

Evaluation of the efficacy of two adjuvant chemotherapeutic regimens against malignant canine mammary tumours

ABSTRACT

Surgical excision is the treatment of choice for mammary tumours in dogs, however it alone yields unsatisfactory results in mammary tumours on account of their high rate of recurrence and metastasis in dogs. So, **considering** to the advantages of different chemotherapeutic drugs the present study was designed to evaluate the efficacy of two adjuvant chemotherapeutic regimens against canine mammary tumours.

This study was conducted on 12 female dogs with a history of mammary tumour irrespective of breed. The animals with carcinoma were randomly divided into two groups containing **a** minimum of six animals each. In both the groups surgical excision was done followed by adjuvant chemotherapy. In group-I Inj. Doxorubicin and Inj. Paclitaxel while in group-II Inj. Doxorubicin and Inj. Carboplatin were administered intravenously on day 14th, 35th and 56th postoperatively. **Haemato-biochemical examination**, histopathological examination, radiographic examination and ultrasonographical evaluation were done at different time intervals. On radiographic examination, lung metastasis was not observed in most of the cases. Ultrasonographic findings **revealed** eight cases were having a malignant lymph node in which two cases were having metastatic lesion in spleen. After chemotherapy, ultrasonography revealed non-reactive lymph nodes and gradual disappearance of metastatic lesion on spleen after second cycle of chemotherapy which was completely disappeared after third cycle of chemotherapy in both the groups. On the basis of different **parameters** evaluated, it can be concluded that both the drug combinations were equally effective against carcinoma but higher rate of hypersensitivity reaction was seen with paclitaxel.

Keywords: Canine mammary tumour, Carcinoma, Doxorubicin, Carboplatin, Paclitaxel

1. INTRODUCTION

The mammary glands are frequent locations for the development of tumours and forms second most common neoplasm in dogs. Appearance of mammary gland tumours in dogs can vary greatly. The tumours can be firm or soft, well-defined lumps or diffuse swellings. Mammary tumours primarily undergo metastasis to the regional lymph nodes or to the lungs [1].

Mostly, canine mammary tumours (CMTs) are hormone dependent and can be prevented if ovariectomy is performed before 1 year of age [2]. The risk of mammary tumours for dogs spayed before their first estrus is 0.05% [3]. In general, sexually intact female dogs have seven times more risk of developing mammary tumours compared to neutered animals [4].

The initial diagnosis of a mammary tumour in female dogs is very vague and based on age, mammary lump, regional lymph node enlargement, dyspnoea and coughing in metastatic spread. On the other hand, in human patients, breast cancer is the most prevalent cancer. In these cases, increased survival is due to the advancements in screening methods, early diagnosis and breakthrough in treatments [5].

To increase the diagnostic accuracy and to eliminate false negative results clinical examination, diagnostic imaging and biopsy are performed simultaneously. In this regard ultrasonography is a useful non-invasive technique for directly imaging the internal and external anatomy of lymph nodes and differentiation of echotexture of benign and malignant tumours.

Classical modalities of cancer therapy include surgery, radiation and chemotherapy [6]. A number of chemotherapeutic drugs have been used for the treatment of mammary tumours. One of the most effective anticancer agents are paclitaxel and docetaxel [7]. A recent veterinary study showed that neo-adjuvant chemotherapy with prednisolone administered to dogs with intermediate grade mast cell tumours resulted in tumour size reduction, ease of excision and microscopically clean margins. Two major taxanes namely paclitaxel and docetaxel are widely used for management of breast cancer in women as adjuvant and neo-adjuvant therapies [8].

Carboplatin is a second-generation platinum chemotherapeutic agent which reacts within and between DNA strands by forming DNA adducts. Carboplatin has a nonspecific effect on the cell cycle phase [9].

Surgery remains the treatment of choice for all the dogs with mammary gland tumours except inoperable cases such as inflammatory carcinomas, distant metastasis and patients with poor general condition. The scope of surgery is determined by the size of tumourous mass, its location and the presence of lymphatic drainage from the affected mammary gland. Malignant tumors tend to be substantially larger than benign ones and most patients are reported to present with multiple mammary tumors. These tumors often behave as independent primary tumors, each exhibiting distinct histopathological characteristics [10-11]. The aim of surgery is to remove all tumors with full surgical margins and/or prevent the formation of new mammary tumor.

A significant advantage of surgical excision of mammary tumors is that it provides opportunity for histopathological examination of the tissue. This approach also prolongs survival time and quality of life for patients. In some instances, it may even be curative, particularly for benign tumors, malignant tumors with a low histological grade or cases detected in their early stages. Several techniques have been described including lumpectomy, simple mastectomy, regional mastectomy and unilateral or bilateral mastectomy [11-12]. Surgical excision alone yields unsatisfactory results in dogs with malignant mammary tumours exhibiting lymphatic or vascular invasion because these tumours have high rates of recurrence and metastasis [13].

