Original Research Article

An evaluation of the systemic impact and clinical efficacy of sequential vincristine sulfate versus doxorubicin in the post-operative management of Canine Mammary Tumours (CMT)

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ABSTRACT

The present study was conducted to evaluate the systemic impact and clinical efficacy of sequential vincristine sulfate compared to doxorubicin in the post-operative management of Canine Mammary Tumours (CMT) on 18 clinical cases of mammary tumour of various breed, irrespective of their age, sex and divided into three groups consisting of 6 animals in each.Group Surgery was treated with surgical removal of tumouronly while animals of Group SurgDox and Group SurgVin were treated with surgical excision followed by administration of Doxorubicin (30mg/m^2) BSA and Vincristine sulfate (0.025 mg/kg) intravenously alongwith DNS at 7^{th} and 14^{th} post-operative days respectively. The haemato-biochemical parameters and wound evaluation were done at different time intervals. Systemic impact on haematological and biochemical parameters were briefly altered and chemotherapeutic medications does not induce any adverse reactions or detrimental effects on vital organs, but these changes remained within the normal physiological range.Doxorubicin and vincristine sulfate therapy showed minimum to no reoccurrence of tumour with few adverse reactions such as inappetance, vomition, anaemia and alopecia. However, these conditions were managed by supportive therapy. Therefore, it could be concluded that sequential vincristine therapy is best for the post-operative management of canine mammary tumours as it effectively regressed the tumour without relapse.

Keywords:- Canine, CMT, Doxorubicin, Mammary tumour, Chemotherapy, Vincristinesulfate

INTRODUCTION

Canine mammary tumors are second most common neoplasm of unspayed female dog representing approximately 40-50% of all neoplasms (Khimta *et al.*, 2010). However, male dogs are also affected but the prevalence is only 1% (Rutterman *et al.*, 2000). The last two sets of glands (4^{th} and 5^{th} pair) are most commonly affected. 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 lymphnodes 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. Bitches spayed prior to their first estrous cycle have about 0.5% risk of developing mammary tumours, while the risk is about 8% in bitches spayed between their first and second estrous and after the second estrous, the risk of mammary tumour development is 26 percent

(Brodey *et al*., 1983). The age group at which mammary tumours occurred most frequently was 8-10 years followed by 10-12 years (Kishor *et al*., 2016). In general, sexually intact female dogs have seven times more risk of developing mammary tumours compared to neutered animals (MacPhail, 2013).

Classical modalities of cancer management include surgery, radiation and chemotherapy (Kumar and Pawaiya, 2010).Surgery remains the treatment of choice for most canine tumours except diffuse, unapproachable and non-exteriosable, inflammatory carcinomas with distal metastasis. The extent of surgery should be determined by location, tumour size and the presence of some single or multiple tumours (Fossum *et al*., 1997).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).A number of chemotherapeutic drugs like Cyclophosphamide, Chlorambucil, Vincristine, Vinblastine, Carboplatin, Cisplatin, Methotrexate, 5-Fluorouracil and Doxorubicin etc have been used for the treatment of canine mammary tumours. Not a single chemotherapy protocol has been reported to be completely effective. Surgery remains the best way to treat most tumours while some tumours are too large and deeply situated where complete removal is not possible. In such cases, radical surgery is advocated followed by chemotherapeutic measures to arrest the recurrence of cancer cellsresulting from remnants. The importance of chemotherapy has been emphasize and was reported that survival could be prolonged after chemotherapy in cancer patients (Maiti *et al*., 2011). Therefore, adjunct chemotherapy i.e. surgical excision along with chemotherapy is a newer approach for the treatment of tumours.Doxorubicin is produced by *Streptomyces peucetius var*. *caesius* and exerts its cytotoxic effect as a DNA-intercalating agent to inhibit further DNA and RNA biosynthesis (Lori *et al*., 2010).Vincristine sulfatebelongs to antimicrotubule agents which areextracted from *Vinca rosea* that cause mitotic arrest by interfering with polymerization or depolymerization of microtubules which plays a critical role in cell function and division (Gustafson and Page, 2013). Since, the reports regarding the use of doxorubicin and vincristine sulfate in adjunct chemotherapy for treatment of canine malignant mammary tumour in clinical cases are limited. Therefore,looking to the advantages of adjunct chemotherapy,the currentstudy was designed to compare the systemic impact and clinical efficacy of sequential vincristine sulfate versus doxorubicin in the post-operative management of Canine Mammary Tumours (CMT).

