Original Research Article

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, looking 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 the history of mammary tumour irrespective of breed. The animals with carcinoma were randomly divided into two groups containing 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 given on day 14, 35 and 56 postoperatively. Haemato-biochemical, 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 revelaed 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 paramenters 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 (Moulton, 1990).

Mostly, canine mammary tumours are hormone dependent and can be prevented if ovariohysterectomy (OH) is performed before 1 year of age. The risk of mammary tumours for dogs spayed before their first estrus is 0.05%. In general, sexually intact female dogs have seven times more risk of developing mammary tumours compared to neutered animals (MacPhail, 2013).

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 (Nounou *et al.,* 2015).

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 (Kumar and Pawaiya, 2010). 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 (Benavente *et al.*, 2016). 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 (Crown *et al.,* 2004) as adjuvant and neo-adjuvant therapies.

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 (Lavalle *et al.*, 2012).

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. Several techniques have been described including lumpectomy, simple mastectomy, regional mastectomy and unilateral or bilateral mastectomy (Novosad, 2003). 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 (Simon *et al.*, 2006).

2. MATERIAL AND METHODS

2.1 Selection and preparation of animals

A total of 12 female dogs having carcinoma was selected out of 22 cases bought 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. 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 was administered @ 0.2 mg/kg body weight intramuscular as a preanesthetic. General anesthesia was induced using Inj. Atropine sulphate @ 0.04 mg/kg body weight intramuscular, Inj. Diazepam @ 1 mg/kg body weight intravenous followed by Inj. Propofol @ 4.0 mg/kg body weight intravenously. Anesthesia was maintained by Inj. Propofol as per the requirement.

2.2 Experimental design

The animals having malignant carcinomas were divided into two groups containing six animals each. In Group I, chemotherapeutic drugs Inj. Doxorubicin @ 30mg/m^2 body

surface area intravenously and Inj. Paclitaxel @ 165mg/m^2 body surface area intravenously was administered on day 14th, 35th and 56th after performing surgical excision of mammary tumour mass. In Group II, chemotherapeutic drugs inj. Doxorubicin @ 30mg/m^2 body surface area intravenous and inj. Carboplatin @ 300mg/m^2 body surface area intravenous was administered in on day 14th, 35th and 56th after performing surgical excision of mammary tumour mass.

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. The dead space formed after excision was obliterated by subcutaneous sutures using Polyglactin 910 no. 0. Skin was sutured by using black braided silk no. 0 or 1 (Fig 01).

