STUDIES ON EFFICACY OF NANOPARTICULATE INDUCED DRUG DELIVERY SYSTEM FOR CHEMOTHERAPY OF SUPERFICIAL TUMOURS IN DOG

THESIS

Submitted
in partial fulfillment of the requirements for the Degree of

MASTER OF VETERINARY SCIENCE
IN
VETERINARY SURGERY & RADIOLOGY

BY
INGLE RUPESH SAKHARAM
ENROL. NO. V1/07/045

NAGPUR VETERINARY COLLEGE, NAGPUR

MAHARASHTRA ANIMAL AND FISHERY SCIENCES UNIVERSITY, NAGPUR - 440 001.

(INDIA)

2014
DECLARATION OF STUDENT

I hereby declare that the experimental research work and interpretation of the thesis entitled "STUDIES ON EFFICACY OF NANO PARTICULATE INDUCED DRUG DELIVERY SYSTEM FOR CHEMOTHERAPY OF SUPERFICIAL TUMOURS IN DOG" or part thereof has not been submitted for any other degree or diploma of any university, nor the data have been derived from any thesis/publication of any university or scientific organization. The sources of materials used and all assistance received during the course of investigation have been duly acknowledged.

Date : 27/08/2014
Place : Nagpur

Signature

(Ingle Rupesh Sakharam)
Enrol. No. V/07/045

Counter signed by
Chairman,
Advisory Committee with date
DECLARATION OF ADVISORY COMMITTEE

Ingle Rupesh Sakharam has satisfactorily prosecuted his course of research for a period of not less than one semester and that the thesis entitled, "STUDIES ON EFFICACY OF NANOPARTICULATE INDUCED DRUG DELIVERY SYSTEM FOR CHEMOTHERAPY OF SUPERFICIAL TUMOURS IN DOG" submitted by him is the result of research work and is sufficient to warrant its presentation to the examination in the subject of Veterinary Surgery and Radiology for the award of Master of Veterinary Science degree by the Maharashtra Animal and Fishery Sciences University, Nagpur.

We also certify that the thesis or part thereof has not been previously submitted by him for a degree of any other university.

Place: Nagpur

Date: 27-8-2014

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Advisor/Guide
Associate Professor,
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<tr>
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CERTIFICATE

This is to certify that the thesis entitled, "STUDIES ON EFFICACY OF NANOPARTICULATE INDUCED DRUG DELIVERY SYSTEM FOR CHEMOTHERAPY OF SUPERFICIAL TUMOURS IN DOG" submitted by INGLE RUPESH SAKHARAM to the Maharashtra Animal and Fishery Sciences University in partial fulfillment of the requirement for the degree of Master of Veterinary Science has been approved by the Student's Advisory Committee after examination in collaboration with the External Examiner.

Name & Signature of External Examiner

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Associate Dean
Nagpur Veterinary College, Nagpur
ACKNOWLEDGEMENT
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Success is not possible lonely without involvement of many minds and hands to beautify it. Emotions cannot be adequately expressed in words because than emotions are transformed into formality. Thanks giving are not a mere formality but it is the fitness to recollect all the people and their kind officious at this juncture. My acknowledgement are many times more than that I am expressing here.

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Place: Nagpur

Date: 27-8-2014

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<td>&amp;</td>
<td>And</td>
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<tr>
<td>@</td>
<td>At the rate of</td>
</tr>
<tr>
<td>b. wt.</td>
<td>Body weight</td>
</tr>
<tr>
<td>cm</td>
<td>Centimeter</td>
</tr>
<tr>
<td>cumm</td>
<td>Cubic millimeter</td>
</tr>
<tr>
<td>DLC</td>
<td>Differential Leucocyte Count</td>
</tr>
<tr>
<td><em>et al.</em></td>
<td>et ali /alia, And others</td>
</tr>
<tr>
<td>EDTA</td>
<td>Ethylene Diamine Tetra Acetic Acid</td>
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<td>Fig.</td>
<td>Figure</td>
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<td>Gr</td>
<td>Group</td>
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<tr>
<td>Hb</td>
<td>Haemoglobin</td>
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<tr>
<td>HCL</td>
<td>Hydrochloride</td>
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<tr>
<td>i.e.</td>
<td>id est/that is</td>
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<tr>
<td>inj.</td>
<td>Injection</td>
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<tr>
<td>i.m.</td>
<td>Intra muscular</td>
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<tr>
<td>i.v.</td>
<td>Intra venous</td>
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<tr>
<td>Kg</td>
<td>Kilogram</td>
</tr>
<tr>
<td>Ltd. Co.</td>
<td>Limited Company</td>
</tr>
<tr>
<td>Mg</td>
<td>Milligram</td>
</tr>
<tr>
<td>mg/dl</td>
<td>Milligram per deciliter</td>
</tr>
<tr>
<td>ml</td>
<td>Milliliter</td>
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<td>viz.</td>
<td>for example</td>
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<tr>
<td>No.</td>
<td>Number</td>
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<td>PCV</td>
<td>Packed Cell Volume</td>
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<td>%</td>
<td>Per cent</td>
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<tr>
<td>±</td>
<td>Plus Minus</td>
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<td>®</td>
<td>Registered</td>
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<tr>
<td>S.D.</td>
<td>Standard Deviation</td>
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<td>S.E.</td>
<td>Standard Error</td>
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<td>TVCC</td>
<td>Teaching Veterinary Clinical Complex</td>
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</tr>
<tr>
<td>TLC</td>
<td>Total Leucocyte Count</td>
</tr>
<tr>
<td>RBC</td>
<td>Red Blood Cell</td>
</tr>
<tr>
<td>WBC</td>
<td>White Blood Cells</td>
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<tr>
<td>etc.</td>
<td>Excea</td>
</tr>
<tr>
<td>μ</td>
<td>Micron</td>
</tr>
<tr>
<td>μg</td>
<td>Microgram</td>
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Chapter 1

INTRODUCTION
INTRODUCTION

A tumour is an abnormal growth of tissue resulting from uncontrolled, progressive multiplication of cells and serving no physiological function also known as neoplasm. A tumour does not always mean cancer, tumours can be benign (not cancerous), pre-malignant (pre-cancerous), or malignant (cancerous). There are many different types of tumours with various names. Their names usually reflect their shape and the kind of tissue they appear in.

Superficial tumours are classified as lipomas, benign tumours, atypical mammary tumours and malignant tumours. They are most frequently found in the superficial layers of the skin or on any part of the body. Frequently, there will be ulceration over the area of the tumour and the dog may scratch or bite at the affected area. The appearance of the tumour does not reveal its potential for spread or recurrence with any certainty. The tumours are usually singular, but dogs may present with multiple nodules, or recurrent ones. Dogs often suffer from superficial tumours and as they do not hamper the body function they are generally unnoticed by the owner as well as the veterinarians. But when they invade into different systems and affect body functions, then they create a problem for owners and veterinarians resulting in a poor prognosis in operative procedures.

Classical modalities for cancer therapy include surgery, radiation and chemotherapy. Cryosurgery, immunotherapy, hyperthermia and use of biological response modifiers are few others amongst the new modalities for cancer therapy (Riley and Riley, 1982). The current focus in the development of cancer therapies is on targeted drug delivery to provide therapeutic concentrations of anticancer agents at the site of action and spares the normal tissues (Javid et al., 2011).

Chemotherapy is the treatment for neoplasm with the help of various drugs. Now a day’s various chemotherapeutic drugs are available for treatment of various types of tumours but they create serious problems particularly in geriatric patients due to their hazardous side effects.

Doxorubicin is an anthracycline glycoside antibiotic originally produced by Streptomyces peucetius var. caesius (Morrison, 1998). It exerts its cytotoxic effect as a DNA-intercalating agent to inhibit further DNA and RNA biosynthesis.
Doxorubicin is widely used either as single agent or in combination with other chemotherapeutic regimens for various types of solid tumours. However, dose limiting toxic side effects such as cardiotoxicity, myelosuppression, myositis and alopecia limit its clinical applications, owing to non-specific distribution to healthy normal tissue. Over the past few decades the studies have focused on development of drug delivery systems and administration routes to increase tissue selectivity and improves its toxicity profile.

Intratumoural administration of chemotherapeutic agent is potentially more effective modality to overcome the described limitation and this has been extensively evaluated using a number of anticancer drugs. Such targeted delivery may realize drug localization within the tumour tissue, while decreasing the incidence and the intensity of side effects (Yanhui et al., 2012).

Although intratumoural administration is a promising approach for the treatment of various solid tumour with minimal systemic toxicity, its efficacy is highly dependent on the timing and frequency of the drug injections because of its rapid clearance from the tumour site. It can be a useful approach to treat the geriatrics and compromised patients with poor anaesthetic and surgical risk. The drug delivery system is required to ensure that the drug is properly localized and released in a controlled way. Several polymeric drug delivery systems have been developed for intratumoural drug delivery, including hydrogels, microparticle, nanoparticles and nanofibers.

Magnetic nanoparticles have been investigated in drug delivery systems because of their high magnetic responsiveness, biodegradability, biocompatibility, high delivery efficacy and potential targeting function. Magnetic nanoparticles while further functionalized with drugs and bioactive agents such as peptides, from distinct particulate systems that penetrate cells and tissue barriers and offer organ specific therapeutic and diagnostic modalities (Javid et al., 2012). Nanoparticles have a variable magnetic field and are able to produce heat (42-45°C) which has selective effect on fast dividing cancer cells than normal tissues (Aliabadi et al., 2012).

Prevention, diagnosis and treatment of cancers have always been a formidable medical challenge. Since the tumour cells are not dissimilar enough from the healthy cells to distinguish one from the other, the drug used against
tumours may cause wide damage to systems, can be toxic to healthy cells and affect overall health of the patient by its side effects.

The integration of nanotechnology in medicine leads to the development of nanomedicine aiming for disease diagnosis and treatment with unprecedented precision and efficacy (Tong and Cheng, 2007). The application of nanotechnology in cancer therapy, also known as cancer nanotechnology is being used clinically to treat the cancer by delivering the drug to target cancer cells.

The surgical procedures in animals suffering from cancer involve risk because of the age factor as they mostly develop cancers at the age of seven and above commonly referred as "cancer age" (Moulton et al., 1990) in a total life span of 12 to 15 years. So, it is logical to think of alternative approaches like the use of local chemotherapy with nanoparticles may reduce the side effects of systemic chemotherapy. In chemotherapy using nanoparticles the doses tried were one-tenth times smaller than those used in chemotherapy. The nanoparticles can be introduced directly to the tumour cells, rather than passing into disease-free tissues and organs.

Magnetic nanoparticles are submicron moieties (diameter ranging from 1 to 100 nm) functionalized by binding them to various substances, including chemotherapeutic agents, radionuclides, nucleic acids, and antibodies (Wu et al., 2008). The magnetic responsiveness of iron oxide (Fe₃O₄) core enables magnetic targeting with a locally applied external magnetic field leading to retention at tumour site (Jayakumar et al., 2009). Once targeted and taken up by the tumour cells, the release of the drug carried by the particle over an extended period result in a desired clinical response. Recently it has been reported that Fe₃O₄ nanoparticles could remarkably enhances the uptake or diffusion efficiency of anticancer drug doxorubicin in leukemic K562 cells (Wang et al., 2006).

The aim of cancer therapy is to prolong life at all costs and to achieve a good quality of life. Intratumoural injection of chemotherapeutic agent, doxorubicin with Fe₃O₄ core enables delivery of drugs to the target tissues with the help of a locally applied external magnetic field which enhances the uptake and diffusion efficiency of doxorubicin by the target cells. The chemotherapeutic effect of doxorubicin could be considerably enhanced at a reduced dose rate.
whereby limiting the drug delivery to the target cells will be a novel approach in cancer therapy. Considering the patient factors such as age and health status of dogs suffering from tumours of skin and soft tissues, the present study could be useful as a curative/palliative measure in prolonging the lifespan and improving the quality of life of the patient.

Objectives:

1) To ascertain the efficacy of nanoparticle drug delivery system for intratumoural administration of Doxorubicin for the treatment of tumour in dogs.

2) To study the efficacy of intravenous administration of Doxorubicin and radical surgery for the superficial tumours in dogs.
Chapter 2

REVIEW OF LITERATURE
2.1. Epidemiology of Superficial Tumours

2.1.1 World health organization (WHO) classification of tumours of domestic animals

The WHO published the original histological classification of tumours of domestic animals in 1974 with the purpose of establishing a sound basis for comparative oncology by providing a widely accepted standard nomenclature of tumours of domestic animals and in order to advance veterinary pathology (Weiss and Frese, 1974).

In 1980, the first Tumour-lymph node-metastasis (TNM) classification of tumours in domestic animals was published by the WHO, in support of the WHO histological classification of tumours of domestic animals. This classification provides the stages of animal tumours to aid veterinary clinicians in planning the treatment, indications about prognosis, to assist in evaluating of treatment results, to facilitate the exchange of information between treatment centers, and to contribute information on comparative values between man and animal (Owen, 1980).

Skin tumours were divided into mesenchymal, epithelial and melanocytic tumours as in the WHO classification, and some authors in their statistics used the term "tumour-like lesion" as a separate term (Goldschmidt and Shofer, 1992).

Yamagami et al. (1996) reported that a combination practice of TNM classification system and histopathological evaluation was useful in determining the prognosis of canine mammary tumours.

2.1.2. Incidence

Brodey et al. (1983) reviewed on survey of animal neoplasm in Alameda and Contra Costa countries, California and stated that mammary gland tumours accounted for 42% of all tumours in bitches. The incidence rates were 105 cases of mammary tumours per 1,00,000 dogs from July 1963 to June 1966, as contrasted to 12.8 cases per 1,00,000 cats which underlined the fact that the dog had a higher incidence than man or any domestic animal.
Dobson et al. (2002) studied that the skin and soft tissues were the most common sites for tumour development in dog with incidence rate of 1437 per 100000 dogs/yr followed by alimentary (20010), mammary (205) and urogenital system (139).

Dhami et al. (2010), in a retrospective study on the incidence of neoplasms in a total of 2070 canine cases, reported 158 cases of neoplasms. The incidence of canine mammary gland tumours was 0.75 per cent of the total canine cases in Gujarat with highest occurrence of tumour in dogs aged between 8 to 12 years. It was concluded that among the tumours in canines, mammary tumours were more common and were benign or malignant and could occur anywhere along the mammary chain mainly in females.

Praveena (2010) reported an incidence of 61 tumours in dogs from the period of December 2008 to December 2009 at Thrissur, out of which 40 (66 per cent) were benign and 21 (31 per cent) were malignant. The location or system wise classification of the tumours showed that the skin and soft tissue tumours were in maximum number i.e., 20 cases (32.8 per cent), mammary gland ranked second i.e., 18 cases (29.51 per cent) and rest of 23 tumours were from genital system, alimentary system, hemolymphatic system, eye and ear origin, and bone.

Kumar et al. (2011) stated that the mammary tumour constituted 0.39 per cent of the total clinical cases of tumours reported in Chennai.

Simeonov et al. (2011) reported that out of 430 cutaneous neoplasms, 223 (51.86 per cent) were malignant, and 207 (48.14 per cent) were benign. Among the biopsy specimens, 250 cases were diagnosed as skin epithelial and melanocytic tumours and 78 cases as mesenchymal tumours of skin and soft tissues.

Sostaric-Zuckermann et al. (2013) observed that the skin and subcutis tumours were diagnosed most frequently in male dogs. Skin and subcutis neoplasms were 60.34 per cent, genital system neoplasms were 11.26 per cent and oral cavity neoplasms were 5.63 per cent. Among female dogs, tumours of the mammary gland (44.87 per cent), skin and subcutis (29.83 per cent), hemopoietic cells (4.39 per cent), and genital tract (4.39 per cent) were most
dominant. It was also reported that among the dogs with tumour 59.1 per cent were malignant, 35.4 per cent were benign and the remaining were not specified.

2.2. Nanoparticles and its Use in Cancer Therapy

According to Curtis and Wilkinson (2001) biocompatibility, size and magnetic properties was made Fe$_3$O$_4$ nanoparticles a primary choice for numerous biomedical applications such as drug delivery, cell separation, immunoassay, MRI, contrast enhancement, tissue repair and hyperthermia.

Once the nanoparticles were targeted and taken up by the tumour cells, the release of the drug carried by the particles over extended period resulted in a desired clinical response (Ferrari, 2005).

Gupta and Gupta (2005) suggested that magnetic nanoparticles could bind to drugs, proteins and enzymes, antibiotics or nucleotides and could be directed to an organ, tissue or tumour using an external magnetic field and also reported that sizes between 10 and 100 nm were the most effective for drug delivery because it could evade the reticuloendothelial system (RES).

Divakaran et al. (2011) reported that oral administration of d-aminoacid oxidase and d-alanine along with magnetic targeting with Fe$_3$O$_3$ nanoparticle significantly suppressed tumour growth in mice.

Kayal and Ramanujan (2010) stated that the magnetic drug targeting is a drug delivery system that can be used in locoregional cancer treatment. Coated magnetic particles, called carriers, are very useful for delivering chemotherapeutic drugs. Magnetic carriers were synthesized by co-precipitation of iron oxide followed by coating with polyvinyl alcohol. Doxorubicin drug loading and release profiles of polyvinyl alcohol coated iron oxide nanoparticles showed that up to 45% of adsorbed drug was released in 80 hours. They conclude that doxorubicin loaded polyvinyl alcohol coated iron oxide nanoparticles is promising for magnetically targeted drug delivery.

