout the observation period. All animals recovered uneventfully and were followed for two months postoperatively. Tumour recurrence was observed only in two animals treated using the continuous mode, whereas no recurrence occurred in the super pulsed group. The findings indicated that CO₂ laser is an effective modality for bovine ocular tumour resection and that the super pulsed mode provided superior surgical precision with reduced collateral thermal damage and improved clinical outcomes.

Keywords: CO₂ laser, Continuous mode, Dermoids, OSCC, Superpulsed mode, Thermal damage

Ocular diseases adversely affect animal health, welfare, and productivity, thereby causing economic losses to livestock owners. Among these, bovine ocular neoplasia (BON) is of particular importance and includes both benign and malignant tumours affecting the eyeball and associated ocular structures (Perino *et al.* 1993). Dermoids are the most common benign ocular tumours and may cause irritation, corneal damage, eyelid deformities, and impaired vision (Badanes and Ledbetter 2019) while, ocular squamous cell carcinoma (OSCC) are the most common malignant ocular tumour in cattle, and may involve conjunctiva, cornea, limbus, sclera, and eyelids, resulting in substantial economic losses due to reduced productivity and premature culling (Patel *et al.* 2019). Surgical excision remains the primary treatment for most ocular tumours, although conventional techniques may be associated with excessive haemorrhage, tissue trauma, postoperative discomfort, and prolonged healing. Alternative therapeutic approaches such as cryotherapy, carbon dioxide (CO₂) laser ablation,

hyperthermia, immunotherapy, and radiation therapy have also been reported (Ng *et al.* 2023). Among these, CO₂ laser surgery has gained increasing acceptance in both human and veterinary medicine because of its excellent haemostatic properties, reduced postoperative pain, minimal tissue manipulation, and improved wound healing (Khalkhal *et al.* 2019, Winkler 2019).

CO₂ laser systems operate primarily in continuous and super pulsed modes. The super pulsed mode delivers short bursts of high peak energy with reduced thermal spread, whereas the continuous mode produces uninterrupted laser output (Fisher 1993, Winkler 2019). Although CO₂ lasers have been used for ocular tumour excision in horses, dogs, and bovines (Clode *et al.* 2012, Dees *et al.* 2015, Patel 2023, Solanki 2024), comparative information regarding continuous and super pulsed modes in bovine ocular surgery is limited, particularly with respect to quantification of collateral thermal damage. Therefore, the present study was undertaken to compare continuous and super pulsed modes of CO₂ laser for resection of bovine ocular tumours and to evaluate the associated thermal tissue damage. It was hypothesized that the super pulsed mode would produce significantly less thermal injury while maintaining effective tumour excision and favourable postoperative outcomes.

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Table 1. Clinical examination of group I animals (Growth resection using continuous mode of CO₂ laser) (N=9)

Group	Case No.	Eye	Conjunctival mucous membrane	Location of growth	Size (mm)	Volume (cm ³)
1	1	OD	Pink	Third eyelid	14×9×5	0.59
		OS	Pink	Medial canthus	16×8×6	0.54
				Upper eyelid and palpebral conjunctiva	19×15×11	2.24
1	2	OD	Pink	Third eyelid and lower palpebral conjunctiva	20×15×10	2.34
		OS	Pink	Medial canthus and lower bulbar conjunctiva	35 ×15×10	4.12
1	3	OD	Pink	Medial canthus	20×15×10	2.36
				Third eyelid, medial canthus with upper and lower palpebral conjunctiva	52×22×15	13.17
		OS	Pink	Third eyelid	15×20×10	3.14
				Upper eyelid and palpebral conjunctiva	20×10×10	1.05
				Medial canthus and lower palpebral conjunctiva	56×26×15	19.81
1	4	OS	Pink	Third eyelid	40× 16× 12	5.36
1	5	OD	Congested	Third eyelid	24×12×7	1.81
1	6	OD	Congested	Third eyelid	45×20×10	9.42
1	7	OS	Pink	Upper palpebral conjunctiva and bulbar conjunctiva	35×20×10	7.33
				Lower palpebral conjunctiva and bulbar conjunctiva	30×18×15	5.09
				Medial canthus	10×8×3	0.33
1	8	OS	Pink	Upper eyelid	32×18×12	5.43
1	9	OD	Pink	Medial canthus	18×14×8	1.85
					Mean	4.78±1.19

