STUDIES ON CHEMOSURGERY IN VETERINARY PRACTICES

A thesis

Submitted to the

West Bengal University of Animal and Fishery Sciences.

In partial fulfilment of the requirements for the Degree of

Master of Veterinary Science

in

VETERINARY SURGERY & RADIOLOGY

BY

DR. ANGSHUMAN RAKSHIT

B.V.Sc. & A. H.

DEPARTMENT OF VETERINARY SURGERY & RADIOLOGY
FACULTY OF VETERINARY
AND ANIMAL SCIENCES WEST BENGAL UNIVERSITY OF ANIMAL AND FISHERY SCIENCES
68, KHUDIRAM BOSE SARANI, CALCUTTA - 700 037

DECEMBER, 1999.

Dedicated to

My

Beloved Grandfather Late Sri Sudhir Kumar Brahma

APPROVAL OF EXAMINERS FOR THE AWARD TO THE DEGREE OF MASTER OF VETERINARY SCIENCE.

We, the undersigned, having been satisfied with the performance of Dr. Angshuman Rakshit, in the viva - voce examination, conducted today the 14th January 2000, recommend that the thesis be accepted for the award of the Degree.

	Name	Signature
1.	Dr. P.K. Samanta, Advisor (Chairman, Advisory Committee)	P. K. Samanta 14/1/2000
2.	Dr. A. A. Khan, MVSc., Ph.D., FRVAC. (External Examiner)	A. A. Khan 14/1/2000
3.	Dr. T.B. Sen. (Member, Advisory Committee)	T.B. Sen 14.1.2000
4.	Prof. (Dr.) P. K. Bose (Member, Advisory Committee)	14.1.2000

West Bengal University of Animal & Fishery Sciences
DEPARTMENT OF VETERINARY SURGERY & RADIOLOGY
FACULTY OF VETERINARY & ANIMAL SCIENCES
37 & 68, KSHUDIRAM BOSE SARANI, CALCUTTA - 700 037

Ref. No..... *Date.....11.12.99.*

Dr. P. K. Samanta
M.Sc. (Vet.); Ph.D. (Cal)
Reader
Deptt. of Veterinary Surgury and Radiology
W.B.U.A.F.Sc.

CERTIFICATE

This is to certify that the work recorded in the thesis entitled " STUDIES ON CHEMOSURGERY IN VETERINARY PRACTICES." submitted by Angshuman Rakshit in partial fulfilment of the requirements for the Award of the Degree of Master of Veterinary Science in Veterinary Surgery and Radiology of the West Bengal University of Animal and Fishery Sciences, is the faithful and bonafide research work carried out under my personal supervision and guidance. The results of the investigation reported in the thesis have not so far been submitted for any other Degree or Diploma. The assistance and help received during the course of investigation have been duly acknowledged.

P. K. Samanta

Dated
The .....11.12.99....., 1999.

(Dr. P. K. Samanta)
Chairman, Advisory Committee

ACKNOWLEDGEMENT

It is a gratifying moment for the author to convey his deep sense of gratitude, indebtness and heartful thanks to Dr. P.K. Samanta, M.Sc. (Vet), Ph.D. (Cal), Reader, Department of Veterinery Surgery and Radiology, West Bengal University of Animal and Fishery Sciences, 68, Khudiram Bose Sarani, Calcutta - 700037 for his benevolent guidance, constructive criticism and whole hearted encouragement throughout the entire course of research work which enabled the author to execute the experiment and preparation of the thesis smoothly.

The author is highly obliged and very much grateful to Dr. T.B. Sen, M.V. Sc., Ph. D., Head, Department of Veterinary Surgery & Radiology for his active help, suggestions and constant inspiration during the course of the study and research work.

The author is particularly indebted to Prof. (Dr.) P. K. Bose, M.V. Sc., Ph.D., Professor, Department of Veterinary Surgery & Radiology for his judicial & helpful discussions during different phases of the study.

The author is indebted to Dr. J. Sengupta, Ex - professor, Department of Pathology & Biochemistry, National Medical College, Calcutta and his clinical & reasearch Laboratory Pathowynd, 29A, Balaram Ghosh Street, Calcutta - 700 004 for his valuable suggestion, guidance and all round help in conducting haematological & biochemical studies.

The author is grateful to Dr. A. Bhattacharya, Vice Chancellor, West Bengal University of Animal & Fishery Sciences, Dr. D. P. Banerjee, Dean, Faculty of Veterinary and Animal Sciences, Dr. D.N. Jana Registrar & Director of Research & Extension of Farms, for extending academic facilities to the author.

The author expresses his heartiest thanks and gratefulness to Dr. Senapaty, Department of Animal Genetics and Breeding, West Bengal University of Animal & Fishery Sciences & Dr. Nabendu Nag, V.S. Govt. of West Bengal, for their wholehearted & sincere help during different phases of the study.

The author expresses his sincere thanks to all the staffs of Horse ward, Police Training College, Barrackpore & Gokul, Ramkrishna Mission Sangha, Adyapeath, Calcutta - 700 076., for their help in completing the study.

The author feels immense pleasure in expressing his heartiest thanks and

gratefulness to Dr. Ananda Maity, Dr. Avijit Dutta, Dr. Subrata Sanki, Dr. Bhupinder Singh, Dr. Samar Haldar, Dr. Paresh Biswas, Dr. Prasant Bhagat, Dr. Paresh Ghorui, Dr. Keshab Dhara, Fellow M.V. Sc. Students of our University for their inspiration, co - operation & help in completing the study.

The author desires to express his heartiest thanks to Dr. Shailesh Kumar, Dr, Partha Sarathi Nag, Dr. Arnab Chakraborty, Dr. Swapan Dass, Dr. Kingshuk Das, Dr. Tapas Biswas, Junior M.V.Sc. students, Department of Veterinary Surgery & Radiology for their all round help.

The assistance and help rendered by the staff members of the central library of the University at both Mohanpur & Belgachia campus, and the central library of the Department of Animal Resources Development, Govt. of West Bengal at Belgachia and all the staff members, Department of Veterinary Surgery & Radiology, West Bengal University of Animal & Fishery Sciences are fully acknowledged.

The author records his deep sense of gratitude to the Dr. H. Mukherjee & Samiran Roy for their sincere cooperation & help.

The author expresses his thanks to Mrs. Joyati Samanta, M.A., B.Ed. for her good wishes, encouragement, cooperation and inspiration during the course of studies.

The author wishes to record his sincere gratitude to Mr. Abhijit Bose, for the immense trouble he took for typing out the thesis within the specified time.

The gratitude of author knows no bound when he mentaion about the cooperation and help extended by shri Debayan Ganguli, cousins Shri Pulak Ranjan Roy & Sanjoy Ranjan Roy, childhood friends Shri. Angshuman Bhattacharya & Dipankar Deb, throughout his academic career.

In fine, the author found no words to express his indebtness to his beloved mother & father, uncle shri Santosh Kumar Rakshit, Aunt Smt. Lina Rakshit, brother Shri Anupam Rakshit, sister Shri Arpita Rakshit & grand mother Smt. Prova Brahma for their constant inspiration, patience & sacrifice without which it would not have been possible to achieve this goal.

Lastly the author expresses his sympathy from the core of his heart to those fellows who are neglected everywhere.