2. MATERIAL AND METHODS

2.1 Selection and preparation of animals

A total of 12 female dogs having carcinoma were selected out of 22 cases brought to Department of Veterinary Surgery and Radiology, Veterinary Clinical complex (VCC), Nanaji Deshmukh Veterinary Science University (N.D.V.S.U), Jabalpur, Madhya Pradesh for the treatment of canine mammary tumours. Complete history of the cases was recorded including age, sex, breed, reproductive status, duration of disease and previous excision of growth anywhere in the body. The screening of the affected animal was done on the basis of physical examination of tumourous mass and affected gland. Owner's consent was also taken into consideration before grouping of these animals for surgico-chemotherapy.

2.2 Pre-surgical evaluation

Before initiating the regimens, a pre-surgical evaluation of all the animals was conducted to assess their physiological and hemato-biochemical profiles, ensuring their overall health status. These dogs were randomly divided into two groups, consisting of 6 animals in each group. The animals were kept off feed for 12 hours and off water for 6 hours prior to anesthesia. Inj. Butorphanol (Aristo Pharmaceutical Pvt. Ltd., Bhopal, Madhya Pradesh, India) was administered at the rate of 0.2 mg/kg body weight intramuscular as a preanesthetic. General anesthesia was induced using Inj. Atropine sulphate (Rathi Laboratories Pvt. Ltd., Patna, Bihar, India) at the rate of 0.04 mg/kg body weight intramuscular, Inj. Diazepam (Lori, Neon Laboratories Limited, Mumbai, Maharashtra, India) at the rate of 1 mg/kg body weight intravenous followed by Inj. Propofol (Troypofol, Torikaa Pharmaceuticals Ltd., Dehradun, Uttarakhand, India) at the rate of 4.0 mg/kg body weight intravenously. Anesthesia was maintained by Inj. Propofol as per the requirement.

2.3 Surgical procedure

2.3.1 Simple lumpectomy

The tumour was excised by giving elliptical skin incision. The mass was dissected bluntly from the surrounding healthy tissue and all the major blood vessels were ligated. Thereafter, the tumour mass was excised and area was washed thoroughly with Povidone iodine solution (Kemecos India Pvt. Ltd. West Bengal, India). The dead space formed after excision was obliterated by subcutaneous sutures using Polyglactin 910 (Solus 910®, Lotus Surgicals Pvt. Ltd., Uttarakhand, India) no. 0. Skin was sutured by using black braided silk no. 0 or 1 (Fig 1).



Fig 1: Simple Lumpectomy: (a) Aseptic Preparation of surgical site, (b) Elliptical incision on skin, (c) Blunt dissection and ligation of vessels, (d) Suturing of incision line

2.3.2 Regional mastectomy

Surgical excision was done with elliptical incision, made around the glands to be excised. Subcutaneous tissue was incised to expose the abdominal fascia. Subcutaneous tissue was separated from the fascia by blunt dissection. All the major blood vessels were ligated. Skin edges were advanced to the center of the defect with subcuticular sutures. The dead space formed after excision was obliterated by subcutaneous sutures using polyglactin 910 (Solus 910®, Lotus Surgicals Pvt. Ltd., Uttarakhand, India) no. 0. Skin edges were sutured by using black braided silk no. 0 or 1 (Fig 2).

