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Exploring the impact of intralesional cisplatin on blood biochemical parameters in bovine squamous cell carcinoma

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Abstract

The dairy industry is pivotal for the livelihoods of farmers, with dairy cattle serving as their primary asset. However, tumours affecting cattle not only compromise their appearance but also raise welfare concerns, particularly squamous cell carcinomas (SCCs), which are prevalent among domestic animals in their productive years. In regions like India, where small-scale and marginal farmers dominate, mixed agriculture is crucial for sustenance. Here, SCCs pose a threat to annual earnings, especially since the vulvar and ocular regions are susceptible to these carcinomas. Ocular and vulvar tumours are notably common among animals raised with semi-intensive management and grazing access. Cancer is characterized by uncontrolled cell proliferation; hence, it remains a longstanding concern, especially due to post-surgical recurrence. This underscores the need for additional therapy. Considering cisplatin's cytotoxic mechanisms, including DNA damage and apoptosis induction, this makes it effective against various cancers and hence can turn out to be useful in preventing reoccurrence in bovine SCC. Therefore, a study was conducted to investigate the effects of intralesional cisplatin administration on biochemical parameters in bovines with SCCs. Results showed fluctuation in biochemical parameters but within normal ranges, suggesting intralesional cisplatin administration as adjunct therapy after excision did not notably affect these parameters. However, postoperative complications, such as abscess formation at the surgical site, were observed in animals treated with a cisplatin-sesame oil emulsion.

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

Introduction

Dairy cattle are the life line of the dairy farmers. Tumours present on cattle are visually unattractive and frequently lead to notable welfare concerns, even prior to progressing to a life-threatening stage. Squamous cell carcinomas are one of the widely reported type of carcinoma at productive age of Domestic animals. (Pandey *et al.* 1989)^[10]

India has a higher proportion of small-scale and marginal farmers than large landowners, so mixed agriculture plays an important role in their livelihood. Looking at this scenario SCC affected cattle can become a threat to their annual earnings. Vulvar region of the reproductive tract and ocular region are more likely to develop Squamous cell carcinoma. Nitya *et al.* (2022)^[19] noted that occurrence of ocular tumours is more common in animals that are raised using a semi-intensive management approach and were given access to grazing in open pastures. In India most of the small scale or marginal farmers also perform similar managemental approach for cattle rearing, hence it makes them more prone for Squamous cell carcinoma (SCC).

Cancer is characterized by the uncontrolled proliferation of cells hence has been a persistent matter of concern since ages. Its recurrence in animals that have previously undergone surgical removal is a major problem. Therefore, there is a need of additional therapy in the cases of Bovine Squamous cell carcinomas.

Going back to 1978, a platinum-based antineoplastic agent that gained approval remains a crucial and effective treatment for various cancers, "Cisplatin" stands as a leading metal-based chemotherapeutic drug with a rich history.

Michele Peyrone originally developed cisplatin in 1845, and its properties were later elucidated by Dr. Barnett Rosenberg in 1965. Cisplatin's ability to kill tumour cells is attributed to a range of cytotoxic mechanisms, with its well-recognized DNA-damaging effect being prominent. It exerts its cytotoxicity by forming DNA adducts, including mono, inter, and intra-strand cisplatin DNA cross-links, leading to cell cycle arrest at various stages (S, G1, or G2-M) and ultimately inducing apoptosis. Additionally, cisplatin disrupts cytoplasmic organelles, particularly the endoplasmic reticulum and mitochondria, while also triggers cellular damage through the activation of apoptotic pathways, oxidative stress, and inflammation (Manohar and Leung, 2018) [6].

Considering the above facts, research was conducted with the objective to study effects of intra-lesional administration of Cisplatin on biochemical parameters in bovines.



Fig 1: Preparation of site for epidural nerve block

Materials and Methods

Screening and Selection of Animals

Total 12 cases were diagnosed with SCC based on tumour cytology (Vara Prasad *et al.*, 2016) [18]. These cases were treated with intra-lesional Cisplatin administration and were studied for variations in biochemical parameters.

Surgical Excision

All the 12 animals underwent surgical excision of tumours. Feeding and watering was withheld for 12 -14 hrs prior to surgery for the patient with ocular tumour whereas animals with vulvar tumour were operated under epidural anaesthesia hence no fasting was advised. Pre operative preparations included shaving and scrubbing of surgical site with 7.5% povidone iodine solution.