Mu et al. (2011) studied that the superhigh-magnetization nanocarriers comprised of a magnetic Fe$_3$O$_4$ core which have high drug loading capacity of doxorubicin. They observed that the supermagnetic property with magnetization conjugates the anticancer drug doxorubicin to the nanocarriers and enhances the
drugs thermal stability and maximizes the efficiency. They observed that this advanced drug delivery system promises to provide more effective magnetic therapy and tumour treatment using lower therapeutic doses and potentially reducing the side effects of cardio toxicity caused by doxorubicin.

2.3. Doxorubicin and its Effects

Madwell and Grant (1977) reported that anticancer drugs inhibit neoplastic cell growth either by blocking cell division (reproductive death) or by causing more general biochemical lesion (metabolic death). The main antineoplastic function of doxorubicin was to bind to DNA thereby inhibiting nucleic acid synthesis.

Bristow et al. (1980) evaluated the acute hemodynamic effects of doxorubicin in the open chest dog. Doxorubicin at doses of 1 to 4 mg/kg administered over two minute produced profound hemodynamic changes. These changes persisted despite administrating the drug as a slow infusion. Histamine release in peripheral tissues was documented by a marked increase in venous histamine levels following doxorubicin administration. A dose of doxorubicin (1 mg/kg) that released histamine and catecholamine produced primary cardiac effects acutely and a cardiomyopathy when administered chronically.

Ogilvie et al. (1989a) studied one hundred eighty five dogs with histologically confirmed measurable malignant tumours to determine the toxicity of anthracycline antitumour antibiotic, doxorubicin which was administered once or twice (at 21 day interval) at rate of 30 mg/m² of body surface area, intravenously. During this study, seven dogs died as a direct result of doxorubicin-induced toxicosis and 16 died as a direct result of the malignant neoplastic disease. Each dog was evaluated for signs of toxicosis and 16 died as direct result of the malignant neoplastic disease. Each dog was evaluated for signs of toxicosis for three weeks after the last dose was administered (15 dogs received one dose, 170 received two doses) or until the dog died, whichever came first. The most common signs of toxicosis were vomiting, diarrhoea, colitis, anorexia and pruritus.

Ogilvie et al. (1989b) studied one hundred eighty-five dogs with histologically confirmed, measurable malignant tumours in a prospective study to
determine the response to two doses of the anthracycline antitumour antibiotic, doses of doxorubicin were administered (30 mg/m²) 21 days apart. A partial or complete remission was obtained in 41 % (64/157) of all evaluable dogs: 26% (11/43) of the dogs with carcinoma, 67% (42/63) of the dogs with lymphoma, and 22% (11/51) of the dogs with sarcoma. Tumours in which there was at least a 50% volume reduction (partial or complete remission) included malignant lymphoma (42/63), fibrosarcoma (1/14), solid follicular thyroid carcinoma (3/13), mammary adenocarcinoma (2/8), hemangiosarcoma (2/8), osteosarcoma (1/6), circumlanal carcinoma (3/5), synovial cell sarcoma (2/3), undifferentiated sarcoma (2/3), nasal adenocarcinoma (1/2), liposarcoma (1/2), infiltrating lipoma (1/1), sclerosing mesothelioma (1/1), and neurofibrosarcoma (1/2).

Arrington et al. (1994) studied the pharmacokinetics and toxicity of single dose of doxorubicin, at dosages of 30 mg/m² of body surface area and 1 mg/kg of body weight, in 17 dogs. The clinical sign of doxorubicin toxicosis at 30 mg/m² dosage revealed that six of seven small dogs (<or=10 kg) became ill, where as seven of ten large dogs (>10 kg) remained clinically normal. Small dogs that received doxorubicin at a dosage of 30 mg/m² had higher peak plasma concentrations and more clinical signs of toxicosis than had large dogs (P<or=0.05). Five of 9 small dogs that received doxorubicin at a dosage of 30 mg/m² developed severe myelosuppression (<1x1093) granulocytes/microliters). In contrast to the toxicoses with body surface area-based dosing, myelosuppression was not induced in small dogs that received doxorubicin at dosage of 1 mg/kg. In small and large dogs given doxorubicin at dosage of 1 mg/kg, pharmacokinetic characteristics and clinical signs of toxicosis were similar.

Doxorubicin (Adriamycin), an anthracycline glycoside antibiotic originally produced by *Streptomyces peucetius var. caesiuss*, was widely used either as a single agent or in combination with other chemotherapeutic regimens for curative, adjuvant, and palliative treatment in cancer patients (Lal et al., 2010).

Pongprom (2011) reported that doxorubicin derivatives that could be given locally and concentrated to the draining lymphatic basin of the breast of humans was developed using lymphatic carriers such as hyaluronan (HA). It was reported that HA- Doxorubicin injection given subcutaneously at weekly interval was beneficial over standard dosing regimen.
2.3.1 Toxicity of doxorubicin

Bostock and Owen (1972) reported that chemotherapeutic agents yielded best results when used to treat rapidly growing tumours, and were much less effective where the tumour was growing slowly and contains many resting cells. The smaller the number of malignant cells present, the greater was the chance of obtaining a 100% kill, thus surgery and radiotherapy should be employed whenever possible in order to reduce the cell numbers before initiating the chemotherapy.

Gilbertson et al. (1983) studied 232 dogs which underwent mastectomy for mammary epithelial neoplasms and revealed the use of the dog as a model for the development of new therapeutic modalities and immunoprophylaxis of human mammary tumours.

Edington et al. (1984) studied that the intra arterial administration of doxorubicin may lead to cardiac toxicity at lower cumulative dose than noted with peripheral intravenous administration.

Haga et al. (2000) observed that a single intravenous administration of 2 mg/kg doxorubicin induced emesis within 24 hours of administration in some dogs, while delayed emesis was observed 24 hours after administration in all dogs. Hypophagia, decreased frequency of drinking and increase frequency of frequency of defecation were induced shortly after delayed emesis.

Talekar (2001) treated cases of mammary tumour with doxorubicin hydrochloride at the dose rate 30 mg/m² and found to be effective with success rate of 66.66%. Side effects like anaemia, vomition and inappetance were reported.

Gille et al. (2002) reported that doxorubicin was a potent cytostatic drug which was applied for the treatment of various kinds of malignant diseases. In spite of routine use of that drug, its major adverse effect and dose-dependant cardiotoxicity could not be prevented. However, several clinical trials indicated that iron chelators were able to moderate the noxious effect more efficiently than radical cavanging and capsulants antioxidants. This in turn supported the idea that doxorubicin iron complexes were involved in triggering the cardiotoxicity of that drug by catylising the formation of oxygen radicals.
Simon et al. (2006) studied the post operative adjuvant treatment of invasive malignant mammary tumour in dogs and found that treatment with doxorubicin led to toxicosis in three dogs: grade 1 diarrhoea in one dog after the first treatment and grade 1 to 2 vomiting and diarrhoea in a second dog after the fourth, fifth, and sixth treatments. Slight alopecia (grade 1) and cutaneous hyperpigmentation (grade 1) were seen in another dog. One dog exhibited a below-normal neutrophil count ($3.3 \times 10^3$ mL) but otherwise myelosuppression was not reported.

Patil et al. (2008) reported that the doxorubicin remained in the first line of cancer therapy even though it produced cardiotoxicity, nephrotoxicity and palmar-plantar erythrodysesthesia commonly referred as hand foot syndrome. It was also reported that engineered nanocarriers with high specificity to cancer cells were also developed to use doxorubicin in clinical use.

Dervisis et al. (2011) reported that hemangiosarcoma is an aggressive disease that is fairly common in the dog. Doxorubicin was administered on day 1 toxicity and efficacy was assessed by clinical and laboratory evaluation by questionnaires completed by the owners of the 24 dogs. Significant toxicities were noted including 41 high grade hematologic and 12 high-grade gastrointestinal toxic events. Five dogs discontinued treatment due to chemotherapy-related toxicities.

Li et al. (2011) reported of no behavioural changes on liver following administration in Beagle dogs with Fe$_3$O$_4$@Au composite magnetic nanoparticles loaded with daunorubicin and there was no significant difference in body weights between the experimental and control groups and the toxicity of Fe$_3$O$_4$@Au composite magnetic nanoparticles have a potential to be used as a safe optical and thermal agent allowing the combination of cancer detection and cancer specific hyperthermic treatments was classified as grade one, suggesting that it has good biocompatibility for cellular application.

Akbarzadeh et al. (2012) stated that with the ability to utilize magnetic attraction and/or specific targeting of disease biomarkers, magnetic nanoparticles offered an attractive means of remotely directing therapeutic agents specifically to a disease site, while simultaneously reducing dosage and the deleterious side effects associated with non-specific uptake of cytotoxic drugs by healthy tissue.
and also stated that the fate of these magnetic nanoparticles upon intravenous administration was highly dependent on their size, morphology, charge, and surface chemistry.

2.4 Engineering the Magnetic Properties

2.4.1. Iron oxide nanoparticles

Lu et al. (2007) reported that magnetic nanoparticles which retained sufficient hydrophilicity and, with coating, not exceeding 100 nm in size, was preferable to avoid rapid clearance by the reticulo-endothelial system.

Wu et al. (2008) stated that Fe₃O₄ nanoparticles were not stable under ambient conditions and were easily oxidized to Fe₃O₄ or dissolved in acidic medium. It was suggested that in order to avoid the possible oxidation in air the synthesis of Fe₃O₄ nanoparticles had to be done in an anaerobic condition.

Wang and Jiang (2009) reported that the Fe₃O₄ nanoparticles prepared in the presence of polymer polyethylene glycol (PEG) 4000 Da by thermal decomposition of iron acetyl acetonate product was nearly spherical, with sizes of the particles ranging from 100–200 nm. Magnetization measurement and Mossbaner spectrum indicate that these particles were nearly super paramagnetic at room temperature. It was concluded that these Fe₃O₄ spherical nanoparticle would be promising materials for applications in advanced magnetic materials.

Singh et al. (2010) stated that iron oxide nanoparticles were found naturally in the environment as particulate matter in air pollution and in volcanic eruptions but could not be generated as emission from traffic, industry and power station. However it could be specifically synthesized chemically for a wide variety of applications.

Divakaran et al. (2011) studied the antitumour activity of the D-Aminoacid oxidase (DAO) complex by oral administration of the complex and the substrate D-alanine to tumour bearing Swiss albino mice and by targeting the complex to the tumour site, using an externally applied magnetic field. Fe₃O₄.DAO along with D-alanine showed remarkable cytotoxicity in a substrate concentration-dependent manner. Both morphological examination and comet assay revealed that Fe₃O₄-
DAO/D-alanine induced apoptosis. Oral administration of Fe₂O₃-DAO and D-alanine along with magnetic targeting significantly suppressed tumour growth in mice. The report provided the first evidence for the promising application of enzyme bound nanoparticles for targeted oxidation therapy.

Wang et al. (2011) suggested that optimized daunorubicin (loaded magnetic nanoparticles formulation, (DNR – MNP's) possessed good aqueous dispersion, small particle size, high drug loading capacity, encapsulation efficiency, sustained drug release and favourable antitumour properties, which could be used as an excellent systemic chemotherapy for leukemia as possibly for other careers.

Akbarzadeh et al. (2012) used polymer coated magnetic nanoparticles for encapsulation of doxorubicin under mild conditions and opined that it could be used for drug delivery. It was also reported that the modified Fe₃O₄ nanoparticles had no cytotoxicity and were biocompatible.

Aliabadi et al. (2012) reported that magnetic nanoparticles in a variable magnetic field are able to produce heat. This heat (42-45°C) has more selective effect on fast dividing cancer cells than normal tissues. Biological results showed that magnetite nanoparticles alone were not cytotoxic at respiratory tract, while in the alternative magnetic field more than 50% of cells were dead. Doxorubicin alone was not cytotoxic during 30 min, but in combination with magnetite more than 80% of the cells were killed. They concluded that doxorubicin and magnetite nanoparticles in an AC magnetic field had combinatory effects against cells.

Javid et al. (2012), Undertaken a study to synthesize doxorubicin carrier, for the human ovarian cancer treatment, using chitosan loaded iron oxide nanoparticles. Doxorubicin loaded and chitosan modified iron oxide nanoparticles were prepared by a co-precipitation and emulsification cross-linking method. They observed that the iron oxide nanoparticles have a potential as a sustained drug delivery system. These results indicate that anticancer drug loaded chitosan-modified Fe₃O₄ nanoparticles have potential use as anticancer drug carriers and can also have an enhanced anticancer effect with lower toxicity as compared to the drug itself.
Iron oxide nanoparticles were the only magnetic nanoparticles approved for clinical use by the United States Food and Drug Administration (Jia et al., 2012).

Yanhui et al. (2012) assessed the antitumour activity by single intratumoural injection of doxorubicin + Magnetic nanoparticles into mice with subcutaneous lewis lung carcinoma. This study showed that the intratumoural administration of anticancer drugs represents a growing trend for maximizing local tumour control with minimal systemic toxicity; however, it requires a novel drug delivery system for treatment efficacy and ease of administration. MNPs have been widely used in the delivery of chemotherapeutics, achieving promising results.

Philip et al. (2013) observed the regression of tumour including mammary tumour without any toxic effects when treated with iron oxide (Fe₃O₄) nanoparticles - Doxorubicin complex into tumour tissue at different locations.

2.4.2. Synthesis of iron oxide nanoparticles

According to Lu et al. (2007), the strategies to develop different coatings could be classified into two groups: nanoparticle coating with inorganic component such as silica, carbon, precious metals or oxides; and nanoparticle coating with organic shells.

The most common methods of synthesis of shape controlled, stable, biocompatible and monodispersed iron oxide nanoparticles include coprecipitation, thermal decomposition, hydrothermal synthesis, microemulsion, sonochemical synthesis (Wu et al., 2008).

Wang and Jiang (2009) described the synthesis of Fe₃O₄ nanoporous particles in the presence of polymer poly ethylene glycol (PEG) 4000 Da by thermal decomposition of iron acetylacetonate.

The Fe₃O₄ nanoparticles were prepared by chemical co-precipitation of Fe²⁺ and Fe³⁺ ions under alkaline conditions (Akbarzadeh et al., 2012).

Hilger and Kaiser (2012) studied the use of nanoparticles in Magnetic Resonance Imaging and magnetic hyperthermia demonstrated that since naked iron oxide cores were prone to aggregate, be oxidized at the nanoparticles
surface, different coating strategies had been developed to circumvent these undesired effects during biomedical applications. Iron oxide nanoparticles were particularly versatile tools for the allocation of multiple functionalities.

2.4.3 Drug delivery using nanoparticles

Fonesca et al. (2002) used poly (D, L-lactide co-glycolide) nanoparticles for delivering paclitaxel to tumours and found that incorporation of paclitaxel in the PGLA nanoparticles strongly enhances its antitumoural efficacy as compound to the free drug.

Celikoglu et al. (2008) described the technique for intratumoural injection of one or more cytotoxic drugs directly into tumour tissue through a flexible bronchoscope by means of endo-bronchial intratumoral chemotherapy (ETIC). They stated that the intratumoral delivery of cytotoxic drugs may be regarded as improved neoadjuvant therapy for use prior to irradiation and/or surgery. ETIC differs significantly from conventional intravenous chemotherapy by virtue of the localized non-systemic route of drug delivery. They further stated that the advantages of this system are precise delivery of a drug superdose directly to the tumour mass (dose impossible to deliver safely by normal systemic chemotherapy) and little systemic drug toxicity (in contrast to systemic intravenous drug delivery which is severely dose limited due to general toxicity).

Jayakumar et al. (2009) stated that Fe₃O₄ nanoparticles, which were biocompatible and stable, could be directed to the site of the tumour by using external magnetic field, after oral administration of the magnetic nanoparticle – doxorubicin complex and the chemotherapy effect of doxorubicin could be considerably enhanced.

High concentration of chemotherapeutic or radiological agents could be achieved near the target sites without any toxic effects to normal surrounding tissue when tagged with ferromagnetic iron dextran reagents and subjected to magnetic targeting (Lokwani et al. 2011).

Mahmoudi et al. (2011) explained the term “drug delivery” as pharmaceutical agents of interest which were entrapped within, or attached to, an organic polymer matrix or inorganic particles, and in that case, drug safety and efficacy could be greatly improved and new targeted therapies were possible.
Akbarzadeh et al. (2012) demonstrated that doxorubicin-loaded, modified Fe₃O₄ nanoparticles had a potent antigrowth effect in an A549 cancer cell line and inhibited cell growth in a time dependent manner. Therefore, these nanoparticles could be natural potent chemopreventive and chemotherapeutic agents for patients with lung cancer, and constituents of these nanoparticles might be appropriate for drug development.

2.4.4. Fate of magnetic nanoparticles in the tissue

Metz et al. (2004) had analyzed the phagocytosis of various approved iron-oxide contrast agents by human monocytes in vitro and suggest that subsequent in vivo cell targeting applications in specific targeting of inflammatory process.

By comparing the concentrations of Fe₃O₄ magnetic nanoparticles at all time points between the experimental and control groups, there were statistically significant differences in heart and bone marrow tissue distribution in the liver, small intestine, spleen, brain, lungs, kidneys and stomach after administration, suggesting that Fe₃O₄ magnetic nanoparticles were distributed widely in various organs in vivo (Wang et al., 2010).

Settles et al. (2011) studied the different capacity of monocyte subsets to phagocytose iron oxide nanoparticles and concluded that the two main subsets of human monocytes differentially take up iron oxide based nanoparticles which leads to different T₁ and T₂ relaxation time when the cells were investigated in vivo.

