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Impact Of Carbon Dioxide Laser On Biochemical Parameters In Bovine Ocular Growths

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Abstract

Twelve bovines with ocular growths were selected and randomly divided into two groups with six animals in each group. In Group 1 saline soaked gauze piece while, in Group 2 saline soaked sterile wooden spoon was used for corneal protection during surgery. All the 12 animals were sedated with xylazine hydrochloride @0.1 mg/kg b.wt. i/m. The anaesthetic induction and maintenance with double drip solution using guaifenesin @50 mg/kg bwt and ketamine @2 mg/kg bwt i/v in 5% DNS was followed. The 9-10 W power output in continuous mode used in both groups in excising all ocular growths in bovines and left unsutured. Postoperative treatment was given for 5 days. In group 1 healing was uneventful in four animals while ocular discharge was observed in two cows. In group 2, majority of the animals (5/6) showed uneventful recovery except one cow which showed corneal opacity and discharge at 3rd day after surgery due to self mutilation. All 12 excised tumor samples were examined microscopically and diagnosed as squamous cell carcinoma (SCC). Histopathologically all 12 cases were well to moderately differentiated sqaumous cell carcinoma with mitotic index ranged from 0.5-2.5. And Ki67 index ranged from 29-37%. All 12 animals did not show reoccurrence at least 3 month post surgery.

Keywords: Squamous cell carcinoma (SCC), Ocular growth, carbon dioxide laser, Biochemical parameters

Introduction

Livestock play an integral role in India's agricultural landscape. It is having the world's largest population of bovine animals (535.78 million), which encompasses 192.50 million cattle and 109.9 million buffaloes (DAHD, 2019). Despite each animal's relatively low productivity, India leads in global milk production, contributing 20% of the world's output (DAHD, 2018). The prevalence of eye cancer among cattle is notablec, with higher occurrences observed in those aged above 5 years, followed by the 2-5 year age group (Heeney and Valli, 1985). This may be due to factors such as exposing adult animals to radiation outdoors or tying them outside. An analysis of breed-specific patterns in cattle reveals that Holstein Friesian crossbred cows exhibit the highest incidence of eye cancer, followed by Jersey crossbred cows and non-descript cows (Carvalho *et al*., 2005; Gharagozlou *et al*., 2007; Daryoush *et al*., 2011; Fornazari *et al*., 2017; Radhakrishnan *et al*., 1999). Notably, female cattle show a higher prevalence of bovine eye cancer, likely due to stress factors like gestation, lactation, and aging (Gharagozlou et al., 2007; Schulz and Anderson, 2010), while male cattle are usually directed towards fattening and meat production.

The acronym LASER stands for Light Amplification by Stimulated Emission of Radiation. The CO_2 laser is having precise tissue ablation and homeostasis capabilities that has led to its widespread use in surgical and medical procedures, often considered an alternative to the traditional scalpel. Operating at a wavelength of 10.6 μm in the far-infrared range, the CO_2 laser is efficiently absorbed by water, leading to effects like desiccation, vaporization, and ablation (Wang *et al*., 2005). The CO_2 laser functions as a potent surgical tool, with applications ranging from incisions to tissue vaporization. The CO_2 laser's benefits include accurate incisions, instant sealing of vessels, reduced surgery time, decreased post-operative inflammation and pain, and an antiseptic effect due to microorganism evaporation. However, drawbacks include high costs, extensive training, and additional safety equipment requirements (Carreira and Azevedo, 2016). Laser therapy has three primary effects on animal tissue: reducing inflammation, alleviating pain, and accelerating healing. (Pryor and Millis, 2015; Tata and Waynant, 2011). Importantly, these effects are due to photochemical changes at the cellular level, similar to photosynthesis or vitamin D synthesis (Anders *et al*., 2014).
The first instances of lasers in veterinary medicine were in larynx surgery for dogs, laying the ground-work for their broader use in canine surgeries (Calin *et al*., 2010). The CO_2 laser has been used in horses to remove cutaneous squamous cell carcinoma (McCauley *et al*., 2002, Hawkins and McCauley, 2005) as well as ocular carcinomas (Sanchez and Cabrera, 2006). However, literatures regarding use of CO_2 laser for surgical ablation of ocular tumor in cattle are scarce, hence present study was undertaken with following objectives.

Materials and methods

The 12 animals diagnosed with ocular growth having diameter < 44 mm were divided in two groups, six cases in each group for its surgical resection using CO_2 laser. Group I – Saline soaked gauze piece for eye protection (N=6) Group II – Saline soaked sterile wooden spoon for eye protection (N=6).

