



ISSN: 2456-2912

NAAS Rating (2026): 4.61

VET 2026; 11(9): 05-10

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www.veterinarypaper.com

Received: 07-06-2026

Accepted: 10-07-2026

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Evaluation of pegylated liposomal doxorubicin as neoadjuvant and adjuvant chemotherapy in dogs with stage IV mammary tumours: Clinical response, toxicity and surgical outcomes

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DOI: <https://www.doi.org/10.22271/veterinary.2026.v11.i9a.3760>

Abstract

Background: Canine mammary tumours are common neoplasms in female dogs, and advanced tumours with regional lymph-node involvement present considerable therapeutic challenges. Surgical excision remains the principal treatment; however, large and locally advanced tumours may increase the risk of residual microscopic disease. Pegylated liposomal doxorubicin (PLD) is a liposomal formulation designed to prolong drug circulation and modify tissue distribution while potentially reducing systemic toxicity.

Aim: This study evaluated clinical response, tumour-volume reduction, histopathological changes, treatment-associated toxicity, and surgical outcomes following a multimodal protocol incorporating neoadjuvant and adjuvant PLD in dogs with Stage IV mammary tumours.

Materials and Methods: Six female dogs aged 6-11 years with Stage IV canine mammary tumours classified as T3N1M0 were included. PLD was administered intravenously at 1 mg/kg as neoadjuvant chemotherapy, followed by definitive surgical excision and postoperative chemotherapy. Tumour dimensions were recorded before and after treatment, and tumour-volume reduction was calculated. Response was assessed using RECIST and Modified Karnofsky Performance Status. Histopathological, haematological, biochemical, and adverse-effect assessments were performed serially.

Results: All six dogs demonstrated measurable tumour-volume reduction, ranging from 4.62% to 68.58%, with a mean reduction of 34.25%. Four dogs achieved $\geq 30\%$ reduction. At 21 days, five dogs showed stable disease and one partial response. At 3 and 6 months, all six dogs showed complete response and remained alive. Histopathology revealed extensive necrosis, epithelial degeneration, apoptosis, stromal fibrosis, and inflammatory infiltration. Haematological changes were mild and transient, while ALT showed a transient increase and creatinine remained stable. Cutaneous effects occurred in four dogs and mild gastrointestinal effects in two, with no cardiotoxicity or treatment-related mortality.

Conclusion: The PLD-based multimodal protocol demonstrated promising short-term tumour control, pathological response, and manageable toxicity. Larger controlled studies with longer follow-up are warranted.

Keywords: Canine mammary tumour, pegylated liposomal doxorubicin, neoadjuvant chemotherapy, adjuvant chemotherapy, mastectomy, RECIST, chemotherapy toxicity, canine oncology

1. Introduction

Canine mammary tumours (CMTs) represent an important group of neoplasms in female dogs, particularly in middle-aged and older animals. Their biological behaviour ranges from relatively indolent benign lesions to highly invasive malignant tumours with regional lymphatic and distant metastatic potential. Advanced tumour size and regional lymph-node involvement are important prognostic considerations. In the present study, the therapeutic cohort consisted exclusively of dogs classified as T3N1M0, corresponding to Stage IV disease according to the TNM system used in the study.

Surgical excision remains the principal treatment for canine mammary tumours. The extent of surgery is generally determined by tumour distribution, gland involvement and lymphatic drainage. However, advanced and large mammary tumours may present challenges in achieving satisfactory surgical margins and may be associated with substantial local tumour burden. Consequently, systemic therapy administered before surgery may have a role in reducing tumour burden and facilitating definitive surgical management.

Neoadjuvant chemotherapy refers to systemic treatment administered before definitive local treatment with the objective of reducing tumour burden and potentially improving subsequent surgical management. Anthracyclines, particularly doxorubicin, have established antineoplastic activity in veterinary oncology. However, conventional doxorubicin is associated with cumulative toxicity, including myelosuppression and cardiotoxicity.

Pegylated liposomal doxorubicin is a liposomal formulation in which doxorubicin is encapsulated within a polyethylene-glycol-coated liposomal carrier. The formulation prolongs circulation time and alters tissue distribution. Liposomal anthracyclines have been investigated in veterinary oncology, with previous studies reporting relatively limited haematological toxicity and identifying cutaneous reactions as an important adverse effect.

Previous work has also demonstrated the potential value of chemotherapy in advanced canine mammary tumours. However, clinical information specifically evaluating PLD as part of a neoadjuvant-surgical-adjuvant protocol remains limited.

