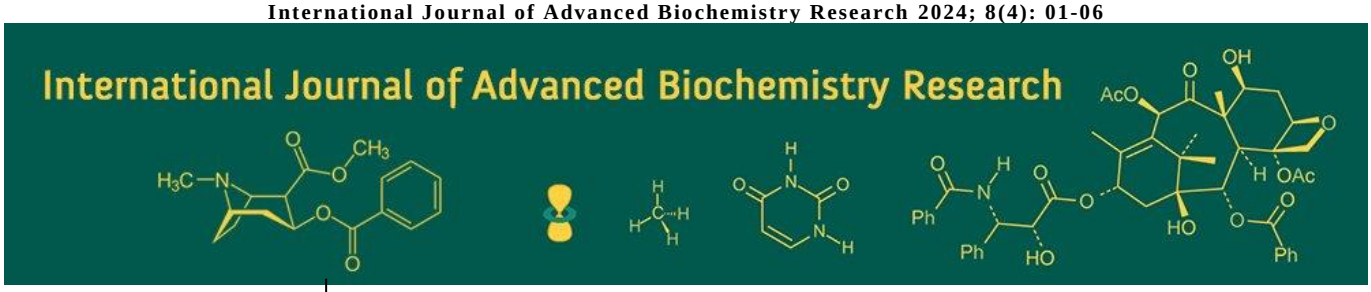

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Optimizing cisplatin dosage for intra lesional administration in bovine squamous cell carcinoma: A comprehensive study in livestock farming

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Abstract
Livestock farming is integral to the livelihoods of Indian farmers, but it is often impacted by bovine squamous cell carcinoma (SCC), a common malignant neoplasm characterized by uncontrolled squamous cell proliferation. This cancer typically originates from epithelial tissues, particularly those exposed to sunlight or chronic irritation. SCC predominantly affects areas such as the eyelids, vulva, prepuce, and regions around the eyes and muzzle. Treatment options for bovine SCC vary depending on factors like tumour location, size, and extent, with surgical excision, cryosurgery, laser ablation, and topical therapies being employed for localized lesions. In cases of advanced or metastatic disease, systemic chemotherapy or radiation therapy may be considered. Drawing from insights in human medicine, where cisplatin has been extensively studied in the context of squamous cell carcinoma treatment due to its effectiveness in disrupting tumour cell DNA and inducing apoptosis in those cells. Present study was conducted on bovines diagnosed with SCC to establish the appropriate dosage of cisplatin for intralesional administration following tumour removal. The precise dosing of cisplatin emulsified in sesame oil for post-tumour excision treatment was facilitated by carefully considering assumed shapes to calculate the volume of surgical beds, ensuring accurate administration of the chemotherapeutic agent. Additionally, proactive measures like obtaining written consent from owners to prohibit milk consumption for 14 days post-chemotherapy administration were implemented to safeguard public health and the well-being of other animals. This comprehensive approach to bovine SCC management emphasizes the importance of tailored treatment strategies and proactive measures in ensuring successful outcomes and minimizing adverse effects.

Keywords: Squamous cell carcinoma (SCC), cisplatin, intralesional administration, ocular tumours, vulvar tumours

Introduction
Dairy cattle are vital to the livelihood of Indian dairy farmers. Bovine squamous cell carcinoma (SCC) is a prevalent malignant neoplasm affecting cattle, characterized by the uncontrolled proliferation of squamous cells. It commonly arises from epithelial tissues, particularly those exposed to sunlight or chronic irritation, such as the skin, mucous membranes, and ocular surfaces. SCC predominantly affect the areas such as the eyelids, vulva, prepuce, and regions around the eyes and muzzle, the multifactorial etiology of bovine SCC includes exposure to ultraviolet (UV) radiation, chronic irritation, viral infections (such as papillomavirus), and nutritional deficiencies. Economic considerations in animal husbandry, the cost-effectiveness of treatment and maintenance of livestock becomes pivotal for profitable husbandry. The productive age of cattle coincides with the most susceptible age of cattle for the occurrence of Squamous cell carcinoma i.e., 6-10 years. (Pandey *et al*. 1989) [8]. Hence SCC emerges as a significant threat to the profitability and sustainability of animal husbandry

Clinically, bovine SCC lesions are typically present as firm, raised, ulcerative masses with irregular borders and varying degrees of inflammation. Depending on the location and extent of the tumour, affected animals may exhibit signs of discomfort, including epiphora, ocular discharge, difficulty in eating or drinking, lameness (in cases involving the hooves), and weight loss. Treatment options for bovine SCC vary depending on the location, size, and extent of the tumour.