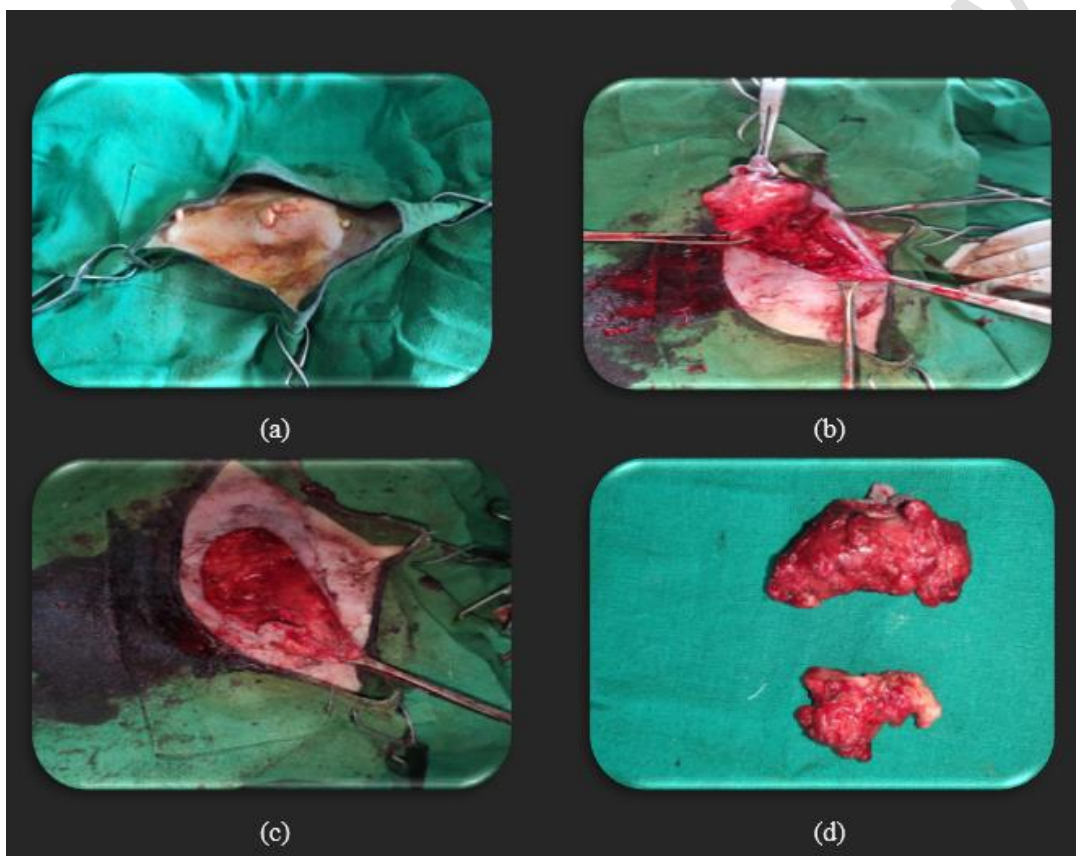


Fig 2: Regional mastectomy of right caudal abdominal mammary gland: (a) Aseptic Preparation of surgical site, (b) Elliptical incision on skin, (c) Blunt dissection and ligation of vessels, (d) Removal of glands along with tumorous mass

2.4 Chemotherapy procedure

The animals having malignant carcinomas were divided into two groups containing six animals each. Adjuvant combination chemotherapy was performed in all the animals under study after 12 days of operation. Dogs in group I were treated with chemotherapy consisting Doxorubicin and Paclitaxel, while dogs in group II treated were with Doxorubicin and Carboplatin. In Group I, chemotherapeutic drugs Inj. Doxorubicin (Doxozest 50, ZEE Laboratories Ltd., Himachal Pradesh, India) at the rate of 30mg/m² body surface area and

Inj. Paclitaxel (Z-taxel 260, ZEE Laboratories Ltd., Himachal Pradesh, India) at the rate of 165mg/m² body surface area was administered intravenously in lateral saphenous vein (*vena saphena lateralis*) or in cephalic vein (*vena cephalica*) on day 14th, 35th and 56th after performing surgical excision of mammary tumour mass. In Group II, chemotherapeutic drugs inj. Doxorubicin @ 30mg/m² body surface area and inj. Carboplatin (Kemocarb®, Fresenius Kabi Oncology Ltd., Nalagarh, Solan Himachal Pradesh, India) at the rate of 300mg/m² body surface area was administered intravenously in lateral saphenous vein (*vena saphena lateralis*) or in cephalic vein (*vena cephalica*) on day 14th, 35th and 56th after performing surgical excision of mammary tumour mass.

The anti-neoplastic agents were administered after diluting with normal saline by slow intravenous infusion. Before the start of chemotherapy all the animals were administered with antiallergic/antiemetic and supportive treatment was given in all the animals included antacids, hepatoprotective and haematinics.

2.5 Haemato-biochemical examination

Approximately 3 ml of blood was collected aseptically from dog's lateral saphenous vein (*vena saphena lateralis*) or in cephalic vein (*vena cephalica*) in the vials containing ethylene diamine tetra acetic acid (EDTA) before surgery (0th day) and on the 14th, 35th and 56th day after surgery and following the chemotherapeutic regimen. Abacus automatic haematology analyzer was used to estimate hemoglobin (gm/dL), total erythrocyte count (10³/μL) and total leukocyte count (10⁶/μL). Serum was separated for the estimation of serum urea nitrogen (mg/dL), serum creatinine (mg/dL), aspartate aminotransferase (U/L) and serum calcium (mg/dL) using CHEM-5 plus semi-autoanalyzer and commercially available standard kits (ERBA Mannheim Transasia biochemical (India) Pvt. Ltd.).

2.6 Histopathological analysis

Histopathological analysis was conducted in the Department of Veterinary Pathology, Nanaji Deshmukh Veterinary Science University (N.D.V.S.U), Jabalpur. Tissue samples from surgically excised tumours were collected and rinsed with normal saline to remove extraneous blood and other debris. Multiple 0.5 cm thick slices of tissue of each tumour were cut and fixed in 10% neutral buffered formalin, routinely processed and embedded in paraffin as per standard protocol. Thin (4-6 μm) sections were cut with the help of microtome and stained with hematoxylin and eosin stain (H and E) according to the standard protocol for microscopic analysis to obtain details of the histopathology [14]. Based on the histological diagnosis, the tumours were divided into benign and malignant tumours.

2.7 Radiographic examination

Two radiographs of thorax in left lateral and ventro-dorsal (VD) views were taken by using Multi Mobil 10 Simens Ltd., X ray machine (100mA) at Department of Veterinary Surgery and Radiology, Nanaji Deshmukh Veterinary Science University (N.D.V.S.U), Jabalpur for the detection and pattern of metastasis preoperatively and postoperatively on day 14th, 35th and 56th in all the groups.