Angshuman Rakshit

(ANGSHUMAN RAKSHIT)

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LIST OF ABREVIATIONS USED

ANOVA	=	Analysis of Variance.
BUN	=	Blood Urea Nitrogen
B. wt.	=	Body weight
$CaCl_2$	=	Calcium Chloride
c.c.	=	Cubic centimeter.
Cu. mm.	=	Cubic millimeter
Cm.	=	Centimeter
Conc.	=	Concentration
dl	=	Deciliter
Deptt.	=	Department
°C	=	Degree Centigrade
°F	=	Degree Fahrenheit
gm	=	Gram,
Gr/gr	=	Group
HCL	=	Hydrochloride
I/M	=	Intra - Mascular
Inj.	=	Injection.
Kg.	=	Kilogram
Ltd.	=	Limited.
mg %	=	Milligram Percentage
ml.	=	Milliliter
min	=	Minute
No./ no.	=	Number
%	=	Percentage
S/C	=	Subcutaneously
S.E. / SE	=	Standard errors.
WBUAFS	=	West Bengal University of Animal & Fishery Sciences.
W/V	=	Weight by Volume.

Chapter I

Introduction

INTRODUCTION

It is true that the world is preparing to keep its foot in the new milliennium, therefore, scientists in all the disciplines of science, are striving hard day and night, to keep up the pace and to meet the challenges of the coming century Veterinary surgery in particular, and Veterinary science in general, are not laging behind. Many important new lanes and by lanes are constructed by the mordern medical science, and accordingly it is advancing through extensive research in experimental medicine & surgery. In this regard, veterinarians are playing key role in it through collaborative researches .Obviously, newer and newer surgical techinques are occupying the field of mordern surgical practice.

Available literatures indicate that, chemosurgery, in human practice, is still a new venture and its practice is being limited only in the highly developed countries. This field of specialised surgery has its own special features but has not been widely explored by veterinary researchers, with the exception of topical chemosurgical practices related to dehorning pastes and solutions (Koger, 1976) The basic principle behind chemosurgery is to perform surgery not by the use of conventional surgical instruments & medicaments but by administering chemical agents for achieving desired results. Further that, for chemical debridement, though veterinarians are using some common drugs which may also come under chemosurgical practice, but standardisation of this therapy as " chemosurgery " has not yet been evaluated scientifically.

In one particular field of chemosurgery, debudding has been a routine and regular feature in most of the organized farms all over the world including many of the states in India, unfortunately in West Bengal, debudding is not being practised even in organized Government farms, inspite of its numerous advantages. This is mainly due to its non availability of chemosurgical agents and also non familiarity of the veterinary surgeons to the technique. So, in the present study, necrotizing property of calcium chloride in different solutions, as observed by some workers, has been utilized for debudding operations in crossbred calves.

An effort has also been made to judge the ability of calcium chloride to slough off granulomatous growths by utilizing its widespread necrotizing property. It has been aimed on the back ground of facts collected on many situations particularly in complicated surgical lesions a "good slough " is desireable

where surgical incision creates a lot of problems & complications.

Further that, there are several pathological conditions where surgical or enzymatic debridement is not feasible and has to be treated with chemical debriding agents The objective behind selecting calcium chloride as a chemical debriding agent is to treat such complicated lesions as better alternative than other agents which are familiar to the veterinary surgeons. So to test the efficiency of this drug for removing dead, devitalized and necrosed tissue, promoting healing by subsiding infection, inciting growth of healthy granulation tissue , minimising loss of blood, body proteins and other vital body components within a reasonable period of time and to help the animal for overcoming the stress early.

There fore the present study has been undertaken for evaluating the functional efficiency of calcium chloride as a chemosurgical agent with human placental extract (Placentrex ®) injection as an adjunct therapy to it. Necessary parameters have , therefore been chalked out accordingly.

The present day informations have been presented under "Review of literature" in chapter II.

Chapter II

Review of Literature

REVIEW OF LITERATURE

Under the review of literature an attempt has been made to cite all important references in relation to the studies of Chemosurgery and its therapeutical adjuncts in Veterinary practices, with particular reference to the function of Calcium Chloride, and Placentrex as biogenous stimulators.

Works have also been reviewed for Chemosurgery in human applications and topical chemosurgery in veterinary practices. Accordingly, gross, anatomical, clinical, biochemical, haematological & morphological features of the study have been presented.

2.1. EFFECTS OF CALCIUM CHLORIDE.

Available literature, searched for, indicated that calcium choloride ($CaCl_2$) has been used not only as a chemosurgical agent but also as a chemosterilizing agent.

The chemical agent calcium chloride , both fused and anhydrous,has the molecular weight of 100.09, melting point 825°c and minimum assay of 98%.

Withford (1976) injected small amounts of calcium chloride solution into the mass of a draining fibromatous lesion on a ram's jaw. The lesion sloughed away and healed by second intension in a few weeks. He also stated that the use of calcium chloride solution resulted in a good slough better than surgical incision. He stated that there has been "no bad effect" although occasionally there was more tissue destruction than needed.

Koger & Pullman (1976) experimented intra-dermal injection of different concentrations of calcium chloride solutions ranging from 12-75% in calte, horse, sheep & dog in volumes 0.05 to 1.0 ml resulting in sharply defined concentric areas of necrosis up to 5 cm in diameter. Biopsies of dermal sections revealed areas of coagulating necrosis surrounded by an inflammatory reactions. The nuclei and cell membranes were remarkably well preserved for several days after the injection, but both nuclei & cytoplasm were shrunken. Thrombi were seen in many vessels in necrotic area and in the living tissue on its border.

Daniel *et al.* (1983) studied the effects of hypocalcaemia on QT (QT interval adjusted for heart rate). It was found that the regression of plasma

calcium level in QT was highly significant throughout the induction and recovery stages. Results from combined induction and recovery stages showed that plasma calcium decreased 0.010 $\pm$ 0.0003 ($\pm$ sb) mmol/1 for an increase of 1 m/sec in QTc.

Kvari (1983) studied the effects of calcium infusion on electrocardiogram of parturient paretic cows. He stated that some form of cardiac abnormality was detected during treatment of all the cows examined, but those with severe hypocalcaemia had the highest incidence of arrhythmias. He concluded that the highest incidence of arrhythmias and the occurrence of post infusion hypercalcaemia raises doubts the suitability of this type of therapy.

Dutta (1991) stated that 7.5%-10% calcium chloride may safely be used by single intratesticular injection for sterilization of male dogs. The adopted technique was safe, economical and can be carried out with confidence even under field condition.

Tucker *et al.* (1992) studied the influence of calcium chloride on systemic acid base status and calcium metabolism in dairy heifers. The animals were fed on diets containing (0%), 0.5%, 1%,1.5% calcium chloride followed by calcium chloride free diet for a week. They observed that free protein concentration in blood increases and blood bicarbonate decreased with increasing dietary calcium chloride. They observed that plasma calcium were unaffected but urinary calcium excretion increased. Increasing dietary chloride increased plasma chloride and urinary chloride excretion. During the readjustment week all differences caused by calcium chloride disappeared. They concluded that feeding of 1% calcium chloride to dry cows for 3 weeks prepartum could prevent parturient paresis, without causing detrimental acid-base disturbances.