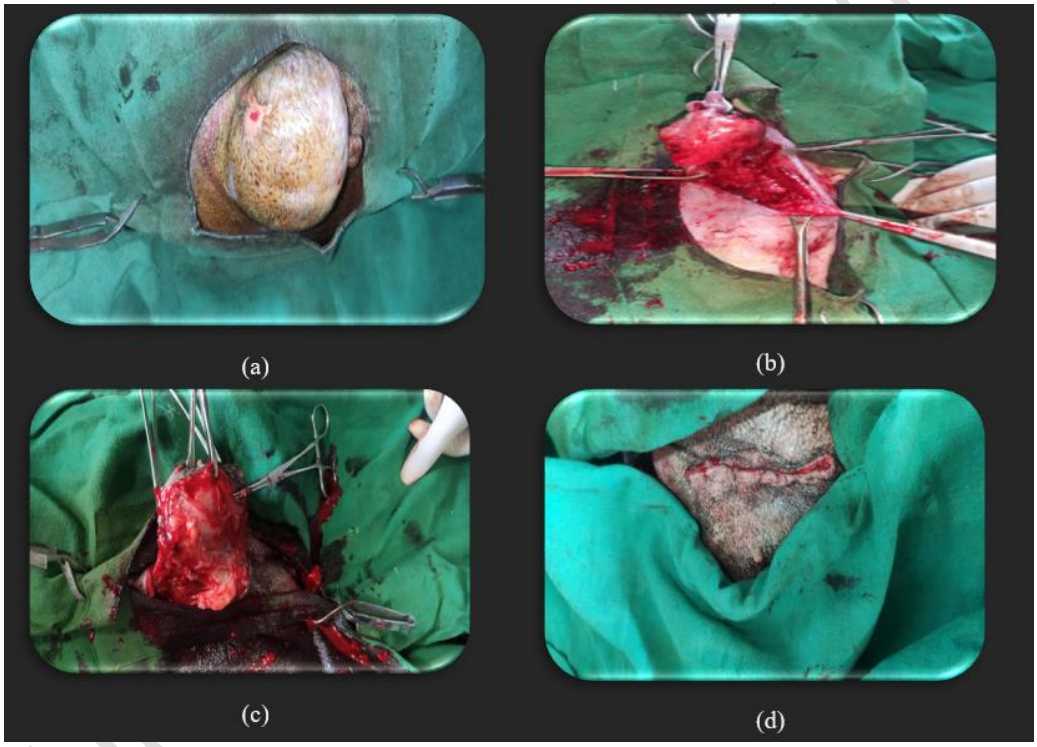


Fig 01: Simple Lumpectomy: (a) Aseptic Preparation of surgical site, (b) Elliptical incision on skin, (c) Blunt dissection and ligation of vessels, (d) Suturing of incision line

2.3.1 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 advance to the center of the defect with subcuticular sutures. The dead space formed after excision was obliterated by subcutaneous sutures using polyglactin 910 no. 0 (MacPhail, 2013). Skin edges were sutured by using black braided silk no. 0 or 1 (Fig 02).