Fig.1 Limbal ocular growth present in left eye

Fig.2 Third eyelid tumor present in left eye

The eye lashes were clipped with scissor and surgical site was prepared aseptically prior to anesthesia by irrigating with copious normal saline. The animals were kept off feed and water for 12 hours before anesthetic induction. The preoperative stabilization was carried out by intravenous administration of normal saline and 5% dextrose normal saline. Xylazine given @ 0.1mg/kg bwt I/M and restrained in lateral recumbency. General anesthesia with double drip Guaifenesin @50mg/kg and ketamine @2mg/kg I/V.

Surgical technique:
In group 1 saline soaked gauzes were used to digitally shield the cornea prior to initiation of the excision of the mass was carried out using CO_2 laser power output of 9-10 W in continuous wave mode, along with a 0.2 mm spot diameter. The laser beam was kept approximately 2 cm above the surgical site. Throughout the procedure, a fume evacuator was used to eliminate the plume generated by the laser treatment, following the recommendations of the manufacturer. During the excision process, the mass was retracted to ensure appropriate tissue tension. Stay sutures with cotton thread were taken to fully elevate the ocular growth and for contactless excision. To prevent collateral thermal damage to the surrounding tissues, any char formation was carefully eliminated using saline-soaked gauze. Constant application of laser energy was applied to the base of the mass until complete excision was achieved. Vessels were cauterized using the CO_2 laser itself, employing a power output of 4-5 W. To ensure that no untreated tissue remained, a crosshatched pattern was employed to cover the tumor bed. Multiple passes of the CO_2 laser beam were made in perpendicular directions till caramelization of tissues was evident, utilizing a power output of 4 W in CW mode and a spot diameter of 0.4 mm. Minimal to no hemorrhage was observed during the procedure. The surgical sites were allowed to

heal naturally without any closure method (second intention healing) (Paczuska *et al.* 2014). In group 2 a moistened autoclaved disposable wooden spoon was used to shield the eyeball from incidental damage to cornea by laser. The spoon was applied on cornea after coating with sterile lignocaine gel.

Fig.3 stay sutures with cotton thread

Fig.4 Ablation of ocular growth using CO2 laser

Postoperative care:

Postoperatively, Inj. Strepto-penicillin @ 10000 IU/kg b.wt. and Inj. Meloxicam @ 0.5 mg/kg b.wt. were given for 5 days. Eye drops Ofloxacin applied up to the recovery. Post-operative complications visually corneal opacity of eyeball, and corneal ulcer were observed. Telephonic follow up was taken to note recurrence for minimum 3 months.

Blood collection:

The blood collection was done for hematological and biochemical studies. 3 ml of blood sample was obtained from the jugular vein in K3EDTA vial before surgery, after surgery and 12^{th} days of surgery for hematological analysis and 6 ml blood was collected for clot activator vial for biochemical analysis. The blood samples were analyzed using an automated animal hematology analyzer.

To confirm the diagnosis of a resected growth, the tumor tissues underwent a histopathological examination.Tissue samples, were collected from the tumor beds viz., Dorsal, Ventral, Lateral, Medial to assess the presence of tumor cells

Results:

In both groups CO_2 laser power output of 9 W in continuous wave mode, along with a 0.2 mm spot diameter was used effectively to remove the ocular growths having dimension less than 34x 16 mm (8/12 animals) while, the power output had to be increased to 10 W in 4 animals with higher dimensions of growth for effective ablation of tumor. The 9-10 W power output in continuous mode with working distance of approximately 2 cm was efficient in excising all ocular growths in bovines. In group 1 the saline soaked gauzes used to digitally shield the cornea during laser surgery required repeated manual adjustments to shield the cornea while, the wooden spoon in group 2 was easy to manipulate and shield the cornea due to its long handle. Furthermore the wooden spoon did not suffer perforation but only black discolouration was noted at end of surgery on the surface facing the laser beam. After removal of the tumor cross hatch pattern was followed, this helped to control bleeding and ensured through ablation till visualization of light brown discoloration of tissue called caramelization. In all the animals the surgical wound was allowed to heal by second intension without primary closure.

In group 1 healing was uneventful in four animals while ocular discharge was observed in two cows which resolved after 4 days of therapy. In group 2, majority of the animals (5/6) showed uneventful recovery except one cow which showed corneal opacity and ocular discharge at 3rd day after surgery due to self mutilation. All three cows with corneal opacity and ocular discharge by local irrigation with powder boric acid solution in normal saline and eye drops chloramphenicol leading to complete recovery.