Wentink *et al.* (1992) studied the deleterious side effects in oral administration of calcium chloride containing products. They observed calcium chloride either in aqueous solution or as a gel was highly caustic for ruminal mucosa but an oil emulsion was relatively safe.

Mitra (1993) experimented with calcium chloride as a chemosterilizing agent on 36 healthy adult bulls in varrying concentration ranging from 10%, 20%, 30%, 40%, & 50%. To avoid pain reactions he advocated the drug in combination with 2% Lignocaine HCl, intratesticularly. He concluded that $CaCl_2$ has a powerful role in inducing extensive or total coagulative necrosis on the testicular parenchyma in the strength of 40% or 50% solution.

Dutta & Samanta (1993) stated that intratesticular injection of calcium chloride ranging fram 7.5%-10% may be used for chemosterilization of male dogs. To avoid any pain reaction they advocated the drug in combination with 2% Lignocaine HCl. They concluded that testicular parenchyma will undergo complete necrosis and will be replaced by fibrous tissue within a month or so.

Samanta (1998) injected intratesticularly 1.5 ml of calcium chloride in different concentrations e.i. 5%, 7.5%, 10%, & 12.5% in combination with 0.5 ml of 2% Xylocaine for chemosterilization of male dogs. He reported that the testes reduced in size and volume after 2 weeks, and continued to shrink. The effect increased with the concentration of calcium chloride. After 8 weeks, histological examination of the testes revealed that 10% & 12.5% calcium chloride solution had the highest necrotizing effects on seminiferous tubules and interstitial tissues of the testicular parenchyma. The structural & functional characteristics of the testes were lost and the dogs were parmanently sterile. He concluded that the chemosterilization technique was simple, economical, free from side effects and could be used on large scale sterilizing programme.

2.2 EFFECTS OF PLACENTREX.

Gupta *et al.* (1952) tried placental extracts in 50 different human ophthalmological cases of high myopia with choroido-retinal changes, early optic atrophy of unknown etiology, old corneal opacities and retinitis pigmentosa. He found the therapy to be definitely valuable.

Dasgupta *et al.* (1954) did a clinical trial with Placentrex in 25 cases of corneal ulcers & corneal ulcers with hypopyon caused by streptococci & staphylococci. They applied Placentrex injection-2 ml I/M on alternate days and with 15 injections ulcers healed and pus disappeared without leaving any trace of scar. Out of 25 cases they got successful results in 23 cases.

Roy (1967) conducted a preliminary clinical trial with placentrex combined with siolan 12 in 10 human cases of Salpingitis and non specific Leucorrhoea. He reported that out of 10 cases all cases got relief with 12-15 injections without any side reactions & pain sensation was far less. He concluded that biogenous stimulators in Placentrex is responsible for better results in all the cases.

Purandare *et al.* (1970) administered placental extract injection in 32 cases of tubal block in females. They were put on alternate day injections of placental extracts-2ml with non-specific protein along with B_{12}. The total

injection each patient received were twenty. 75% of the tubes were opened after the course of 20 injections. Among the open tubes, 2 became pregnant during the course of treatment. In 25% of the cases, the tubes remain blocked in spite of the therapy. The injection therapy was highly efficacious even in correcting menstrual disorders. They concluded that Placentrex was very favourable in sterility cases, tubal blocks & menstrual irregularities.

Sukla (1972) studied 23 cases of superficial burns in humans and found that smallest area of burn in group I is 63 sqcm and has taken 12 days to heal showing obvious advantage of placental extract which he had given to all the groups as therapy. In the range of 30-34 days time maximum area which healed in group-I was 364 sqcm, in group-II 328 sqcm and in group-III only 127 sqcm.

He evaluated the effects of placental extracts on non-healing ulcers in 5 cases. Out of these, 4 cases healed when given placental extract as local dressing as well as I/M injection.

He also evaluated the effect of placental extract on grossly infected wounds. Out of the total 3 cases only one healed completely, making the success rate 33%.

He also mentioned the effects of placental extract on 7 selected bedsore cases. He concluded that placental extract enhances the rate of healing, contraction of bed sores and larger area of trial groups healed in lesser time that the control groups.

Sarkar (1975-80) studied the effects of Placentrex on female infertility. The worker analysed 300 cases of infertility out of which 208 cases were primary and 92 cases were of secondary infertility. In 92 cases of secondary infertility Placentrex was given without doing the tube test and the results were very satisfactory i.e. 38%. She concluded that the biogenous stimulating potency of Placentrex has not only the positive result on bringing back the tubal patency in blocked tube but also improved the metabolism on the reception decidua and recipient fertilised ovum resulting in pregnancy in a good percentage of cases.

Punshi (1976) studied the effects of placental extracts in vitiligo. The number of patients treated were 35, 25 were females & 10 males of varrying age groups between 12-45 years. Injections of placental extracts were given I/M daily except Sundays. The duration of treatment varried from 3 months to 9 months. Average number of injections given are 150. 71.1% cases showed

good to excellent recovery. He concluded that placental extract contains tylosine, a precursor of melanin. It also contains copper which acts as a catalyst in formation of melanin pigment.

Dabral (1976) administered Placentrex intra-articularly in 21 human patients suffering from osteoarthritis of knee joint. The results recorded were 47.9% excellent, 28.5% good and 35.5% fair. He concluded that intra-articular placenta extract therapy is painless and easy to administer and does not have any contraindications. No adverse effect was noticed in any of the cases. The results obtained were quite encouraging for early relief of pain, stiffness and swelling.

Mukherjee (1976) tried Placentrex in 20 cases of indolent ulcers produced during and after deep x-ray therapy. 2 ml Placentrex was given I/M on alternate days & simultaneously 2 ml Placentrex solution was applied on the ulcers. He reported that out of 20 cases, 19 cases got complete healing with 20 injections of Placentrex I/M on alternate days.

Sundaramma et al (1976) undertook a clinical trial with Placentrex in cases of pulmonary Tuberculosis. He concluded that Placentrex has a definite role as an adjuvant in the treatment of tuberculous cavity diseases. Sputum conversion and closure of the cavities was highly encouraging along with chemotherapy.

Basu (1976) studied the effects of Placentrex in the treatment of chronic Dacryocystitis in 27 patients. 1 ml of Placentrex was used for syringing the naso-lacrimal passage and the rest 1 ml was injected I/M every week. Out of 27 patients treated only 3 did not show any improvement (11.11%) & rest 24 patients showed definite improvements (88.89%).

Mittal (1977) studied the role of placental extract in the therapy of urticaria in 26 selected cases of humans. There was quick complete relief in 69.2% of cases, showed relief in 23.1% of cases and in 7.7% of the cases there was no good results.

Sinha (1978) undertook a symptomatological study of parenteral placental extract therapy in human Atropic Rhinitis. He reported that there was marked clinical improvement after local administration of placental extract. It relieved epistaxis in 100% cases, crusting in 88.4% cases, headache in 87.6% cases and nasal obstruction in 96.5% cases. Ear & throat symptoms were also relieved completely.