2.8 Ultrasonographic examination

An abdominal ultrasound in B-mode was done preoperatively and postoperatively in dog having mammary tumour at the Department of Veterinary Surgery and Radiology, Nanaji Deshmukh Veterinary Science University (N.D.V.S.U), Jabalpur on day 14th, 35th and 56th with linear array transducer (8-12 MHz) and curvilinear transducer (3-5 MHz) using Phillips HD7 XE Ultrasound Machine (Philips and Nuesoft Medical Systems Co. Ltd., Neusoft Park,

Hun Nan Industrial Area, Shenyang, China) for deep seated organs with special reference to liver and spleen.

The transducer was placed in the central superficial region of the neoplasms, adjusting the focus, gain, and depth adjusted as necessary. Following these adjustments, the nodules and masses were measured in longitudinal sections (length and height) and transverse sections (width).

2.9 Statistical analysis

Statistical analysis of data was carried out to find the mean \pm SE. One way analysis of variance (ANOVA) technique (by using SPSS software package) was taken to interpretate the effect of treatment on various parameters. The effects of treatment were estimated by using general linear model's procedures of statistical software SPSS 17.0 (SPSS, Chicago, IL, USA). Treatment means were separated by applying Duncan's test and differences were considered as statistically significant at $p < 0.05$.

3. RESULTS AND DISCUSSION

Total 22 cases of canine mammary tumour in dogs were registered in Department of Veterinary Surgery and Radiology, Veterinary Clinical complex (VCC), Nanaji Deshmukh Veterinary Science University (N.D.V.S.U), Jabalpur, Madhya Pradesh during the study period. They were aged between 3-13 years and weighed between 9-21 kgs. The breed wise distribution of dogs for canine mammary tumour was as follows: 08 German shepherd, 06 Mongrel, 04 Spitz, 03 Labrador retriever and 01 Dobermann pinscher.

The physiological profile including rectal temperature, respiratory rate and heart rate was within the normal range; however, the haematological profile revealed some variations. Total leucocyte count was elevated, while the values of haemoglobin and packed cell volume were below the normal range. Therefore, all the animals were stabilized with antibiotic treatment and supportive therapy, including oral haematinics, prior to surgery.

3.1 Haemato-biochemical examination

3.1.1 Haemoglobin (g/dL)

Mean values of haemoglobin (g/dL) in group I varied from 9.45 ± 0.34 to 13.80 ± 0.56 . A slight decrease was observed on day 14th followed by significant ($p < 0.05$) decrease on day 35th and day 56th. Similarly, the mean value of haemoglobin (g/dl) in group II varies from 10.38 ± 0.30 to 13.98 ± 0.28 which showed significant ($p < 0.05$) decrease on day 14th, 35th and 56th (Table 1).

The decrease in haemoglobin values was in accordance with the findings of Ravikumar (1998) who reported significant decrease in haemoglobin after chemotherapy in dogs while Sharma *et al.* (2010) and Yadav *et al.* (2013) reported non-significant reduction in haemoglobin concentration [15-17].

The common side effect of chemotherapy is anaemia. Chemotherapeutic drugs damage the bone marrow cells because of their high growth fraction which ultimately decreases the haemoglobin.

Table 1: Mean ± SE values of haemoglobin (g/dL) in group I and group II

Days	Group I	Group II
0	13.80 ^A ± 0.56	13.98 ^A ± 0.28
14	12.75 ^A ± 0.62	12.80 ^B ± 0.36
35	10.78 ^B ± 0.37	11.58 ^C ± 0.27
56	9.45 ^B ± 0.34	10.38 ^D ± 0.30

A & B: Values within group with different superscript differ significantly ($p < 0.05$)

3.1.2 Total leukocyte count ($10^3/\mu\text{L}$)

The mean values of total leukocyte count ($10^3/\mu\text{L}$) ranged from 10.83 ± 0.78 to 13.05 ± 0.74 in group I which was within the physiological limit. Slight increase in total leukocyte count was observed by day 14th while overall decrease was seen by day 35th and 56th of chemotherapy in group I. Slight decrease in total leukocyte count was noted on day 14th while significant ($p < 0.05$) decrease was observed on day 35th in group II (Table 2). Similarly, Sharma *et al.* (2020) reported that there was a significant reduction in total leukocyte count after chemotherapy [16]. A study on effect of doxorubicin on haematological and blood biochemical profile of healthy dogs revealed that doxorubicin was responsible for significant decrease in leucocyte count in dogs. This leukocytopenia reported after chemotherapy might be due to tendency of chemotherapeutic drug to target rapidly dividing cells and unable to differentiate tumour cells and healthy cells.