Therefore, the present study evaluated the clinical response, tumour-volume reduction, histopathological changes, haematological and biochemical alterations, treatment-associated adverse effects and short-term clinical outcome following PLD-based multimodal treatment in six dogs with Stage IV canine mammary tumours.

2. Materials and Methods

2.1 Study design and animals

The therapeutic component of the study was conducted at the Department of Veterinary Surgery and Radiology, Veterinary College Hospital, Hebbal, Bengaluru.

Six dogs diagnosed with advanced mammary neoplasia were selected for detailed therapeutic evaluation. The dogs ranged from 6 to 11 years of age, with a mean age of approximately 8.4 years. All animals were female. Five were intact and one was spayed. The breeds represented were Labrador Retriever, German Shepherd, Shih Tzu, Boxer, non-descriptive and Golden Retriever.

All six cases were classified as T3N1M0 (Stage IV) based on the TNM staging system used in the study.

Table 1: Baseline characteristics of the six dogs

Animal	Age (years)	Breed	Sex	Reproductive status	BCS
MNI1	6	Labrador Retriever	Female	Intact	8/9
MNI2	10	German Shepherd	Female	Intact	7/9
MNI3	10	Shih Tzu	Female	Spayed	7/9
MNI4	10	Boxer	Female	Intact	8/9
MNI5	11	Non-descriptive	Female	Intact	6/9
MNI6	6	Golden Retriever	Female	Intact	6/9

The case characteristics are reported in the original study table.

2.2 Clinical examination and staging

A detailed physical examination was performed in each animal. Tumour location, dimensions and regional lymph-node involvement were recorded. Assessment for distant pulmonary metastasis was undertaken as part of staging. Haematological and serum biochemical investigations were performed before and during treatment.

2.3 Neoadjuvant chemotherapy

Pegylated liposomal doxorubicin was administered intravenously at 1 mg/kg as neoadjuvant chemotherapy. Tumour measurements were obtained before treatment and following neoadjuvant chemotherapy before definitive surgery.

Tumour-volume reduction was calculated from the recorded tumour dimensions. The study documented measurable tumour-volume reduction in all six animals.

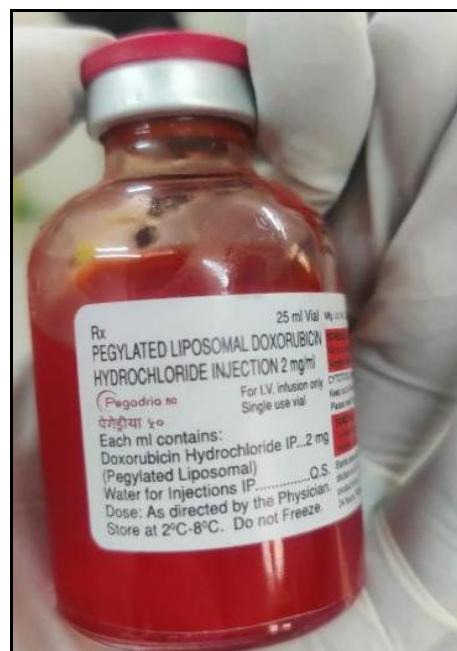


Fig 1: Pegadria injection (A) diluted in 5%dextrose infusion bottle (B)

2.4 Surgical management

Following neoadjuvant treatment, definitive surgical excision was performed according to tumour distribution and mammary gland involvement.

Regional mastectomy was the principal procedure. MNI3 required regional mastectomy with additional mastectomy for a contralateral inguinal mammary mass, while MNI4 underwent regional mastectomy combined with additional mastectomy of the ipsilateral inguinal mammary gland. MNI1, MNI2, MNI5 and MNI6 were managed by regional mastectomy.

Mammary tissue was dissected from the underlying fascia with ligation of associated vascular pedicles. In regional mastectomy, the associated lymph node was removed en bloc. Wounds were closed routinely in anatomical layers.

2.5 Anaesthesia

Anaesthesia consisted of midazolam premedication at 0.25 mg/kg IM and butorphanol at 0.4 mg/kg IM, followed by propofol induction at 4 mg/kg IV, titrated to effect, and maintenance with isoflurane at 1-2% in oxygen.

2.6 Postoperative and adjuvant treatment

Postoperative management included wound dressing and pressure bandaging. Ceftriaxone was administered at 25 mg/kg IM and buprenorphine at 0.04 mg/kg IM for postoperative analgesia. Following satisfactory wound healing, postoperative adjuvant PLD was administered as part of the multimodal treatment protocol.

2.7 Assessment of tumour response

Tumour response was evaluated using tumour-volume reduction and RECIST criteria.