MATERIALS AND METHODS

The current investigation was carried out at the Teaching Veterinary Clinical Complex (TVCC) and Department of Veterinary Surgery and Radiology, College of Veterinary Science & A.H., Anjora,

Durg (C.G.) from July 2017 to July 2018 on 18 clinically suspected cases of canine mammary tumour (17 female and 1 male) and divided randomly into three groups viz**.,** Group Surgery, Group SurgDox and Group SurgVin**,** each comprising of 6 animals. Mammectomy was performed in animals of all the three groups under general anaesthesia using atropine, xylazine and ketamine. Post-operatively, dogs were given intravenous fluid therapy (DNS 500 ml), antibiotic (inj. Ceftriaxone 500 mg I/V) and analgesic (inj. Meloxicam 2 ml I /M) for five consecutive days. Antiseptic dressing was done with Povidone iodine liquid and Povidine ointment. Sequential chemotherapy drug (Doxorubicin and Vincristine) was given in Group SurgDox and Group SurgVin after 7 days of operation as post-operative management. Group Surgery was treated with only surgical removal of tumour whereas group SurgDox, Doxorubicin (30mg/m^2) BSA was given intravenously alongwith DNS at 7^{th} and 14^{th} post-operative days respectively while in group SurgVin, Vincristine sulfate (0.025 mg/kg) was administered intravenously alongwith DNS at 7^{th} and 14^{th} post-operative days. Supportive therapy using Tribivet @ 0.05-0.2 mg/kg b.wt. I/M was given for 5 days followed by Polybion syrup one tsf bid and Tab Liv-52® every day to dogs of Group SurgDoxand SurgVinduring course of chemotherapy.

I) Evaluation of Systemic Effect

a) Haematological parametes

Using an automated haematology blood cell counter (B. C. 2800 Vet. Mindray), blood samples (approximately 1 ml) were drawn from dogs' cephalic or saphenous veins in vials containing ethylene diamine tetra acetic acid (EDTA) before and on 10^{th}, 30^{th} and 60^{th} day post surgery for the estimation of Haemoglobin (gm/dl), Packed Cell Volume (%)**,** Total Erythrocyte Count (millions /mm^3), Total Leukocyte Count (thousand /mm^3) and Total Platelet Count (lac /mm^3). The Differential Leukocyte Count (DLC) was performed manually by making blood smears from the drawn blood and after methanol fixation; thesmear was stained with Geimsa stain. A percentage was given for each count.

b) Biochemical parameters

For estimation of biochemical parameters, 3 ml of peripheral venous blood were drawn from the dog in a sterile, clean vial before and at 10^{th}, 30^{th} and 60^{th} day post surgery. Serum was separated for estimation of serum glucose (mg/dl), total serum proteins (gm/dl), serum urea nitrogen (mg/dl), serum creatinine (mg/dl), aspartate aminotransferase (U/L), alanine aminotransferase (U/L), and alkaline phosphatase (U/L) using semi-automated biochemistry analyzer (Diasil-100 Systronics make).

II) Evaluation of Clinical Efficacy of sequential chemotherapy

This was doneon the basis of wound examination and recurrence of tumour following 2 weeks of chemotherapyin post-operative management of canine mammary tumour. Wound was assessed on

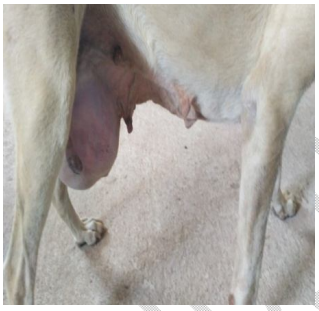
0, 3rd and 10th day following surgery to determine the nature of wound (normal/oedematous) and alsotype of discharge (bloody/serosanguinous/fetid/pussy). Side effects of the chemotherapy if any were also recorded.