Magnetic particles smaller than four μm were eliminated by cells of the reticuloendothelial system, mainly in the liver (60%–90%) and spleen (3%–10%). Particles larger than 200 nm were usually filtered to the spleen, the cutoff point of which extended up to 250 nm, while particles up to 100 nm were mainly phagocytosed by liver cells (Akbarzadeh et al., 2012).

2.5. Drug Delivery to Resistant Tumour Cells

According to Brodey et al. (1983) in case of malignant mammary tumour, by radiographic evidence of lung metastases, a solitary metastasis could be observed on rare occasions and pulmonary lobectomy could be performed along
with mastectomy but adjuvant immunotherapy or chemotherapy appeared essential postoperatively, as other subclinical tumour foci were surely present.

Ambudkar et al. (1999) reported that cancer-cell resistance could be considered as one of the major reasons for failure of chemotherapy for the majority of cancer patients.

2.6. Local Chemotherapy

Smith et al. (1999) studied the drug metabolism and distribution after intratumoural chemotherapy with flurouracil / epinephrine injectable gel in human pancreatic cancer xenograft and reported that one potential advantage of this approach compared with systemic administration was the possibility of achieving significantly higher local drug retention in the target tumour and circumventing various physiological barriers, such as simultaneous drug excretion during the drug transportation process.

Goldberg et al. (2001) compared percutaneous tumour ablation using a combination of radio frequency ablation and intratumoural doxorubicin injection in rats found to cause increased coagulation in solid tumours in intratumoural injection of doxorubicin in comparison with the radiofrequency ablation alone.

Almond et al. (2003) reported that intratumoural injection of anti neoplastic drug mitoxantrone could provide high localized drug concentration with greatly reduced systemic toxicity.

Transcellular transport of large hydrophilic molecules could be facilitated transporters, adsorpive or receptor-mediated transcytosis, which would be enhanced by high local concentrations of nanoparticles (Berry and Curtis, 2003).

Intratumoural injection of chemotherapeutic agents was a potentially more effective alternative to systemic administration because direct delivery of the anticancer drug to the target may improve both the stability and efficacy of anticancer drugs (Tong and Cheng, 2007).

Xie et al. (2007) and Al-Abd et al. (2010) suggested that intratumoural administration of chemotherapeutic agents was a potentially more effective modality to overcome the limitation of conventional intravenous chemotherapy and this had been extensively evaluated using a number of anticancer drugs.
Jain et al. (2008b) reported that when therapeutics (drugs or genes) attached to the magnetic iron oxide nanoparticles were injected at or near a target site and an external magnetic field applied, the therapeutic agents could be effectively concentrated in the target cells or tissues.

Patil et al. (2008) reported that engineered nanocarriers of doxorubicin referred to carriers modified to escape recognition by reticuloendothelial system and/or functionalized with target specific ligands for selective accumulation at the target site.

Kang et al. (2011) indicated that intratumoural injection of doxorubicin-loaded magnetic particle gels might allow doxorubicin to effectively accumulate in the tumour and induce long lasting inhibition of tumour growth.

Jia et al. (2012) stated that although intratumoural administration was a promising approach for the treatment of various solid tumours with minimal systemic toxicity, its efficacy was highly dependent on the timing and frequency of the drug injections because of its rapid clearance from the tumour site.

2.7. Magnetic Field

Alexiou et al. (2000) reported that "magnetic drug targeting" offers a unique opportunity to treat malignant tumours locoregionally without systemic toxicity. It was also suggested that it might be possible to use magnetic particles as a "carrier system" for a variety of anticancer agents such as radionuclides, cancer specific antibodies and genes.

Jayakumar et al. (2009) found that in animals experimentally induced cancer were given magnetic treatment following administration of the nanoparticle - doxorubicin complex, the tumour failed to reappear on termination of the drug administration but tumour reappeared and started growing in animals given no magnetic treatment.

Min et al. (2010) stated that transcellular transport of heparin-magnetic iron oxide nanoparticles could be enhanced by magnetic field to some degree, but the effect of the magnet at high concentration seemed to be much smaller than at low concentration because particles could form large aggregates, upon interacting with the cells and with each other and also considered the possibility
that transport of magnetic iron oxide nanoparticles across cell monolayers might be facilitated by an external magnet field by attracting the particles and facilitating their passage between the cells.

2.8. Haematological Changes

Medway et al. (1969) reported that decreased in haemoglobin values in highly vascular malignant neoplasm is due to loss of blood during surgery. They also observed that the leucopenia was often accompanied by a thrombocytopenia while thrombocytopenia was associated with spontaneous haemorrhages causing anaemia.

Benjamin (2010) reported that the total erythrocytes count decreases in malignancies; whereas, leucopenia, neutropenia, lymphocytosis, monocytosis and eosinopenia is observed which is due to replacement of bone marrow. Haemoglobin value decreases due to anaesthetic and surgical stress; while thrombocytopenia is due to myelosuppressive drugs like adriamycin.

MacEwen et al. (1981) used combination chemotherapy consisting of vincristine, L-assparginase, cyclophosphamide and methotrexate to treat lymphosarcoma in dog. The toxic effects noticed were anaemia (p<0.05≤30%) leucopenia (WBC-4,000 cells/cm3), myelosuppression, vomiting, diarrhoea and anorexia.

Coles (1986) attributed that decrease in total erythrocyte count and haemoglobin values is due to loss of blood during surgery from highly vascular malignant neoplasms; while, lymphocytes, monocytosis and eosinopenia were affected not only by alternations in renal function, but may be altered by certain physiologic factors or disease not primarily of renal origin.

Shepelevtseva et al. (1986) studied the toxicity of doxorubicin prepared with an original chemical method from rubomycin (daunomycin) in albino rats and dogs. The antibiotic was administered intravenously in multiple doses. A significant loss of body weight, a decreased in the relative weigh of spleen, thymus and ovary and increase in the relative weight of heart, kidneys and adrenal gland were observed in rats after daily administration of doxorubicin in various doses for five times. The dose of 0.5 mg/kg doxorubicin was lethal for the dogs after five days, seven days and ten days administrations. Multiple
administration of the antibiotic in doses of 0.2 mg/kg and 0.125 mg/kg did not result in death of the dogs. There were areas of the alopecia on the belly and joints, ulcers, body weight loss, increase urea level in serum and diarrhoea before death. The faces contained significant admixtures of blood. A doxorubicin had an inhibitory effect on haemopoiesis of the albino rats and dogs. Bone marrow aplasia was recorded before death after discontinuation doxorubicin administration, the inhibitory on haemopoiesis persisted for 2 to 3 days. Histological examination of organs and tissues of the animals killed at different period after multiple intravenous administration of doxorubicin in various doses showed that doxorubicin had mainly the damaging effect on the gastrointestinal epithelium, heart muscle, epithelium of the proximal tubuli of the kidneys, lymphoid organs and testis. The damage depended on the dose of doxorubicin and duration of its use.

Tilmant et al. (1986) studied the treatment of dog suffering with synovial cell sarcoma with doxorubicin hydrochloride at a dose rate of 20 mg/m² intravenously every three weeks for a total of seven administrations with combination of cyclophosphamide at the dose rate of 50 mg/m² orally on days 3, 4, 5 and 6 of each week for seven consecutive weeks. The side effect included bone marrow suppression, thrombocytopenia and cardiac arrhythmia in dog.

Dobson and Gorman (1993) recorded that neutropenia was the most common and the most serious complication of chemotherapy and cytotoxic drugs, as it suppresses the bone marrow resulting in reduced production of platelets.

Arrington et al. (1994) studied the pharmacoknetics and toxicity of single dose of doxorubicin, at dosages of 30 mg/m² of body surface area and 1 mg/kg of body weight, in 17 dogs. They reported that the mean WBC counts and granulocyte counts for all dogs were lower on day 7 with 30 mg of doxorubicin/m² (n=17), compared with that for 1 mg of doxorubicin/kg (n=14; P<or=0.01).

Ahaus et al. (2000) studied the hematological toxicity of doxorubicin containing protocols in dogs with spontaneously occurring malignant tumour and concluded that haematological toxicity was not a contraindication for using doxorubicin based protocols in dogs with malignant tumours, since its prevalence was less than 30% with each protocol.
Sivakumar (2000) Treated cases of canine transmissible venereal tumour with methotrexate and cyclophosphamide and observed that haemoglobin percentage was less than normal value with slight decrease in PCV percentage. The differential leucocytic count showed non-significant variation. Pulse rate varied within physiological range while increased in respiratory rate, decreased erythrocyte and leucocyte count, neutropenia, lymphocytosis and monocytosis.

Talekar (2001) treated cases of mammary tumour with doxorubicin hydrochloride at the dose rate 30 mg/m². He observed decrease in mean TEC upto third day of treatment. The mean TLC value increased on third day and seventh day and became normal on 21st day. Neutrophilia, frank lymphocytosis was observed whereas haemoglobin value progressively declined.

Kerr (2002) reported that anaemia is caused due to low packed cell volume with free haemoglobin, abnormal platelet count and neoplastic infiltration of bone marrow; whereas, neutropenia occurs due to decrease bone marrow production, bone marrow aplasia, anaemia, and thrombocytopenia and due to treatment with cytotoxic anticancerous drugs.

Brar et al. (2002) recorded that anaemia occurred due to bone marrow depression results in progressive decrease in erythrocyte count and haemoglobin concentration. Serum glutamic pyruvic transaminase enzyme was present in large quantities in the hepatocytes cytoplasm of dog also known as liver specific enzyme. Other sources of serum glutamic pyruvic transaminase include cardiac and skeletal muscles, pancreas and renal cells. Damage to these tissue due to chemotherapeutic agent results in higher value of serum glutamic pyruvic transaminase.

Todorova et al. (2005) studied six bitches with mammary tumour in which chemotherapeutic drug doxorubicin, was given intravenously @ 120/30 mg/m² once weekly, for three consecutive weeks and cyclophosphamide, intravenously @ 100 mg/m² three days after the doxorubicin administration, for three consecutive weeks. Toxic effects were lethargic, uncoordinated movements, difficult respiration, anorexia vomiting, diarrhoea and alopecia. The blood analyses showed significantly decreased RBC and WBC count during the second time period. They opined that the chemotherapy should be postponed until the minimum WBC count was restored.
Gadmade (2006) treated the vaginal tumours with Doxorubicin hydrochloride @ 30 mg/m² at interval of 10 days for three occasions and methotrexate @ 0.3 mg/m² at interval of ten day at four occasions. The author noted the side effects of doxorubicin hydrochloride as anaemia, vomition, anorexia, alopecia and dullness. In both the treatment groups, there was significant decrease in TEC, TLC, haemoglobin, neutrophill percentage and platelet count and monocytosis. The serum glutamic oxalate transaminase, serum glutamic pyruvic transaminase, serum glucose, blood urea nitrogen, serum creatinine and serum calcium levels were significantly elevated.

According to Wang et al. (2010) the results of atomic absorption spectrophotometry showed that the highest concentration of iron magnetic nanoparticles in the peripheral blood of mice at six hours in the experimental group after intragastric dosing, and decreased slowly but remained at a higher level until it reached another distribution peak on day five and then gradually declined again.

2.9. Biochemical Changes

Cornellus (1957) reported that serum glutamic pyruvic transaminase level elevated in severe anaemia occurred.

Medway et al. (1969) reported the increase in serum glutamic oxalate transaminase in serum calcium in highly vascular malignant neoplasms.

Benjamin (2010) reported that there is increase in serum glutamic oxalate transaminase in hepatocellular disease and skeletal and cardiac muscle necrosis.

Pond and Morrow (1982) reported a case of maxillary osteosarcoma in dog which was treated with combination of doxorubicin, Methotrexate and cyclophosphamide. The dog was healthy but after that the leg became anorectic, developed icterus, ascites, and sub acute hepatic and renal failure, elevation of bilirubin level, blood ammonia level, mild elevation in serum glutamic pyruvic transaminase (SGPT), blood urea nitrogen (BUN), creatinine and phosphorus. There was mild non-regenerative anaemia, leucocytosis and neutrophilia shift to left and they concluded that since methotrexate was a known as hepatotoxin in man, it was considered the probable cause of hepatic necrosis in dogs.
Brar et al. (2002) recorded that the serum glutamic pyruvic transaminase enzyme was present in large quantities in the hepatocytes cytoplasm of dog also known as liver specific enzyme. Other sources of serum glutamic pyruvic transaminase include cardiac and skeletal muscles, pancreas and renal cells. Damage to these tissues due to chemotherapeutic agent result in higher values of serum glutamic pyruvic transaminase.

Todorova et al. (2005) studied six bitches with mammary tumour in which chemotherapeutic drug was given. They observed not significant changes in serum urea, creatinine, ASAT, ALAT and total protein.

Jain et al. (2008a) observed that intravenous administration of magnetic nanoparticles neither caused any long term changes in the liver enzyme levels nor induced oxidative stress in rats.

2.10. Prognosis of Tumour

2.10.1. Tumour size

Vail et al. (1997) measured the tumours in millimeters for each dog before the treatment and then again after 21 days following each drug administration which consisted of three dimensional caliper measurements in assessable tumours.

According to Vail et al. (1998), tumour volume measurements were made immediately before each treatment and categorized on the basis of their response to chemotherapy as: complete response, complete regression of all measurable lymph nodes; partial response and no response.

Matos et al. (2012) stated that the tumour size or volume was one of the most studied characteristics in prognostic studies and the size was measured at pre-operative physical examination by palpation or with calipers, the largest diameter determining the tumour diameter.

2.10.2 Metastasis

Brodey et al. (1983) explained that some mammary tumours were characterized by ill defined borders suggestive of local invasion into adjacent mammary tissue, skin and fascia and strongly suggested malignancy which might
have a history of gradual enlargement over a long period followed by a short, sudden spurt of growth accompanied by local invasion and possible spread or a short history of rapid progressive growth.

Jia et al. (2012) stated that the incidence of metastasis to lungs, kidney, and occasionally liver and heart of mice was highest in mice treated without magnetic nanoparticles and lowest in mice receiving doxorubicin with magnetic nanoparticles with an external magnetic field.

2.11. Other Superficial Tumours

Misdorp and Hart (1976) explained that canine mammary carcinomas showed different biologic behaviour depending on whether the tumours were simple or complex.

Tumours of the mammary gland were a common clinical problem in the adult bitch, comprising up to 52% of all neoplasm (Bostock, 1977).

According to Brodey et al. (1983), the caudal two glands were far more often affected than the cranial three glands which were firm to hard nodules markedly with softer mammary parenchyma.

Sharma et al. (1997) reported that anaesthesia was maintained by administration of ketamine (2 to 3 mg/kg) or xylazine (1 mg/kg) + ketamine (2 to 3 mg/kg) intravenously. Induction was smooth recovery was uneventful. Analgesia and muscle relaxation were optimum for surgical intervention has also observed.

Malignant tumours of nail bed epithelium were most commonly seen in dogs between seven and 11 years of age. A single digit or multiple digits on the same animal might be involved and there was often loss of the nail with secondary infection of the nail bed and metastasis occurred via lymphatics to regional lymph nodes and lungs (Goldschmidt and Hendrick, 2002).

Dernell (2005) reported that mast cell tumours (MCT) are the most common cutaneous tumour in the dog. Surgical removal is the treatment of choice for local MCT disease. Regional resection may also be reconsidered. Complete surgical resection for dogs with no evidence of metastasis will result in upwards of 90% 1-year remission.
Dogs bearing high-grade mammary tumours with an associated high risk of metastasis would benefit from adjuvant chemotherapy, and in vitro studies suggest that both doxorubicin and carboplatin kill neoplastic canine mammary cells (Simon et al., 2006).

Approximately 50% of all canine mammary tumours were malignant, of which epithelial tumours, including carcinomas and adenocarcinomas, were the most common histological types (Kumaraguruparan et al., 2006).

Sebaceous hyperplasia accounts for most sebaceous gland tumours in the dog characterized histologically by an accumulation of nearly mature sebaceous glands and were found on the limbs, trunk, and eyelids and were found peripheral to and phasing into sebaceous adenomas or adenocarcinomas whereas sebaceous adenomas were relatively uncommon that were similar in appearance and behaviour to hyperplastic lesions (Vail and Withrow, 2007).

London (2008) stated that the wide surgical excision is indicated for all canine mast cell tumour (MCT). He further studied the prognostic factors and stated that the 83% of dogs with grade 1 MCT, 44% of dogs with grade 2 MCT, and 6% of dogs with grade 3 MCT were alive 1500 days after surgical excision. In another study, 100% of dogs with grade I, 44% of dogs with grade 2 and 7% of dogs with grade 3 were alive two years after surgical excision.

Julie et al. (2013) reported a case of liposarcoma in a dog with its confirmatory diagnosis and successful surgical management. They stated that the liposarcomas are rare tumours in canine.
Chapter 3

MATERIALS & METHODOLOGY
MATERIALS AND METHODS

The studies were undertaken on eighteen clinical cases of the malignant superficial tumour and were divided in three equal groups. Six dogs with the tumour size more than 3 cm were subjected to the surgical treatment; whereas 12 dogs with smaller tumour size were subjected to the chemotherapy.

Data regarding incidence of superficial tumours were obtained from the cases recorded at Teaching Veterinary Clinical Complex, N.V.C, Nagpur from December 2013 to July 2014. The detailed history was obtained in each case. The selected dogs were subjected to detailed general clinical, haematological and serum biochemical examination on the first day of treatment. The dogs were subjected to the following treatment regimen.