The RECIST categories used in the study were:

- **Complete response (CR):** disappearance of all target lesions.
- **Partial response (PR):** $\geq 30\%$ reduction in target lesion measurement/volume.
- **Stable disease (SD):** neither sufficient reduction for PR nor sufficient increase for progressive disease.
- **Progressive disease (PD):** $\geq 20\%$ increase in target lesion measurement/volume.

MKPS was used to assess functional status. Grade 0 represented full activity without clinically evident functional limitation.

2.8 Histopathological examination

Tumour tissue obtained following chemotherapy and surgical excision was subjected to histopathological examination. Sections were evaluated for tumour-cell degeneration, necrosis, inflammatory infiltration, fibrosis, mitotic activity and other treatment-associated pathological changes.

2.9 Haematological and biochemical monitoring

Haemoglobin, platelet count, total erythrocyte count, total leukocyte count and differential leukocyte counts were assessed at baseline and during follow-up.

Serum ALT and creatinine were evaluated as indicators of hepatic and renal biochemical status.

2.10 Assessment of adverse effects

Treatment-associated adverse effects were recorded throughout the treatment period and graded using the Veterinary Cooperative Oncology Group-Common Terminology Criteria for Adverse Events (VCOG-CTCAE).

2.11 Follow-up

Clinical follow-up assessments were performed at 3 and 6 months following treatment. RECIST response, functional status and survival status were documented.

2.12 Statistical analysis

Statistical analysis was performed on serial clinical laboratory parameters. The thesis reports that the observed haematological and biochemical changes were not statistically significant ($P > 0.05$). The exact statistical software, statistical test and version should be inserted from the original analysis record before submission, rather than inferred.

3. Results

3.1 Tumour-volume response

All six dogs showed measurable reduction in tumour volume following neoadjuvant PLD. Percentage reduction ranged from 4.62% to 68.58%, with a mean reduction of 34.25%.

Table 2: Tumour-volume response following neoadjuvant PLD

Animal	Initial volume (cm ³)	Final volume (cm ³)	Reduction (cm ³)	Reduction (%)	Overall effect
MNI1	320.103	248.208	71.895	22.46	+++
MNI2	470.596	299.299	171.297	36.40	+++
MNI3	20.662	12.937	7.725	37.39	+++
MNI4	161.8635	50.847	111.0165	68.58	+++
MNI5	749.770	715.131	34.639	4.62	+
MNI6	423.360	270.739	152.621	36.05	+++

All six dogs demonstrated reduction in tumour volume. Five of six dogs showed $>10\%$ reduction and four of six showed $\geq 30\%$ reduction.

Data correction: The original thesis table contains an incorrect variation value for MNI4. The correct reduction calculated from $161.8635 - 50.847$ is 111.0165 cm^3 ; the reported 68.58% reduction is mathematically consistent with these volumes.

3.2 RECIST response

At the 21-day assessment, five dogs were classified as having stable disease and one dog (MNI4) demonstrated partial response.

At the 3-month assessment, all six dogs were classified as having complete response. Complete response was similarly recorded in all six dogs at 6 months.

All six dogs were alive at the 6-month assessment.

Table 3: RECIST response and clinical outcome

Animal	TNM	Surgery	RECIST Day 21	RECIST 3 months	RECIST 6 months	6-month status
MNI1	T3N1M0	Regional mastectomy	SD	CR	CR	Alive
MNI2	T3N1M0	Regional mastectomy	SD	CR	CR	Alive
MNI3	T3N1M0	Regional mastectomy + additional mastectomy	SD	CR	CR	Alive
MNI4	T3N1M0	Regional mastectomy + additional mastectomy	PR	CR	CR	Alive
MNI5	T3N1M0	Regional mastectomy	SD	CR	CR	Alive
MNI6	T3N1M0	Regional mastectomy	SD	CR	CR	Alive

3.3 Histopathological response

Post-treatment tumour sections demonstrated extensive treatment-associated pathological changes. These included multifocal necrosis, connective-tissue proliferation, fibrosis, mononuclear inflammatory-cell infiltration, degeneration and necrosis of neoplastic epithelial cells, epithelial detachment and sloughing, calcification within necrotic debris and nuclear pyknosis and chromatin condensation.

MNI4 demonstrated moderate epithelial pleomorphism accompanied by reduced mitotic activity compared with the pre-chemotherapy biopsy. MNI1 also demonstrated markedly reduced mitotic figures. MNI2 showed extensive necrosis with abundant cellular debris and dense inflammatory infiltration.

3.4 Functional status

All six dogs had MKPS grade 0 at the 3- and 6-month assessments, indicating preservation of normal functional activity during follow-up.