III) Statistical analysis

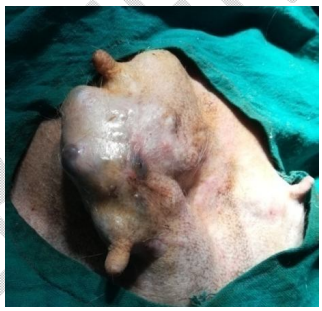
A computerized statistical software program SPSSv17.0 was used to analyze all of the data in order to compare the mean value within each group at various time intervals as well as among groups at different time intervals, which were represented as mean±standard error.The level of significance was set at $P<0.05$.

RESULTS AND DISCUSSION

The goal of the present investigation was to evaluate the systemic impact and clinical efficacy of sequential vincristine sulfate versus doxorubicin in the post-operative management of Canine Mammary Tumours. All 18 cases of mammary tumour affected dogs presented to Department of Veterinary surgery and Radiology were unspayed and uncastrated. On clinical examination, the shape of mammary tumours varied from ovoid, elongated, rounded to irregularly nodular (**fig. 1**). Most of the tumours were solitary and pedunculated. Grossly, tumours were soft to firm in consistency.



a. Intact mammary tumour involving 5th inguinal gland

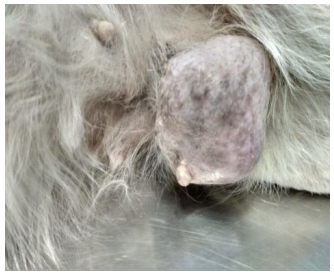


b. Mammary tumour involving both 4th and 5th mammary gland

c. Ulcerative mammary tumour with maggots in female dog

d. Intact mammary tumour

e. Ulcerative mammary



f. **Intact mammary tumour**

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in female dog **tumour with maggots in male dog**

Fig. 1. Showing location of canine mammary tumour involving mammary glands

I)Systemic Effect of sequential vincristine sulfate vs doxorubicin in post-operative management of Canine Mammary Tumours.

(A) Haematological parameters:Harmatological paramaters did not show much variation in canine mammary tumour dogs before, post-surgery and after chemotherapeutic regimens in different groups at various time intervals (Table 1). There was non-significant (P>0.05) decrease in haemoglobin values in group Surgery at 10^{th} day after surgical excision which might be due to surgical stress as reported by Benjamin (2010) and due to loss of blood from highly vascular malignant tumour during surgery. Thereafter, values showed increasing trend upto 60^{th} day. This could be due to haemato-concentration of red cells because of tissue alteration in acute inflammation and increased viscosity of blood (Vegard, 2019). Whereas in group SurgDox and SurgVin, haemoglobin showed non-significant decrease upto 30^{th} day which was less than 0 day value and indicated anaemia. Thereafter, haemoglobin values increased upto 60^{th} day of observation period. The decrease in haemoglobin value after doxorubicin and vincristine administration might have resulted due to chemotherapeutic damage to red blood cell formation in the bone marrow. Similarly, Brar *et al*. (2002) recorded anaemia due to bone marrow depression resulting in progressive decrease in haemoglobin concentration. Non-significant decrease in packed cell volume and TEC was recorded in all the three groups which could be due to cytotoxic drug that suppress the replicating precursor cells of the bone marrow leading to reduced production of erythrocyte. Nak *et al*. (2005) reported that decreasedHb, PCV and TEC may be attributed to the tumoral bleeding and myelosuppression induced by vincristine drug. There was non-significant decrease in TEC in group SurgDox and SurgVin which could be due to chemotherapy induced erythrocytopenia resulting from myelosuppression. Similar observation was also recorded by Brar *et al.* (2002) and Todorova *et al.* (2005). There was significant (P<0.05) decrease in TLC in group SurgDox and SurgVin upto 30^{th} day, as these cytotoxic drugs suppress the replicating precursor cells of bone marrow, thus, resulting in reduced production of leucocytes. Todorova *et al.* (2005) reported a dose dependent reversible leukopenia as the predominant manifestation of doxorubicin bone marrow haematologic toxicity.Total platelet count showed non-significant decrease in group Surgery and SurgDox upto 30^{th} day but to its contrary in group SurgVin, platelet count increased (thromcytosis) upto 30^{th} day and declined which was statistically non-significant. In group SurgDox (surgical removal alongwith doxorubicin therapy) where two doses of therapy were given in dogs suffering with mammary tumour revealed decreased in TPC (thrombocytopenia) which was