<table>
<thead>
<tr>
<th>Group</th>
<th>Number</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>6</td>
<td>Inj Doxorubicin(^1) @ 30 mg/m(^2) intravenously in 150 ml of 5% Dextrose saline solution at scheduled interval i.e. on 1(^{st}) and 21(^{st}) day</td>
</tr>
<tr>
<td>Group II</td>
<td>6</td>
<td>Iron oxide (Fe(_3)O(_4)) nanoparticle(^2) - Doxorubicin complex @ 3 mg/kg body weight intratumoural at 2 - 3 different sites at scheduled interval i.e. on 1(^{st}), 7(^{th}) and 21(^{st}) days</td>
</tr>
<tr>
<td>Group III</td>
<td>6</td>
<td>Surgical excision of the growth by following standard operative procedure</td>
</tr>
</tbody>
</table>

3.1 Clinical Examination

The animals under study were examined thoroughly and data were recorded on following parameters.

3.1.1 Location and size of tumour

The measurement of the tumour was obtained on the aforementioned days using a vernier calliper.

3.1.2 Visual examination of lesion i.e. ulcerative, wound etc....

The observations with regard to external appearance of tumour viz. Ulcerative, Smooth, intact etc. were recorded.

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1. Adriamycin - Doxorubicin Hydrochloride for injection I.P. rapid dissolution 50 mg., Pfizer
2. Iron Oxide Nanoparticles - Sigma Aldrich Ltd., USA
3.2 **Haematological Parameters**

Two ml blood was collected in sterilized glass vials containing EDTA (Ethylene Diamine Tetracetic acid - 2 mg/ml blood) on 0\(^{th}\), 10\(^{th}\), 20\(^{th}\) and 30\(^{th}\) days interval for haematological examinations and freshly prepared blood smear was used for differential leucocyte count (DLC).

3.2.1 **Haemoglobin**

The haemoglobin was estimated as per standard method suggested by Schalm *et al.* (1975) by using Sahli’s haemoglobinometer. The results were expressed as gm/dl of blood.

3.2.2 **Total erythrocyte count**

Total erythrocyte count was determined as per the standard procedure described by Schalm *et al.* (1975) by using Neubauer’s chamber and the values were expressed in millions/ cumm of blood.

3.2.3 **Total leucocyte count**

Total leucocyte count was determined as per the standard procedure described by Schalm *et al.* (1975) by using Neubauer’s chamber and the values were expressed in thousands/ cumm of blood.

3.2.4 **Packed cell volume**

It was estimated by the Wintrobe’s Microhaematochrit method and the values are expressed in percentage.

3.2.5 **Differential leucocyte count**

The thin fresh blood smear was prepared and stained with Leishman’s stain. A total 100 cells were counted and expressed in percentage (%). Enumeration of neutrophils, lymphocytes (large and small), monocytes, eosinophils and basophils was done using ‘Battlement’ method described by Schalm *et al.* (1975).
3.2.6 **Total platelet count**

Platelets were counted on Microx-60 automated cell counter and the values were expressed in lac/cumm of blood.

3.3 **Serum Biochemical Parameters**

Four ml blood from the cephalic or saphenous vein was collected in a dry glass tube without anticoagulant for serum separation. Blood was centrifuged after 15 minutes of collection at 1000 RPM for 10 minutes. The biochemical studies serum glucose, alkaline phosphatase, liver function test (SGOT and SGPT) and kidney function test (BUN and serum creatinine) were performed with the help of Semi-autoanalyser (STAR 21) using standard kits supplied by the Avantor diagnostic Ltd, India on 0\textsuperscript{th}, 10\textsuperscript{th}, 20\textsuperscript{th} and 30\textsuperscript{th} days interval in all the animals.

3.3.1 **Serum glutamic oxaloacetate transaminase (SGOT)**

SGOT values were estimated as per the method suggested by Frankel and Reitman (1963) and the values were expressed in U/L.

3.3.2 **Serum glutamic pyruvate transaminase (SGPT)**

SGPT values were estimated as per the method suggested by Frankel and Reitman (1963) and the values were expressed in U/L.

3.3.3 **Serum glucose**

The serum glucose values were estimated by using the method suggested by Frankel and Reitman (1957) and were expressed in mg/dl.

3.3.4 **Blood urea nitrogen (BUN)**

The BUN values were estimated by enzymatic urease method and values were expressed in mg/dl.

3.3.5 **Serum creatinine**

The serum creatinine values were estimated by alkaline picrate method and values were expressed in mg/dl.
3.3.6 **Alkaline phosphatase**

Serum alkaline phosphatase was estimated by using semi auto analyzer (Avantor Diagnostic Ltd.) using standard biochemical kit. The values were expressed in IU/L.

3.4 **Regression of Growth**

The observations on regression of growth in group I and II animals were recorded with the help of Vernier Calliper (Plate 6).

3.5 **Recurrence/ Metastasis**

The recurrence of growth was assessed by palpation of the site; whereas the metastasis was ascertained by thoracic radiographic examination.

3.4 **Histomorphological Examination**

The core biopsy tissue samples were collected by using biopsy gun and the tissue samples were preserved in 10 % neutral buffered formaline (Plate 4-5). The tissues were processed by rapid paraffin embedding technique. Sections of 5 \( \mu \) thickness were cut and stained by Hematoxyline-Eosin stain (H & E) (Bancroft and Stevens, 1982).

3.5 **Preparation of PVP Coated Iron Nanoparticles**

The iron oxide nanoparticles (ferric chloride and ferrous chloride at the rate of 2:1 molar ratio and 50-100 \( \mu \) size) used in the study was coated with the Poly vinyl pyrrolidone\(^3\) (PVP). The nanoparticles were dissolved in milli-Q water. Ammonia solution (28\%) was added drop wise into the metal chloride solution with vigourous stirring until the pH became 11 to 12, and subsequently heating the slurry at 80°C for one hour. Poly vinyl pyrrolidone (PVP\(^\text{3}\)) was added to the above slurry and further heated for one hour and cooled to room temperature. The salt solution was decanted and the precipitate was repeatedly washed to remove any impurity ions.

3.5.1 **Preparation of iron nanoparticles-doxorubicin complex**

Equal volumes of one per cent PVP coated iron nanoparticles and one per cent solution of Poly Oxy Ethylene 25- propylene glycol Stearate\(^4\) (POES) were

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3. Poly-vinyl Pirolidone - Sigma Aldrich Ltd., USA
4. Poly oxy Ethylene- Sigma Aldrich Ltd., USA
mixed and sonicated to form a brownish yellow solution of iron nanoparticles coated with PVP and POES. The solutions were mixed in 10:1 ratio using 10 parts of doxorubicin and 1 part of iron oxide nanoparticles. The binding of doxorubicin with iron nanoparticles was facilitated by means of ultrasonication using sonicator (Plate 3) (Jayakumar et al., 2009).

3.5.2 Storage of iron nanoparticles-doxorubicin complex

The prepared drug complex was later stored under refrigeration. Since the iron nanoparticles are easily oxidized on exposure to light, the vials were stored away from sunlight in amber coloured bottle (Plate 2).

3.6 Treatment Protocol

3.6.1 Group I

In this group, dogs underwent chemotherapy by administration of the Inj. Doxorubicin @ 30 mg/m² intravenously in 150 ml of 5% Dextrose saline solution at scheduled interval i.e. on 1st and 21st days (Plate 1).

3.6.2 Group II

The prepared iron oxide (Fe₃O₄) nanoparticles - doxorubicin complex was injected aseptically into the tumour at a dose rate of 3 mg/kg body weight. The treatment was repeated on day 1, 7 and 21.

The biometry of the tumours was measured using Vernier callipers before every treatment. The local changes and tissue reactions to the iron oxide nanoparticles (Fe₃O₄) - doxorubicin complex injection was observed. Side effects and other observation, if any were also recorded.

3.6.3 Group III

The radical surgery was performed to remove the tumour mass. The animals were aseptically prepared. Premedication with atropine sulphate at the rate of 0.045 mg/kg body weight intramuscularly and after 15 minutes with xylazine and inj. betnesol at the rate of 1 mg/kg body weight intramuscularly.

5. 5% Dextrose, Nirflife Ltd Health are Division, Gujarat
6. At-vet-Atropine Sulphate 1 mg/ml, Dotcom Pharma, Mumbai
7. Xylavin- Xylazine Hydrochloride, 20 mg/ml, Indian Immunologicaalcs Ltd, Mumbai
Plate 1. Inj. Adriamycin (Doxorubicin hydrochloride)

Plate 2. Inj. Doxorubicin-Iron oxide nanoparticle Complex

Plate 3. Ultrasonicator
Anaesthesia was induced and maintained using ketamine\textsuperscript{9} at the rate of 5 mg/kg body weight and inj. diazepam\textsuperscript{10} at the rate of 1-2 mg/kg body weight intravenously. Local anaesthesia was used wherever necessary. An elliptical skin incision was made around the tumour. The tissues were bluntly dissected using mayo scissors and by peeling of the tumour mass and the bleeding vessels were ligated using catgut size 1/0 along the way. After the tumour was excised, the subcutaneous tissues were sutured using chromic catgut no. 0; followed by skin sutures using monofilament nylon.

3.7 Post operative care

Post operatively, in all the cases amoxicillin-cloxacillin\textsuperscript{11} at the rate of 20 mg/kg bodyweight was administered intramuscularly and the wounds were dressed. Anti-inflammatory meloxicam\textsuperscript{12} inj. at the dose rate of 0.5 mg/kg body weight was administered intramuscularly. Follow up examinations were scheduled post operatively according to the requirement.

3.8 Complications

Post surgical and other complications, if any were recorded in all the groups.

3.9 Statistical Analysis

The data recorded during the study was statistically analysed by using Analysis of Variance as per the Snedecor and Cochran (1994).

\textsuperscript{9} Aneket- Ketamine Hydrochloride, 50mg/ml, Neon Laboratories Mumbai
\textsuperscript{10} Calmose- Diazepam, 5 mg/ml Ranbaxy Laboratories Ltd, H.P.
\textsuperscript{11} Intamox-D3.5 - Amoxicillin- Cloxacillin, Intas Pharmaceutical Ltd. Ahmedabad (Guj.)
\textsuperscript{12} Meloxnex- Meloxicam 5 mg/ml, 100 ml. Intas Pharmaceutical Ltd. Ahmedabad (Guj.)
Chapter 4

RESULTS & DISCUSSION
RESULTS AND DISCUSSION

The clinical study was conducted on superficial tumours in dog with reference to various treatment regimens during the period of December 2013 to July 2014. During this period the clinical cases in animal reported to the Teaching Veterinary Clinical Complex, Nagpur were screened for superficial tumour irrespective of age, sex and breed. Out of them 18 clinical cases were selected for the study and were divided in three equal groups. The surgical excision, intravenous chemotherapy and the intratumoral administration of iron oxide nanoparticles - Doxorubicin complex were undertaken according to the size of tumour.

The results obtained during the study are discussed and presented as below.

4.1 History

All the cases subjected for study were brought to the hospital with a history of a mass on the superficial part of animal. The mass was of varied size and location with or without pain and ulceration. The duration of illness was also recorded. The age, breed and sex-wise distribution of the cases during the study is depicted in Table 1.

Table 1: Age, Breed and Sex-wise distribution of dogs with superficial tumours

<table>
<thead>
<tr>
<th>History</th>
<th>Number</th>
<th>Per cent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Up to 8 yrs</td>
<td>1</td>
<td>5.55</td>
</tr>
<tr>
<td>8 and above</td>
<td>17</td>
<td>94.44</td>
</tr>
<tr>
<td>Breed</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-descript</td>
<td>11</td>
<td>61.11</td>
</tr>
<tr>
<td>Pomeranian</td>
<td>3</td>
<td>16.66</td>
</tr>
<tr>
<td>German shepherd</td>
<td>2</td>
<td>11.11</td>
</tr>
<tr>
<td>Rottweiler</td>
<td>1</td>
<td>5.55</td>
</tr>
<tr>
<td>Labrador</td>
<td>1</td>
<td>5.55</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>8</td>
<td>44.44</td>
</tr>
<tr>
<td>Female</td>
<td>10</td>
<td>55.56</td>
</tr>
<tr>
<td>Duration of illness</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Up to 3 months</td>
<td>4</td>
<td>22.22</td>
</tr>
<tr>
<td>3 to 6 months</td>
<td>6</td>
<td>33.33</td>
</tr>
<tr>
<td>6 months and above</td>
<td>8</td>
<td>44.44</td>
</tr>
<tr>
<td>Location</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Head region</td>
<td>2</td>
<td>11.11</td>
</tr>
<tr>
<td>Limbs</td>
<td>4</td>
<td>22.22</td>
</tr>
<tr>
<td>Thoracic region</td>
<td>4</td>
<td>22.22</td>
</tr>
<tr>
<td>Cranial/ventral abdominal region</td>
<td>3</td>
<td>16.16</td>
</tr>
<tr>
<td>Caudal/ventral abdominal region</td>
<td>5</td>
<td>27.77</td>
</tr>
<tr>
<td>Size</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Below 3 cm</td>
<td>6</td>
<td>33.33</td>
</tr>
<tr>
<td>3 to 8 cm</td>
<td>5</td>
<td>27.77</td>
</tr>
<tr>
<td>More than 8 cm</td>
<td>7</td>
<td>38.88</td>
</tr>
<tr>
<td>Visual examination of lesion</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ulcerative</td>
<td>8</td>
<td>44.44</td>
</tr>
<tr>
<td>Non-ulcerative</td>
<td>10</td>
<td>55.55</td>
</tr>
</tbody>
</table>
4.1.1 Age

The study indicated that the highest incidence of superficial tumour was amongst older dogs above 8 years of age (94.44 %) (Table 1 and Figure 1).

In the present study, the age group of the animals ranges from 8 to 15 years with the mean age of ten and half years. This age has been always considered the “cancer age” which is a risk age for general anaesthesia and surgical excision. The present observations are in accordance with the findings of Moulton et al. (1990), Yamagami et al. (1996) and Dhami et al. (2010), Simeonov et al. (2011).

4.1.2 Breed

The highest incidence of superficial tumour was observed in non-descript (61.11%) followed by Pomeranian (16.66%), German shepherd (11.11%), Rottweiler and Labrador (5.55%) breeds of dog (Table 1 and Figure 2).

Brodey et al. (1983) and Kumar et al. (2011) reported that the incidence of tumour in pure breeds were higher than in cross breeds.

However, in the present study the highest incidence in the non-descript dogs might be due to more number of non-descript dogs reported to the T.V.C.C., Nagpur during the observation period.

4.1.3 Sex

During the present study, the incidence of superficial tumour was observed more in female (55.55 %) as compared to male (44.44 %) (Table 1 and Figure 3).

According to Brodey et al. (1983) the incidence of mammary tumours was low in males which were only one percent of the total population of dogs. Conroy (1983) stated that there was no significant difference in the incidence by sex in the case of cutaneous tumours.

The higher incidence in females observed during the study could be attributed to hormonal correlation or due to more number of female dogs reported to the T.V.C.C. Nagpur during the observation period.
4.1.4 **Duration of illness**

During the study the duration of illness was also recorded. The higher percent of dogs with 6 months above (44.44 %) illness history was observed followed by 3 to 6 months (33.33 %) and below 3 months (22.22 %). (Table 1 and Figure 4).

In the present study, the late reporting of the dogs for treatment could be due to more number of stray dogs (Non-descript) brought by the N.G.O.s or could be due to owners negligence. Since the cutaneous or mammary tumour do not cause any health problems and many a times are not observed by the owners due to their ventral location and hair coat, they are often neglected.

4.2 **Clinical Examination**

4.2.1 **Location and size of tumour**

During the present study, the location and size of tumour was recorded. The higher percent of the tumour was observed on caudal abdominal region (27.77 %) followed by limb and thoracic region (22.22 %), cranial abdominal region (16.16 %) and head region (11.11%). However, the tumour with more than 8 cm size was observed more as compare to 3 to 8 cm (27.77 %) or less than 3 cm (33.33 %) (Table 1, Figure 5 and Plate 7-12).

The higher percent of tumour in caudal abdominal region could be attributed to the higher incidence of mammary tumour particularly of caudal two glands as also stated by Brodey *et al.* (1983).

4.2.2 **Visual examination of lesion**

The tentative diagnosis was done on the basis of gross appearance, clinical history, gross morphology, the duration and rate of tumour growth. This was in agreement to the findings of Vail and Withrow (2007). In the present study, the skin over the tumour was found ulcerated in eight cases that showed no tendency to heal, which might be due to the change in the condition of the skin over the tumour mass. Similar findings were also reported by Goldberg *et al.* (2001).
Different locations of tumours

Plate 7. Head region
Plate 8. Hind limb

Plate 9. Cranio-ventral abdominal region
Plate 10. Medial aspects of thigh region

Plate 11. Caudo-ventral abdominal region
Plate 12. Right forelimb
4.3 Haematological Parameter

4.3.1 Haemoglobin (Hb %)

In the present study, the haemoglobin (gm/dl) was recorded in all the animals on 0\(^{th}\), 10\(^{th}\), 20\(^{th}\) and 30\(^{th}\) day. The mean values are depicted in Table 2 and graphically presented in Figure 6.

During the present study, the changes in mean haemoglobin values were statistically non-significant; however, the values were progressively declined in all the groups. During the present study, the values were found within the normal physiological range in all the groups.