Kameswaran (1978) studied the role of biogenic stimulants in otorhinolaryngology. For this study, 100 cases of different ear & nose problems were selected at random. In cases of Tinnitus aurium 50% of the patients improved. 6 out of 8 vestibular neuronitis cases became free from symptoms after 20 injections. In atropic rhinitis 8 out of 10 cases showed improvement. The drug was free from any allergic accidents.

Jain (1980) described the role of Placentrex in the treatment of burns in man. 40 cases were studied in this series, out of which 10 were taken as control and rest 30 were treated with Placentrex in different ways. All cases were investigated for Hb%, urine sugar & albumin. He concluded that there was tremendous decrease in the incidence of infection (43%) in patients treated by Placentrex as compared to those by conventional methods. Patients treated by Placentrex showed significant decrease (11%) in time required for complete healing. Rate of healing was faster (31.05%) in patients on Placentrex treatment (10.17 sq. cm. per day) as compared to control cases (7.76 sq cm per day). Local Placentrex application gave better results (10.61 sq. cm. per day) whereas the combination of local and parenteral was most effective (12.80 sq. cm. per day).

Bertone *et al.* (1982) mentioned that Placentrex is active in many chronic inflammatory conditions commonly encountered in gynaecology such as post radiation cervico-vaginitis, Dystrophic and chronic cervico vaginitis. With placental extract they noticed the following effects:-

1. Disappearance of leucocyte infiltration.

2. Decrease of pH-down to normal range.

3. Increase in cellular glycogen.

4. Improvement in colposcopic & macroscopic appearance.

Girotto *et al.* (1982) reported that placental extract injection gave very good results in chorio-retinal dystrophy of either myopic or senile origin. They concluded that the beneficial effects of placental extracts therapy was due to:-

1. High protein content, polypeptides of low molecular weight and presence of free amino acids such as Alanine, Leucine, Lysine & Valine.

2. Possible effect of the placental extract on the microcirulation in the reti-

nal arteries and the chorio, capillary vessels.

Lolli (1982) came to the conclusion that local application of placental extract favourably influence tissue formation after gingivectomy and rapid epithelisation of the wound. He also observed similar beneficial effects in cases of slow cicatrisation of wounds.

Marchegiani *et al.* (1982) described that the results obtained with placental extracts in eschars, slow cicatrization of wounds, post phlebitic ulcers, were very favourable in 120 cases.

Rosenthal (1982) concluded that intraarticular application of placental extract in the arthritic joints of patients with rheumatoid arthritis resulted in a significant improvement.

In a separate study, he reported that intra-articular application of placenta extract prolongs local arthritic remissions induced by steroids, thus limiting use of the latter.

Bigliardi (1982) reported that a semi-greasy placenta ointment was shown to be at least as effective as topical steroids in the treatment of first & second degree radio dermatitis consecutive to dermatoroeutgen therapy. Unpleasant symptoms such as burning, pruritus were quickly soothed.

Shibasaki *et al.* (1982) evaluated corticotropin releasing factor-like activity in human placental extracts.

Duc *et al.* (1983) reported that placental FSF (fibrin stabilizing factor) can be used therapeutically for factor XII deficiency & for the acceleraion in wound healing following major surgery. Several placental proteins have been isolated with immunostimulatory and immunodeviatory properties.

Rajasimha (1983) evaluated the efficacy of Placentrex in 24 human patients suffering from chronic Bronchial Asthma. 2 ml I/M Placentrex was given daily for 30 days to all the patients and thereafter a follow - up in them for the next 30 days was made. He observed that at the end of 30 days & in the follow-up period, 75% of the patients showed no need for IV aminophylline with their sleep hours increasing to 4 - 8 hours, uninterrupted. 25% of patients showed no response and continued to require IV aminophylline daily during Placentrex therapy and in the subsequent follow-up period. No untoward effect due to Placentrex inj. was observed in this study.

He concluded that since some PG series have been shown to be bronchial smooth muscle relaxants, it is a conjecture at this stage, whether Placentrex through its biogenic stimulating effect raises PG levels in chronic wheezers. It was also observed that 5% of the patients in this series who were chronic IV aminophylline users felt no need for IV aminophylline by the 15th day and switched on to occasional use of oral bronchodilators in the follow-up period.

Amarlal (1983) undertook a clinical trial with Placentrex on 218 patients of vitiligo. The response was assessed over a period of 6 months to one year. The drug proved very effective giving excellent to good results in 80.2% and was well accepted by patients with no side-effects.

Lehninger (1984) mentioned that human placental extract contains significant quantities of DNA & RNA. The nucleotides are known for their tissue regeneration & effect through their unique process of protein synthesis.

Reddy (1985) undertook a 1 year study consisting of 100 patients suffering from Poliomyelitis. He advocated the following treatment.

1. Bed rest with support of pillows in functional position was advised during the first 3 weeks. No injections were allowed.

2. Inj. Placentrex 2ml I/M was given in 3 courses. From 4th to 6th week : Inj. Placentrex on every day combined with electrical stimulation, followed by a free interval of 1 week.

3. From 8th to 10th week : Inj. Placentrex on alternate days combined with electrical stimulation, followed by a free interval of 1 week.

4. From 12th to 14th week. Inj. Placentrex was given twice a week combined with electrical stimulation.

After 1 year, he obtained the following results 18% of patients had Good to Excellent recovery. 36% of patients had fair recovery. 44% of patients had poor recovery.

He concluded that Inj. Placentrex possibly acts by liberating Biogenic stimulators in the body. The stimulations are taken up by the diseased tissues. These substances stimulate the cellular metabolism and thereby regenerate the diseased tissues.

Another school of thought is that these biogenous stimulators will give rise in Acetylcholine production which in turn stimulates neuromuscular excitability & tissue repair.

Dhir (1985) undertook a trial study with intra-articular injection of aqueous extract of human placenta (Placentrex). One ampoule (2ml) of Placentrex was injected at a time under strict asceptic conditions. Physiotherapeutic measures & antinflammatory drugs were continued as supportive measures in all the patients. Effect of the injection were assessed weekly following the injection. If required the injection was repeated after 2 weeks. No patient received more than 4 injections consecutively. He evaluated that 11 knees had good results. These included 3 patients with bilateral knee affection. Other 9 knees in 8 patients had fair results. The remaining 4 knees in 4 patients had poor results. He concluded that Placentrex was not only safe but also gave significant relief from pain, swelling & stiffness of the knee joint in osteoarthritis.

Moscatelli *et al.* (1986) tested extracts of human placenta for enhancement of proliferative growth of primary cultures of human keratinocytes. The study proved that extract contains a water soluble activity that is a potent promoter of proliferative growth in primary cultures of human epidermal keratinocytes. He presumed that the factor comes from the placental parenchyma.

Lodi (1986) found excellent result in psoriasis with placental extract. 20 patients affected by psoriasis of light & medium severity were treated with a topical placental extract. Results were evaluated with PASI method and it demonstrated a clear reduction of extension and severity of lesions.

Vadarajan (1990) gave placental extracts injection in cases of bronchial asthma andurticaria in 100 selected cases for a period of 50 days. He reported excellent results as shown by complete cessation of signs in 58% of cases. Moderate results with drastic reduction in the requirements of antiasthmatic drugs were seen in 20% of cases. Poor result were seen in 22% of cases with those of strong family incidence and co-existing chronic bronchitis.