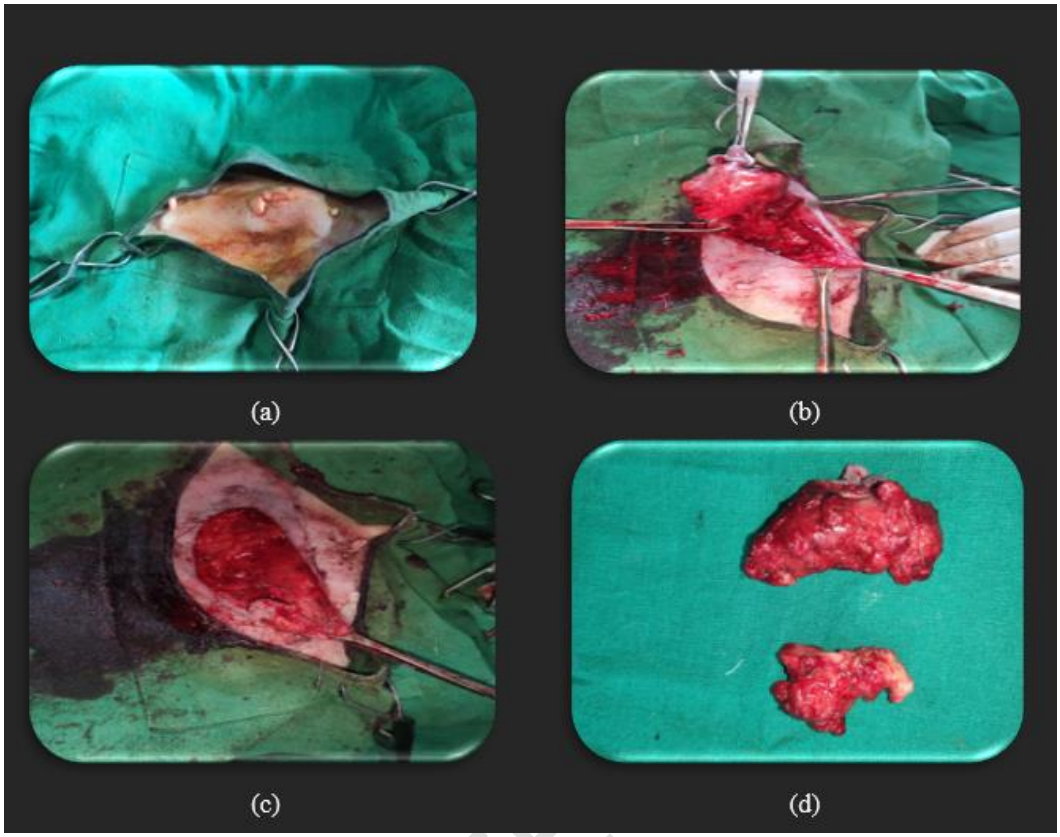


Fig 02: Regional mastectomy: (a) Aseptic Preparation of surgical site, (b) Elliptical incision on skin, (c) Blunt dissection and ligation of vessels, (d) Removal of glands along with tumours mass

2.3 Chemotherapy procedure

Adjuvant combination chemotherapy was performed in all the animals under study after 12 days of operation. All 12 dogs were treated with chemotherapy consisting Doxorubicin and Paclitaxel in group I, while in group II treated with Doxorubicin and Carboplatin as per the protocol described. 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.

2.4 Haemato-biochemical examination

Approximately 3 ml of blood was collected aseptically from the saphenous vein or cephalic vein for estimation of haematological and biochemical parameters by automatic blood analyzer and biochemical analyzer (ERBA) using commercially available standard kit preoperatively and postoperatively on the day 14, 35 and 56.

2.5 Radiographic examination

Two radiographs of thorax in left lateral and ventro-dorsal (VD) views were taken for the detection and pattern of metastasis preoperatively and postoperatively on day 14th, 35th and 56th in all the groups.

2.6 Ultrasonographic examination

An abdominal ultrasound was done preoperatively and postoperatively on day 14, 35 and 56 with linear array transducer (8-12 MHz) and curvilinear transducer (3-5 MHz) for deep seated organs with special reference to liver and spleen.

3. RESULTS AND DISCUSSION

3.1 Haemato-biochemical examination

3.1.1 Haemoglobin

Mean values of haemoglobin in group I varies from 9.45 ± 0.34 to 13.80 ± 0.56. A slight decrease was observed between day 0 and day 14 followed by significant ($p<0.05$) decrease on day 35 and day 56 while mean value of haemoglobin in group II varies from 10.38 ± 0.30 to 13.98 ± 0.28 showed significant ($p<0.05$) decrease on day 14, 35 and 56 (Table 01).

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.

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 01. 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/µL)

The mean values of total leukocyte count (10^3/µL) ranged from 10.83 ± 0.78 to 13.05 ± 0.74 in group I which was within the physiological limit. Slight increase in TLC value was showed by day 14 while overall decrease was seen by day 35 and 56 of chemotherapy in group I. Slight decrease in total leukocyte count was noted on day 14 while significant ($p<0.05$) decrease was observed on day 35 in group II (Table 02). Similarly, Sharma *et al.* (2010) reported that there was a significant reduction in TLC count after chemotherapy. Sindhur *et al.* (2019) conducted a study on effect of doxorubicin on haematological and blood biochemical profile of healthy dogs and stated 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 02: Mean ± SE values of total leukocyte count (10^3/µL) 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/µL)

The mean values of total erythrocyte count (10^6/µL) ranged from 5.41 ± 0.11 to 6.86 ± 0.36 in both groups. The mean values of TEC decreased significantly over day 14 followed by a gradual decrease over a period of day 35 to 56 in group I. While in group II there was a gradual decrease in TEC count by day 14 followed by significant decrease on day 35 (Table 03). These findings are in accordance with Sharma *et al.* (2010) and Sindhur *et al.* (2019), who observed significant decrease in total erythrocyte count after chemotherapy. This decreases in erythrocyte count after chemotherapy might be due to myelosuppression induced by chemotherapy.