Surgical excision, cryosurgery, laser ablation, and topical therapies (such as chemotherapy agents or immunomodulators) may be employed for localized lesions. In cases of advanced or metastatic disease, systemic chemotherapy or radiation therapy may be considered, although the efficacy of these treatments in cattle is variable. Cisplatin is commonly employed in the treatment of human squamous cell carcinoma (SCC) of the skin, lungs, oesophagus, cervix, etc. As a highly effective chemotherapeutic agent, cisplatin is utilized either as a single agent or in combination with other drugs or treatments to combat SCC.

Cisplatin's ability to disrupt DNA and induce apoptosis in cancer cells makes it particularly effective against rapidly dividing tumour cells, such as SCCs. It plays a crucial role in the management of squamous cell carcinoma, offering patients a valuable treatment option.

As mentioned by Le, X., and Hanna, E. Y. (2018) [4], chemotherapy has been incorporated into the multimodal treatment of squamous cell carcinoma of the head and neck (SCCHN) in human medicine with the goal of improving cure rates and functional outcomes. Cisplatin has been extensively studied as a concurrent chemotherapy agent for five decades in the context of cancers, particularly SCCHN, due to its role in sensitizing tumours to radiation therapy. Madhulaxmi *et al.* (2017) [5] noted that among various chemotherapy drugs, cisplatin is commonly utilized for oral squamous cell carcinoma treatment in different capacities, including as an induction, adjuvant, neoadjuvant, or palliative therapy.

Therefore, taking into account the aforementioned facts, a study was carried out on bovines diagnosed with SCC, with a particular focus on establishing the appropriate dosage of cisplatin for intralesional administration following tumour removal.

Material Methods

Selection

Total 12 cases were diagnosed with Squamous Cell Carcinoma (SCC) using tumour cytology analysis (Vara Prasad *et al.* 2016) [13]. Following diagnosis, these cases were treated with intralesional cisplatin after surgical excision. Tumours were grossly examined for any presence of ulceration, maggots, or infection.

Surgical Excision

Feeding and watering were ceased for 12–14 hours before surgery for all patients with ocular tumours, while no fasting was implemented for cases of Vulvar SCC. Both surgical sites underwent preparation by washing with antiseptic solution, thorough cleaning, and proper shaving. Subsequently, the area was scrubbed using a 7.5% povidone iodine solution.

Sedation for animals with ocular SCC was achieved using xylazine hydrochloride at a dosage of 0.1 mg/kg intramuscularly in combination with Peterson's nerve using 2% lignocaine hydrochloride. Additionally, local infiltration with a 2% lignocaine hydrochloride solution was conducted at the upper and lower eyelids for eyeball extirpation. tarsorrhaphy was performed, followed by elliptical incision over eyelid. The sharp surgical dissection was followed by transection of ocular muscles. The entire soft tissue structure in the eye and the ocular squamous cell carcinoma in the orbit were removed, and the haemorrhage was stopped by ligating optic artery using chromic catgut no.1. Cisplatin emulsion was infiltrated in the tumour bed. Horizontal mattress sutures were taken using cotton thread to appose the skin. Bandages soaked in tincture benzoin were used to fill the orbital cavity and one suture at medial canthus was left open for regular dressing.

Cows with perineal/vulvar tumours received caudal epidural anaesthesia at the 1st and 2nd intercoccygeal spaces. A precise surgical incision was created and the tumours were removed with 0.5 to 1.0 cm margins of healthy tissue. Digital pressure and ligation with chromic catgut was used to stop the bleeding. Cisplatin emulsion was infiltrated in the surgical bed. The muscles were closed by a simple continuous pattern using chromic catgut no.2 and the subcutaneous layer with a walking suture pattern using chromic catgut no.1. The skin was closed with a simple interrupted pattern using silk no.2.