Modak & Samanta (1993) reported that local administration of a combination of long acting Terramycin and Placentrex yielded satisfactory result as a post operative treatment and management of medial patellar desmotomy in bovines.

2.3 CHEMOSURGERY IN HUMAN APPLICATIONS.

Mikhail (1984) untertook microscopically controlled excision technique (Mohs' chemosurgery) in 110 patients suffering with sublingual epidermoid carcinoma. He concluded that Mohs' chemosurgery was ideal for this tumours. Amputation of the affected digits was too drastic, especially when the thumb, the most common site, is involved.

Snyder *et al.* (1984) reported a case of osteomyelitis complicating Mohs' surgery for recurrent basal cell carcinoma of the fore head. They concluded that open, bone exposed defects allowed to heal by secondary intention require meticulous wound care and follow-up.

Buecker *et al.* (1986) stated that the external auditory canal represents an area of high risk for recurrence of tumours and concluded that carcinomas involving this region should be removed by Mohs' chemosurgery.

Cruickshank *et al.* (1986) undertook a retrospective study of 27 patients treated for stage Isquamous cell carcinoma of the floor of the month and assessed the effectiveness of therapy. He concluded that Toluidine blue O mucosal staining and Mohs' chemosurgery are helpful adjuncts in determining the tumour margins.

Brody *et al.* (1986) described a technique for treatment of actinic degeneration, acne scarring, rhytids and pigmentary aberration. The technique was to apply superficial trichloro acetic acid followed by medium-depth chemical peeling of skin and lastly deep phenolic cauterant application. They also reported that combining solid CO_2, with trichloroacetic acid promotes both epidermal & dermal regeneration.

Mohs (1996) developed a method for the total microscopic control of excising external cancers by first excising the affected tissues in successive layers and then using frozen sections for the systematic microscopic scanning for the entire undersurface of each layer, He concluded that this method permitted the precise detection and selective removal of "silent" cancerous ramification and obviated the need to sacrifice a margin of normal appearing tissue. The reliability of the method for treating eyelid cancers is manifested by high 5 - year cure rates of 98% for basal cell carcinoma and 98.1% for squamous cell carcinoma.

Bingham (1986) discussed in detail the use of single agent chemotherapy, combination chemotherapy, combined irradiation & surgery as well as the

advantages of adjuvant chemosurgery.

Larson (1987) treated 43 kerato can thomas with Mohs' micrographic surgery with the use of fresh tissue technique. He mentioned that out of the 43 cases, one aggressive kerato canthoma recurred, yielding an overall recurrence rate of 2.4% . He concluded that micrographic surgery is an expedient treatment that allows for complete microscopic examination of the keratocanthoma, maximum preservation of normal tissue and a high degree of assurance of cure.

Sillman *et al.* (1987) undertook a study of anogenital papilloma virus infection and neoplasia in immunodeficient women. They conducted 5 - Fluoro - uracil chemosurgery, followed by main-tainence of 5 - fluorouracil therapy. They concluded that this therapy is effective and provided field suppression against recurrent HPV infection and neoplasia with minimal damage to affected organs.

Takahashi *et al.* (1988) reported the application of trichloro acetic acid to inferior turbinates as a chemosurgical approach against carcinoma and found good results.

Larson *et al.* (1988) stated that topical haemostatic agents are very helpful in attaining capillary & small vessel hemostasis in dermatologic surgery. They reviewed the commonly used topical haemostatic agents including oxidized cellulose, absorbable gelatin & thrombin along with newer agents such as microfibrillar collagen, fibrin sealants and acrylates. They recommended certain agents for certain situations.

Brody (1988) concluded that medium depth chemical peeling of skin is a variation of superficial chemosurgery.

Balducci (1988) mentioned that chemosurgery may reduce local relapse of extra - mammary Paget's disease.

Goldberg *et al.* (1989) treated basal cell carcinoma, the most common type of skin cancer, by a variety of methods. The techniques which he employed included curettage with electrodesiccation, scalpel excision, Mohs' chemosurgery, cryosurgery, radiation therapy and discussed in detail the advantages and disadvantages of all these techniques.

Sillman *et al.* (1991) in an updated study of anogenital papilloma virus infection and neoplasia in immunodeficient women reported that people with

deficient cell mediated immunity have an increased susceptibility to viral infections and certain cancers, particularly non - Hodgkin's lymphomas and cancers of the skin and anogenital regions.

Anogenital neoplasms in immunodeficient patients show a strong association with HPV infections; often occurs at relatively young ages ; involve multifocal locations ; and tend to persist ; recur; and progress rapidly despite standard therapy. Special treatment is required i.e, 5 - Fluorouracil chemosurgery, followed by maintenance of 5 - Fluorouracil therapy is often effective and provides field suppression against recurrent HPV infection and neoplasia, with minimal damage to affected organs. After removal of all detectable HPV and neoplastic lesions ; immuno deficient patients require close surveillance of the entire anogenital tract.

Gulya *et al.* (1992) reported that carcinomatous neural infiltration and subsequent neural dysfunction are phenomena associated with the facial nerve and parotid gland turmours. They presented the case history of a man who successively developed facial and trigeminal neural dysfunction after Mohs' chemosurgery of a primary cutaneous squamous cell carcinoma. Their paper documented histologically the occurrence of such neural invasion and illustrated the utility of gadolinium-enhanced magnetic resonance scanning in patient management.

Chaudhuri (1993) discussed in detail about the clinical consideration following uremia in humans. He stated that in renal failure, large scale derangement of body metabolism occur. This includes accumulation of waste products like urea. Normal blood urea concentration is about 15 - 40 mg/100 ml in humans. In renal failures, blood urea rises and may attain such values like 200 mg/ 100 ml or so. Although urea is almost nontoxic, blood urea determination offers a valuable and at the same time an easy diagnostic & prognostic procedure, because the level of blood urea gives an idea of the intensity of the renal failure. He also stated that one of the early signs of renal failure is rise of creatinine concentration of the blood.

Sillman *et al.* (1997) undertook a study to evaluate the risk factors for presistance of vaginal intraepithelial neoplasia, its invasion & management. They reviewed records and histopathological studies of 94 patients with vaginal intra epithelial neoplasia diagnosed from 1986 to 1997. They found that 64 out of 94 patients (88%) had prior concurrent anogenital squamous neoplasia, including 21 with invasive and 43 with intraepithelial nature. 23 had prior radiotherapy, 10 had anogenital neoplastic syndrome & 11 were immu-

nosuppressed. In 52 of 74 treated patients (70%) vaginal intraepithelial neoplasia went into remissions after a single treatment. In 18 patients (24%) of recurrent vaginal intraepithelial neoplasia went into remission after 5 - Flurouracil Chemosurgery. In 4(5%) it progressed to invasion. They concluded that although most vaginal intra - epithelial neoplasia got into remission after a treatment 5% of cases may progress from occult foci to invasion in spite of close follow- up.

2.4 TOPICAL CHEMOSURGERY IN VETERINARY PRACTICE.

Koger & Pullman (1976) stated that injection of $CaCl_2$ solution induced necrosis of hornbuds in calves. Various concentrations and volumes of $CaCl_2$ solutions were injected under the horn buds of young claves less than 2 months old, with an overall success rate of 70% (92 of 131 horn buds). Results indicated 1ml of 50% $CaCl_2$ solution injected under the centre of horn buds will prevent the horn growth in calves upto the age that the horn buds begins to fuse with the periosteum.