Table 2: Mean ± S.E. of haemoglobin (gm %) recorded in different treatment groups

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>0(^{th}) day</th>
<th>10(^{th}) day</th>
<th>20(^{th}) day</th>
<th>30(^{th}) day</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>6</td>
<td>10.65 ± 0.68</td>
<td>9.76 ± 0.58</td>
<td>9.13 ± 0.85</td>
<td>8.90 ± 0.82</td>
</tr>
<tr>
<td>II</td>
<td>6</td>
<td>11.56 ± 0.75</td>
<td>11.43 ± 0.66</td>
<td>11.33 ± 0.75</td>
<td>11.33 ± 0.72</td>
</tr>
<tr>
<td>III</td>
<td>6</td>
<td>11.80 ± 0.81</td>
<td>11.30 ± 0.80</td>
<td>10.93 ± 0.77</td>
<td>10.83 ± 0.82</td>
</tr>
</tbody>
</table>

The mean haemoglobin values on day 0 were 10.65 ± 0.68 gm/dl, 11.56 ± 0.75 gm/dl and 11.80 ± 0.81 gm/dl in group I, II and III, respectively. Thereafter, the values showed a continuous and progressive decline in all the groups up to 30\(^{th}\) day, except in group II, where the value remains constant on 20\(^{th}\) and 30\(^{th}\) day.

These findings are in accordance with the observations reported by MacEwen et al. (1981), Talekar (2001), Brar et al. (2002) and Todorova et al. (2005).

In the present study, the progressive declining trend of haemoglobin percent in group I and II could be due to effect on reticuloendothelial system and myelosuppression results in erythrocytopenia in terms of reduction in haemoglobin percent. However, the decrease in haemoglobin values in group III could be due to loss of blood during surgery in highly vascular malignant neoplasm which is in agreement with the Medway et al. (1969) or due to anaesthetic and surgical stress as reported by Benjamin (2010).
4.3.2  Packed cell volume (per cent)

In the present study, the packed cell volume (per cent) was recorded in all the animals on 0, 10th, 20th and 30th day. The mean values are depicted in Table 3 and are graphically presented in Figure 7.

Table 3: Mean ± S.E. of packed cell volume (per cent) recorded in different treatment groups

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>0th day</th>
<th>10th day</th>
<th>20th day</th>
<th>30th day</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>6</td>
<td>47.50 ± 1.81</td>
<td>43.16 ± 1.51</td>
<td>38.00 ± 1.66</td>
<td>30.50 ± 1.38</td>
</tr>
<tr>
<td>II</td>
<td>6</td>
<td>45.45 ± 1.57</td>
<td>44.50 ± 1.88</td>
<td>45.50 ± 1.94</td>
<td>45.23 ± 1.67</td>
</tr>
<tr>
<td>III</td>
<td>6</td>
<td>47.67 ± 0.42</td>
<td>46.83 ± 0.31</td>
<td>47.17 ± 0.31</td>
<td>47.83 ± 0.48</td>
</tr>
</tbody>
</table>

Figures with different superscript differ significantly

The mean value of packed cell volume on day 0 was 47.50 ± 1.81 per cent, 45.45 ± 1.57 per cent and 47.67 ± 0.42 per cent in group I, II and III, respectively. In group I, the significant decrease in packed cell volume was observed and the value on 30th day was 30.50 ± 1.38 per cent, whereas; non-significant changes were observed in group II and III.

In group II, the packed cell volume showed undulating trend. The value decreases slightly on 10th day and thereafter increases on 20th day. The value on 30th day was 45.23 ± 1.67 per cent. In group III, the continuous declining trend was observed and the value on 30th day was 47.83 ± 0.48 per cent.

Kerr (2002) reported that causes of anaemia i.e. low packed cell volume with free haemoglobin, abnormal platelet count and neoplastic infiltration of bone marrow; in the present study in group I, the significant decrease in packed cell volume could be due to cytotoxic drug doxorubicin which suppress the replicating precursor cells of the bone marrow resulting in reduced production of erythrocytes.

However, in group II, non significant and undulating changes in the mean packed cell volume could be attributed to the less cytotoxic effect of the doxorubicin, whereas; no specific reason could be attributed non-significant decrease in group III animals.
Fig. 7. Mean value of packed cell volume (per cent) in different treatment groups

Fig. 8. Mean value of total erythrocyte count (million/cumm) in different treatment groups
4.3.3 Total erythrocyte count (million/cumm)

The total erythrocyte count (million/cumm) was recorded in all the animals on 0<sup>th</sup>, 10<sup>th</sup>, 20<sup>th</sup> and 30<sup>th</sup> day. The mean values are depicted in Table 4 and graphically presented in Figure 8.

**Table 4: Mean ± S.E. of total erythrocyte count (million/cumm) recorded in different treatment groups**

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>0&lt;sup&gt;th&lt;/sup&gt; day</th>
<th>10&lt;sup&gt;th&lt;/sup&gt; day</th>
<th>20&lt;sup&gt;th&lt;/sup&gt; day</th>
<th>30&lt;sup&gt;th&lt;/sup&gt; day</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>6</td>
<td>5.80±0.24</td>
<td>5.45±0.21</td>
<td>4.37±0.22</td>
<td>3.92±0.24</td>
</tr>
<tr>
<td>II</td>
<td>6</td>
<td>5.49±0.26</td>
<td>5.48±0.26</td>
<td>5.32±0.28</td>
<td>5.34±0.30</td>
</tr>
<tr>
<td>III</td>
<td>6</td>
<td>5.67±0.41</td>
<td>5.75±0.40</td>
<td>5.44±0.35</td>
<td>5.31±0.28</td>
</tr>
</tbody>
</table>

Figures with different superscript differ significantly

The mean value of TEC on day 0 was 5.80±0.24 million/cumm and 5.49±0.26 million/cumm in group I and II, respectively. In group I, the significant decrease in TEC was observed up to 30<sup>th</sup> day and the value on 30<sup>th</sup> day was 3.92±0.24 million/cumm, whereas; non-significant decrease in TEC was observed in group II.

The group III animal revealed the mean TEC of 5.67±0.41 million/cumm on day 0, which increases slightly on 10<sup>th</sup> day i.e. 5.75±0.40 million/cumm. The value thereafter showed declining trend up to 30<sup>th</sup> day and the value was 5.31±0.28 million/cumm. The values during the present study were found within the normal physiological limits.

Similar finding were reported by Talekar (2001), Brar et al. (2002) and Todorova et al. (2005). Dobson and Gorman (1993) stated that the cytotoxic drugs suppress the replicating precursor cells of the bone marrow resulting in reduce production of erythrocytes.

In the present study, the decrease in total erythrocyte count could be attributed to chemotherapy induced erythrocytopenia due to myelosuppression.

In group I, significant decrease in mean TEC indicates the cytotoxic effect of the doxorubicin, however, the cytotoxic effect was not intensely observed in group II animals where the mean TEC showed non-significant decrease.
4.3.3 **Total leucocyte count (thousands/cumm)**

The total leucocyte count (thousands/cumm) was recorded in all the animals on 0, 10\textsuperscript{th}, 20\textsuperscript{th} and 30\textsuperscript{th} day. The mean values are depicted in Table 5 and graphically presented in Figure 9.

**Table 5: Mean ± S.E. of total leucocyte count (thousands /cumm) recorded in different treatment groups**

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>0\textsuperscript{th} day</th>
<th>10\textsuperscript{th} day</th>
<th>20\textsuperscript{th} day</th>
<th>30\textsuperscript{th} day</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>6</td>
<td>14.13\textsuperscript{a} ± 0.95</td>
<td>13.76\textsuperscript{a} ± 0.91</td>
<td>11.53\textsuperscript{b} ± 0.33</td>
<td>10.23\textsuperscript{b} ± 0.44</td>
</tr>
<tr>
<td>II</td>
<td>6</td>
<td>14.08 ± 1.74</td>
<td>14.36 ± 0.95</td>
<td>13.15 ± 1.48</td>
<td>12.68 ± 1.01</td>
</tr>
<tr>
<td>III</td>
<td>6</td>
<td>13.43 ± 0.71</td>
<td>14.56 ± 0.89</td>
<td>15.00 ± 0.99</td>
<td>14.18 ± 1.05</td>
</tr>
</tbody>
</table>

Figures with different superscript differ significantly.

In group I, the mean total leucocyte count on 0\textsuperscript{th} day was 14.13 ± 0.95 thousands/cumm, which showed significant decreasing trend up to 30\textsuperscript{th} day and the value was 10.23 ± 0.44 thousands/cumm. While in group II, the mean value on 0\textsuperscript{th} day was 14.08 ± 1.74 thousands/cumm. The value increased on 10\textsuperscript{th} day and subsequently showed the declining trend up to 30\textsuperscript{th} day and the value on 30\textsuperscript{th} day was 12.68 ± 1.01 thousands/cumm. However, non significant increase was observed up to 20\textsuperscript{th} day in group III. The value decreases thereafter on 30\textsuperscript{th} day and was 14.18 ± 1.05 thousands/cumm.

These findings are in agreement with Medway et al. (1969), Benjamin (2010), Arrington et al. (1994) and Todorova et al. (2005).

Dobson and Gorman (1993) reported that cytotoxic drugs suppress the replicating precursor cells of the bone marrow resulting in reduced production of leucocytes. In the present study, this declining trend is attributed by a myelosuppressive action of doxorubicin. Therefore, it could be stated that the intravenous doxorubicin therapy would induced leucocytopenia, whereas; intratumoural doxorubicin therapy does not have any significant effect on mean total leucocyte count.

4.3.4 **Differential leucocyte count**

The differential leucocyte count was recorded in all the animals on 0, 10\textsuperscript{th}, 20\textsuperscript{th} and 30\textsuperscript{th} day.
Fig. 9. Mean value of total leucocyte count (thousand/cumm) in different treatment groups

Fig. 10. Mean value of neutrophil count (per cent) in different treatment groups
4.3.4.1. Neutrophil (%)

In the present study, neutrophil count was recorded in all the animals on 0, 10th, 20th and 30th day. The mean values are depicted in Table 6 and graphically presented in Figure 10.

Table 6: Mean ± S.E. of neutrophil (%) recorded in different treatment groups

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>0th day</th>
<th>10th day</th>
<th>20th day</th>
<th>30th day</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>6</td>
<td>60.17a ± 3.24</td>
<td>55.33ab ± 3.54</td>
<td>48.67b ± 2.76</td>
<td>47.00b ± 2.08</td>
</tr>
<tr>
<td>II</td>
<td>6</td>
<td>70.18 ± 1.72</td>
<td>67.66 ± 1.92</td>
<td>70.18 ± 1.80</td>
<td>70 ± 1.61</td>
</tr>
<tr>
<td>III</td>
<td>6</td>
<td>79.17 ± 2.23</td>
<td>81.33 ± 3.33</td>
<td>77.33 ± 2.64</td>
<td>78.00 ± 2.93</td>
</tr>
</tbody>
</table>

Figures with different superscript differ significantly

In group I, significant decrease in neutrophil count was observed. The mean neutrophill count recorded on day 0 was 60.17a ± 3.24 per cent and was decreased up to 47.00b ± 2.08 per cent on 30th day. However, in group II, the mean neutrophil count showed non-significant changes and was 70.18 ± 1.72 per cent on day 0, which decreases up to 20th day. The value thereafter slightly increases on 30th day and was 70.00 ± 1.61 per cent.

The mean neutrophil count in group III showed non-significant and undulating trend during the observation period. The mean value on 0 day was 79.17 ± 2.23 per cent. The mean values of neutrophil count were observed within the normal physiological limits.

The results in group I and II were in agreement with the findings of Benjamin (2010), Dobson and Gorman (1993) and Kerr (2002).

Kerr (2002) reported that neutropenia occur due to decreased bone marrow production, bone marrow aplasia, anaemia, thrombocytopenia and due to treatment with cytotoxic anticancer drugs. Invariably neutrophils are generated in bone marrow. However, in response to the bacterial infection, first body guard cells are liberated to blood stream.

In the present study, there was no bacterial infection but the chemotherapeutic agent doxorubicin was administered 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 doxorubicin therapy would induced neutropenia, whereas; intratumoural doxorubicin therapy does not have any significant effect on mean neutrophil count.

In group III, surgical trauma incited inflammation at the site of surgery may lead to an elevated neutrophil percentage. However, addition of antibiotic Amoxycillin - cloxacillin at the rate of 20 mg/kg body weight combated secondary bacterial infection and wound was maintain in fully aseptic condition without much of inflammatory changes therefore neutrophil percentage remain within normal range.

4.3.4.2. **Lymphocyte (%)**

In the present study, lymphocyte count was recorded in all the animal's on 0, 10th, 20th and 30th day. The mean values are depicted in Table 7 and graphically presented in Figure 11.

**Table 7: Mean ± S.E. of lymphocyte (%) recorded in different treatment groups**

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>0th day</th>
<th>10th day</th>
<th>20th day</th>
<th>30th day</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>6</td>
<td>35.00 ± 2.35</td>
<td>36.33 ± 3.24</td>
<td>44.33 ± 280</td>
<td>45.33 ± 1.82</td>
</tr>
<tr>
<td>II</td>
<td>6</td>
<td>22.67 ± 2.20</td>
<td>25.67 ± 2.50</td>
<td>28.50 ± 2.39</td>
<td>25.66 ± 1.68</td>
</tr>
<tr>
<td>III</td>
<td>6</td>
<td>13.83 ± 1.92</td>
<td>13.50 ± 3.25</td>
<td>19.33 ± 2.64</td>
<td>16.16 ± 2.68</td>
</tr>
</tbody>
</table>

*Figures with different superscript differ significantly*

In group I, the mean lymphocyte percentage on 0 day was 35.00 ± 2.35 per cent which showed significant increase throughout the observation period. The lymphocyte count on 30th day was 45.33 ± 1.82 per cent. In group II, the mean lymphocyte count showed non-significant changes and the value on 0 day was 22.67 ± 2.20 per cent, which showed inclining trend up to 20th day. The value on 30th day decreased slightly and was 25.66 ± 1.68 per cent. However, this increase in mean lymphocyte count was within the normal physiological range. Similar observations were reported by Benjamin (2010), Talekar (2001), Gadmade (2006) indicating a strong immunoresponse in animals.
Fig. 11. Mean value of lymphocyte count (per cent) in different treatment groups

Fig. 12. Mean value of monocyte count (per cent) in different treatment groups
The mean lymphocyte count in group III showed non-significant and undulating trend during the observation period. The mean value was 13.83 ± 1.92 per cent and 16.16 ± 2.68 on 0 and 30th day, respectively. Sivakumar (2000) claimed that it might be associated with surgical stress. In the present study, aseptic surgical procedure and addition of Amoxicillin-cloxacillin revealed slight inflammatory changes, which has also reduce the level of prostaglandin collagenase, leucocyte migration and suppress leucocyte super oxide production and hence the lymphocyte count was observed within the normal physiological limits.

4.3.4.3. Monocyte (%)

In the present study, monocyte count was recorded in all the animals on 0, 10th, 20th and 30th day. The mean values are depicted in Table 8 and graphically presented in Figure 12.

Table 8: Mean ± S.E. of monocyte (%) recorded in different treatment groups

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>0th day</th>
<th>10th day</th>
<th>20th day</th>
<th>30th day</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>6</td>
<td>3.00 ± 0.68</td>
<td>5.33 ± 1.45</td>
<td>3.33 ± 0.61</td>
<td>3.66 ± 0.80</td>
</tr>
<tr>
<td>II</td>
<td>6</td>
<td>4.66 ± 1.05</td>
<td>4.83 ± 1.32</td>
<td>2.00 ± 0.51</td>
<td>3.00 ± 1.29</td>
</tr>
<tr>
<td>III</td>
<td>6</td>
<td>3.83 ± 0.47</td>
<td>2.66 ± 0.79</td>
<td>1.50 ± 0.76</td>
<td>3.50 ± 1.38</td>
</tr>
</tbody>
</table>

In group I, the mean monocyte count showed non-significant changes and the value on 0 day was 3.00 ± 0.68 per cent. On 10th day the value elevated and was 5.33 ± 1.45 per cent. In group II, the mean monocyte count on 0 day was 4.66 ± 1.05 per cent, which showed undulating trend and the value on 30th day was 3.00 ± 1.29 per cent. The observations in group I were in agreement with the findings of Benjamin (2010). Similar observations were also reported by Talekar (2001) and Kerr (2002).

In the present study, increase in mean monocyte count in group was associated with corresponding neutropenia and lymphocytosis. Therefore, it could be stated that addition of chemotherapeutic agent in blood stream might have elevated monocyte percentage.
Fig. 13. Mean value of eosinophil count (per cent) in different treatment groups

![Bar chart showing eosinophil count across different treatment groups and days.]

Fig. 14. Mean value of total platelet count (lakhs/cumm) recorded in different treatment groups

![Bar chart showing total platelet count across different treatment groups and days.]

Group III animals did not show much alteration in the mean monocyte count. The mean value on 0 day was 3.83 ± 0.47 per cent. There was undulating irregular trend. Statistically values were not found significant and values of the mean monocyte percentage were found within normal physiological range. Similar observation was reported by Talekar (2001) and Gadmade (2006).

4.3.4.4. **Eosinophil (%)**

In the present study, non-significant changes were recorded in all the animals on 0, 10\textsuperscript{th}, 20\textsuperscript{th} and 30\textsuperscript{th} day in eosinophil count when compared within the group. The normal eosinophil count ranges from 2 to 10 per cent (Chauhan and Agarwal, 2008). During the present study, the values were observed within the normal physiological limit. The mean values are depicted in Table 9 and graphically presented in Figure 13.