Table 2: Mean ± SE values of total leukocyte count ($10^3/\mu\text{L}$) in group I and group II

Days	Group I	Group II
0	11.48 ± 1.66	12.66 ^A ± 0.70
14	13.05 ± 0.74	12.16 ^A ± 0.73
35	12.03 ± 0.92	10.08 ^B ± 0.91
56	10.83 ± 0.78	9.58 ^B ± 0.43

A & B: Values within group with different superscript differ significantly ($p < 0.05$)

3.1.3 Total erythrocyte count ($10^6/\mu\text{L}$)

The mean values of total erythrocyte count ($10^6/\mu\text{L}$) ranged from 5.41 ± 0.11 to 6.86 ± 0.36 in both groups. The mean values of total erythrocyte count decreased significantly over day 14th followed by a gradual decrease over a period of day 35th to 56th in group I. While in group II there was a gradual decrease in total erythrocyte count by day 14th followed by significant decrease on day 35th (Table 3). Likewise, significant decrease in total erythrocyte count after chemotherapy was also reported in previous studies [16,18]. This decreases in erythrocyte count after chemotherapy might be due to myelosuppression induced by chemotherapy.

Table 3: Mean ± SE values of total erythrocyte count ($10^6/\mu\text{L}$) in group I and group II

Days	Group I	Group II
0	6.70 ^A ± 0.27	6.86 ^A ± 0.36
14	5.83 ^B ± 0.25	6.23 ^A ± 0.35
35	5.58 ^B ± 0.17	5.81 ^B ± 0.25
56	5.41 ^B ± 0.11	5.56 ^B ± 0.19

A & B: Values within group with different superscript differ significantly ($p < 0.05$)

3.2 Serum biochemistry

3.2.1 Blood urea nitrogen (mg/dL)

The mean value of blood urea nitrogen (mg/dL) varied from 17.96 ± 2.03 to 18.26 ± 1.83 in group I and from 17.06 ± 1.57 to 18.65 ± 1.49 in group II at different time intervals during study period. The values showed non-significant gradual increase in blood urea nitrogen after chemotherapy (Table 4). The findings of current study were in accordance with Karayannopoulou *et al.* (2001) who tested serum biochemical parameters during adjuvant postoperative chemotherapy in female dogs with mammary tumour and recorded non-significant increase in blood urea nitrogen [19].

Similarly, Kumar (2017) performed clinical diagnostic and therapeutic management of certain tumours in dogs and found non-significant increase in blood urea nitrogen after chemotherapy [20]. The increase in level of blood urea nitrogen represents the nephrotoxicity of associated drug which included loss of renal epithelium and damage to brush border cells of kidney.

Table 4: Mean \pm SE values of blood urea nitrogen (mg/dL) in group I and group II

Days	Group I	Group II
0	17.96 ± 2.03	17.06 ± 1.57
14	17.98 ± 1.88	17.15 ± 1.55
35	18.12 ± 1.57	18.10 ± 1.18
56	18.26 ± 1.83	18.65 ± 1.49

3.2.2 Creatinine (mg/dL)

The mean values of creatinine (mg/dL) varied from 1.15 ± 0.12 to 1.88 ± 0.10 in group I while in group II, it ranged from 1.03 ± 0.17 to 1.29 ± 0.07 at different time intervals. Non-significant difference was recorded in both the groups while the values showed increase in creatinine level by 35th day followed by decrease on 56th day in group I. A gradual increase in creatinine level was recorded by day 56 in group II (Table 5).

Karayannopoulou *et al.* (2001) observed that creatinine value increased significantly but the mean values of creatinine remain under the physiological range [19]. The elimination of the drugs through the kidneys is negligible but the slight increase in creatinine might be due to the moderate nephrotoxicity of drug seen in older age of dogs with kidneys not adequately eliminating the accumulated drug.

Table 5: Mean \pm SE values of creatinine (mg/dL) in group I and group II

Days	Group I	Group II
0	1.15 ± 0.12	1.03 ± 0.17
14	1.66 ± 0.09	1.15 ± 0.13
35	1.88 ± 0.10	1.23 ± 0.06
56	1.28 ± 0.13	1.29 ± 0.07

3.2.3 Alanine transaminase (U/L)

The mean value of serum alanine transaminase (U/L) varied from 51.06 ± 9.25 to 64.03 ± 8.43 in group I while in group II it ranged from 55.10 ± 4.04 to 61.55 ± 4.57 at different time

intervals. Non-significant increase was noticed in **alanine transaminase** values in both the groups after chemotherapy (**Table 6**). The findings of present study are similar to Karayannopoulou *et al.* (2001) and Parameswara (2016) who reported the increased value of serum alanine transaminase and serum alkaline phosphatase [19, 21]. The metabolism of chemotherapeutic drugs take place in liver by means of hydroxylation in presence of cytochrome p450 enzyme which might have resulted stress on liver and brought moderate change in **alanine transaminase** values.

Table 6: Mean ± SE values of alanine transaminase (U/L) in group I and group II

Days	Group I	Group II
0	51.06 ± 9.25	55.10 ± 4.04
14	54.53 ± 9.06	55.46 ± 4.60
35	59.56 ± 8.63	58.18 ± 4.59
56	64.03 ± 8.43	61.55 ± 4.57

3.2.4 Calcium (mg/dL)

The mean values of calcium (mg/dL) varied from 10.21 ± 0.34 to 10.46 ± 0.48 in group I and 9.45 ± 0.12 to 10.05 ± 0.42 in group II. Non-significant decreases were noticed in both the groups after administration of chemotherapeutic drugs (**Table 7**). Similar observations were found by Sharma *et al.* (2014) who evaluated adjuvant combination for malignant cutaneous tumors in dogs and recorded slight decrease in serum calcium level after chemotherapy [22].