3.5 Haematological findings

Mean haemoglobin concentration decreased from 14.00 ± 0.80 g/dL at baseline to 11.90 ± 0.83 g/dL on day 28 and subsequently recovered to 13.02 ± 0.76 g/dL on day 57.

Total erythrocyte count decreased from $6.08 \pm 0.39 \times 10^6/\mu\text{L}$ at baseline to $4.88 \pm 0.31 \times 10^6/\mu\text{L}$ on day 28 and recovered to $5.29 \pm 0.28 \times 10^6/\mu\text{L}$ by day 57.

Platelet counts ranged from $336.7 \pm 48.9 \times 10^3/\mu\text{L}$ to $399.0 \pm 95.0 \times 10^3/\mu\text{L}$ during follow-up.

Total leukocyte count decreased from $17.22 \pm 2.00 \times 10^3/\mu\text{L}$ at baseline to $10.63 \pm 0.74 \times 10^3/\mu\text{L}$ on day 36 and recovered to $12.10 \pm 1.32 \times 10^3/\mu\text{L}$ by day 57. The thesis reports that these changes were not statistically significant.

Table 4: Serial haematological parameters

Day	Hb (g/dL)	Platelets ($\times 10^3/\mu\text{L}$)	TEC ($\times 10^6/\mu\text{L}$)	TLC ($\times 10^3/\mu\text{L}$)
0	14.00 ± 0.80	381.5 ± 67.3	6.08 ± 0.39	17.22 ± 2.00
7	13.63 ± 0.57	368.0 ± 64.9	5.87 ± 0.37	14.12 ± 2.44
21	12.35 ± 0.68	399.0 ± 95.0	5.41 ± 0.26	17.86 ± 2.95
28	11.90 ± 0.83	336.7 ± 48.9	4.88 ± 0.31	20.56 ± 4.18
36	12.85 ± 0.92	349.3 ± 69.2	5.17 ± 0.33	10.63 ± 0.74
43	12.75 ± 0.82	374.7 ± 76.3	5.22 ± 0.34	13.26 ± 2.75
57	13.02 ± 0.76	360.0 ± 70.3	5.29 ± 0.28	12.10 ± 1.32

3.6 Serum biochemical findings

ALT increased from 28.42 ± 4.54 U/L at baseline to 53.30 ± 9.06 U/L on day 36 and subsequently decreased to 48.30 ± 9.12 U/L on day 57.

Serum creatinine remained relatively stable, ranging from 0.94 ± 0.04 mg/dL to 1.09 ± 0.07 mg/dL during the observation period.

Table 5: Serial biochemical parameters

Day	ALT (U/L)	Creatinine (mg/dL)
0	28.42 ± 4.54	1.02 ± 0.06
7	30.93 ± 3.86	0.94 ± 0.04
21	28.35 ± 4.62	1.01 ± 0.03
28	41.38 ± 9.62	1.09 ± 0.07
36	53.30 ± 9.06	1.06 ± 0.09
43	44.47 ± 8.84	1.07 ± 0.11
57	48.30 ± 9.12	1.07 ± 0.08

3.7 Treatment-associated adverse effects

PLD was generally tolerated during the treatment period. Cutaneous adverse effects were recorded in four dogs and gastrointestinal signs in two dogs. The dermatological findings included alopecia, erythema, pruritus, hyperpigmentation, moist desquamation and ulcerative lesions. Gastrointestinal signs included anorexia, vomiting and diarrhoea.

No treatment-associated cardiotoxicity or mortality was recorded.

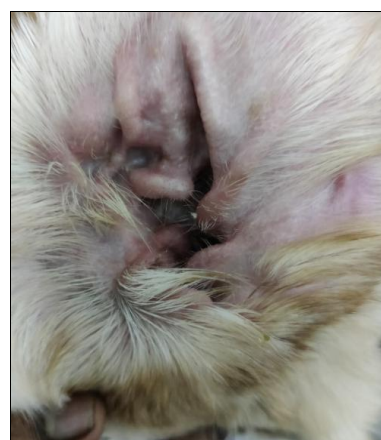


Fig 2: Palmaro plantar erythrodysesthesia like syndrome evident in the animal no. 1 having cutaneous toxicity evident has erythematous ulcerative lesions in the abdominal area (A), alopecia and thickening of skin near elbow (B), additionally this animal also have otitis externa (C)

4. Discussion

The present study evaluated a multimodal therapeutic protocol incorporating neoadjuvant PLD, surgical excision and postoperative chemotherapy in six dogs with Stage IV canine mammary tumours. The most notable finding was measurable tumour-volume reduction in all six dogs following neoadjuvant PLD, with reductions ranging from 4.62% to 68.58%.