Koger (1976) modified the method of dehorning by injection of $CaCl_2$. Calves aged 13 days were restrained with the aid of I/M injection of 10 mg xylazine. 1.5 ml of 50% $CaCl_2$ solution was injected slowly under the horn bud. This resulted in inflammation and in about 4 days the area become dry & gangrenous. The gangrenous area later seperated and eventually normal hair covered the small scar.

Nag *et al.*(1977) worked on sialic acid and sialidase activity in rat testis and epididymis in relation to age. They observed the action of different antifertility agent like cyproterone acetate, mono- chlorhydrin & WIN 18446. All the drugs increased the sialidase activity. It was observed that the sialic acid content decreased after treatment with cyproterone acetate & monochlorhydrin but increased after treatment with WIN 18446.

Raman *et al.*(1977) studied the effects of cyproterone actate and flutamine on testicular phosphatase activity in rats.

Bose *et al.* (1977) studied the effects of cyproterone acetate on accessory sex organs and on the fertility performance of the hamster.

Wallace C.E. (1980) has described the method of injecting $CaCl_2$ under the developing horn buds. He supported koger & Pullman (1976) in the application of 1ml of 50% calcium chloride under the horn bud in injectable form. But he specified the age group of calves to be injected, should be one week

old. Analgesia & physical restraints are required to make an accurate injection. During the following several weeks the horn bud necroses and the tissue sloughs.

Wallace (1980) also reported the application of caustic substances as satisfactory methods of dehorning. Caustic substances such as pastes can be applied directly over the horn buds or injections of caustic substance can be given under the horn bud. He concluded that the pastes must be used carefully. Calves should be seperated so that they cannot lick each other and care must be taken to prevent the paste from getting into the calf's eye.

Hardie (1986) stated that elevations of blood urea nitrogen in canines is due to water deprivation,water deprivation in association with nephrogenic diabetes insipidus due to chronic gm (-ve) sepsis & due to pyelonephritis.

Breitschwerdt (1986) reported that acute renal failure in canines occurs due to infectious agents (bacteria & viruses), certain chemical agents, renal ischemia, obstructive uropathy & post traumatic reasons. He also discussed in detail about the diagnostic procedures related to acute renal failure. Out of the several diagnostic features, elevation of BUN & creatinine was one of them. Suppression of infection results in gradual diminution of BUN associated with increased renal function.

Zukowski *et al.* (1993) studied the histologic and bacteriologic differences that various commonly used occlusive dressings have upon the initial burn depth and the subsequent healing of peeled skin, using Yucatan minipig as animal model. They also compared chemical peel with dermabrasion and chemabrasion. Their results showed to statistical difference in peel depth between "wet" versus "moist" phenol application or between occluded versus non - occluded dressings. Based upon that animal model, they recommended that phenol solutions should be applied moist rather than wet and that an occlusive dressing other than adhesive tape be used and maintained for a minimum of 4 days.

Singh J. (1993) reported that potassium or sodium hydroxide sticks should be applied with firm pressure over the buds in a circular fashion. He concluded that the method was painful and cause much tissue damage. It should also be ensured that the horn bud is completely removed otherwise a crooked horn may develop.

Singh J (1993) agreed with koger that 1 to 3 ml of 50% solution of calcium chloride injected under the horn bud will prevent its growth in 70% of

the animals so treated. However, he added to the conclusion that if the bud appears to grow after 7 to 10 days the injection should be repeated.

Singh *et al.* (1994) mentioned that the physiological range of BUN in dogs is from 10 mg% to 20 mg%. Greater or lower the limits indicates abnormality.

Banerjee (1992), Prasad (1992), Venugopalan (1994) supported Singh & Wallace in that potassium hydroxide sticks can be used to destroy horn buds. Banerjee added that a ring of petroleum jelly should be applied near the base of the horn button to protect the eyes & neighbouring skin from the excess caustics. Prasad in addition to this reported that zinc oxide should be dusted after the application of the caustic.

Chakraborty (1994) described about the physiological limits of temperature, heart rate, respiration rate of calves and this are as follows -

A) Temperature Range

Calves	°F	°C
	101.5 - 103.5	38.6 - 39.8

B) Respiration range.

Calves 27 - 30 /min

C) Heart rate

Calves 80 - 100/min.

He also mentioned that neutrophilia in animals occurs due to bacterial infection, pneumonia, lung abscess, cardiac damage, traumatic pericarditis, peritonitis, & pyogenic meningitis.

Chapter III

Materials and Methods

MATERIALS & METHODS

3. OVERALL SURGICAL PLANNING :

The present study has been conducted in two diversified directions and accordingly it has been broadly divided into two groups namely, group A for chemical debudding and group B for chemo - surgery.

Group A - Cross bred calves were utilized for chemical debudding.

Group B - Horses & Dogs were utilized for chemosurgery and for this purpose group B was sub-divided into B_1 - for horses & B_2 for Dogs.

Group B_1 - Where chemosurgery on horses were conducted for the treatment of granulomatous lesions that developed from unhealthy wounds, with chronic irritation and defied usual therapy.

Group B_2 - Where chemosurgery on canine patients were conducted for the treatment of suppurative lesions.

3.1. CHEMICAL DEBUDDING : GROUP A

3.1.1. Selection and grouping of experimental animals.

In this approach, the study was conducted with 21 male calves in 'Gokul', Ramkrishna Mission Sangha, Adyapeath, Calcutta - 76 and formed group A. The group A was sub-divided into A1, A2 & A3 according to age. The age of the calves varied from 3 days to 1 month. Out of the 21 calves, 10 calves were of 3 to 11 days old and the rest 11 calves were of 12 to 30 days old. Out of the 10 calves of the age group 3 - 11 days, 7 calves were selected randomly and placed in group A_1 and the remaining 3 animals were placed in group A_3.

Among the 11 calves belonging to the age group of 12 to 30 days, 7 calves were selected randomly and placed in group A_2 and the rest 4 animals were placed in group A_3. Therefore,

Group A_1 → Consisted of 7 calves of the age group 3 -11 days, serially numbered from 1 to 7.

Group A_2 → Consisted of 7 calves of the age group 12 - 30 days., serially numbered from 8 to 14

Group A_3→ Consisted of 7 calves of ages varying from 3 days to 1 month and was considered as the control group, serially numbered from 15 to 21.

Before the onset of the study, each animal was observed closely for 2 to 28 days, according to the ages of the calves, in order to check up the condition of their health. Preoperatively, routine examination in respect of temperature, pulse & respiration as well as haemogram and biochemical analysis of serum were done of all the animals in order to assess the physical status of individual calves. The stool of individual calves were also assessed for the presence of any intestinal helminths and were corrected accordingly.

3.1.2. : Drugs & chemicals

(i) Calcium chloride - E. Merck (India) Ltd., Bombay.

(ii) Placentrex injection - 2 ml ampoule containing aqueous extract of fresh human placenta 0.1mg/ml, Albert David, Calcutta.

(iii) 2% Xylocaine injection - 30 ml vial containing 2% lignocaine HCl., Astra IDL (I) Ltd., Bangalore.