Fig.5 Caramelization of surgical bed post surgery **Fig.6 ocular discharge**

BIOCHEMICAL PARAMETERS

Various biochemical parameters viz, ALP, ALT, AST, creatinine, BUN, albumin and TP were recorded on pre-operative, post-operative and 12th postoperative day.

Table1: Mean ± SE values of biochemical parameters in bovines with ocular growth (n=12)

Parameters	Groups	Pre-operative	Post-operative	12th day	P value
ALP	G1	25.45 ± 8.15	19.82 ± 10.42	16.92 ± 5.16	0.759
	G2	57.75 ± 16.37	95.02 ± 40.79	39.88 ± 1.53	0.339
	P value	0.108	0.127	0.089	
ALT	G1	12.67±4.11	8.11±2.55	22.41±13.90	0.494
	G2	16.72±4.13[a]	26.45±9.24[a]	50.50±8.34[b]	0.018
	P value	0.503	0.085	0.114	
AST	G1	24.85±7.86[a]	19.60±5.87[a]	158.88±41.12[b]	0.001
	G2	45.13±13.08	91.90±33.01	129.66±15.96	0.055
	P value	0.213	0.080	0.523	
Creatinine	G1	0.40±0.09[a]	0.32±0.09[a]	1.25±0.14[b]	0.000
	G2	1.19±0.58	0.80±0.21	1.39±0.15	0.522
	P value	0.207	0.055	0.526	
BUN	G1	9.05±2.40[a]	7.23±1.77[a]	39.87±4.90[b]	0.000
	G2	17.09±4.69[a]	11.77±2.95[a]	40.98±4.99[b]	0.001
	P value	0.158	0.216	0.319	

Mean bearing different subscripts (a, b) differ significantly ($p < 0.05$) within column

1 Alkaline phosphatase (U/L): In group 1, the mean values of alkaline phosphatase (ALP) on pre-operative, postoperative was 25.45 ± 8.15, 19.82 ± 10.42 and 16.92 ± 5.16 while on 12th postoperative day it significantly dropped to 86.16 ± 9.13. In group 2, the mean values of alkaline phosphatase (ALP) on pre-operative, post-operative and 12th postoperative day were 57.75 ± 16.37, 95.02 ± 40.79 and 39.88 ± 1.53, respectively. No significant differences were observed when comparing between and within groups at pre-operative, post-operative and 12th postoperative day. Furthermore, in present study the mean value of ALP remained within the normal range of 0-200 (U/L) as reported by Constable *et al*. (2017). Gautam *et al*. (2016) and Priyanka *et al*. (2021) observed ALP within the normal range in cattle operated for squamous cell carcinoma.

2 Alanine aminotransferase (U/L): In group 1, the mean values of alanine aminotransferase (ALT) on pre-operative, postoperative and 12th postoperative day was 12.67 ± 4.11, 8.11 ± 2.55 and 22.41 ± 13.90, respectively. While, in group 2, the mean values of alanine aminotransferase (ALT) on pre- operative, post-operative and 12th postoperative day were 16.72 ± 4.13, 26.45 ± 9.24 and 50.50 ± 8.34, respectively. In group 1 no significant difference was observed at pre-operative, post-operative and 12th postoperative day, however in group 2, mean values of ALT on 12th postoperative day was significantly higher than preoperative day. In group 2 the value on 12th postoperative day was slightly increased than normal reference range of 11-40 (U/L) given by Constable *et al*. (2017). Similar, within normal range fluctuations in ALT values have been observed by Naik (2010), Gautam *et al*. (2016), Swamy (2016), Podarala *et al*. (2020), and Priyanka *et al*. (2021) in cattle operated for squamous cell carcinoma. On comparing 1 and 2 no significance difference in mean ALT values was observed at different time intervals.

3 Aspartate aminotransferase (U/L): In group 1, the mean values of aspartate aminotransferase (AST) on pre-operative, postoperative and 12th postoperative day was 24.85 ± 7.86, 19.60 ± 5.87 and 158.88 ± 41.12, respectively. While in group 2, the mean values of aspartate aminotransferase (AST) on preoperative, post-operative and 12th postoperative day was 45.13 ± 13.08, 91.90 ± 33.01 and 129.66 ± 15.96, respectively. In group 1 and 2 increase in mean AST value on 12th operative day was observed, this value was significantly higher in group 1 and non significantly high as compared to pre operative value. In group 2 the value fluctuate within the normal range of 78-132 (U/L) as given by Constable *et al*. (2017), while in group 1 the mean value on 12th post operative day was slightly higher than reference range. Furthermore as the animal shows normal feeding hence, liver damage might not be likely. Swamy (2016) reported that AST non significantly increased throughout the study at various time intervals. Gautam *et al*. (2016) and Priyanka *et al*. (2021) observed a non significant difference in AST within the normal range in cattle with squamous cell carcinoma. On comparing 1 and 2 no significance difference in mean AST values was observed at different time intervals.