**Table 9: Mean ± S.E. of eosinophil (%) recorded in different treatment groups**

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>0\textsuperscript{th} day</th>
<th>10\textsuperscript{th} day</th>
<th>20\textsuperscript{th} day</th>
<th>30\textsuperscript{th} day</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>6</td>
<td>2.16 ± 0.47</td>
<td>3.00 ± 0.73</td>
<td>3.00 ± 0.95</td>
<td>4.00 ± 0.03</td>
</tr>
<tr>
<td>II</td>
<td>6</td>
<td>2.50 ± 0.56</td>
<td>1.83 ± 0.30</td>
<td>2.33 ± 0.66</td>
<td>1.33 ± 0.21</td>
</tr>
<tr>
<td>III</td>
<td>6</td>
<td>3.16 ± 0.87</td>
<td>2.33 ± 0.84</td>
<td>1.83 ± 0.60</td>
<td>2.33 ± 0.88</td>
</tr>
</tbody>
</table>

In group I, the mean eosinophil count recorded on 0 day was 2.16 ± 0.47 per cent. The values were increased on 10\textsuperscript{th} day. The values thereafter showed undulating trend. In group II, the mean eosinophil count recorded on 0 day was 2.50 ± 0.56 per cent, which decreases on 10\textsuperscript{th} day. The values thereafter showed undulating trend in both group I and II. In group III, the mean eosinophil count on 0 day was 3.16 ± 0.87 per cent, which showed undulating trend. No specific reason could be attributed to non-significant changes in mean eosinophil count during the present study.

4.3.5 **Platelet count (lakhs/cumm)**

The total platelet count (lakhs/cumm) was recorded in all the animals on 0, 10\textsuperscript{th}, 20\textsuperscript{th} and 30\textsuperscript{th} day. The mean values are depicted in Table 10 and graphically presented in Figure 14.
Table 10: Mean ± S.E. of total platelet count (lakhs/cumm) recorded in different treatment groups

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>0\textsuperscript{th} day</th>
<th>10\textsuperscript{th} day</th>
<th>20\textsuperscript{th} day</th>
<th>30\textsuperscript{th} day</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>6</td>
<td>4.05\textsuperscript{a} ± 0.32</td>
<td>3.59\textsuperscript{ab} ± 0.21</td>
<td>2.83\textsuperscript{b} ± 0.24</td>
<td>1.72\textsuperscript{c} ± 0.30</td>
</tr>
<tr>
<td>II</td>
<td>6</td>
<td>4.47 ± 0.32</td>
<td>4.28 ± 0.34</td>
<td>4.01 ± 0.32</td>
<td>4.28 ± 0.29</td>
</tr>
<tr>
<td>III</td>
<td>6</td>
<td>4.31 ± 0.55</td>
<td>4.01 ± 0.47</td>
<td>4.03 ± 0.50</td>
<td>3.85 ± 0.63</td>
</tr>
</tbody>
</table>

Figures with different superscript differ significantly

In group I, the mean platelet count on 0 day was 4.05 ± 0.32 lakhs/cumm. The value decreased on 10\textsuperscript{th} day i.e. 3.59 ± 0.21 lakhs/cumm. This decrease in mean platelet count was found statistically significant, while the values again decreased on 20\textsuperscript{th} day i.e. 2.83 ± 0.24 lakhs/cumm, and on 30\textsuperscript{th} day i.e. 1.72 ± 0.30 lakhs/cumm. These decreased values of mean platelet count were found statistically highly significant but within the normal physiological range. Similar observations were reported by Tilmant \textit{et al.} (1986) and Gadmade (2006). Benjamin (2010) reported thrombocytopenia due to myelosuppressive drugs like adriamycin. Dobson and Gorman (1993) stated that cytotoxic drugs suppress the replicating precursor cells of the bone marrow resulting in reduced production of platelets.

In group II, the mean platelet count showed non-significant changes and was 4.47 ± 0.32 lakhs/cumm on 0 day. The value decreases up to 20\textsuperscript{th} day and was 4.01 ± 0.32 lakhs/cumm. The value thereafter increases slightly and was recorded as 4.28 ± 0.29 lakhs/cumm on 30\textsuperscript{th} day. In the present study, non-significant decrease up to 20\textsuperscript{th} day could be attributed to suppression of replicating precursor cells of the bone marrow resulting in reduced production of platelets. It can be stated that the intravenous administration of doxorubicin results in more reduction of platelet production than intratumoral administration of doxorubicin, indicating the lesser toxic effect of the intratumoral route of administration.

Animals in group III did not show much alteration in the mean platelet count. The mean value on 0 day was 4.31 ± 0.55 lakhs/cumm and the values thereafter revealed irregular trend. The changes in the mean total platelet count were non-significant. However, these values were within the normal physiological range.
4.4 Biochemical Parameter

4.4.1 Serum glutamic oxaloacetate transaminase (IU/L)

The mean values of serum glutamic oxaloacetate transaminase of various treatments were recorded in all the animals on 0, 10th, 20th and 30th day and are presented in Table 11 and Figure 15.

Table 11: Mean ± S.E. of serum glutamic oxaloacetate transaminase (IU/L) recorded in different treatment groups

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>0th day</th>
<th>10th day</th>
<th>20th day</th>
<th>30th day</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>6</td>
<td>27.39 ± 3.04</td>
<td>29.22 ± 2.68</td>
<td>32.47 ± 3.01</td>
<td>39.33 ± 2.00</td>
</tr>
<tr>
<td>II</td>
<td>6</td>
<td>26.22 ± 3.42</td>
<td>26.88 ± 3.35</td>
<td>26.54 ± 3.22</td>
<td>27.00 ± 3.04</td>
</tr>
<tr>
<td>III</td>
<td>6</td>
<td>26.29 ± 1.73</td>
<td>26.53 ± 1.79</td>
<td>25.99 ± 1.94</td>
<td>26.61 ± 1.61</td>
</tr>
</tbody>
</table>

Figures with different superscript differ significantly

In group I, the mean serum glutamic oxaloacetate transaminase on 0 day was 27.39 ± 3.04 IU/L which showed significant inclining trend throughout the observation period. The values on 30th day were 39.33b ± 2.00 IU/L. However, this increase in mean values of serum glutamic oxaloacetate transaminase was within the normal physiological range. Similar observations were reported by Pond and Morrow (1982), Brar et al. (2002), Todorova et al. (2005), Gadmade (2006) and Benjamin (2010) who reported non-significant changes in Serum glutamic oxaloacetate transaminase.

However, in group II, the mean serum glutamic oxaloacetate transaminase on 0 day was 26.22 ± 3.42 IU/L. The values showed slightly inclining trend thereafter. The value on 30th day was 27.00 ± 3.04 IU/L. This increase in mean serum glutamic oxaloacetate transaminase values were found statistically non-significant and within the normal physiological range.

Medway et al., (1969) and Benjamin (2010) attributed that increase in Serum glutamic oxaloacetate transaminase in hepatocellular disease and skeletal and cardiac oxaloacetate transaminase in hepatocellular disease and skeletal and cardiac muscle necrosis in the present study doxorubicin get deposited in bone marrow and detoxified by liver, therefore liver was loaded much for secretion of transaminase. It has also caused the regression of growth and
Fig. 15. Mean value of Serum glutamic oxaloacetate transaminase (IU/L) recorded in different treatment groups

Fig. 16. Mean value of serum glutamic pyruvic transaminase (IU/L) recorded in different treatment groups
degeneration of muscle fiber including cardiac muscles, thus the level of serum glutamic oxaloacetate transaminase was increased.

From the present study, it can be stated that deposition of doxorubicin in the bone marrow was less when given by intratumoral route than intravenous route, suggesting that there is less absorption of the doxorubicin in blood stream and less ill effect on hepatic system by intratumoral administration of doxorubicin.

The group III did not show much alterations in the mean serum glutamic oxaloacetate transaminase. The mean serum glutamic oxaloacetate transaminase on 0 day was 26.29 ± 1.73 IU/L. The mean values revealed irregular trend and was found statistically non-significant. However, the values were within the normal physiological limits. No specific reason could be attributed to non-significant changes in group III animals.

### 4.4.2 Serum glutamic pyruvic transaminase (IU/L)

The mean values of serum glutamic pyruvic transaminase of various treatments were recorded in all the animals on 0, 10th, 20th and 30th day and are presented in Table 12 and Figure 16.

**Table 12: Mean ± S.E. of serum glutamic pyruvic transaminase (IU/L) recorded in different treatment groups**

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>0th day</th>
<th>10th day</th>
<th>20th day</th>
<th>30th day</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>6</td>
<td>36.98 ± 5.54</td>
<td>39.80 ± 6.95</td>
<td>46.56 ± 5.57</td>
<td>53.75 ± 5.29</td>
</tr>
<tr>
<td>II</td>
<td>6</td>
<td>44.33 ± 11.62</td>
<td>56.16 ± 9.96</td>
<td>49.83 ± 10.29</td>
<td>41.33 ± 10.92</td>
</tr>
<tr>
<td>III</td>
<td>6</td>
<td>23.81 ± 2.88</td>
<td>23.35 ± 2.56</td>
<td>25.38 ± 2.98</td>
<td>26.33 ± 3.39</td>
</tr>
</tbody>
</table>

In group I, the mean serum glutamic pyruvic transaminase recorded on 0 day was 36.98 ± 5.54 IU/L. The value thereafter showed continuous inclining trend upto 30th day. The value on 30th day was 53.75 ± 5.29 IU/L. This increase in mean serum glutamic pyruvic transaminase was found statistically non-significant but within the normal physiological limits. Similar findings were reported by Todorova et al., (2005) and Gadmade (2006).

In group II, the mean serum glutamic pyruvic transaminase recorded on 0 day was 44.33 ± 11.62 IU/L. The mean value on 10th day was found increased
i.e. 56.16 ± 9.96 IU/L. Thereafter the mean value showed declining trend up to 30th day. The value on 30th day was 41.33 ± 10.92 IU/L.

Brar et al. (2002) reported that serum glutamic pyruvic transaminase enzyme was present in the hepatocytes cytoplasm of dog in large quantities, also known as liver specific enzyme. Other sources of serum glutamic pyruvic transaminase include cardiac, skeletal muscles, pancreas and renal cells. Damage to these tissues due to chemotherapeutic agent results in higher values of serum glutamic pyruvic transaminase.

In the present study, the increase in serum glutamic pyruvic transaminase values in group I could be attributed to the damage to cardiac, skeletal muscles, pancreas and renal cells due to doxorubicin. However, it can also be stated that the intratumoral administration of doxorubicin has less damage to these cells as compared to intravenous administration, suggesting that intratumoral route is safer route of administration because of its less ill effects.

In group III, the mean serum glutamic pyruvic transaminase values did not showed much alteration. The value on 0 day was 23.81 ± 2.88 IU/L, which showed undulating trend thereafter. The value on 30th day was 26.33 ± 3.39 IU/L. The mean values were within the normal physiological limits. No specific reason could be attributed to non-significant changes in group III animals.

4.4.3 **Serum glucose (mg/dl)**

The mean values of Serum glucose of various treatments were recorded in all the animals on 0, 10th, 20th and 30th day and are presented in Table 13 and Figure 17.

**Table 13: Mean ± S.E. of serum glucose (mg/dl) recorded in different treatment groups**

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>0th day</th>
<th>10th day</th>
<th>20th day</th>
<th>30th day</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>6</td>
<td>79.33 ± 4.68</td>
<td>81.33 ± 3.89</td>
<td>85.83 ± 3.97</td>
<td>92.66 ± 6.03</td>
</tr>
<tr>
<td>II</td>
<td>6</td>
<td>70.00 ± 4.94</td>
<td>70.50 ± 4.78</td>
<td>76.33 ± 4.17</td>
<td>72.83 ± 4.74</td>
</tr>
<tr>
<td>III</td>
<td>6</td>
<td>74.00 ± 4.71</td>
<td>74.33 ± 4.18</td>
<td>79.50 ± 5.75</td>
<td>74.66 ± 4.50</td>
</tr>
</tbody>
</table>
In group I, the mean serum glucose on 0 day was 79.33 ± 4.68 mg/dl. The mean values thereafter showed continuous inclining trend throughout the observation period. The value on 30th day was 92.66 ± 6.03 mg/dl. The increase in the mean values of serum glucose was non-significant. In group II, the mean serum glucose value on 0 day was 70.00 ± 4.94 mg/dl, which showed undulating trend thereafter. The value on 30th day was 72.83 ± 4.74 mg/dl. Similar observations were recorded by Pond and Morrow (1982) and Gadmade (2006).

In the present study, administration of chemotherapeutic agent doxorubicin would create stress on the body which would stimulate the adrenal cortex to secrete glucocorticoids and mineralocorticoids and medulla secretes epinephrine and nor-epinephrine. This release of epinephrine, nor-epinephrine, glucocorticoids, mineralocorticoids and adrenaline are responsible for increase in glucose level.

In group III, the mean serum glucose value on 0 day was 74.00 ± 4.71 mg/dl. The value showed inclining trend up to 20th day and decreases thereafter near to normal on 30th day. The value on 30th day was 74.66 ± 4.50 mg/dl. However, the changes in the mean serum glucose value during the study were statistically non-significant and the values were within the normal physiological limit. No specific reason could be attributed to non-significant changes in group III animals.

4.4.3 Blood urea nitrogen (mg/dl)

The mean values of blood urea nitrogen of various treatments were recorded in all the animals on 0, 10th, 20th and 30th day and are presented in Table 14 and Figure 18.

Table 14: Mean ± S.E. of blood urea nitrogen (mg/dl) recorded in different treatment groups

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>0th day</th>
<th>10th day</th>
<th>20th day</th>
<th>30th day</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>6</td>
<td>23.33 ± 2.97</td>
<td>24.00 ± 2.62</td>
<td>23.50 ± 2.70</td>
<td>28.66 ± 3.27</td>
</tr>
<tr>
<td>II</td>
<td>6</td>
<td>12.83 ± 2.07</td>
<td>14.16 ± 2.18</td>
<td>13.50 ± 2.30</td>
<td>13.25 ± 2.63</td>
</tr>
<tr>
<td>III</td>
<td>6</td>
<td>19.50 ± 1.83</td>
<td>18.83 ± 1.90</td>
<td>18.50 ± 2.30</td>
<td>19.00 ± 1.89</td>
</tr>
</tbody>
</table>
In group I, the mean blood urea nitrogen on 0 day was 23.33 ± 2.97 mg/dl which increases on 10th day. The value thereafter showed undulating trend and the mean value on 30th day was 28.66 ± 3.27 mg/dl. This increase in mean blood urea nitrogen was statistically non-significant.

In group II, the mean blood urea nitrogen on 0 day was 12.83 ± 2.07 mg/dl. The mean value was increased on 10th day and the value was 14.16 ± 2.18 mg/dl. The mean value thereafter showed declining trend and the value on 30th day was 13.25 ± 2.63 mg/dl.

However, the changes in mean blood urea nitrogen were within the normal physiological range. Similar observations were reported by Pond and Morrow (1982) and Shepelevtseva et al. (1986). Todorova et al. (2005) reported non-significant changes in blood urea nitrogen. Benjamin (2010) reported that increase values of blood urea nitrogen are due to shock and pseudo hyperparathyroidism.

Pond and Morrow (1982) reported that the elevated blood urea nitrogen was due to renal failure. Coles (1986) reported that urea nitrogen level in the blood are affected not only by alterations in renal function, but may be altered by certain physiologic factors or diseases not primarily of renal origin. In the present study, in group I and II, chemotherapeutic agent i.e. Doxorubicin being a cytotoxic catabolic agent which causes breakdown of protein within body and presence of malignancy thus urea concentration of blood was increase.

In group III, the mean value did not show much alteration in the mean blood urea nitrogen. The mean blood urea nitrogen value on 0 day was 19.50±1.83 mg/dl. The mean value thereafter showed declining trend up to 20th day and then slightly increases on 30th day and the value on 30th day was 19.00 ± 1.89 mg/dl.

Statistically values were found non-significant but within the normal physiological range. No specific reason could be attributed to non-significant changes in group III animals.
4.4.3 Serum creatinine (mg/dl)

The mean values of serum creatinine of various treatments were recorded in all the animals on 0, 10\textsuperscript{th}, 20\textsuperscript{th} and 30\textsuperscript{th} day and are presented in Table 15 and Figure 19.

\begin{table}[h]
\centering
\begin{tabular}{|c|c|c|c|c|}
\hline
Group & n & 0\textsuperscript{th} day & 10\textsuperscript{th} day & 20\textsuperscript{th} day & 30\textsuperscript{th} day \\
\hline
I & 6 & 1.17 ± 0.12 & 1.16 ± 0.12 & 1.35 ± 0.18 & 1.41± 0.20 \\
II & 6 & 1.11 ± 0.20 & 1.14 ± 0.20 & 1.17 ± 0.18 & 1.13 ± 0.19 \\
III & 6 & 0.95 ± 0.20 & 0.99 ± 0.20 & 1.00 ± 0.21 & 1.05 ± 0.22 \\
\hline
\end{tabular}
\caption{Mean ± S.E. of serum creatinine (mg/dl) recorded in different treatment groups}
\end{table}

In group I, the mean serum creatinine value on 0 day was recorded as 1.17 ± 0.12 mg/dl. The mean value on 10\textsuperscript{th} day was slightly decreases. The mean value thereafter showed increasing trend up to 30\textsuperscript{th} day and the value on 30\textsuperscript{th} day was 1.41± 0.20 mg/dl.

In group II, the mean serum creatinine value recorded on 0 day was 1.11 ± 0.20 mg/dl, which showed slightly inclining trend up to 20\textsuperscript{th} day. The value on 30\textsuperscript{th} day was decreased and was 1.13 ± 0.19 mg/dl. The changes in mean serum creatinine were found statistically non-significant and the values were within the normal physiological limits.