Table 03: Mean ± SE values of total erythrocyte count (10^6/µL) 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 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 04). 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.

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. The increase in level of BUN represents the nephrotoxicity of associated drug which included loss of renal epithelium and damage to brush border cells of kidney.

Table 04: Mean ± 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 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 05).

Karayannopoulou *et al.* (2001) observed that creatinine value increased significantly but the mean values of creatinine remain under the physiological range The elimination of the drugs through the kidneys is negligible but the slight increases 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 05: Mean ± 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 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 ALT values in both the groups after chemotherapy (Table 06). The findings of present study are similar to Karayannopoulou *et al.* (2001) and Parameswara (2016) noticed increased value of serum alanine transaminase and serum alkaline phosphatase 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 ALT values.

Table 06: 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 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 07). 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.

Table 07: Mean ± SE values of calcium (mg/dL) in group I and group II

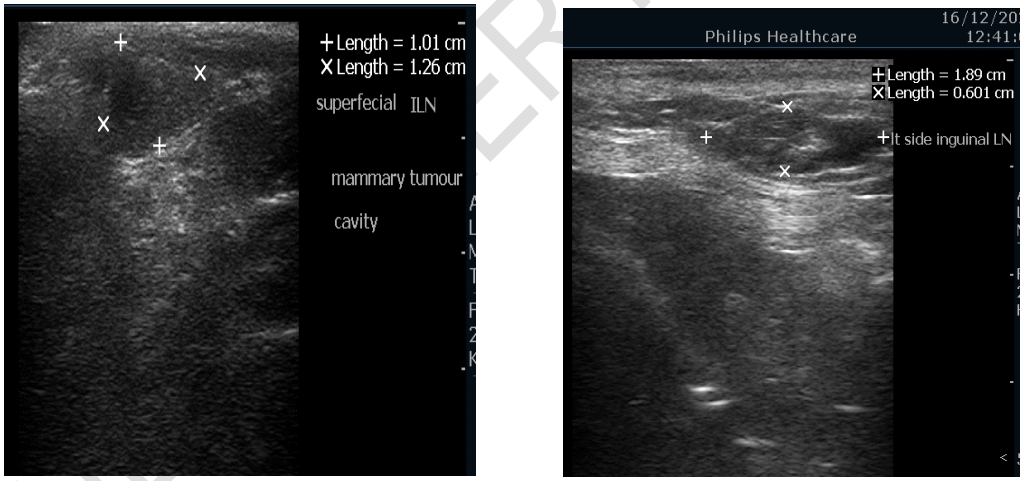
Days	Group I	Group II
0	10.46 ± 0.48	10.05 ± 0.42
14	10.40 ± 0.47	9.96 ± 0.48
35	10.31 ± 0.45	9.83 ± 0.40
56	10.21 ± 0.34	9.45 ± 0.12