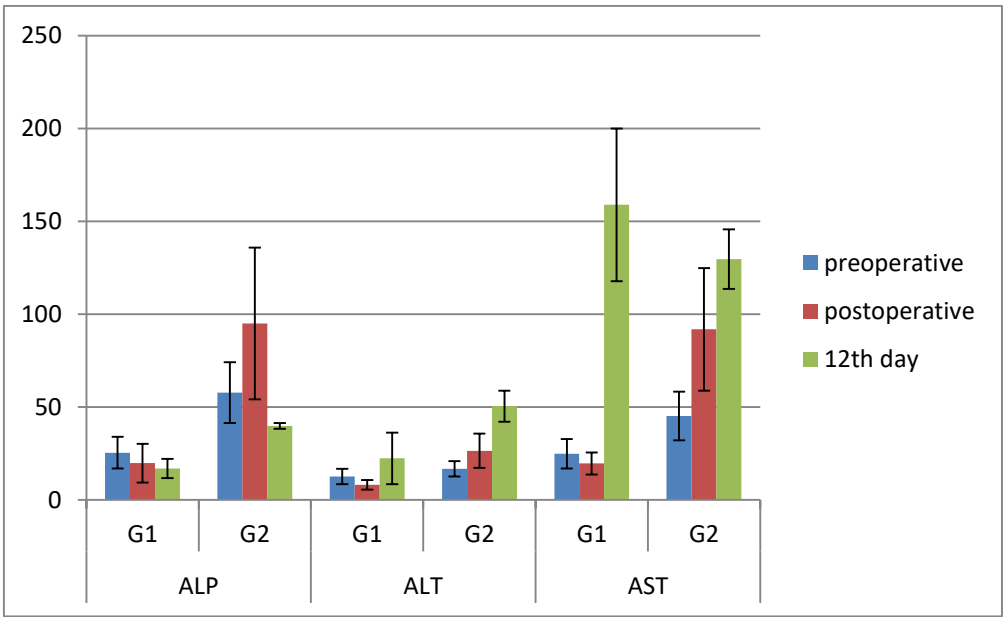

Figure 7: Column graph showing ALP, ALT, and AST in bovines

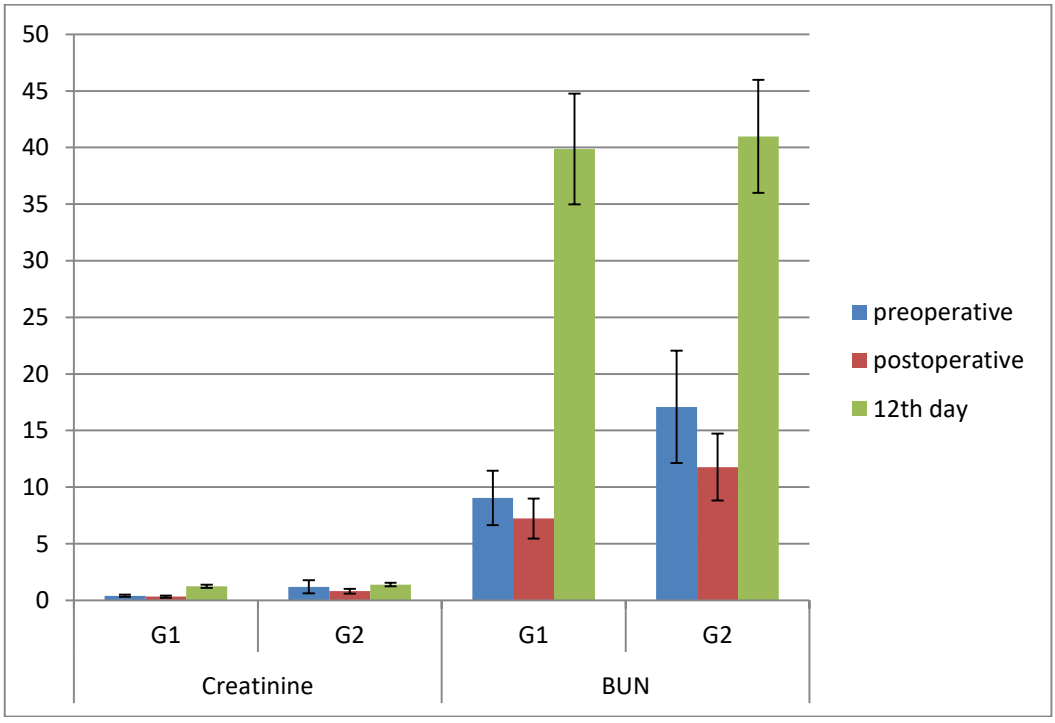

Figure 8: Column graph showing Creatinine and BUN in bovines

4 Creatinine (mg/dl): In group 1, the mean values of creatinine on pre-operative, post-operative and 12th postoperative day was 0.40 ± 0.09, 0.32 ± 0.09 and 1.25 ± 0.14, respectively. In group 2, the mean values of creatinine on pre-operative, post-operative and 12th postoperative day was 1.19 ± 0.58, 0.80 ± 0.21 and 1.39 ± 0.15, respectively. In both group 1 and 2 non significant decrease in creatinine was observed post operatively. However, on 12th post operative day significant increase in creatinine was observed in group 1 and non significant increase was noted in group 2. However, the mean value of creatinine varied within the normal range of 0.5-2.2 (mg/dl) as given by Bullers (2016). No significant differences were observed when comparing between groups at all time intervals. Contrary to our findings, Swamy (2016) reported non significant increase in the mean values of creatinine at different intervals while, Gautam *et al*. (2016) and Priyanka *et al*. (2021) observed creatinine within the normal range with non significant variation in cattle operated for squamous cell carcinoma. On comparing 1 and 2 no significance difference in mean creatinine values was observed at different time intervals.

5 Blood urea nitrogen (mg/dl): In group 1, the mean values of blood urea nitrogen (BUN) on pre-operative, postoperative and 12th postoperative day was 9.05 ± 2.40, 7.23 ± 1.77 and 39.87 ± 4.90, respectively. While in group 2, the mean values of blood urea nitrogen on pre-operative, post-operative and 12th postoperative day were 17.09 ± 4.69, 11.77 ± 2.95 and 40.98 ± 4.99, respectively. In both group 1 and 2 non significant decrease in BUN value was observed post operatively however, the mean value showed a significant increase at 12th post operative day. In accordance to our findings, Swamy (2016) reported non significant increase in mean values of BUN at different intervals throughout the study on bovine SCC. Gautam *et al.