(iv) Nebasulf powder - Pfizer (I) Pvt. Ltd., Bombay.

(v) Himax ointment - Indian Herbs, Saharanpur.

(vi) 70% rectified spirit - Bengal chemical, Calcutta.

(vii) Sodium chloride - 500 ml containing sodium chloride 0.9% w/v.

Mount Mettur Pharmaceuticals Ltd., Chennai.

(viii) Betadine - 100 ml bottle containing Povidone Iodine 5% w/v (U.S.P.), Wockhardt Ltd., Aurangabad.

(ix) Novalgin injection - 30 ml vial containing analgin 0.4% w/v U.S.P., Hoechst India Ltd.

3.1.3. : Other requisites :

(i) Hypodermic needles - 16 gauze, 18 gauze & 21 gauze (3/4 inch)

(ii) Disposable syringes 2ml & 10 ml.

(iii) Absorbent cotton.

(iv) Shaving blade.

(v) Surgical drapes.

(vi) Sterile test tubes.

3.1.4. : Method

3.1.4.a. Preparation of the chemosurgical agent

For debudding operation 50% & 70% solution of calcium chloride were prepared asceptically with sterile normal saline and 2% Xylocaine HCL.

The composition of the reconstituted calcium chloride solution is given in table - 1.

TABLE - 1 : Composition of reconstituted $CaCl_2$ solution.

% of $CaCl_2$	mg/ml $CaCl_2$	Lignocaine	Ratio of $CaCl_2$ & Lignocaine	other constituents of lignocaine HCL inj. mg/ml
50	500	20	25 :1	Sodium chloride - 6 Sodium metabisulphate -0.5 Methyl paraben - 1.0
70	700	20	35 : 1	- do -

3.1.4.b.Preparation of the animal just before debudding operation.

The area of jugular vein was clearly shaved to facilitate the collection of blood sample for examination. According to the method, the area around the horn buds was shaved & cleaned with 70 % alcohol and covered up with drapes. The animals were carefully restrained and secured. The site of operation was again washed and cleaned with 70% alcohol & then painted with Betadine. Before administration of the drugs related physical parameters were recorded and blood samples taken for haematological & biochemical studies. Design of the experiment chalked out for group A is presented in table - 2.

3.1.4.c. : TABLE - 2 **Design of the experiment chalked out for group -A**

SL NO.	GR. NO.	TOTAL NO. OF ANIMALS	METHODS OF DEBUDDING
1 - 7	A_1	7	1ml of 50% reconstituted solution of $CaCl_2$ was injected under the centre of horn bud. A 18 gauze needle connected to a 10ml disposable syringe was a good combination. The site of injection was dorso - medial or lateral to the horn bud at a distance from the centre of the bud equal to the length of the needle, so that the hub of the needle will prevent accidental outward penetration of the needle. The needle penetrates the skin at an oblique angle & firm digital pressure is applied over the puncture site as the needle is withdrawn preventing leakage. The calves were injected 1ml of Placentrex daily for 7 days along with Novalgin 2ml for 3days post operatively.
8 - 14	A_2	7	1ml of 70% reconstituted solution of $CaCl_2$ was injected under the centre of hornbud. The procedure of the injection and the following follow-up being the same as Group-A_1.
15 -21	A_3	7	1ml of normal saline solution was injected under the centre of hornbud inexactly the same procedure as followed with group - A_1 animals.

3.1.4.d : Observations :

All the animals of group A_1, A_2 & A_3 were observed for a period of 6 months following debudding operation. They were kept in same natural condition with uniform standard diet and liberal supply of drinking water.

Clinical, haematological, biochemical studies & studies on Inflammatory & morphological changes were undertaken to assess the effectiveness of the drug.

Clinical Studies :

Clinical examination of all the animals were done at regular intervals upto 45 days of post debudding operations.Clinical evaluation of all the animals were assessed by rectal temperature, heart rate, respiration rate. Defecation, micturition & appetite were also observed & recorded closely during the first week and then at regular intervals upto 6 months.

Haematological Studies :

Blood was collected at pre - operative day and post operative 1,3,5,7,15,30, & 45 days. Haematological investigation like total count (T.C.) & differential count of leucolyte were done in routine manner.

Biochemical studies :

Blood serum was collected at preoperative day and post operative 1,3,5,7,15,30 & 45 days & checked for B.U.N. & blood creatinine levels in routine manner.

Studies on Inflammatory reactions and morphological features :

Sequence and extent of inflammation, necrosis, pain reaction on touch, any further growth of horn & other morphological features were evaluated throughout the study period.

3.1.4.e. Statistical Analysis :

The results were statistically analysed by using Fisser's 'F' test and Analysis of Variance (ANOVA) were done.

3.2. GROUP B_1

3.2.1. : Experimental Animals :

In this study, the experiment was carried out in Horse ward, Police training college, Barrackpore, West Bengal. The experiment was performed on a group of 7 horses, serially numbered from 22 to 28 having granulomatous growths and the group was identified as group B_1. Observations of the horses prior to chemosurgery were exactly identical to that described in 3.1.1.

3.2.2. & 3.2.3. : Drugs, Chemicals & other requisites.

The drugs, chemicals & other requisites used in this study are same as described in 3.1.2. & 3.1.3.

3.2.4. : Method

*3.2.4.a.: **Preparation of the chemosurgical agent*** - 50% of calcium chloride solution was prepared asceptically with sterile normal saline and 2% Xylocaine HCl in the method described in table -1

*3.2.4.b.: **Preparation of the animal just before chemosurgery***

The area of jugular vein was clearly shaved to facilitate the collection of blood sample. Also a wide area around the granulomatous growths were shaved & cleaned with alcohol (70%). The animals were carefully restrained & secured. The site of operation was again cleaned with 70% alcohol and then painted with Betadine. Before administration of the drug related physical parameters were recorded & blood samples taken for haematological & biochemical studies. These pre-operative parameters were regarded as control.

*3.2.4.c.: **Design of the experiment chalked out for group B_1 is presented in table 3.***

TABLE - 3 : Design of the experiment chalked out for Group B_1

SL NO.	GR. NO.	TOTAL NO. OF ANIMALS	METHODS
22 - 28	B_1	7	A total of 2 - 4 ml of 50% reconstituted solution of $CaCl_2$ according to the size of the growth, was injected deep into the tissues, in order to reach the base of the growth. A total number of 4 sites of injection were selected encircling the growth e.i. dorsal, ventral, anterior & posterior to the growth. At each site 0.5 - 1 ml of the solution was injected with the help of a 21 gauze sterile needle attached to a 10ml disposable syringe. The needle penetrated the skin at 60^o angle each time. Placentrex was injected at a dose rate of 10ml. I/M along with Inj. Trineurosol - H - 5ml I/m on every alternate day & 5-7 such injections were given to every animals.

3.2.4.d. Observations.

All the animals of group B_1 were observed for a period of 45 days postoperatively. They were kept in same natural condition with uniform standard diet and liberal supply of drinking water

Clinical, Haematological, Biochemical, Inflammatory & morphological studies were undertaken to assess the effectiveness of the drug.

Preoperative data of all the parameters were considered as control.