Cisplatin Adjunct Therapy

For administration, cisplatin (1 mg/ml) was emulsified in sesame oil @ 0.2 ml/mg cisplatin (Patel, 2022) [11]. Sesame oil used for emulsification was filtered using a sterile 0.22 mm syringe filter. The calculated amount of sesame oil and cisplatin were drawn in a 20ml syringe separately. Both the syringes were connected to a two way stop cock and then sesame oil and cisplatin were rapidly pumped to and fro through the stopcock to form a milky white emulsion. (Goodrich and Semvolos, 2000) [4]

After excision of tumour, this milky white emulsion was injected @ 1 mg/cm³ into the surgical bed in parallel row

fashion approximately 1cm apart (Desai, 2022) [2]. The volume of surgical bed was counted using $V=2/3\pi a^2b$ formula for ocular tumours and $V= \pi abh$ for vulvar tumours.

Post-Operative Care

Post-operative treatment included, injections of streptopenicillin 8 (10000 IU/kg body weight) and meloxicam (0.5 mg/kg body weight) for 5 days and antiseptic dressing throughout the healing process with liquid povidone iodine. The sutures were taken out 12 days after surgery.

Blood Collection

For all the animals biochemical tests were performed, prior to surgery, immediate post-operatively and on 12th post operative day the, blood was drawn from the jugular vein into a clot activator (6 ml) vacutainer. The blood that had been collected in the clot activator vial was left in stable position until the serum separated at room temperature. After centrifuging the vial at 5000 rotations per minute (RPM) for 10 minutes, the serum was obtained and utilised to examine the biochemical parameters.

Biochemical Parameters Studied:

- Alanine Aminotransferase (ALT):** The levels of alanine aminotransferase were measured and represented in U/L.
- Aspartate Aminotransferase (AST):** The aspartate aminotransferase levels were measured and expressed in U/L.
- Alkaline Phosphatase (ALP):** The measured alkaline phosphatase values were stated in U/L.
- Creatinine:** The results of the measurement of creatinine were expressed in mg/dl.
- Blood Urea Nitrogen (BUN):** The levels of blood urea nitrogen were measured and represented in mg/dl.

Statistical Analysis

Using paired t-test, the data were analysed for biochemical parameters. The analysis of parameters included comparisons between different time intervals. Statistical software SPSS version 20 was used for data analysis

Results and Discussion

Screening and Selection of Animals

Twelve cases were diagnosed with SCC based on tumour cytology findings (Vara Prasad *et al.*, 2016) [18]. These individuals received treatment involving intra-lesional Cisplatin administration, and their biochemical parameters were subsequently examined for variations.

After gross clinical examination, FNAC was taken to confirm the type of tumour, and based on which 12 positive cases for SCC were selected for further treatment procedures. The microscopic examination of these smears revealed nuclear pleomorphism, increased nuclear: cytoplasmic ratio and Keratin pearl formation suggesting the presence of SCC. Similarly, Podarala *et al.* (2020) [12] undertook a study involving 12 instances of Ocular Squamous Cell Carcinoma that were selected based on preoperative FNAC findings. Likewise, Regmi *et al.* (2018) [14] documented a case involving a four-year-old Murrah buffalo, which was diagnosed as SCC based on FNAC results indicating the presence of scattered malignant squamous cells with dense cytoplasm. Additionally, Mathewos *et al.* (2020) [7] noted that FNAC of SCC-positive

cases revealed distinct features such as irregular nuclear outline, nuclear enlargement, coarse nuclear chromatin, hyperchromasia, and prominent nucleoli.

All 12 animals presented were clinically healthy in terms of watering, feeding, and lactation. Gautam *et al.* (2016) [3] also mentioned that the SCC affected animals are alert and active

with normal feeding. The majority of the presented animals (8/12 animals) had pale mucous membrane, and in the remaining two-two cases, it was found to be slightly congested and pink accordingly. This might be attributed to poor nutrition as the cases were long standing.