3.3 Radiographic examination

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

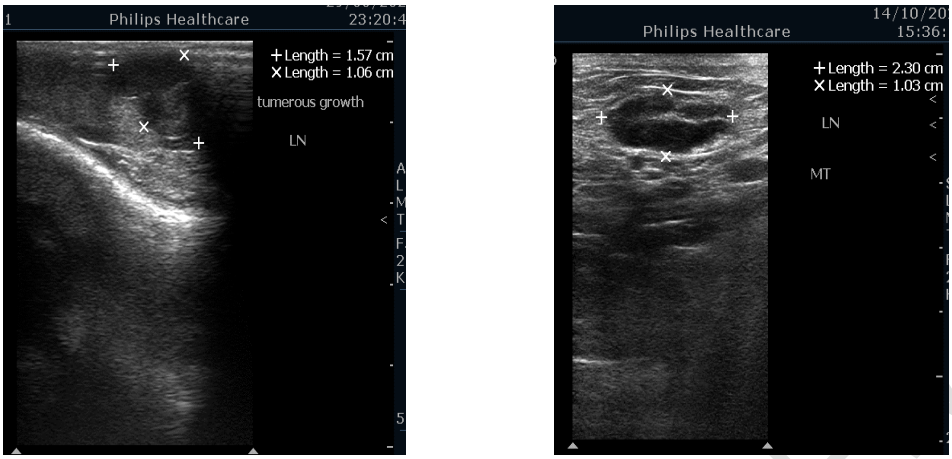
3.4 Ultrasonographic examination

Eight cases were having a malignant lymph node in which two cases were having metastatic lesion in spleen. The malignant lymph nodes were seen to become normal after the 2nd dose of chemotherapy while the metastatic lesion in spleen tend to become normal after 3rd dose of chemotherapy in both the groups (Fig 03-06).



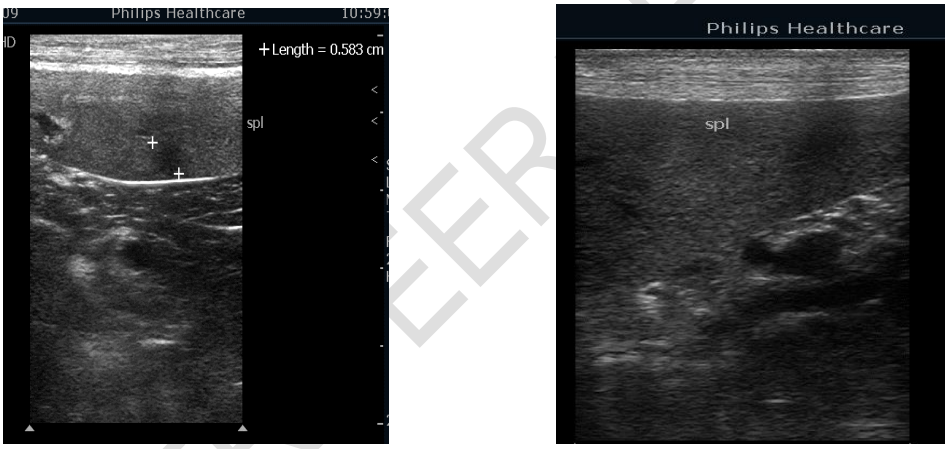
Group I (day 0) **Group I (day 42)**

Fig 03: Ultrasonographic measurement of associated lymph node in group I on day 0 and day 42



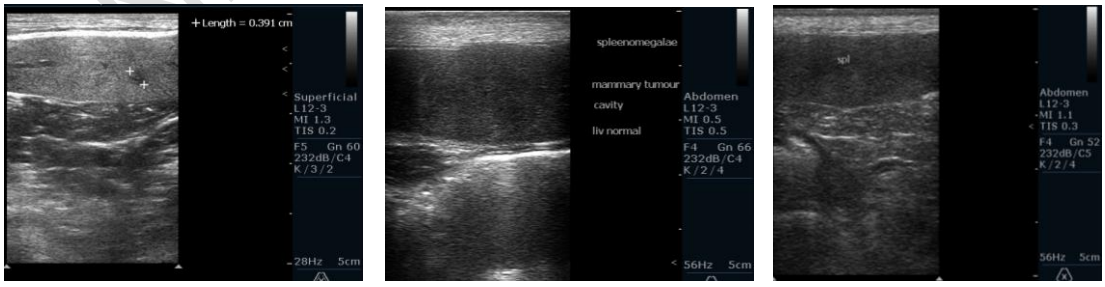
Group II (day 0) **Group II (day 42)**

Fig 04: Ultrasonographic measurement of associated lymph node in group II on day 0 and day 42



(a) **(b)**

FIg 05: Ultrasonograhic 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 06: Ultrasonograhic 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.

Adverse effects

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. 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. 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 negligence. Sorenmo (2003), Novosad (2003) and Philibert *et al.* (2003) 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.

4. CONCLUSION

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.

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