Table 2. Clinical examination of group II animals (Growth resection using superpulsed mode of CO₂ laser) (N=8)

Group	Case No.	Eye	Conjunctival mucous membrane	Location of growth	Size (mm)	Volume (cm ³)
2	1	OD	Pink	Medial canthus	18× 12× 6	1.36
				Lower palpebral conjunctiva	32×18×10	5.43
				Upper eyelid	35×20×9	7.33
		OS	Pink	Third eyelid and medial canthus	29× 16× 8	3.89
				Upper palpebral conjunctiva	10× 8× 6	0.33
				Lower palpebral conjunctiva	16× 12× 8	1.21
2	2	OD	Pink	Third eyelid	28×20×12	5.86
2	3	OS	Pink	Third eyelid	8×4×3	0.07
2	4	OS	Pink	Corneo-conjunctival dermoid	22×16×6	2.95
2	5	OD	Congested	Third eyelid	24×14×7	2.46
		OS	Congested	Third eyelid	28×16×9	3.75
2	6	OS	Pink	Third eyelid	41×20×12	8.58
2	7	OD	Pink	Third eyelid with medial canthus	65×31×12	32.69
2	8	OD	Pink	Third eyelid	22×16×13	2.95
		OS	Pink	Limbus	10×8×5	0.33
				Third eyelid with medial canthus	18×14×10	1.85
					Mean	5.07±1.95

MATERIALS AND METHODS

The present research work was carried out at Department of Veterinary Surgery and Radiology, in collaboration with Department of Veterinary Clinics

and the Department of Veterinary Pathology, College of Veterinary Science and Animal Husbandry, Kamdhenu University, Sardarkrushinagar (Gujarat) – 385 506, during the period from January to June 2025.

A total of 17 bovines presenting with ocular tumours

having a maximum diameter of ≤ 65 mm and considered amenable to complete laser excision were included in the study (Table 1 and 2). Animals with extensive orbital involvement requiring exenteration/extirpation or tumours exceeding 65 mm in maximum diameter were excluded from the study. The presented animals involved 12 indigenous animals (9 Kankrej and 3 Gir) and 5 Holstein Friesian (HF) crossbred cattle. Among them, 11 animals were affected unilaterally, while 6 animals (5 indigenous animals and 1 HF crossbred cattle) were affected bilaterally. Among the unilateral cases, 5 indigenous animals showed multiple growths in a single eye. Consequently, a total of 34 ocular growths from 17 affected animals were examined. To minimise selection bias, animals were randomly allocated to Group I or Group II at the time of presentation. Thus, tumour size, location, breed, age, and tumour type were not considered during group allocation. In Group I (9 animals), ocular tumour resections were carried out using the continuous mode of CO₂ laser (4-10 W power output). In group II (eight animals), tumour resections were conducted using the super pulsed mode of CO₂ laser [(4-8 W power output with 50% duty cycle (1000 microseconds on and 2 milliseconds off)]. Case details such as breed, age, sex, parity, pregnancy status, duration of tumour, site of tumour, and any primary treatment administered were collected from each animal. All animals were examined clinically and details such as location of the growth, presence or absence of vision, consistency of the growth (firm, soft, fragile, or fluctuating), weight and diameter of the tumour, colour of the ocular mucous membranes, number of growths (solitary or multiple), and size of the growth (length $\times$ breadth $\times$ height) using an ocular caliper (≤ 20 mm longest diameter) or scale (> 20 mm) were documented in table 1. The volume of the tumour was calculated using the equation: $V = ab^2 / 6 \text{ cm}^3$ where “a” represents the longest diameter of the tumour and “b” is the diameter perpendicular to it, as mentioned by Forde *et al.* (2015).

The animals with ocular tumour (Fig. 1, 2, 3, 4) were kept off-feed for 24 hours, and water was withheld for 12 hours before induction of anaesthesia. On the day of surgery, the periocular region was prepared under aseptic precautions. Animals were positioned in lateral recumbency with the affected eye uppermost. In both the groups, xylazine hydrochloride was administered as a pre-anaesthetic intramuscularly @0.1 mg/kg body weight. Anaesthesia was induced and maintained using a double drip solution comprising guaifenesin (50 mg/kg body weight, prepared as a 5% solution in lukewarm 5% dextrose normal saline) and ketamine (1.0 mg/ml of the same 5% guaifenesin solution). The double drip solution was administered intravenously at a rate of 0.5 ml/kg for induction and subsequently at 2.5 ml/kg/hour for maintenance throughout the surgical procedure.