Cisplatin Adjunct Therapy

Preparation

Cisplatin (1mg/ml) was emulsified in sesame oil (@ 0.2 ml/mg cisplatin) prior to administration (Patel, 2022) [15]. Before emulsification, sesame oil was filtered through a sterile 0.22mm syringe filter. The calculated doses of sesame oil and cisplatin were drawn into separate 20ml syringes. Both syringes were connected to a stopcock, then sesame oil and cisplatin were rapidly pumped back and forth through it to form a milky white emulsion.

Fig 1: Preparation of Cisplatin-sesame oil emulsion using stopcock.

Fig 2: Administration of Cisplatin-sesame oil emulsion in the surgical bed

Administration

After the excision of ocular tumour, cisplatin emulsified in sesame oil was injected @1mg/cm³ into the hemi ellipsoid surgical bed. The volume of surgical bed was calculated using V=2/3πacb, (where a, and c are semi minor axis and b is semi major axis or can also be called as, a and c are the radius of transverse cross section i.e., orbital radii and b is the depth i.e., orbit). The length of orbital radii and depth were calculated using vernier callipers, geometrical divider and ruler scale.

Similarly, the second volume of the orbit was calculated by increasing length of both the radii by 1 mm and depth by 2 mm, considering it as the height of the layer of residual tumour cells if any remaining. These differences in volumes were used for the dosing of cisplatin @ 1mg/cm^3 as the adjunct chemotherapeutic agent to be injected at the surgical bed.

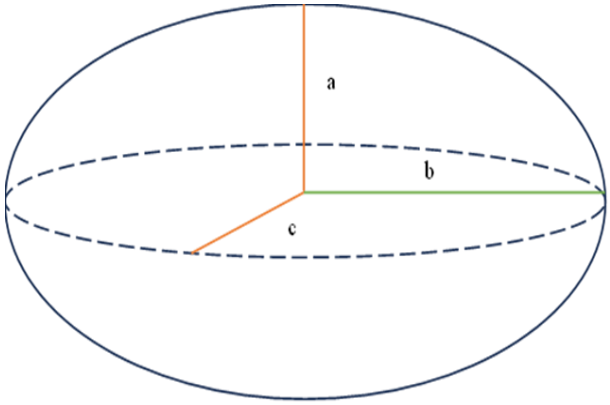

Diagram 1: Ellipsoid

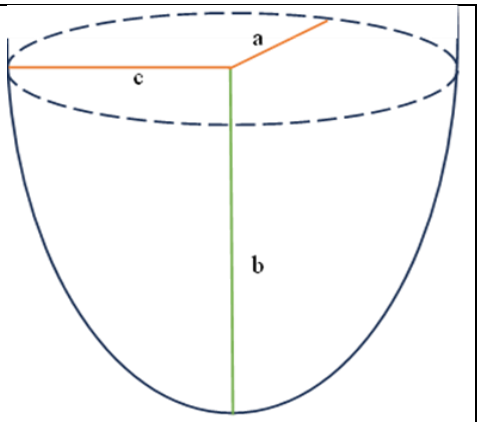

Diagram 2: Hemi-ellipsoid

∴ Volume of Ellipsoid (V_E)= 4/3 πacb
∴ Volume of hemi-ellipsoid (V)= (4/3 πacb) / 2
∴ Volume of hemi-ellipsoid (V)= 2/3 πacb
(If measures of radii a and c are found to be apparently equal, then it can be calculated as V=2/3πa²b or 2/3πc²b)

In the animals operated for vulvar tumours, the volume of elliptical cylinder like surgical bed was calculated using V= πabh, where 'a' is length of semi major axis, 'b' is length of semi minor axis and 'h' is height of the cylinder (assumed height of residual tumour cells), depending upon which the dose for cisplatin to be administration was calculated.

The cisplatin emulsion was administered in the surgical bed in a parallel row, approximately 1cm apart (Desai, 2022) [2]. Written consent was obtained from the owners that, after administration of a chemotherapeutic agent, milk consumption shall be prohibited for any living being for 14 days.