statistically non-significant. Thromobocytopenia was observed due to bone marrow suppression. Doxorubicin hydrochloride localizes in the bone marrow of long bones and with progression of chemotherapy, the ability of bone marrow to produce platelets is diminished. Immune mediated thrombocytopenia occurs when antibody attaches to platelets promoting the destruction by the mononuclear phagocytic system. However, in group SurgVin where surgical excision alongwith two doses of vincristine sulfate therapy was given in dogs suffering with mammary tumour revealed increased in TPC (thrombocytosis) which was statistically non-significant.Therefore, it could be stated that chemotherapeutic agent vinca alkaloid vincristine sulfate is a safe drug since thrombocytosis was evident with minimum haematological alterations.

There was significant ($P<0.05$) decrease in neutrophils count in group SurgDox and SurgVin, after administration of chemotherapeutic agent doxorubicin and vincristine sulfate which usually localized into the bone marrow of long bone and its myelosuppressive action reduced the neutrophil percentage therefore, it could be stated that the intravenous chemotherapy induces neutropenia. Whereas, in group Surgery, neutrophil decrease was negligible. Similarly, Ravikumar *et al.* (1999) observed a progressive decrease in neutrophils count till the end of vincristine therapy which resulted due to decreased bone marrow production. Whereas, lymphocyte count in group SurgDox and SurgVin showed significant ($P<0.05$) increase at 30^{th} day intervals which decreased significantly ($P<0.05$) on 60^{th} day whereas in group Surgery there was marginal variation in lymphocyte count during period of observation. The significantly ($P<0.05$) reversible leukopenia and neutropenia observed following chemotherapy (doxorubicin and vincristine) in the present study was probably due to the action of cytotoxic drugs which suppressed the replicating precursor cells of bone marrow and created myeloid toxicity. There was non-dignificant variation in the values of eosinophils and monocytes before and after surgicochemotherapeutic regimens and all the haematological values remained within normal physiological range. Srivastava *et al.* (2009) reported that during the course of vincristine therapy, reticuloendothelial system depresses which causes the changes in haematological parameters in dogs affected with mammary tumours as observed in the present study. Similarly, Khan *et al.* (2017) also observed a significant ($P<0.05$) decrease in haematological parameters viz., haemoglobin, PCV, TEC, TLC, Platelet count, neutrophilis and eosinophils after surgical excision along with doxorubicin treatment in canine affected with mammary tumourwhich could be due to total suppression of the bone marrow activity, accidental infection and allergic reaction.

Table 1: Haematological parameters (Mean±S.E) in different groups at various time interval

Parameters	Groups	0 day	10^{th} day	30^{th} day	60^{th} day
Haemoglobin	**Surgery**	12.40^{aA}±0.93	12.15^{aA}±0.44	12.20^{aB}±0.30	12.50^{aB}±0.50