During laser surgery, protective eyewear specific for the 10,600 nm wavelength of the CO₂ laser was used by all operating personnel to prevent accidental ocular exposure to reflected laser radiation. For resection of tumour, a CO₂



Fig. 1. Ocular dermoid on the third eyelid in a Kankrej



Fig. 2. Third eyelid tumour in HF cross cattle

laser system of wavelength 10,600 nm equipped with an articulated arm delivery unit, a 0.8 mm ceramic tip, and a 0.2 mm focal spot was used. Following surgical draping, the cornea was protected using two autoclaved, lignocaine gel-coated disposable wooden spoons (160 mm $\times$ 30 mm $\times$ 1 mm) positioned with their broad concave surfaces against the corneal surface. In cases involving corneal or corneo-conjunctival dermoids, additional protection was provided using saline-soaked sterile gauze placed over the adjacent healthy cornea to minimise inadvertent thermal injury during laser application. A stay suture was applied to the growth using cotton thread for elevation.

The resections were conducted under two modes of CO₂ laser, i.e., continuous mode (Fig. 5) and super pulsed mode (Fig. 6). A fume evacuator was used to remove the plume generated during resection. Tissue tension was applied by retracting the mass throughout the resection process. Saline-soaked sterile gauze was used to remove char to minimise lateral thermal spread. Laser energy was applied to the tumour base until the tumour was completely resected. In both groups, CO₂ laser was also used for haemostasis, sealing small blood vessels during resection. A crosshatch pattern was applied at the tumour bed to eliminate any residual tumour tissue using 4 W power in continuous mode with a 0.4 mm spot diameter. The laser beam was passed repeatedly in perpendicular directions until visible tissue caramelisation appeared. The surgical wound was allowed to heal by secondary intention without suturing.

Following tumour resection, Strepto-Penicillin was administered at a dose rate of 10,000 IU/kg body weight and meloxicam at 0.5 mg/kg body weight intramuscularly for a period of five days. Eye drops moxifloxacin (0.5%) and flurbiprofen (0.03%) were applied topically to prevent secondary infection and reduce inflammation, respectively. Animals were closely monitored for post-operative complications, particularly for the excessive ocular discharge (watery or pus-like) and recurrence of growth. To check the recurrence of the growth, a telephonic follow-up was taken for a minimum of two months.

The Schirmer tear test (STT-I) was conducted preoperatively, immediately after surgery, and on the 12th postoperative day using Whatman No. 41 filter paper strips (5 mm $\times$ 35 mm) (Featherstone and Heinrich 2013)



Fig. 3. Ocular dermoid on the medial canthus in a Kankrej



Fig. 4. Ocular dermoid on lower bulbar conjunctiva in a Kankrej



Fig. 5. Continuous mode of laser for tumour resection in group I



Fig. 6. Superpulsed mode of laser for tumour resection in group II

to remove tear production. The fluorescein dye test was performed before surgery, immediately after surgery, and again on the 12th postoperative day 15 minutes after completing STT I. The physiological parameters, such as heart rate, respiration rate, and rectal temperature, were recorded preoperatively and on the 12th postoperative day.

The resected tumours were weighed using an electronic balance and then processed for histopathological examination to confirm the diagnosis as per standard procedure given by Luna (1968). For evaluation of thermal injury and residual tumour cells, four surgical margin samples (dorsal, ventral, medial, and lateral) were collected from each resected tumour. Thus, a total of 136 margin samples (34 tumours $\times$ 4 margins) were subjected to histopathological examination. The objective was to compare the extent of thermal damage produced by continuous and super-pulsed modes of CO₂ laser. The presence of a vaporised zone along with coagulative necrosis confirmed thermal injury caused by the laser. The depth of thermal damage was measured in μ m. The results obtained in the present study were subjected to further statistical analysis.

RESULTS AND DISCUSSION

Ocular tumours were recorded in 12 indigenous cattle [Kankrej (n=9) and Gir (n=3)] and five Holstein Friesian (HF) crossbreds. Of the 17 affected animals, eight were males and nine were females, indicating no marked sex predisposition. The age of affected animals ranged from 35 days to 12 years. Dermoids were predominantly observed in nulliparous animals below four years of age and were congenital in origin, whereas OSCC was mainly encountered in adult HF crossbred cattle above four years of age. Similar age- and tumour-related patterns have been reported previously (Sarangom *et al.* 2015; Bodinga

et al. 2020; Nithya *et al.* 2022; Sharma *et al.* 2024; Solanki 2024).