Table 7: Mean ± SE values of calcium (mg/dL) in group I and group II

Days	Group I	Group II
0	10.46 ± 0.48	10.05 ± 0.42
14	10.40 ± 0.47	9.96 ± 0.48
35	10.31 ± 0.45	9.83 ± 0.40
56	10.21 ± 0.34	9.45 ± 0.12

3.3 Histopathological analysis

Out of 22 samples analyzed, 6 cases (27.27%) were benign and 16 cases (72.72%) were malignant as shown in table 8. Canine mammary adenocarcinoma (14) was the most prevalent tumors in the current investigation. The benign CMTs were comprised of 13.64% (3/22) adenomas and 3.64% (3/22) fibroadenoma. Likewise, adenocarcinoma displayed the highest proportion (63.63%) among all malignant CMTs followed by fibro adenocarcinoma (09.09%).

The present study is in accordance with the Suryawanshi (2022) who reported 13 cases (72.22%) out of 18 cases were malignant in nature and remaining 5 cases (27.77%) were benign type [23]. Zheng *et al.* (2021) reported 48.41% cases (244/504) were benign tumors and 51.59% (260/504) were malignant tumors based on the histopathological classification during their study period [24]. Similarly, Soler *et al.* (2016) reported that out of 104 mammary gland tumours, 34.62% were found benign while 65.38% tumours were considered malignant [25].

Table 8: Histopathology of canine mammary tumours

S.No.	Histopathological type	Histopathological classification	Number	Percent (%)
1.	Adenocarcinoma	Malignant	14	63.63
2.	Fibro adenocarcinoma	Malignant	2	09.09
3.	Adenoma	Benign	3	13.64
4.	Fibroadenoma	Benign	3	13.64
Total			22	100.00

Several publications reveal that dogs with histopathological pre-malignant lesions or dogs with previous history of malignant canine mammary tumours showed an increased risk of developing a new malignant canine mammary tumour. This might be explained by the fact that the entire mammary tissue is under the same hormonal exposure [26-28]. Consequently, multiple tumours may develop over time or may be present in various stages of transformation at the same time [29].

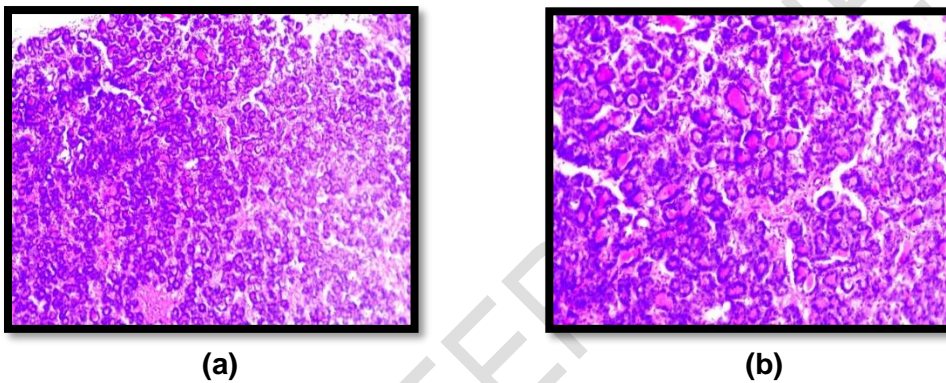


Fig 3: Microscopic section of mammary tumour showing adenocarcinoma (a) 10X (b) 20X

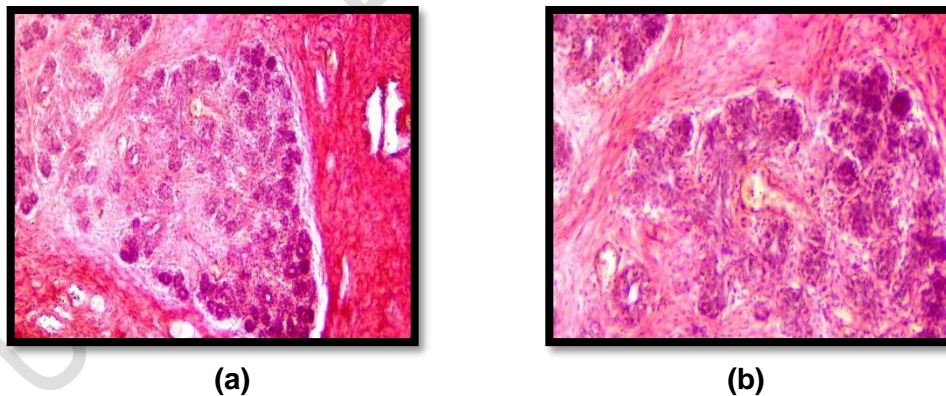


Fig 4: Microscopic section of growth on mammary gland of dog showing fibroadenoma. (a) 10X (b) 20X

3.4 Radiographic examination

On radiographic examination, lung metastasis was not observed in most of the cases.