Fig 2: Extirpated tumour mass with intact eyeball



Fig 3: Administration of Cisplatin-sesame oil emulsion in the surgical bed

Cisplatin Adjunct Therapy

The mean surgical bed volumes for ocular SCC measured around 10.66 ± 0.29 and for vulvar tumours it measured around 11.21 ± 1.79 . After removal of entire tumorous mass, intra lesional administration of milky white emulsion @ 1 mg/cm^3 was done as mentioned by Theon *et al.* (1994) [17], Goodrich and Semevolos (2000) [4] and Patel. (2022) [11].

The treatment involved injecting the solution into the tumour bed using a sterile hypodermic needle, following the parallel row technique described by Desai (2022) [2]. Theon *et al.* (1994) [17] noted that administering cytotoxic drugs directly through intralesional injection facilitates achieving higher drug concentrations at the site of tumour.

All animals exhibited complete healing of the surgical wound within a timeframe of 12 to 14 days, with suture removal typically done on 12th postoperative day. This observation aligns with findings reported by Sarangabani *et al.* (2017) [15], Kuma and Sharif (2018) [5], and Desai (2022) [2]. Furthermore, the obliteration of the orbital cavity was observed between the 18th and 23rd day post-surgery, consistent with observations made by Nair *et al.* (2013) [9] and Priyanka *et al.* (2021) [13].

Post-Operative Care and Complications

Four cases operated for OSCC developed abscess which was managed with antiseptic dressing combined with sprinkling of cephalixin powder and in one case operated for vulvar tumour recto-vaginal fistula occurred which was managed by re-suturing using of six bites sutures technique.

Biochemical Parameters

Table 1: Biochemical parameters

Parameters	Pre operative	Post operative	12 th day post operative	P value
ALT (U/L)	12.74 ± 2.12^a	13.45 ± 2.12^a	32.14 ± 0.87^b	0.0001
AST (U/L)	41.39 ± 6.52^a	40.16 ± 5.99^a	66.8 ± 6.28^b	0.007
ALP (U/L)	43.76 ± 6.85^a	43.76 ± 6.85^a	72.22 ± 11.50^b	0.039
Creatinine (mg/dl)	0.73 ± 0.09	0.73 ± 0.09	0.77 ± 0.04	0.902
BUN (mg/dl)	11.1 ± 1.8	11.1 ± 1.8	11.91 ± 0.86	0.913

ALT (U/L) (Alanine Aminotransferase)

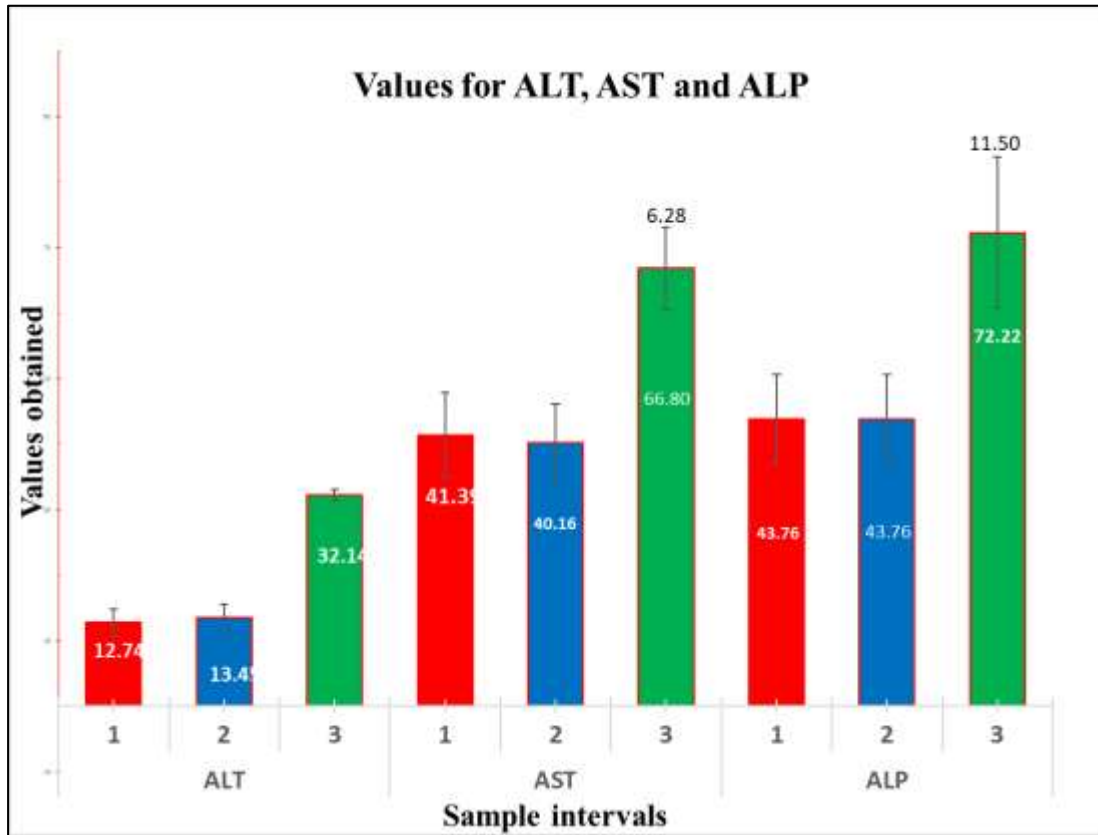
The mean values of ALT (U/L) of all the animals pre operative, post operative and on 12th post operative day were 12.74 ± 2.12 , 13.45 ± 2.12 and 32.14 ± 0.87 respectively. The ALT values increased non-significantly in the post operative