Among the 17 animals, tumours were located in the left eye (n=6), right eye (n=5), and both eyes (n=6). A slightly higher involvement of the left eye was noted, in agreement with previous reports (Nithya *et al.* 2022; Solanki 2024), although right eye predominance has also been documented (Priyanka *et al.* 2021). Bilateral occurrence, particularly in dermoids, has been reported in crossbred cattle (Ismail 1993; Alam and Rahman 2012). A total of 34 tumours were evaluated. In unilateral cases (OD and OS), tumours were fewer and predominantly well demarcated, mainly affecting the third eyelid and medial canthus, with occasional involvement of the eyelids. In bilateral (OU) cases, 21 tumours were recorded, with 13 well-demarcated and eight spreading lesions involving multiple ocular structures. Overall, well-demarcated lesions were more frequent than spreading types. These findings were consistent with earlier reports indicating the third eyelid, limbus, medial canthus, and conjunctiva as common sites (Sarangom *et al.* 2015, Kumar *et al.* 2020, Nithya *et al.* 2022, Solanki 2024), possibly due to increased exposure to ultraviolet radiation. Clinically, the conjunctival mucous membrane was pink in 14 animals and congested in three, similar to findings reported by Patel (2023) and Solanki (2024). Tumour size ranged from 8–65 mm, while was comparable to previous studies (Patel 2023; Solanki 2024). Vision was normal in most animals, except two cases showing corneal opacity, which aligned with earlier observations (Solanki 2024), indicating limited functional impairment in early-stage lesions.

The mean power output for tumour resection in group I (continuous mode) was 7.17 ± 0.26 W, whereas in group II (super pulsed mode), it was 6.63 ± 0.17 W (50% duty cycle), showing a significantly higher ($P < 0.05$) power requirement in the continuous mode. Similar trends have also been reported by Patel (2023) and Solanki (2024). The mean surgical time was also significantly ($P < 0.05$) higher in group I (6.78 ± 0.41 min) compared to group II (6.56 ± 0.72 min), indicating improved efficiency with super pulsed mode. The relationship between tumour dimensions and laser settings used for resection is presented in Table 3.

Postoperative recovery was uneventful in all animals (Fig. 7 and 8). Recurrence was observed only in two animals of group I (continuous mode), while no recurrence was noted in group II, supporting the earlier findings (Patel 2023; Solanki 2024). Physiological and ophthalmic parameters remained within normal reference limits throughout the study. No significant differences ($P > 0.05$) were observed between groups in heart rate, respiration rate, rectal temperature, STT-I values, or fluorescein dye test results at different observation intervals. A transient postoperative reduction in STT-I values was observed in both groups, which returned towards preoperative values by the 12th postoperative day. The fluorescein dye test remained negative in all animals except one case in Group II that required partial keratectomy at the corneo-conjunctival

Table 3. Comparison of tumour size and laser parameters used for tumour resection

Group	Tumour size range (mm)	Power output range (W)	Mean power output (W)	Mean excision time (min)	Mean thermal damage (μm)
Continuous mode (Group I)	10×8×3 to 56×26×15	4–10	7.17±0.26	6.78±0.41	454.94±38.85
SuperPulsed mode (Group II)	8×4×3 to 65×31×12	4–8	6.63±0.17	6.56±0.72	179.38±12.72

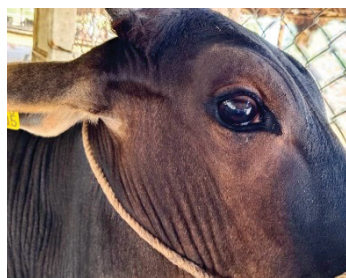
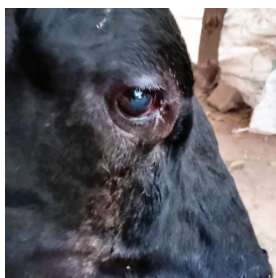
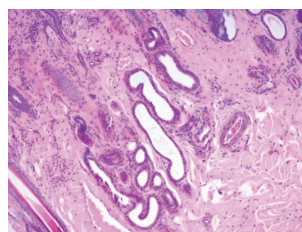
Fig. 7. Complete recovery at 12th day postoperatively in case of dermoidFig. 8. Complete recovery at 12th day postoperatively in case of OSCC