Similar findings were reported by Gadmade (2006). Hurria \textit{et al.} (2005) reported that increase in serum creatinine level was associated with increase in risk of fever. However, Pond and Morrow (1982) and Medway \textit{et al.} (1969) reported that the elevated level of serum creatinine was due to renal failure.

In the present study, increase in serum creatinine could be related with catabolic action of chemotherapeutic agent. Cytotoxic and catabolic activity would increase the level of creatinine. Therefore, it is stated that administration of chemotherapeutic agent - Doxorubicin would increase creatinine level.

In group III, the mean serum creatinine value recorded on 0 day was 0.95 ± 0.20 mg/dl. The mean value thereafter showed steady and continuous increasing trend throughout the observation period.
Fig. 19. Mean value of serum creatinine (mg/dl) recorded in different treatment groups

<table>
<thead>
<tr>
<th>Treatment groups</th>
</tr>
</thead>
<tbody>
<tr>
<td>0th day</td>
</tr>
</tbody>
</table>

Fig. 20. Mean value of alkaline phosphatase (u/l) recorded in different treatment groups

<table>
<thead>
<tr>
<th>Treatment groups</th>
</tr>
</thead>
<tbody>
<tr>
<td>0th day</td>
</tr>
</tbody>
</table>
4.4.3 **Alkaline Phosphatase (U/l)**

The mean values of alkaline phosphatase were recorded in all the animals on 0, 10th, 20th and 30th day and are presented in Table 16 and Figure 20.

**Table 16: Mean ± S.E. of alkaline phosphatase (U/l) recorded in different treatment groups**

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>0th day</th>
<th>10th day</th>
<th>20th day</th>
<th>30th day</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>6</td>
<td>117.50 ± 11.19</td>
<td>177.83 ± 16.65</td>
<td>273.33 ± 36.41</td>
<td>371.00 ± 52.30</td>
</tr>
<tr>
<td>II</td>
<td>6</td>
<td>102.85 ± 12.63</td>
<td>107.33 ± 12.23</td>
<td>127.58 ± 16.00</td>
<td>132.66 ± 16.36</td>
</tr>
<tr>
<td>III</td>
<td>6</td>
<td>99.16 ± 9.45</td>
<td>113.00 ± 11.67</td>
<td>127.50 ± 19.98</td>
<td>137.50 ± 20.66</td>
</tr>
</tbody>
</table>

Figures with different superscript differ significantly.

In group I, the mean alkaline phosphatase recorded on 0 day was 117.5 ± 11.19 (U/l). The mean value significantly increases throughout the observation period. The mean value on 30th day was 371.00 ± 52.30 (U/l). In group II, the mean alkaline phosphatase value recorded on 0 day was 102.85 ± 12.63 (U/l). The value thereafter showed continuous increasing trend up to 30th day and the value on 30th day was 132.66 ± 16.36 (U/l). The changes in the mean alkaline phosphatase in group II were found statistically non-significant.

Alkaline phosphatase activity increases in sarcoma and carcinoma (Chauhan and Agarwal, 2008). The increase in the alkaline phosphatase level observed during the present study could be attributed to presence of malignancy and addition of chemotherapy induced stress, which is intensely reflected by significant rise in alkaline phosphatase activity in group I.

In group III, the mean alkaline phosphatase recorded on 0 day was 99.16 ± 9.45 (U/l). The mean value thereafter showed inclining trend throughout the observation period. The mean value recorded on 30th day was 137.50 ± 20.66 (U/l). These changes in the mean alkaline phosphatase were found statistically non-significant; however, the values were within the normal physiological range.
### 4.4 Regression of Growth

<table>
<thead>
<tr>
<th>Animal No.</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Regression</td>
<td>Symptoms associated with toxicity</td>
<td>Regression</td>
</tr>
<tr>
<td>1.</td>
<td>++</td>
<td>V A</td>
<td>++</td>
</tr>
<tr>
<td>2.</td>
<td>+</td>
<td>V AL</td>
<td>++</td>
</tr>
<tr>
<td>3.</td>
<td>++</td>
<td>A AL</td>
<td>+</td>
</tr>
<tr>
<td>4.</td>
<td>+++</td>
<td>V A AL</td>
<td>+++</td>
</tr>
<tr>
<td>5.</td>
<td>++</td>
<td>AL</td>
<td>+++</td>
</tr>
<tr>
<td>6.</td>
<td>+</td>
<td>A</td>
<td>++</td>
</tr>
</tbody>
</table>

+ - Minimum regression  
++ - Moderate regression  
+++ - Complete regression  

V - Vomition  
A - Anorexia  
AL - Alopecia

Tumour size is one of the most important characteristics in prognostic studies. The size of the tumour was measured at different scheduled interval with the help of Vernier caliper and the regression study was carried out in all the cases of Group I and Group II.

In group I, mild regression was observed in 2 (33.33 %) cases; whereas moderate regression was observed in 3 (50.00 %) cases and complete regression was observed in only 1 (16.66 %) case (Plates 13-18).

In group II, mild regression was observed in 1 (16.66 %) case; whereas moderate regression was noticed in 3 (50.00 %) cases and complete regression was observed in 2 (33.33 %) cases (Plates 19-24). Philip et al. (2013) also reported regression of tumours including mammary tumours with doxorubicin intratumoural therapy without any side effects.

The study revealed gross reduction in size i.e. length and width of tumour in all cases after intravenous or intratumoral injections with a gradual sloughing and reduction in the vascularity of the tumour mass. However, the cytotoxic effects on the body were also noticed in terms of alopecia, vomition and anorexia only in Group I. Therefore could be stated that the intratumoral injection of iron oxide nanoparticles- doxorubicin complex can be used as an alternative option in selected cases of tumour where radical surgery is at a risk and systemic chemotherapy is not advisable due to poor health status.
Regression of tumour due to Doxorubicin therapy: Group I

Plate 13. Day 0

Plate 14. Day 7

Plate 15. Day 14

Plate 16. Day 21

Plate 17. Day 28

Plate 18. Day 30
Regression of tumour due to Doxorubicin-complex therapy: Group II

Plate 19. Day 0
Plate 20. Day 7
Plate 21. Day 14
Plate 22. Day 21
Plate 23. Day 28
Plate 24. Day 30
It could be stated that even if the surgical removal is indicated, the risk involved can be reduced by initial therapy with intratumoral administration of chemotherapeutic agent. This will also reduce the size and separation of attachments of the tumour with the underlying bony structures where by the surgery become comparatively easy.

4.5 Recurrence / Metastasis

Follow up is one of the most important variables in studying the prognostic studies, which was based on periodic clinical examinations. According to Matos et al. (2012), if any abnormality is detected during these examinations, all available tests should be performed, 5% of detectable lung masses are either non-neoplastic lesions or tumours of different origin (e.g. primary lung carcinoma). Therefore, whenever possible and ethically acceptable, biopsies should be used to confirm metastatic disease. Likewise, in all possible cases, the animals that die during the follow-up period must be submitted to complete necropsies with careful search for metastases.

The recurrence of growth was assessed by palpation of the site and metastasis was ascertained on the basis of thoracic radiographic examination. During the study, all the cases were followed up for 2 months after the completion of treatment for recurrence. The recurrence was not observed in group I and II animals; whereas, it was noted in 1 case in group III animal, where surgery was performed again to remove the tumour mass.

The metastasis was studied by thoracic radiography in all the animals on day 0. The metastatic lesions were not detected in any of the animals.

The overall results of the study suggests that the intratumoral injection of the antineoplastic drug doxorubicin complexed with iron oxide nanoparticles can be adopted as a palliative line of treatment for tumours in terminally ill and compromised patients where surgical treatment is risky or not advisable. This study being a preliminary clinical trial, further investigations are required before being introduced into the field of cancer therapy in dogs on a massive scale.
Response to treatment

The purpose of the study was to find out the efficacy of iron oxide nanoparticles – doxorubicin complex in clinical cases of superficial tumours. The local injection of iron oxide nanoparticles – doxorubicin complex intratumourly was found to produce local reaction at the site of injection followed by discoloration, necrosis, regression and detachment of the tumour tissue from muscle layer. The undesirable side effects noticed included unsightly tissue necrosis and oozing of tissue fluids. The reactions were similar in all cases except the degree of regression. The degree of regression was noticed in four cases out of six which indicated that the local treatment was effective with intratumoral injection of the complex followed by drug targeting into the tumour tissue. This was not consistent with treatment used by Jayakumar et al. (2009) in which the growth of tumour was found inhibited in mice administered with doxorubicin complexes orally during the period of administration as compared to untreated animals but was consistent to the finding by Jia et al. (2012) that the tumour growth rates in mice treated with iron oxide nanoparticles – doxorubicin orally with an external magnetic field was significantly decreased where as free doxorubicin treatment alone could only slightly reduced the tumour growth. The positive response to treatment includes regression in size, detachment and relative easiness in surgical management. The appearance of tissues at the site of reaction showed initial necrosis in and around the site of injection without any visible tissue changes in other places. The tumours showed a grey discoloration at the site of treatment. Goldberg et al. (2001) found that injection of intratumoral doxorubicin alone produced a patchy ill defined zone of coagulation that measured less than the size of tumour before therapy. However greater doses or increased in the volume of injection did not increase the diameter of coagulation. According to Alexiou et al. (2000) animals in the groups treated with ferrous fluids and a magnetic field developed a slight grey discoloration of the skin covering the tumour, in addition, scattered, dark injected vessels were seen in the tumour region. The grey discoloration, caused by the strong magnetic field strength which attracted the ferrous fluids throughout the whole tumour to this layer, was completely reversible and lasted for approximately 48 hours.

Smith et al. (1999) explained that direct intratumoral injection of anticancer drugs can be proposed as one of the treatment options for preventing
tumour recurrence. One potential advantage of this approach as compared with systemic administration was the possibility of achieving significantly higher local drug retention in the target tumour and circumventing various physiological barriers, such as simultaneous drug excretion during the drug transportation process. The use of magnetic nanoparticles tagged to anticancerous drugs like doxorubicin under an externally created magnetic field could make the drug targeting to the tumour cells directly. The observations in the present study also support this procedure.

The overall response of treatment protocol using iron oxide nanoparticles – doxorubicin complex intratumorally indicated that it is an effective modality in skin appendage tumours and papillary adenocarcinoma of mammary gland in canines. The study also suggests that the effectiveness of this protocol of other superficial tumours needs further investigations.

In the present study, the use of nanoparticles could reduce the therapeutic dose and reduce the systemic toxicity. This study suggests further refinement in the route of administration, frequency of treatment in tumour conditions which was found to respond to treatment. In future the treatment protocol can be recommended in superficial tumour confirmed by biopsy in canine.

In group III, all the cases of superficial tumour were subjected to surgical intervention under dissociative anaesthesia (Plates 25-30). The use of inj. Xylazine hydrochloride, inj. Atropine sulphate and inj. Betnesol at dose rate of 1mg/kg body weight through intramuscular route, 0.04 mg/kg body weight through subcutaneous route and 1 to 2 mg/kg body weight through intramuscular route, respectively, was found satisfactory. Anaesthesia was induced and maintained with inj. Ketamine at the rate of 5 to 10 mg/kg body weight and inj. Diazepam at the rate of 1 to 2 mg/kg body weight through intravenous route. The method of dissociative anaesthesia proved to be satisfactory for surgical excision without any complications. This anaesthesia was found more suitable with profound narcosis and degree of muscular relaxation. These findings are in agreement with the findings of Sharma et al. (1997).
Radicle surgery : Group III

Plate 25.
Plate 26.

Plate 27.
Plate 28.

Plate 29.
Plate 30.
4.5 Histopathological Examination

In the present study, the histopathological examination was carried out in all the cases and only after confirmation of malignancy, the cases were included in the study. Each biopsy sample was collected with the help of Bard- Max Core disposable biopsy instrument (Size: 18 guage x 16 cm length, Length of sample notch: 1.8 cm, Penetration depth: 22 mm) (Plate 4). In group I and II, the histopathological examination was again carried out on 30th day of treatment in Group I and Group II, to assess the regression of the tumour on the basis of histomorphological examination.

During the study, various types of tumours e.g. (8) fibrosarcoma, (2) basal cell carcinoma, (5) adenocarcinoma, papillary adenocarcinoma with local lymph node involvement, (2) schirrous adenocarcinoma and (1) mixed tumour were recorded (Plates 35-40).

In group I, histopathological examination on day 0 revealed basal cell carcinoma in two animals with proliferation of basal cells and fibrous connective tissue around it (Plate 31). After treatment-drastic reduction in neoplastic basal cell with huge proliferation of fibrous connective tissue and infiltration of neutrophils around the fibrous connective tissue was observed on day 30 (Plate 32).

In group II, histopathological examination revealed basal cell carcinoma with proliferation of basal cells with dense fibrous connective tissue around it (Plate 33). The histopathological examination on day 30 revealed reduction in multiplying basal cells with reduced nuclear chromacity. The pink homogenous mass indicated necrosis of the multiplying epithelial cells (Plate 34).

4.5.1 Fibrosarcoma

Tumours comprised of well differentiated, spindle shaped tumour cells arranged in interwoven or herringbone patterns, cytoplasm was scanty, and the inconspicuous nuclei were elongate to oval in shape. Highly anaplastic tumours had marked cellular pleomorphism, ovoid, polygonal and multinucleated giant cells with large round to oval nuclei and prominent nuclei was seen. The mitotic figures were frequent. Peripheral aggregates of lymphocyte are occasionally seen over end multiplying cells (Plate 35).
Plate 31. Section of growth from basal cell carcinoma with proliferation of basal cells (H & E, 10X)

Plate 33. Section of basal carcinoma surrounded by thick fibrous connective tissue (H & E, 10X)

Plate 32. Section of same growth after treatment with reminance of fibrous connective tissue (H & E, 10X)

Plate 34. Section of basal cell carcinoma with reduced number of multiplying cells (H & E, 10X)
4.5.2 Basal cell carcinoma

Basal cell carcinoma was extending from the basal cells of the epidermis into the dermis and subcutis, as cords and sheets of small, basophilic cells with hyperchromatic nuclei and little cytoplasm. The nuclei showed little pleomorphism. Necrosis was observed in the center of the invading cords and islands of tumour cells. The nuclei are ovoid with inconspicuous nucleoli, and the number of mitoses found was quite variable. Tumour cells showed no differentiation to squamous epithelium or adnexal structures. There was often a marked dermal fibroblast proliferation around tumorous cells in response to the infiltrating tumour cells (Plate 36).

4.5.3 Adenocarcinoma and Tubular adenocarcinoma

There was heavy proliferation of secretary epithelial cells which were mainly arranged in tubular fashion with minimal proliferation of myoepithelial and connective tissue cells. Present adenocarcinoma mainly composed of tubular epithelial cells with less to moderate proliferation of myoepithelial cells having vacuolated cytoplasm. There were solid sheets of polyhedral to spindle shaped cells having vacuolated cytoplasm. There was considerable deviation of mammary tissue architecture. Growth seemed to be mainly invasive type with neoplastic epithelial cells invading surrounding basement membrane and connective tissue. Neoplastic cells were loosely arranged indicating least cohesion between cells. Numerous pleomorphic neoplastic cells were present in tubular lumen. Mitotic figures were increased and inflammatory reaction mainly with neutrophilic and lymphocytic infiltration was observed in tumours (Plates 37-38).

4.5.4 Schirrous adenocarcinoma

There was heavy proliferation of secretary epithelial cells which were mainly arranged in tubular fashion with minimal proliferation of myoepithelial and connective tissue cells. Simple tubular adenocarcinoma mainly composed of tubular epithelial cells with less to moderate proliferation of myoepithelial cells having vacuolated cytoplasm. There were solid sheets of polyhedral to spindle shaped cells having vacuolated cytoplasm. There was considerable deviation of mammary tissue architecture. Growth seemed to be mainly invasive type with
neoplastic epithelial cells invading surrounding basement membrane and connective tissue. Neoplastic cells were loosely arranged indicating least cohesion between cells. Numerous pleomorphic neoplastic cells were present in tubular lumen. Mitotic figures were increased and inflammatory reaction mainly with neutrophilic and lymphocytic infiltration was observed in tumours. These observation were similar to the adenocarcinoma, however, there was huge proliferation of fibrous connective tissue separating multiplying neoplastic cells indicating the tumour to be schirrous adenocarcinoma (Plate 39).

4.5.5 Pappilary adenocarcinoma

Neoplastic cells were arranged in leaf like or papillary manner, the features of neoplastic cells were similar to tubular epithelial or cuboidal type of cells. Papillary growth of cells was mainly invasive with deranged architecture. Mitotic figures were prominent. Although the inflammatory reaction was seen, it was moderate with predominant lymphocytic and plasma cell infiltration. The local lymph-node revealed metastasis of tumour cells (Plate 40).

4.5.6 Malignant mixed tumour

Neoplastic growth composed of cells of all types of cells linage i.e. luminal epithelial, myoepithelial and connective tissue with their metaplastic growth as cartilage formation with mild degree of calcification. Architecture of tumour seemed to be highly invasive infiltrating the basement membrane. Mitotic figures were moderate along with cellular pleomorphism.
Chapter 5

SUMMARY & CONCLUSION
SUMMARY AND CONCLUSIONS

The study was carried out on 18 cases of dogs with malignant superficial tumour irrespective of age, sex and breed during the period of December 2013 to July 2014. The dogs were divided in three equal groups and were subjected to three different treatment regimens.