period, beyond which significant increase in ALT value was noted on 12th post operative day. All the mean values for ALT varied within the normal range of 11-40 (U/L) as mentioned by Constable *et al.* (2016) [1]. Non-significant

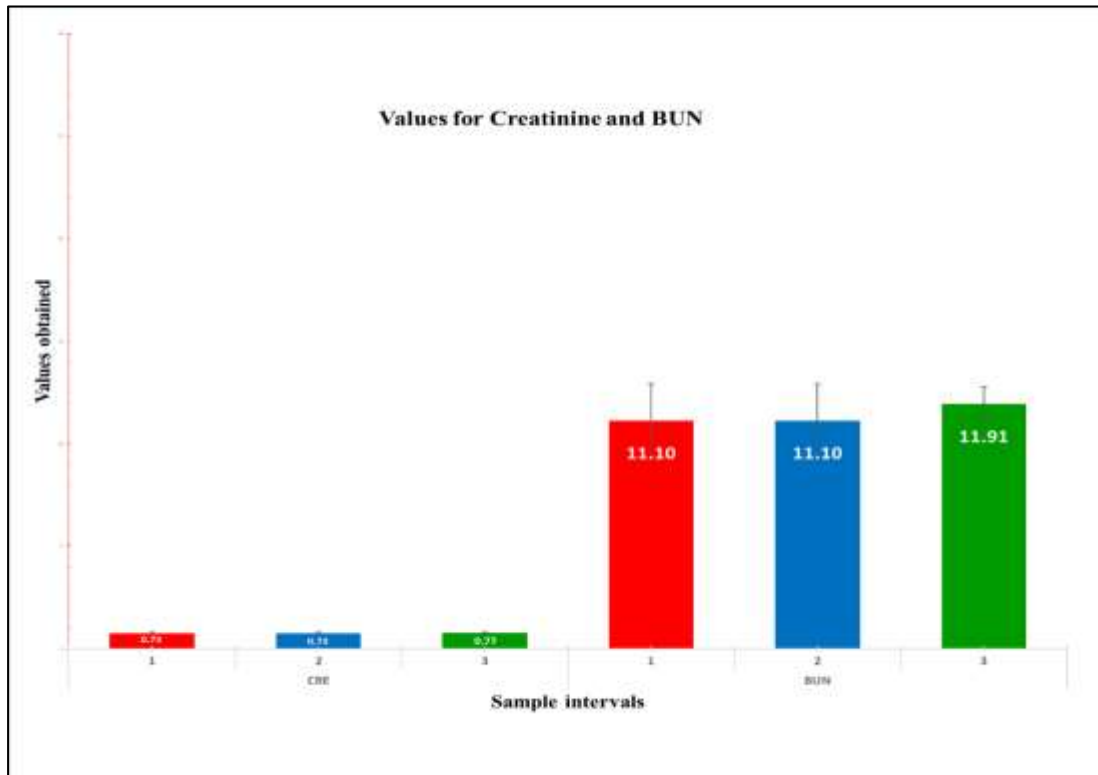
variation in mean value was observed in samples of different time intervals.