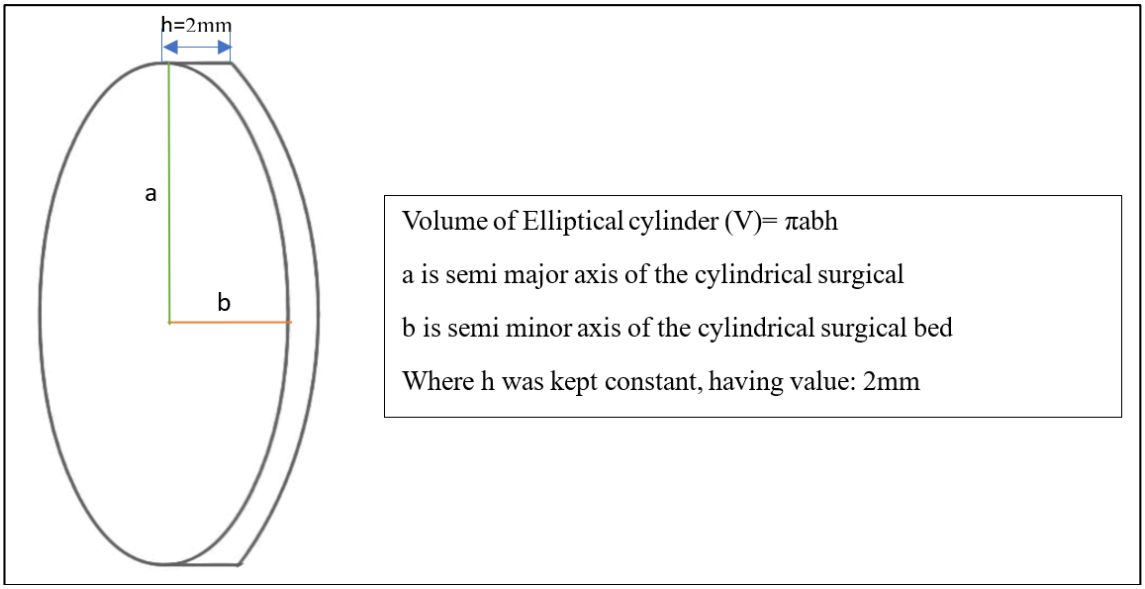

Diagram 3: Schematic representation of assumed elliptical cylinder like surgical bed

Post-Operative Care:

Following surgery, all patients received the same post-operative treatment, for 5-days injections of strepto-penicillin (10000IU/kg body weight) and meloxicam (0.5 mg/kg body weight). The antiseptic dressing was kept on during the healing process with liquid povidone iodine, and the sutures were removed 12 days after surgery. Regular dressing of the suture line and orbital cavity was done until complete recovery.

Complications
Majority of animals administered with cisplatin-sesame oil emulsion developed abscess during post operative phase. This complication was managed by regular antiseptic dressing with betadine and sprinkling of Cephalexin powder.

Results and Discussion
Selection
A total of 12 cases were identified as suffering from Squamous Cell Carcinoma (SCC) through tumour cytology analysis, as reported by Vara Prasad *et al.* in 2016 [13]. Following the diagnosis, these cases underwent treatment utilizing intralesional administration of cisplatin after surgical excision. The objective behind this treatment method is to deliver the chemotherapy drug precisely to the tumour site, maximizing its concentration at the target area while minimizing systemic side effects. This will induce cell death and impede further proliferation and dissemination of the cancerous cells. This localized therapeutic approach can effectively manage SCC in cattle while mitigating the risk of systemic chemotherapy-related adverse effects.
Four out of twelve cattle had ulcerated tumours, and three were infested with maggots. These lesions can be attributed to long-term cases; similarly, Gautam *et al.* (2016) [3] and Sarangabani *et al.* (2017) [10] also mentioned maggot infestation as a common sequel to SCC. Oral *et al*. (2019) [7] described a peri-vulvar ulcerated tumour mass in an eight-year-old HF cross-bred cattle.