The dogs in group I were treated with doxorubicin hydrochloride at the dose rate of 30 mg/m² by slow intravenous route along with 150 ml of 5% dextrose saline solution with interval of 21 days. Group II dogs were administered with Iron oxide (Fe₃O₄) nanoparticle - Doxorubicin complex @ 3 mg/kg body weight intratumoural at 2 to 3 different sites at scheduled interval i.e. on 1st, 7th and 21st days; however, group III dogs were subjected to the radical surgery for excision of the tumour.

The regression of the tumour was assessed on the basis of various parameters. Also the age, breed, sex and duration of illness wise incidence were recorded during the study.

The age wise incidence of superficial tumour was recorded highest amongst older dogs above 8 years of age (94.44%). The highest incidence of superficial tumour was observed in non-descript (61.11%) followed by Pomeranian (16.66%), German shepherd (11.11%), Rottweiler and Labrador (5.55%) breeds of dog; whereas, sex wise incidence revealed higher incidence in female (55.55 %) as compare to male (44.44%).

The higher per cent of dogs with 6 months above (44.44 %) illness history was observed followed by 3 to 6 months (33.33 %) and below 3 months (22.22%).

During the study, the location and size of tumour was also recorded. The higher percent of the tumour was observed on caudal abdominal region (27.77%) followed by limb and thoracic region (22.22%), cranial abdominal region (16.16%) and head region (11.11%). However, the tumour with more than 8 cm size was observed more (38.88%) as compare to 3 to 8 cm (27.77%) and less than 3 cm (33.33%).
During present investigation, in group I use of doxorubicin was found to be effective in four cases (66.66%) with no recurrence and initiation of regression of tumour was observed in two cases along with side effects like anaemia, vomiting, anorexia, alopecia and dullness. In group II, the use of Iron oxide (Fe₃O₄) nanoparticle - Doxorubicin complex @ 3 mg/kg body weight intratumoural at 2 - 3 different sites at scheduled interval was found to be effective in five cases with success rate of 83.33% without any ill effect and recurrence was observed; whereas initiation of regression was observed in one case.

In group III, all the six clinical cases of superficial tumour were subjected to surgical intervention. The use of inj. xylazine hydrochloride, inj. atropine sulphate and inj. betnesol at the dose rate of 1 mg/kg body weight through intramuscular route, 0.04 mg/kg body weight through subcutaneous route and 1 to 2 mg/kg body weight through intramuscular route, respectively was found to be satisfactory. Anaesthesia was induced and maintained with inj. ketamine @ 5 to 10 mg/kg body weight and inj. diazepam @ 1-2 mg/kg body weight through intravenous route. The method of dissociative anaesthesia proved to be satisfactory for required surgical excision without any complications. This anaesthesia was found more suitable with profound narcosis and desired degree of muscular relaxation. The site/ location of tumour were varied and hence the surgical technique was used according to the site of tumour. Out of six cases, recurrence was recorded in only one case after two months of surgical excision.

The clinical haemato-biochemical observations were recorded at the scheduled interval of 0th, 10th, 20th and 30th day of observation in all three groups, while biopsy samples for confirmation of type of tumour before treatment and to study the regression on 30th day were collected from group I and group II dogs; whereas in group III, the biopsy samples were collected after surgical resection.

In the present study, haemoglobin showed a continuous and progressive decline in all the groups up to 30th day, except in group II where the value remains constant on 20th and 30th day. The significant decrease in packed cell volume was observed in group I; whereas undulating trend in group II and non-significant decreasing trend in group III was observed. Total erythrocyte count showed significant decrease in group I, and non-significant changes in group II and group III dogs.
During the study, total leucocyte count showed significant declining trend in group I; whereas, non-significant decrease in group II and undulating trend in group III were observed. Neutrophil percent recorded during the study showed significant decrease in group I, however non significant and undulating trend was observed in group II and III.

In group I, significant increasing trend i.e. lymphocytosis was observed; whereas non-significant and undulating trend was observed in group II and III. The monocyte and eosinophil count during the study revealed non-significant changes in all the groups, but the values were within the normal physiological range.

The total platelet count in group I showed significant declining trend i.e. thrombocytopenia; whereas in group II, non-significant decrease up to 20th day and in group III, non-significant undulating trend was observed.

Serum glutamic oxaloacetate transaminase values in group I showed significant inclining trend; however, in group II and III, non-significant and undulating trend was observed. Serum glutamic pyruvic transaminase and serum glucose revealed non-significant increasing trend in group I and non-significant undulating trend in group II and III. Blood urea nitrogen and serum creatinine showed non-significant changes in all the groups. However, significant increase in alkaline phosphatase in group I and non-significant increasing trend in group II and III was recorded.

During the study, the recurrence and metastasis was also studied. Recurrence of the tumour was observed in only one case of group III; whereas, no recurrence was observed in group I and II dogs. Metastasis was studied with the help of plain radiograph and no metastasis was observed in any of the case during the study.

During the study, various types of tumours e.g. (8) fibrosarcoma, (2) sclerous adenocarcinoma, (5) adenocarcinoma, (2) papillary adenocarcinoma with local lymph node involvement, (2) basal cell carcinoma and (1) mixed tumour were recorded.

In group I, histopathological examination on day 30 revealed drastic reduction in neoplastic basal cell with huge proliferation of fibrous connective
tissue and infiltration of neutrophils around the fibrous connective tissue was observed on day 30. In group II, histopathological examination on day 30 revealed reduction in multiplying basal cells with reduced nuclear chromacity.

The purpose of the study was to find out the efficacy of iron oxide nanoparticles-doxorubicin complex intratumoural and intravenous administration of doxorubicin and radical surgery in clinical cases of superficial tumours in dog. The intratumoural injection of iron oxide nanoparticles-doxorubicin complex was found to produce local reaction at the site of injection followed by discoloration, necrosis, regression and detachment of the tumour tissue from muscle layer. The undesirable side effects noticed included unsightly tissue necrosis and oozing of tissue fluids. The reactions were similar in all cases except the degree of regression. The positive response to treatment includes regression in size, detachment and relative easiness in surgical management. The appearance of tissues at the site of reaction showed initial necrosis in and around the site of injection without any visible tissue changes in other places.

The overall response of treatment protocol using iron oxide nanoparticles-doxorubicin complex intratumorally indicated that it is an effective modality in skin appendage tumours, squamous cell carcinoma and papillary adenocarcinoma in canines. The study also suggests that the effectiveness of this protocol of other superficial tumours needs further investigations.

CONCLUSIONS

1. The treatment protocol using iron oxide nanoparticles-doxorubicin complex intratumorally is an effective modality in tumours of skin appendage and papillary adenocarcinoma in dog without any adverse effect and much alterations in haemato-biochemical indices.

2. The intravenous administration of doxorubicin is effective for regression of basal cell carcinoma and adenocarcinoma but has various adverse effects on clinical and haemato-biochemical parameters. Radical surgery is effective for large sized superficial tumours but has limited utility in senile and poor anaesthetic risk patients.
(A)BIBLIOGRAPHY


(B) Vita
VITA

The author of this dissertation **Mr. Ingle Rupesh Sakharam**, born on 12th October, 1988 at Mangrulpur, Dist. Washim, Maharashtra state.

He has passed his S.S.C. examination with distinction from Jyoti Vidyalaya, Akola in the year 2004 and H.S.S.C. examination from Rajshree Shahu Junior College, Aurangabad in the year 2007.

Author joined Nagpur Veterinary College, Nagpur (Maharashtra Animal and Fishery Sciences University, Nagpur) in the year 2007 and successfully completed the Bachelor of Veterinary Science and Animal Husbandry (B.V.Sc. & A.H.) degree with first division in July 2012.

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Author has actively participated in National Service Scheme camping programme and participated in various animal treatment camps, animal birth control camps, anti-rabies vaccination camps and animal exhibitions held during the study. He has passed the N.C.C. “B” and “C” certificate examinations. He has actively participated in State level cultural programme – “Youth Festival” and represented the university basket ball team at state level event. The author is also a life member of the Indian Society for Veterinary Surgery.
(C) THESIS ABSTRACT
THESIS ABSTRACT

a) Title of thesis : STUDIES ON EFFICACY OF NANOPARTICULATE INDUCED DRUG DELIVERY SYSTEM FOR CHEMOTHERAPY OF SUPERFICIAL TUMOURS IN DOG

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e) Year of award of degree : 2014

f) Major subject : VETERINARY SURGERY AND RADIOLOGY

g) Total number of pages in the thesis : 61

h) Number of words in the thesis abstract : 513

i) Signature of student

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ABSTRACT

The study was carried out on 18 cases of dogs with malignant superficial tumour irrespective of age, sex and breed during the period of December 2013 to July 2014 and were divided in three equal groups.

The dogs in group I were treated with doxorubicin hydrochloride at the dose rate of 30 mg/m² by slow intravenous route along with 150 ml of 5% dextrose saline solution with interval of 21 days. Group II dogs were administered with Iron oxide (Fe₃O₄) nanoparticles-Doxorubicin complex @ 3 mg/kg body weight intratumoural at 2 to 3 different sites at scheduled interval i.e. on 1st, 7th
and 21st days; however, group III dogs were subjected to the radical surgery for excision of the tumour.

The regression of the tumour was assessed on the basis of various parameters. Also the age, breed, sex and duration of illness wise incidence were recorded during the study.

The incidence of superficial tumour was recorded highest amongst older dogs above 8 years of age, in non-descript followed by Pomeranian, German shepherd, Rottweiler and Labrador breeds of dog; whereas, sex wise incidence revealed higher incidence in female as compare to male. The higher per cent of dogs with 6 months above illness history was observed followed by 3 to 6 months and below 3 months.

During the study, the location and size of tumour was also recorded. The higher percent of the tumour was observed on caudal abdominal region followed by limb and thoracic region, cranial abdominal region and head region.

During present investigation, in group I use of doxorubicin was found to be effective in four cases with no recurrence and initiation of regression of tumour was observed in two cases along with side effects like anaemia, vomition, anorexia, alopecia and dullness in. In group II, the treatment protocol was found to be effective in five cases with success rate of 83.33% without any ill effect and recurrence was observed; whereas initiation of regression was observed in one case. In group III, radical surgery was performed with satisfactory results.

The clinical haematopoietic and biochemical observations were recorded at the scheduled interval of 0th, 10th, 20th and 30th day of observation. Significant changes were observed in packed cell volume, total erythrocyte count, total leucocyte count, neutrophil, lymphocyte and total platelet count, serum glutamic oxaloacetic transaminase and alkaline phosphatase. However, non-significant changes were recorded in haemoglobin, monocyte, eosinophil count, serum glutamic pyruvic transaminase, serum glucose, blood urea nitrogen and serum creatinine.

Recurrence of the tumour was observed in only one case of group III; whereas, no recurrence was observed in group I and II dogs. No metastasis was observed in any of the dog during the study.
During the study, various types of tumours e.g. fibrosarcoma, schirrous adenocarcinoma, adenocarcinoma, papillary adenocarcinoma, basal cell carcinoma and mixed tumour were recorded.

The overall response of treatment protocol using iron oxide nanoparticles-doxorubicin complex intratumorally indicated that it is an effective modality in tumours of skin appendage and papillary adenocarcinoma in canines. The study also suggests that the effectiveness of this protocol of other superficial tumours needs further investigations.
(D) प्रबंध सारांश
प्रबंध सारांश

अ. प्रबंधाचे शिरक:\nश्वानामयील बाह्य अबुदांवर रासायनिक
उपचारातील असं सुळ कण्युक्त ओषधऱ्याचा
pद्वारे उपयुक्ततेचा अभ्यास

ब. निदाध्यायचे पूर्ण नाव:\nइंगऱ्टे हपेश सखऱेराम

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ऱ. पदवी प्रदान करण्यात वर्ष:\n2014

व. मुख्य विषय:\nपशुशालनिर्मित्तांिक व क्ष-किरणासंग्रह

ग. प्रवांतातील एकूण पृष्ठ:\n69

ह. सारांशातील एकूण शब्द:\n573

ई. निदाध्यायची सही:\n
ज. अभ्यास सारांश:\nअभ्यास हा डिसेंबर 2013 ते जून 2014 या कालावधीतील दोषुकत बाह्य
अबुदांवर १८ श्वानाम्याचा वय, सिंगव व जात लक्षात ने पेटा करण्यात आला आणि
त्याची तीन समान गटात विभागणी करण्यात आली.

गट क्र. १ माहिल वाणिज्य २१ दिवसांच्या अंतर्गत डॉक्टर्सीसीन हायड्रोक्लोराइड
३० मि.प्र. प्रति मी. वर्ग या प्रमाणाने १५० मि.प्र. क्र. १ डेस्काउंट शारदणुत दवासंचार
नसेवाचे हल्लूहल्लू देण्यात आले. गट क्र. २ माहिल वाणिज्य प्रणाल्यांनुसार
लोहाचे असेल सुकण मिश्रित डॉक्टर्सीसीन ३ मि.प्र. प्रति किली फी.रु. कावनातुसार अबुदांवर अनुमती
२ ते ३ वैद्यविद्या जागेवर पहिल्या, सातव्या व एकवीद्या दिवसी देखाव आलेल्या, तर गट क्र. ३ मधील वानांच्या अरुंदेचे समुद्रु उच्चवाट लक्षित्येत राहणारे करण्यात आलेले. सदर अभ्यासादरम्यान अरुंदेची शालेची मदर वेगवेगळ्या मापदंडजीवन दर्शिका आलेली. काहीही अरुंदेच्या प्रश्नातीच्या नंद (व. विशेष वयोलूड) गावती वानांमध्ये, पठाणपट पॅमेयिवन, जर्मन फार्म, रांटलिवर आणि लंबाडऱ्यांना यांची वानांमध्ये आढळते, तर हिंगाणुसार मादीमध्ये नरस्पिथा जारी प्रामाण्य आढळत. वानांमध्ये ६ महिण्यपाकेचा जारी आजारीपणाचा काळ हा जारी प्रमाणत मिर्जानासारखा आहे. त्यापासून ३ ते ६ महिण्यपाकेचा कमी आढळत. अभ्यासामध्ये अरुंदेच्या आमेज्याच्या आकाराची नोंद करण्यात आलेली. अरुंदेचे जात वास प्रमाण हे पोटाच्या माणील भागावर आढळते, त्यापासून पायव्यांमध्ये, छातीच्या भागवार, पोटाच्या समर्थ्याच्या भागवार व तोडळाच्या आढळते.

सदर पद्धताच्या प्रमाणाने गट क्र. १ मधील ४ वानांमध्ये उजल डॉक्टरकीविअनमारी उपयुक्तता आढळती आणि २ वानांमध्ये अरुंदेच्या घेतलेला सुविधा झालेली दिसून आली परंतु रक्तपेट, उडदी, भुक मंदावणे, केवळ गावली व असंतानता हे दुर परिणाम आढळते. गट क्र. २ मध्ये उपचार पद्धती उपयुक्त आढळती अरुंदेने त्याची यासाची ओळख करण्यासाठी दुर परिणामविषयक व.३.३३ टक्के आढळती, तर एका वानांमध्ये अरुंदेच्या घेतलेला सुविधा झाली. गट क्र. ३ मध्ये समुद्रच्या उच्चवाट लक्षित्येत राहणारे करण्यात आली असताना त्याचे माहितीकारण परिणाम होते.

चिकित्साविभाग, रक्तजीववाणिज्यिक घटकांचे पहिल्या, दहावा, विशुक्या आणि तिसर्या दिवसी नोंद करण्यात आलेली. त्यामध्ये बाहेर पेशीचे आकारानिवण, एकूण लाल रक्तपेटी, एकूण लाल रक्तसर्जी, नुसर्त फक्तफक्त, हिमवर्षाळू, ड्रायमेयनंज, अल्कैलाइन फ्यूस्फोटेजमध्ये लक्षणीय बदल आढळत. त्याचा, हिमावर्षाळू, कोलोसाइड, इलोसोफिल, ड्रायमेयनंज, ग्लुटामिकामिथिफोबिक ड्रायमेयनंज, ब्लू युरिया नाप्रोडिसन, क्रियातयीन आणि रक्तजीववाणिज्यिक परिणाम, अरुंदेच्या नोंद झाली.

गट क्र. ३ मधील एका वानांमध्ये अरुणाची पुऱ्ऱक्या वाढ दिसून आली, तर गट १ व २ मधील वानांमध्ये पुऱ्ऱक्या वाढ आढळली नाही. अभ्यासादरम्यान कोणत्याही वानांमध्ये अरुंदेचे रोग व्यावहार्य आढळते नाही.

अभ्यासादरम्यान वेगवेगळ्या अरुंदेचा जसे फायदोमार्कोमा, स्कर्स ओरीनोकार्सिनोमा, ओरीनोकार्सिनोमा, ग्रेटेले ओरीनोकार्सिनोमा व बेसल सेट कार्सिनोमा आणि विशेष रक्तपेट अरुंदेची नोंद झाली.

प्राणबायुसूक्त लोह व डॉक्टरकीविअन मिश्रणाचा उपचार पद्धती प्रतिसादप्रवल असले दर्शवलेले, ही वानांभोज लच्चन जोडविरूढ अरुंदेचे, कार्सिनोमा आणि पॅपिलार्कार्सिनोमा यांच्याकडे एक उपयुक्त उपचार पद्धती असून. अभ्यासादरम्यान असे सुगत होतांना की, तात्त्विक अरुंदेच्या उपचाराची कोणती वातावरणिक असेल की, त्याचे अरुंदेच्या उपचाराची करते त्याच्या उपयुक्ततेच्या पद्धताच्या गरज आहे.