3.5 Ultrasonographic examination

B-mode ultrasonography revealed eight cases were having malignant lymph nodes in which two cases were having metastatic lesion in spleen.

The ultrasonographic picture of superficial inguinal lymph node in group I (Fig 5) showed cortical widening and appear more rounded on day 0 (before chemotherapy). These lymph nodes have short to long axis ratio more than 0.5 that shows malignancy as reported in previous studies [30-31]. After 2nd dose of chemotherapy on 42nd day, the short to long axis ratio less than 0.5 which indicates recovery.

Similar results had been obtained in group II (Fig 6) also where short to long axis ratio was found more than 0.5 which showed malignancy on day 0 (before chemotherapy) and then it becomes less than 0.5 which indicates recovery after 2nd dose of chemotherapy.

A hypoechoic lesion of 0.583cm and 0.391cm respectively in group I and II (Fig 7 and 8) were observed at 0 day before the start of chemotherapy and after third dose of chemotherapy the splenic parenchyma appeared back to normal in both the groups.

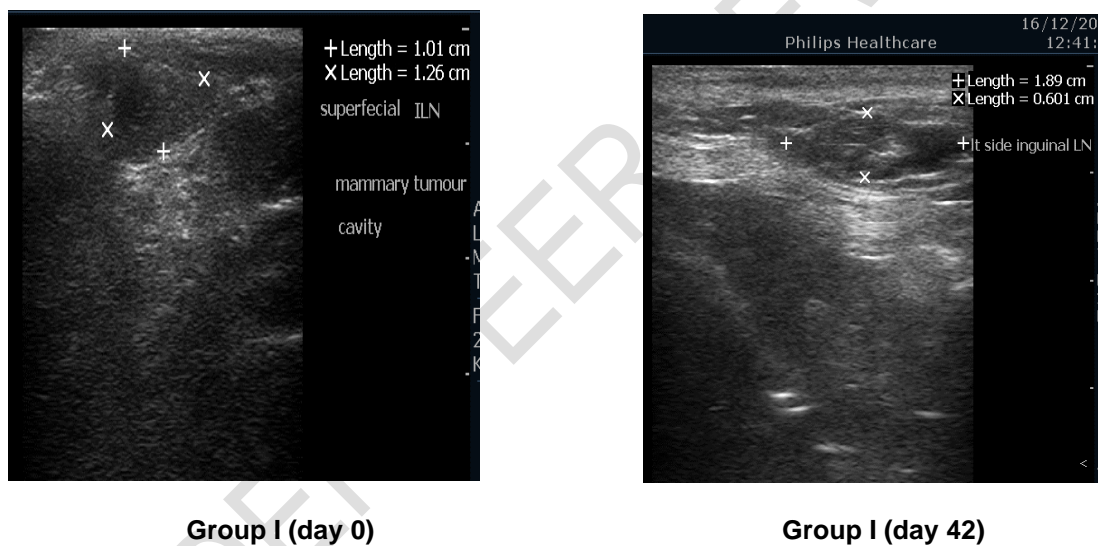
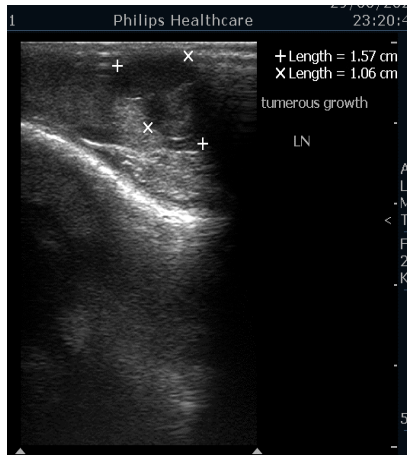
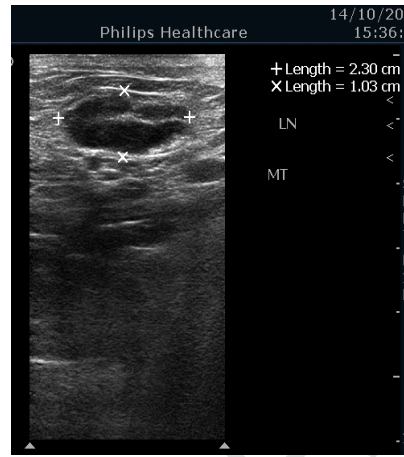


Fig 5: Ultrasonographic measurement of superficial inguinal lymph node in group I on day 0 and day 42

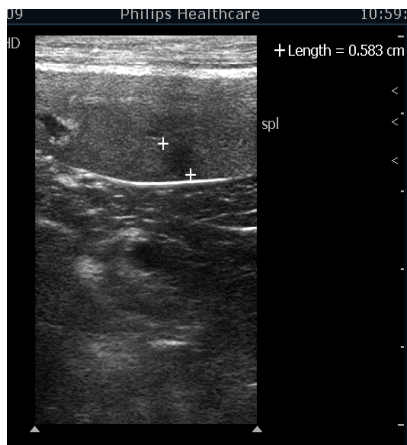


Group II (day 0)