Surgical excision
Shaving and scrubbing represent crucial preparatory measures before surgical interventions, ensuring optimal cleanliness and minimizing the risk of infections. In our study, these steps were meticulously carried out for all 12 cases, encompassing thorough shaving and scrubbing of the incision site using a 7.5% povidone iodine solution. Our approach aligns with established protocols followed by researchers such as Desai (2022) [2] and Chigerwe *et al.* (2017) [1].
The tumours in all 12 animals were surgically removed. For patients with ocular tumours, feeding and watering were stopped 12–14 hours before surgery, Sharma *et al*. (2020) [11] also found that 12hrs of fasting was sufficient for such cases; however, since animals with vulvar tumours were operated under epidural anaesthesia, there was no need for fasting.
Extirpation of the affected eyeball closely followed the protocol outlined by Desai (2022) [2]. Initially, tarsorrhaphy was performed after preparing the surgical site and draping. This was followed by an elliptical skin incision made near the eyelashes. The entire soft tissue mass was excised, and the optic artery was ligated using catgut no. 1. In cases where the tumour margins were fused, complete excision of the tumour was difficult. The volume of the surgical bed was then calculated to determine the dosage of cisplatin required. Subsequently, the entire cavity was packed with bandages soaked in tincture benzoin, and horizontal mattress skin sutures were used to close the cavity, with one suture left open at the medial side for regular dressing. This standardized procedure ensures thorough tumour removal and promotes postoperative wound healing, aligning with established protocols in the field as followed by Yakan *et al.* (2017) [14] and Desai. (2022) [2].
In cows with perineal/vulvar tumours, caudal epidural anaesthesia was administered at the 1st and 2nd intercoccygeal spaces following the technique described by Sarangabani *et al*. (2017) [10]. A meticulous surgical incision was made, and tumours were excised with 0.5 to 1.0 cm margins of healthy tissue. Haemorrhage control was achieved through digital pressure and ligation with chromic catgut. Cisplatin emulsion was infiltrated into the tumour bed as outlined by Patel (2022) [15] and Desai (2022) [2]. Muscle closure was performed using a simple continuous pattern with chromic catgut no.2, while the subcutaneous layer was sutured with a walking suture pattern using chromic catgut no. 1. Finally, the skin was closed with a simple interrupted pattern using silk no. 2.

Cisplatin Adjunct Therapy
Preparation
Following the determination of the tumour bed volume, the doses of cisplatin at 1 mg/cm³ and sesame oil at 0.2 ml/mg cisplatin were calculated referring to Theon *et al*., 1994 [12]. The average volumes of the surgical beds for ocular squamous cell carcinoma (SCC) were found to be approximately 10.66±0.29, while for vulvar tumours, the measured volumes were around 11.21±1.79. The calculated quantity of sesame oil underwent filtration using a sterile 0.22-micron syringe filter and was then collected into a sterile 20 ml syringe. These syringes were connected to a stopcock to facilitate the preparation of an emulsion solution. The solution was emulsified by pumping the syringes back and forth until it attained a milky white appearance (Theon *et al*., 1994; Patel, 2022) [12, 15].

Administration
Following the complete excision of the tumorous mass, the procedure involved the intralesional administration of cisplatin-sesame oil, prepared as a milky white emulsion at a concentration of 1 mg/cm³, in accordance with protocols outlined by Theon *et al*. (1994) [12] and Patel (2022) [15]. This solution was injected into the tumour bed using a sterile hypodermic needle, employing a parallel row fashion technique, as detailed by Desai (2022) [2]. Theon *et al*. (1994) [12] underscored the significance of intralesional injection of cytotoxic drugs, emphasizing its role in achieving higher drug concentrations and ensuring accurate placement of antineoplastic agents within the tumour. Additionally, the use of viscous fluid preparations such as sesame oil or almond oil has been advocated for enhanced therapeutic outcomes, as noted by Theon *et al*. (1994) [12].
Considering the average volumes of surgical beds to be approximately 10.66±0.29 for the ocular surgical bed and 11.21±1.79 (Table 1. and 2.) for the vulvar surgical bed, the mean volume of cisplatin (1 mg/ml) infiltrated into these beds ranged from 10 to 12 ml, where a maximum of 16.8 ml and a minimum of 7.4 ml of cisplatin (1 mg/ml) were used. However, variations in cisplatin volume were observed, with a maximum of 16.8 ml and a minimum of 7.4 ml administered.