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(gm%)	**SurgDox**	$12.63^{bA}\pm0.40$	$11.24^{aA}\pm0.43$	$10.85^{aA}\pm0.54$	$11.20^{aA}\pm0.36$
	SurgVin	$12.10^{bA}\pm0.408$	$11.73^{abA}\pm0.71$	$10.99^{aA}\pm0.36$	$11.50^{abA}\pm0.54$
Packed Cell Volume (%)	**Surgery**	$37.68^{aAB}\pm1.24$	$36.58^{aB}\pm1.50$	$36.07^{aB}\pm1.74$	$36.43^{aB}\pm1.51$
	SurgDox	$34.16^{bA}\pm1.24$	$30.72^{aA}\pm1.50$	$29.60^{aA}\pm1.51$	$31.13^{aA}\pm1.70$
	SurgVin	$39.56^{aB}\pm1.24$	$37.60^{aB}\pm1.50$	$35.71^{aB}\pm1.72$	$36.60^{aB}\pm1.52$
Total Erythrocyte Count (TEC) (x 10^6 cu mm)	**Surgery**	$6.94^{aA}\pm0.20$	$6.89^{aA}\pm0.25$	$6.80^{aA}\pm0.20$	$6.95^{aA}\pm0.28$
	SurgDox	$7.99^{bB}\pm0.20$	$6.80^{aA}\pm0.50$	$6.20^{aA}\pm0.25$	$6.72^{aA}\pm0.70$
	SurgVin	$7.50^{bAB}\pm0.50$	$6.65^{abA}\pm0.60$	$6.06^{aA}\pm0.40$	$6.10^{abA}\pm0.20$
Total Leukocyte Count (TLC) (x 10^3 cu mm)	**Surgery**	$11.50^{aB}\pm0.40$	$11.20^{aB}\pm0.70$	$11.68^{aC}\pm0.60$	$11.78^{aB}\pm0.50$
	SurgDox	$10.70^{cA}\pm0.46$	$9.20^{bA}\pm0.50$	$8.73^{aA}\pm0.30$	$9.10^{abA}\pm0.60$
	SurgVin	$12.05^{cC}\pm0.30$	$11.60^{bB}\pm0.45$	$11.08^{aB}\pm0.60$	$11.28^{bB}\pm0.16$
Total Platelet Count (TPC)	**Surgery**	$3.55^{aB}\pm0.20$	$3.40^{aA}\pm1.10$	$3.10^{aA}\pm1.17$	$3.46^{aA}\pm1.19$
	SurgDox	$3.80^{cB}\pm0.20$	$2.99^{bA}\pm0.30$	$2.30^{aA}\pm0.70$	$2.57^{aA}\pm0.14$
	SurgVin	$3.10^{aA}\pm0.70$	$3.89^{bA}\pm0.11$	$4.10^{bB}\pm0.16$	$3.99^{bB}\pm0.19$
Neutrophils (%)	**Surgery**	$70.85^{bA}\pm0.96$	$69.95^{abA}\pm1.15$	$69.50^{aC}\pm1.10$	$70.20^{abC}\pm1.58$
	SurgDox	$73.55^{cAB}\pm0.67$	$67.16^{bA}\pm1.10$	$60.43^{aA}\pm0.28$	$62.70^{aA}\pm0.50$
	SurgVin	$74.88^{dB}\pm0.96$	$68.67^{cA}\pm1.15$	$61.90^{aB}\pm1.10$	$65.30^{bB}\pm0.58$
Lymphocyte (%)	**Surgery**	$20.57^{aA}\pm1.30$	$22.52^{cA}\pm1.10$	$21.20^{bA}\pm1.09$	$20.45^{aA}\pm0.30$
	SurgDox	$21.15^{aA}\pm0.08$	$31.30^{cB}\pm0.75$	$35.85^{dC}\pm.33$	$27.45^{bB}\pm0.35$
	SurgVin	$22.65^{aB}\pm0.07$	$32.42^{cC}\pm0.90$	$34.63^{dB}\pm0.30$	$29.62^{bC}\pm0.60$
Eosinophil (%)	**Surgery**	$2.98^{bC}\pm0.10$	$2.45^{abA}\pm0.90$	$2.20^{aA}\pm0.11$	$2.40^{abA}\pm0.30$
	SurgDox	$2.65^{aAB}\pm0.60$	$2.22^{aA}\pm0.90$	$2.02^{aA}\pm0.54$	$2.20^{aA}\pm0.40$
	SurgVin	$2.28^{aA}\pm0.15$	$2.05^{aA}\pm0.07$	$1.90^{aA}\pm0.21$	$2.00^{aA}\pm0.03$
Monocyte (%)	**Surgery**	$3.48^{aA}\pm0.10$	$3.85^{aA}\pm0.90$	$4.05^{aA}\pm0.05$	$3.99^{aA}\pm0.20$
	SurgDox	$3.80^{aA}\pm0.20$	$4.10^{aA}\pm0.50$	$4.50^{aA}\pm0.35$	$4.20^{aA}\pm0.11$
	SurgVin	$3.60^{aA}\pm0.55$	$3.85^{abA}\pm0.90$	$4.25^{bA}\pm0.70$	$4.00^{abA}\pm0.30$