Fig. 9. Mass showing well-differentiated pilosebaceous units in the subjacent dense collagenous stroma. H & E 100X

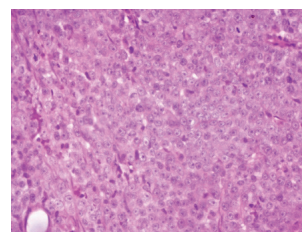


Fig. 10. Poorly differentiated squamous cell carcinoma. H & E 200X

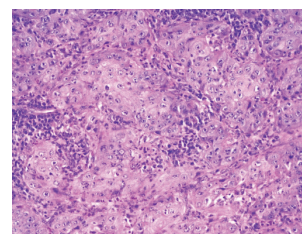


Fig. 11. Moderately differentiated squamous cell carcinoma. H and E 200X

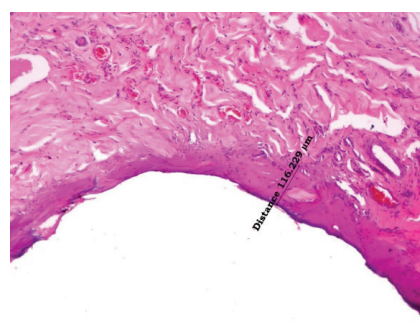
junction. These findings indicated that both laser modalities were safe and did not adversely affect ocular function or general physiological status. Similar observations have been reported by Patel (2023) and Solanki (2024).

Histopathological examination identified 26 dermoids (Fig. 9), five ocular squamous cell carcinomas (OSCC) (Fig. 10 and 11), two cases of lacrimal gland hyperplasia, and one papilloma. Dermoids were characterized by cornified stratified squamous epithelium with well-developed pilosebaceous units embedded within dense collagenous stroma, accompanied by variable inflammatory cell infiltration and occasional melanin pigmentation, consistent with previous reports (Sarangom *et al.* 2015, Shulesko *et al.* 2023). OSCC lesions exhibited varying degrees of differentiation, including well-, moderately-, and poorly differentiated forms, with mitotic figures ranging from 0.6 to 5.9, which was comparable to earlier findings (Carvalho *et al.* 2005, Desai 2024, Patel 2023, Solanki 2024). The papilloma showed marked keratinocyte hyperplasia with intranuclear eosinophilic inclusion bodies in the stratum spinosum, as described by Kumar *et al.* (2013).

All 136 surgical margin samples were free of tumour cells, confirming complete tumour excision in both groups. Histologically, laser thermal injury (Fig. 12) was characterized by a vaporized zone associated with coagulative necrosis. Mean thermal damage was significantly ($P<0.05$) higher in Group I (454.94±38.85 μm) than in Group II (179.38±12.72 μm), indicating a 60.6% reduction in collateral thermal injury with the super pulsed mode. Similar findings have been reported by Cercadillo *et al.* (2010), Li *et al.* (2021), and Solanki (2024). The superiority of the super pulsed CO₂ laser observed in the present study further corroborates the earlier experimental findings of Baggish and ElBakry (1986) and Lanzafame *et al.* (1988), who demonstrated reduced lateral thermal injury and improved wound characteristics with super

pulsed laser delivery as compared with continuous-wave systems. The intermittent delivery of laser energy in the super pulsed mode allows tissue cooling between pulses, thereby minimizing heat diffusion to adjacent tissues while maintaining effective tumour excision.

The indigenous cattle aged between 35 days to 3.5 years were mostly affected with congenital dermoids, while OSCC was more common in adult HF cross cattle. The ocular dermoids and papilloma were firm, while lacrimal gland hyperplasia and OSCC were soft on palpation. The mean power output, mean excision time and mean average thermal damage were higher in the continuous mode than the super pulsed mode of the CO₂ laser. Histopathologically,

Fig. 12. Surgical margins showing 116 μm thermal damage. H and E 100X

dermoids were characterised by cornified stratified squamous epithelium with pilosebaceous units in dense collagenous stroma, accompanied by epithelial hyperplasia and infiltration of inflammatory cells, and sometimes melanin pigmentation within the stratum basale. OSCC were well to poorly differentiated, followed by moderately differentiated, with an average mitotic figure varying from 0.6 to 5.9. The thickening of the epidermis, especially the stratum spinosum, was noticed in papilloma.

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