* (2016) and Priyanka *et al.* (2021) observed a non significant difference in BUN within the normal range in cattle operated with squamous cell carcinoma. The mean BUN value on 12th post operative day in both groups was higher than the normal range of 6-27 (mg/dl) as given by Constable *et al.* (2017). On comparing 1 and 2 no significance difference in mean BUN values was observed at different time intervals.

Histopathology:

All 12 excised tumor samples were examined microscopically and diagnosed as squamous cell carcinoma (SCC). In both groups majority of the SCCs were well differentiated (8/12) having low mitotic index below 0.5-2.5 followed by moderately differentiated (4/12) with mitotic index of 1.5-1.9. In both groups no reoccurrence was observed till three months. Immunohistochemistry was performed and positive immunoreactive cells were counted and the Ki-67 index ranged from 25-37% in as well as moderately differentiated SCC.

Conclusion:

Hence it is concluded that the ocular squamous cell carcinoma was predominantly present in third eyelid of cattle aged from 5-9 years. The ablation of ocular squamous cell carcinomas were satisfactorily performed using 9-10 W CO_2 laser, continuous mode, 0.2 mm spot diameter and approximate 3 cm working distance without drying of eyes and no significant rise in post operative temperature in surgical bed. The physiological (heart rate, respiration rate, rectal temperature), hematological (hemoglobin, packed cell volume, total erythrocyte count, total leukocyte count, differential leukocyte count) and biochemical (alkaline phosphate, alanine amino transferase, aspartate amino transferase) parameters in bovines operated for ocular sqaumous cell carcinoma using CO_2 laser were within normal physiological limits at before, after and 12th day post operative day. Histopathologically all 12 cases were well to moderately differentiated sqaumous cell carcinoma with mitotic index ranged from 0.5-2.5. And Ki67 index ranged from 29-37%. All 12 animals did not show reoccurrence at least 3 month post surgery.

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