abcd-value (Mean±S.E.) bearing different superscript differ significantly (P<0.05) within groups
ABC-value (Mean±S.E.) bearing different superscript differ significantly (P<0.05) between groups

(B) Biochemical parameters:Serum glucose levels in group SurgDox showed significant (P<0.05) increasing trend upto 60th day after doxorubicin but within normal physiological range whereas non-significant decrease upto 60th day was recorded in group SurgVin after vincristine therapy (Table 2). The increase in glucose level in group SurgDox might be due to stress created on body by chemotherapeutic agent doxorubicin which caused the release of glucocorticoid and mineralocorticoid due to stimulation of adrenal cortex and epinephrine and non-epinepherine as a result of stimulation of the medulla. But serum glucose level in group Surgery showed irregular variation throughout the observation period and values remained within normal physiological limits. Total serum proteins value in the animals of group SurgDox, showed significant (P<0.05) decrease at 10th day which furtherincreased significantly (P<0.05) upto 60th day. However, the TSP values in group Surgery and SurgVin showed marginal changes during observation period. The marginal irregular pattern of serum total proteins after chemotherapy could be consequential to gastro-intestinal disorder of cytotoxic drugs (Sandhu and Rampal, 2006). In contrast to our study, Gupta *et al.* (2014) reported a significant

increase (P<0.05) in total protein value of surgically treated animals from preoperative 5.87±0.35g/dl to 6.485±0.33g/dl following surgery. Whereas, in surgical excision followed by vincristine treated group, there was non significant change in total protein values during the course of therapy but it remained towards the higher side of normal range.The animals of group SurgDox showed significant (P<0.05) increase in serum urea nitrogen and serum creatinine levels upto 30th day which furtherdecreasedsignificantly (P<0.05) at 60th day. Whereas group Surgery and SurgVin showed irregular changes throughout the observation period. Increase in BUN may be linked to glomerular filtration rate or increased protein catabolism caused by necrosis of tumour or metabolic side effects of neoplasia and increase in serum creatinine was attributed to the increase in catabolic activity. However, Todorova *et al*. (2005) did not find any significant change in the blood urea nitrogen and serum creatinine level after 2nd dose of doxorubicin. Similarly, Srivastava *et al*. (2009) has reported that the plasma concentration of total protein, urea nitrogen, creatinine and glucose remained within normal range during the entire period of vincristine therapy with surgical excision. Khan *et al*. (2017) also reported significant (P<0.05) increase in BUN, serum creatinine in dog treated with surgery followed with doxorubicin therapy but the values were within physiological limits whereas a non-significant change were recorded in surgically treated mammary tumour dog. The animals of group SurgDox and SurgVin showed significantly (P<0.05) increasing trend in AST levels upto 30th day and thereafter the values decreased on 60th day after doxorubicin and vincristine therapy respectively. However, group Surgery showed non-significant variation after surgical excision. Whereas ALT level showed significant (P<0.05) increasing trend in group Surgery and SurgDoxupto 60th day following surgical removal of tumour and doxorubicin treated group respectively but animals of groupSurgVinshowed non-significant decrease in ALT level at 10th day which later on increased at 30th day and again decreased at 60th day after vincristine therapy. In the present study, the increase in AST and ALT levels following chemotherapy in SurgDox and SurgVin groups may probably be due to the result of detoxification of doxorubicin hydrochloride and vincristine in the liver which gets loaded exclusively for secretion of transaminase (Todorova *et al*., 2005). In contrast to present study, Palta (2000) reported negligible changes in AST and ALT values after neoadjuvant and adjuvant chemotherapy with vincristine in canine mammary neoplasm cases. But Gupta *et al*. (2014) observed a non-significant increase in ALT values after second cycle of therapy in surgical excision followed by vincristine treated group which might be due to increase in metabolic activity of liver for the detoxification of drugs. Similar significant (P<0.05) increase in AST and ALT was also observed in dog treated with surgery and followed sequential doxorubicin therapy whereas as a non-significant