The mean tumour-volume reduction was 34.25%, and four of six dogs demonstrated reductions of at least 30%. MNI4 showed the greatest reduction at 68.58% and was the only dog classified as having partial response at the early 21-day RECIST evaluation. This correspondence between volumetric reduction and early RECIST response provides clinical support for substantial cytoreductive activity in this individual case.

The response was variable, however. MNI5 demonstrated only a 4.62% reduction in tumour volume but was subsequently classified as having complete response at the later assessments. This observation illustrates the limitation of using early tumour-volume reduction as the sole measure of outcome in a multimodal treatment protocol. Surgical excision and postoperative chemotherapy were integral components of treatment and may have contributed substantially to the later clinical response.

Histopathological examination provided additional evidence of treatment-associated tumour injury. Extensive necrosis, epithelial degeneration, apoptotic nuclear changes, fibrosis and inflammatory-cell infiltration were identified in the post-treatment specimens. Reduced mitotic activity was specifically observed in MNI1 and MNI4. These changes are consistent with cytotoxic treatment effects and support the clinical evidence of tumour regression.

The surgical approach was predominantly regional mastectomy, with additional mastectomy required in two dogs because of more extensive glandular involvement. The reduction in tumour burden following neoadjuvant treatment may have contributed to improved delineation of the surgical field, although this study did not formally quantify surgical difficulty or margin quality.

The toxicity profile was predominantly dermatological. Four of six dogs developed cutaneous manifestations, making dermatological toxicity the most frequent adverse effect. Such findings are consistent with previous reports of liposomal doxorubicin, in which cutaneous toxicity has been recognized as an important adverse effect. Gastrointestinal signs were recorded in two dogs and were described as mild.

Haematological monitoring demonstrated a transient decrease in haemoglobin and erythrocyte count followed by recovery. Platelet concentrations remained relatively stable throughout treatment, and the observed haematological changes were reported as statistically nonsignificant. These findings are consistent with the relatively limited myelosuppression reported with liposomal anthracycline formulations.

The biochemical profile was also generally stable. ALT increased during treatment, reaching a maximum mean value on day 36, followed by partial reduction. Creatinine remained stable throughout the observation period. These findings do not suggest clinically important renal toxicity within the six-case series.

The later clinical response was encouraging. Five dogs had stable disease and one had partial response at 21 days, while all six were classified as having complete response at the 3- and 6-month evaluations. All six dogs were alive at six months. However, this finding must be interpreted carefully

because the study was not designed to distinguish the individual contribution of neoadjuvant PLD, surgery and postoperative chemotherapy.

The study has several limitations. First, the sample size was small, comprising only six dogs. Second, there was no untreated control group or comparison with conventional doxorubicin. Third, all animals received multimodal therapy, preventing attribution of later complete responses to PLD alone. Fourth, follow-up was limited to six months. Finally, the study was conducted in naturally occurring tumours, resulting in biological heterogeneity despite uniform clinical staging.

Nevertheless, the study provides preliminary clinical evidence that PLD can be incorporated into a multimodal treatment protocol for advanced canine mammary tumours and can produce measurable preoperative tumour cytoreduction with generally manageable toxicity.

5. Conclusion

In this six-dog clinical series, pegylated liposomal doxorubicin administered as part of a multimodal protocol consisting of neoadjuvant chemotherapy, definitive surgical excision and postoperative chemotherapy was associated with measurable tumour-volume reduction in all dogs with Stage IV canine mammary tumours.

Tumour-volume reduction ranged from 4.62% to 68.58%, with a mean reduction of 34.25%. Histopathological examination demonstrated extensive treatment-associated tumour necrosis and degenerative changes. Treatment-related toxicity was predominantly cutaneous, while haematological and biochemical alterations were generally mild and transient. All six dogs were classified as having complete response at the 3- and 6-month assessments and remained alive at six months.

These findings suggest that PLD may have potential as a component of multimodal management of advanced canine mammary tumours. However, the small sample size, absence of a control group and inability to separate the effects of chemotherapy from surgery and adjuvant treatment necessitate cautious interpretation. Prospective controlled studies with larger cohorts and longer follow-up are required to establish the therapeutic efficacy and survival benefit of PLD in canine mammary neoplasia.

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How to Cite This Article

Narayanamurthy BR, Balappanavar BR, Nagaraja BN, Srinivasamurthy KM, Ganganaik S. Evaluation of pegylated liposomal doxorubicin as neoadjuvant and adjuvant chemotherapy in dogs with stage IV mammary tumours: clinical response, toxicity and surgical outcomes. *International Journal of Veterinary Sciences and Animal Husbandry*. 2026;11(9):05-10.

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