Post-Operative Care
The postoperative management involved the administration of Strepto-penicillin and Meloxicam as antibiotics and analgesics, respectively, to prevent infections and manage pain following surgery. Strepto-penicillin, given intramuscularly at a dose of 10000 IU/kg b.wt., acted as a

broad-spectrum antibiotic to deter bacterial infections at the surgical site, while Meloxicam, administered intramuscularly at 0.5 mg/kg b.wt., served as a nonsteroidal anti-inflammatory drug to alleviate postoperative pain and inflammation. Additionally, wound care included the regular changing of bandages soaked in betadine for animals with ocular tumours to pack the orbital cavity, ensuring a sterile environment, and daily dressing of the suture line with liquid povidone iodine to prevent infection. These protocols were supported by previous studies by Nair *et al*. (2013) [6], Yakan *et al*. (2017) [14], Sarangabani *et al*. (2017) [10], and Priyanka *et al*. (2021) [9].

Complications

There was occurrences of abscesses in the majority of animals administered a milky white emulsion of cisplatin. Patel (2022) [15] suggest a correlation between the combination of sesame seed oil and infiltrated cisplatin and delayed absorption, potentially leading to the development of such abscesses. Regular antiseptic dressings containing betadine, along with the addition of cephalexin powder, worked well in treating the abscess.

Table 1: Dimensions of surgical bed in ocular tumours cattle

Sr. No.	Site	Radius 1 (cm)	Radius 2 (cm)	Depth 1 (cm)	Depth 2 (cm)	Volume 1 (cm^3)	Volume 2 (cm^3)	Bed Volume (cm^3)
		a≈c		b		V=2/3πa²b (a≈c)		(V1-V2)
1	Left Eye	3.1	3.2	5.2	5.4	104	115.10	11.15
2	Right eye	3.05	3.15	5	5.2	97.36	108.09	10.64
3	Right eye	3	3.1	4.8	5	90.43	100.58	10.16
4	Left eye	3.3	3.4	5	5.2	113.98	125.83	11.85
5	Right eye	2.9	3	5.3	5.5	93.35	103.62	10.27
6	Right Eye	3.15	3.25	5	5.2	103.85	114.97	11.12
7	Right eye	2.85	2.95	4.8	5	81.61	99.08	9.47
	Mean & SE	-	-	-	-	-	-	10.66±0.29

Table 2: Dimensions of surgical bed in Vulvar tumour cattle

Sr. No.	Site	Semi-major axis (a) (cm)	Semi-minor axis (b) (cm)	Height (h) (cm)	Bed Volume (cm^3) V =πabh
1	Vulvar tumour	6	3.75	0.2	14.13
2	Vulvar tumour	4.25	2.75	0.2	7.34
3	Vulvar tumour	4.5	3.25	0.2	9.18
4	Vulvar tumour	4.2	3.3	0.2	8.70
5	Vulvar tumour	6.35	4.2	0.2	16.74
	Mean & SE	-	-	-	11.21±1.79

Conclusions

The precise dosing of cisplatin emulsified in sesame oil for post-tumour excision treatment was facilitated by the careful consideration of assumed shapes to calculate the volume of the surgical beds. Utilizing an hemi-ellipsoid for the ocular surgical bed and an elliptical cylinder for the vulvar surgical bed offered a systematic and accurate approach for determining the required dose of the chemotherapeutic agent. The ocular surgical bed volume was determined using the formula for a hemi-ellipsoid (V=2/3πabc), while the vulvar surgical bed volume was calculated using the formula for an elliptical cylinder (V=πabh). This systematic approach ensured precise administration of the chemotherapeutic agent being administered in parallel rows approximately 1 cm apart for optimal distribution. Volume of administered cisplatin @1mg/cm^3 ranged from minimum 7.4 ml to 16.8 ml maximum based on the volumes calculated. Additionally, written consent was obtained from owners, stipulating a 14-day prohibition of milk consumption for any living being following chemotherapy administration. This precautionary measure was implemented to safeguard public health and the well-being of other animals from potential exposure to residual cisplatin.

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