change in surgically treated mammary tumour dog but all values were within physiological limits as reported by Khan *et al*. (2017).The values of alkaline phosphatase (ALP) were towards the higher limit of normal range in all the three groups at time of presentation. Similarly, Bala (2005) reported elevated levels of alkaline phosphatase at presentation of canine mammary tumour. Alkaline phosphatase (ALP) levels in group Surgery, SurgDox and SurgVin showed significant ($P<0.05$) decrease upto 30th day from 85.40±15.35 to 53.87±9.70 U/L, 93.80±18.60 to 50.67±4.68 U/L and 80.55±12.80 to 60.68±18.45 U/L respectively with a further increase upto the end of observation period. This indicates that increase in the alkaline phosphatase level could be attributed to presence of malignancy and addition of chemotherapy (vincristine and doxorubicin) induced stress, which was intensely reflected by rise in alkaline phosphatase activity during the study. Chauhan and Agarwal (2008) recorded that alkaline phosphatase activity increases in case of carcinoma. Gupta *et al*. (2014) reported progressive increase in serum ALP value after first cycle and second cycle of vincristine therapy.

Table 2 : Biochemical parameters (Mean±S.E) in different groups at various time interval

Parameters	Groups	0 day	10th day	30th day	60th day
Glucose (mg/dl)	**Surgery**	69.31^{aB} ±1.04	73.53^{bB}±1.09	72.46^{bB}±0.10	73.38^{bB}±0.48
	SurgDox	73.54^{aC}±0.75	75.69^{bC}±0.63	78.95^{cC}±1.10	83.55^{dC}±1.35
	SurgVin	67.43^{aA}±1.09	66.59^{aA}±1.03	66.14^{aA}±1.45	65.98^{aA}±1.58
Total Serum Proteins (gm/dl)	**Surgery**	6.84^{aA}±2.10	6.43^{aB}±1.05	6.31^{aB}±0.75	6.14^{aA}±1.04
	SurgDox	7.14^{cC} ±0.89	6.74^{aC}±1.10	7.03^{bB}±1.32	7.50^{dC}±0.67
	SurgVin	6.32^{aB}±0.59	6.25^{aA}±0.40	6.30^{aA}±0.10	6.68^{bB}±0.67
Serum Urea Nitrogen (mg/dl)	**Surgery**	11.20^{aA}±2.15	10.47^{aA}±1.39	10.89^{aA}±2.80	11.05^{aA}±1.49
	SurgDox	12.39^{aB} ±0.45	14.18^{bC}±0.30	17.71^{cB}±0.85	15.64^{cB}±0.60
	SurgVin	11.02^{aA}±1.15	11.59^{abB}±1.39	11.10^{aA}±1.20	11.80^{bA}±1.10
Serum Creatinine (mg/dl)	**Surgery**	1.12^{aC}±0.022	1.23^{bB}±0.038	1.25^{bB}±0.026	1.30^{bB}±0.30
	SurgDox	1.02^{aB}±0.22	1.55^{bC}±0.38	1.89^{cC}±0.26	1.28^{abB}±0.60
	SurgVin	0.83^{aA}±0.56	0.88^{aA}±0.84	0.92^{aA}±0.74	0.90^{aA}±0.45
Aspartate aminotransferase	**Surgery**	33.63^{aB}±2.072	34.73^{abA}±1.90	35.54^{bcA}±1.67	36.65^{cA}±1.56
	SurgDox	30.64^{aA}±1.45	36.76^{bB}±1.36	42.42^{cB}±2.70	37.44^{bAB}±1.40
	SurgVin	34.53^{aB}±0.50	39.74^{cC}±0.66	42.84^{dB}±0.89	38.16^{bC}±0.46
Alanine Aminotransferase	**Surgery**	33.34^{aB}±5.40	38.90^{bB}±4.90	43.03^{bcB}±6.74	45.48^{dB}±3.64
	SurgDox	32.43^{aB}±6.56	40.60^{bB}±5.63	44.70^{cB}±3.86	46.90^{dB}±4.60
	SurgVin	30.40^{bA}±4.20	29.12^{aA}±2.92	29.84^{abA}±3.54	29.58^{abA}±2.05
Alkaline phosphatase (U/L)	**Surgery**	85.40^{dB}±15.35	60.70^{bA}±8.06	63.87^{aB}±9.70	75.40^{cC}±13.39
	SurgDox	93.80^{dC}±18.60	65.55^{bB}±11.60	50.67^{aA}±4.68	69.65^{cB}±5.40
	SurgVin	80.55^{dA}±12.80	60.68^{bA}±8.45	58.76^{aA}±15.44	60.77^{cA}±8.89