Podarala *et al.* (2020) [12] noted considerable fluctuations in alanine transaminase (ALT) levels across various time points during their investigation of ocular squamous cell carcinoma in cattle. Conversely, Naik (2010) [8] and Swamy

(2016) [16] observed a negligible rise in ALT levels shortly after surgery, followed by a subsequent minor decline on the 7th day post-operation, without statistical significance. Additionally, Gautam *et al.* (2016) [3] observed non-significant variances in ALT levels within the normal range among cattle affected by squamous cell carcinoma.



Graph 1: ALT, AST and ALP values



Graph 2: Creatinine and BUN values

AST (U/L) Aspartate Aminotransferase

The mean values of AST (U/L) on pre operative, post operative and on 12th post operative day were 41.39±6.52, 40.16±5.99 and 66.80±6.28 respectively.

There was non-significant decrease in post operative AST values that later increased significantly by 12th post operative day. Naik (2010)^[8] observed a non-significant rise in aspartate aminotransferase (AST) immediately after surgery, followed by non-significant declines on the 7th day post-operatively. Swamy (2016)^[16] reported that AST exhibited non-significant increases at different time points throughout their study. In contrast, Gautam *et al.* (2016)^[3] found non-significant variations in AST levels within the normal range among cattle diagnosed with squamous cell carcinoma.

Alkaline Phosphatase (U/L)

The mean values of ALP obtained on pre operative, post operative and on 12th post operative day were 43.76±6.85, 43.76±6.85 and 72.22±11.50 respectively.

Non-significant change in ALP was observed in post operative period, whereas there was a significant increase in ALP noted on 12th post operative day

Looking at the above values it can be concluded that the variations in mean values occurred were within the normal range of 0-200 (U/L) as mentioned by Constable *et al.* (2016)^[1]. Priyanka *et al.* (2021)^[13] did not observe significant deviations from the normal range, while Heranjali (1978)^[20] reported a decrease, and Prajapati (1984)^[21] noted non-significant increases in ALP levels. The variations may be due to different study populations, methodologies, or other factors.

Creatinine (mg/dl)

The mean values of creatinine on pre operative, post operative and on 12th post operative day were 0.73±0.09, 0.73±0.09 and 0.77±0.04 respectively.

The calculated p values for day comparisons suggest that non-significant variation was observed in mean value of creatinine at different levels of observations and the values fluctuated within normal reference range of 0.5-2.2 (mg/dl) as mentioned by Bullers (2016)^[22].

In contrast, Swamy (2016)^[16] documented a statistically insignificant rise in the average creatinine levels at various time points in cattle undergoing surgery for squamous cell carcinoma (SCC). On the other hand, both Gautam *et al.* (2016)^[3] and Desai (2022)^[2] observed that creatinine levels remained within the normal range with non-significant fluctuations in cattle subjected to SCC surgery.

BUN (mg/dl): The mean values of BUN on pre operative, post operative and on 12th post operative day was 11.1±1.8, 11.1±1.8 and 11.91±0.86 respectively.

The calculated p values for different time intervals suggest that non-significant variation was observed in mean value of BUN at different levels of observations and the values fluctuated within normal reference range of 6-27 (mg/dl) as mentioned by Constable *et al.* (2016)^[1]

Gautam *et al.* (2016)^[3], Priyanka *et al.* (2021)^[13] and Desai (2022)^[2] noted that there was non-significant variation in blood urea nitrogen (BUN) levels within the normal range among cattle who underwent surgery for squamous cell carcinoma. In contrast, Swamy (2016)^[16] reported non-

significant increases in the average BUN values at various time points during the study involving bovine SCC.

Conclusions

All the 12 cattle suffering from bovine Squamous cell carcinoma administered with milky white emulsion of cisplatin-sesame oil showed non-significant increase in ALT, AST and ALP immediate post-operatively but had significant increase at 12th post operative day. Whereas, creatinine and BUN showed non-significant increase at 12th post-operative day. Hence, it can be concluded that, the biochemical parameters of HF cross bred cattle affected with squamous cell carcinoma, administered with Cisplatin @ 1 mg/cm³ as adjunct therapy in surgical bed after excision to prevent reoccurrence showed fluctuations at different time intervals but all remained within the normal range suggesting intralesional administration of Cisplatin in surgical bed did not have any major effects over them. Apart from this the animals treated with cisplatin-sesame oil emulsion showed abscess formation at surgical site as postoperative complication.

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