Group II (day 42)

Fig 6: Ultrasonographic measurement of superficial inguinal lymph node in group II on day 0 and day 42



(a)

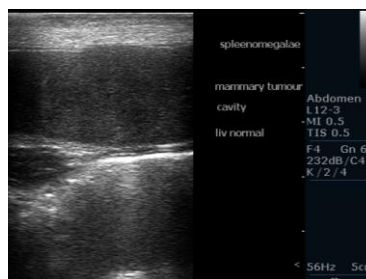


(b)

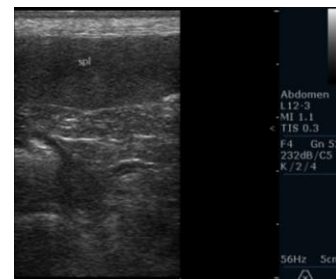
Fig 7: Ultrasonographic finding in spleen in group I (a) Metastatic lesion in spleen on day 0 (b) Normal Spleen after third cycle of chemotherapy



(a)



(b)



(c)

Fig 8: Ultrasonographic finding in spleen in group II (a) Metastatic lesion in spleen on day 0 (b) Splenomegaly on day 0 (c) Normal Spleen after third cycle of chemotherapy

Parmeshwara (2016) compared the efficacy of Paclitaxel and Docetaxel as anticancer drugs for management of malignant mammary tumour in dogs and concluded that both the drugs were having equal spectra of clinical activity on regression of mammary tumor [21].

Adverse effects

The use of paclitaxel has not been frequently described in veterinary medicine owing to the high prevalence of acute hypersensitivity reactions. These reactions are characterized by transient flushing, rash, symptomatic bronchospasm with or without urticaria, anaphylaxis or death [32]. During the administration of paclitaxel high incidence of cutaneous hypersensitivity reaction was seen in majority of cases. However, hypersensitivity did not result in discontinuation of therapy in any dog because repeated premedication and increased infusion time allowed completion of therapy in all dogs. After administration of Doxorubicin, the adverse signs observed were anorexia, vomiting and diarrhoea. Poirier *et al.* (2004) also reported adverse effects like hypersensitivity after administration of paclitaxel [33]. These reactions might be a result of the solubilizing agent Cremophor in which the drug is formulated. Sharma *et al.* (2014) recorded anaphylaxis due to rapid infusion of chemotherapeutic agents' doxorubicin, cyclophosphamide and 5-fluorouracil [22]. In present study, no side effects were observed during and after the administration of Carboplatin.

Recurrence free interval

Two animals were found with recurrence of mammary tumour within 90 days which did not receive chemotherapy because of owner's negligence. Previous studies described that the survival time and the efficacy of chemotherapy depended on various factors viz. stage of the disease, the histological type and the degree of tumour differentiation, tumour size and the early diagnosis [12,34,35].

4. CONCLUSION

The prevalence of mammary tumors in dogs poses a significant concern in small animal practice therefore early investigation and diagnosis is essential for adequate patient care. Despite the use of various adjuvant chemotherapy protocols following surgery for the treatment of CMTs, a standardized and effective treatment regimen has yet to be established. Adjuvant chemotherapy is advised for dogs diagnosed with inflammatory mammary carcinoma or advanced metastatic conditions.

The administration of chemotherapeutic agents in the present study involved a meticulous and prolonged procedure to ensure safety and efficacy of therapy. The process required the patient to remain stabilized on the treatment table, often for more than six hours, due to the slow infusion rate necessary to minimize adverse effects. During this time, close monitoring of the dog is critical, particularly for signs of hypersensitivity reactions which are a known risk with paclitaxel.

On the basis of different parameters evaluated in the present study, it can be concluded that both the drug combinations were equally effective against carcinoma but higher rate of hypersensitivity reaction without any mortality was seen with paclitaxel. Carboplatin was relatively affordable, well tolerated and easy to administer without any hypersensitivity reaction compared to paclitaxel in dogs.

Due to limited research period, a small number of animals were included in the study. To formulate an effective chemotherapy using aforementioned protocols, study should be replicated in a large sample size.

Future research should focus on optimizing dosing regimens, minimizing toxicity, and evaluating long-term efficacy to enhance survival rates and quality of life in affected dogs. Additionally, advancements in molecular diagnostics and personalized medicine could further refine these combination treatments, paving the way for tailored therapeutic approaches in veterinary oncology.

5. CONSENT

All authors declare that written informed consent was obtained from the pet's owner for publication of the research.

6. ETHICAL APPROVAL

The research described in the study was conducted in compliance with the ethical standards and guidelines of the Institutional Animal Ethics Committee (IAEC) and due permission was received from the ethical committee of the University.

7. DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Authors hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

8. ACKNOWLEDGEMENT

The authors are thankful to the Dean, College of Veterinary Science and Animal Husbandry, Jabalpur, M.P. for the successful conduct of the research.

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