abcd-value (Mean±S.E.) bearing different superscript differ significantly ($P<0.05$) within groups

ABC-value (Mean±S.E.) bearing different superscript differ significantly ($P<0.05$) between groups

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2. Clinical efficacy of sequential vincristine sulfate vs doxorubicin in post-operative management of Canine Mammary Tumours.

The nature of wound healing process was normal in all the three groups without any complications or discharge. In all the three groups, complete wound healing was observed in 8 to 10days and wasfollowed for at least three months after surgery and post chemotherapeutic regimens (doxorubicin and vincristine sulfate). The symptoms associated after administration of chemotherapeutic drug were inappetence, vomition, anaemia and alopecia. These above symptoms were comparatively more in animals of group SurgDox as compared to group SurgVin. However, the condition of the animal was managed by supportive therapy with administration of intravenous fluids, antaacids and liver tonics as a palliative measure. There was no recurrence in group SurgVinduring the study period while, minimum recurrence (+) was observed in one case in group SurgDoxwhereas group Surgery showed mild to moderate recurrence (+, ++) in two cases. These findings are in agreement with Karma (2006) and Srivastava *et al*. (2009) who observed that surgery combined with Vincristine therapy has been proven as excellent therapeutic regimen for canine mammary tumour leading to complete regression of the neoplasm without relapse.However, surgical excision following doxorubicin therapy was also effective in management of canine mammary tumour but showed minimum recurrence as observed in one case. The overall response of post- operative management canine mammary tumour using surgical excision alongwith sequential administration of chemotherapeutic drug (doxorubicin and vincristine) indicates that vincristine sulfate had minimum side effect on systemic impact on haematological and biochemical parameters, which proves its superiority over doxorubicin.

Table 3: Showing Clinical efficacy of sequential vincristine sulfate vs doxorubicin in post-operative management of Canine Mammary Tumours.

Animal No.	Group Surgery		Group SurgDox		Group SurgVin	
1	Nil	+ +	V, Al	+	I	0
2	Nil	0	A, I	0	I	0
3	Nil	0	I, A	0	A, V	0
4	Nil	+	I	0	A, V, Al	0
5	Nil	0	I, V, Al	0	V, Al	0
6	Nil	0	A, V	0	I	0

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0 indicates no recurrence, + indicates minimum recurrence, + + moderate recurrence, + + + severe recurrence V= Vomition, ; A= Anaemia ; I= Inappetence ; Al= Alopecia

CONCLUSION

Systemic impacts on haematological and biochemical parameters were temporarily altered, but these alterations remained within the normal physiological range as chemotherapeutic drugs do not

cause any adverse reaction or deleterious effect on vital organs. Sequential vincristine sulfate and Doxorubicin therapy showed minimum to no reoccurrence of tumour with few adverse reactions such as inappetance, vomition, anaemia and alopecia. However, these conditions were managed by supportive therapy. Therefore, it can be concluded that sequential vincristine sulfate therapy ismore effective for post-operative treatment of canine malignant tumour as compared to sequential doxorubicin therapy as it effectively suppressed the development of new tumour cells and metastasis.

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