Clinical, haematological, biochemical, Inflammatory & morphological studies

The studies conducted were similar with that of debudding operations in calves, for 45 post - operative days.

3.2.4.e.: Statistical Analysis -

The results were statistically analysed by using the Fisser's 'F' test and Analysis of Variance (ANOVA) were done.

3.3. : CHEMOSURGERY : GROUP B_2

3.3.1.: Experimental Animals :

In this study, chemical debridement were carried out on a group of 7 canine clinical cases presented at Deptt. of Surgery & Radiology, W.B.U.A.F. Sc., Cal - 37. This group, designated as group B_2 were serially numbered from 29 to 35. The dogs were of both sexes, weighing from 12 - 35 kg and ages varying between 2 to 5 years.

The dogs were presented with a variety of suppurative lesions presented in table - 4

TABLE - 4 : Description of suppurative lesions in canine patients.

SL No.	Affections	Breed Affected	No. of Case	Serial No. Awarded
1.	Capped Elbow	Golden Retriever	1	29
2.	Sinus tract	GSD	2	30 & 31
		Labrador	1	32
3.	Bilateral anal	Spitz	1	33
	fistulla	GSD	2	34 & 35

3.3.2. : Drugs & Chemicals :

Identical as 3.1.2. & 3.2.2. with the addition of

1. Inj. Floxidin - 15 ml vial containing 10% Enrofloxacin w/v, Hoechct India Ltd.
2. Inj. Trineurosol - H- 5 ml vial containing

   Vit B_1 - 100 mg

   Vit B_6 - 50 mg

   Vit B_{12} - 1000 mcg/ml

   Merind Ltd., Bombay

3.3.3.: Other requisites -

Identical as 3.1.3. & 3.2.3. with the addition of -

1. Routine surgical instruments.
2. Ethilon - Suture material (Non absorbable), Johnson & Johnson Ltd., Mumbai.
3. Roll bandage.
4. Sterile gauze
5. Leucoplast adhesive tape (Johnson & Johnson Ltd., Mumbai)

3.3.4.: Method .

3.3.4.a - Preparation of the chemo debriding agent -

For chemical debriding 10% solution of calcium chloride was prepared asceptically with sterile normal saline.

3.3.4.b - Preparation of the animal just before chemodebridement.

The area of radial vein was shaved to facilitate collection of blood sample. A wide area around the wounds were shaved & cleaned with 70% alcohol. The animals were carefully restrained and secured. Before administering the drugs related physical parameters were recorded and blood samples taken for haematological & biochemical studies. These pre-operative parameters were regarded as control.

3.3.4.c. : Design of the experiment chalked out for group - B_2 is presented in table 5.

TABLE - 5 : Design of the experiment chalked out for group B_2.

SL NO.	GR. NO.	TOTAL NO. OF ANIMALS	METHODS
29 - 35	B_2	7	1. The accumulated pus & exudate of the lesion were at first drained & then the site was thoroughly irrigated with luke warm normal saline. 2. After mopping out the area with sterile gauze 2-5 ml of the reconstituted 10% $CaCl_2$ solution, depending on the nature & extension of infection along with 1-2ml placentrex and 1 ml of inj. Floxidin was irrigated locally once a day for the first 2 days and then on alternate days for 4-5 days. 3. When signs of healthy granulation tissue were imminent clinically, the wounds were sutured for healing by delayed primary closure. 4. Routine surgical management of the wounds were done till removal of the sutures. 5. Systemic I/M injection of Placentrex were administered daily for 5 - 7 days. The dose rate being 1ml below 10kg body weight & 2ml above 10kg body weight. 0.5 - 2ml inj. Floxidin I/M daily for 5 - 7 days & inj. Trineurosol - H, 0.5 to 1ml on alternate days I/M were given, depending on body weight.

3.3.4.d : Observations.

All the animals of group B_2 were observed for a period of 45 days post operatively. They were kept in same natural conditions with uniform standard diet & liberal supply of drinking water.

Clinical, Haematological, Biochemical, Inflammatory & morphological studies were undertaken to assess the effectiveness of the drug.

Pre - operative data of all the parameters were considered as control.

Clinical, haematological, biochemical, Inflammatory & morphological studies

The studies conducted were exactly similar, as that followed for debudding operations in calves and chemosurgery for granulomatous wounds for a period of 45 days post chemical debridement.

3.3.4.e : Statistical Analysis.

The results were statistically analysed by using the Fisser's 'F' test and Analysis of Variance (ANOVA) was done.

Fig. 1 - Experimental animals under Group – A.

Fig. 2 - Administration of 50% $CaCl_2$ to Group - A_1 animal.

Fig. 3 - A circle of erythematous swelling surrounded the horn buds, following administration of 50% $CaCl_2$.

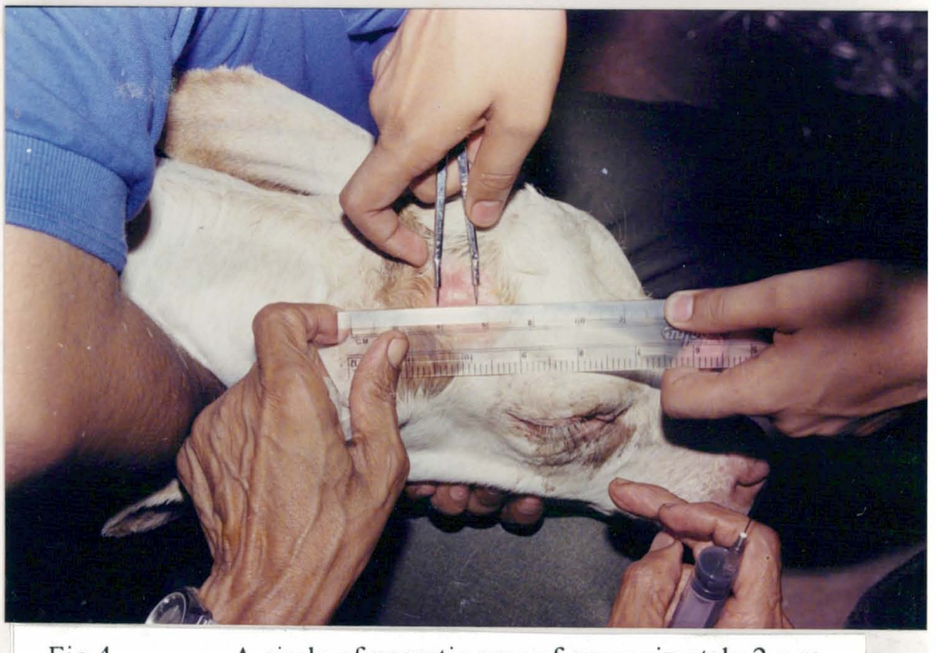


Fig.4 - A circle of necrotic area of approximately 2 c.m. was evident on post injection day 3.

Fig. 5 - Separation of the necrotic mass on 10th post-operative day.

Fig. 6 - Animal resembled a natural poll at 6th month of age.

Fig. 7 - The horn bud appearing vital & growing in another animal of group – A_1.

Fig. 8 - Administration of 70% $CaCl_2$ in group - A_2 animal.

Fig. 9 - The horn bud at 4th post-operative day.

Fig. 10 - The animal resembled a natural poll at 6th month of age.

Fig. 11 - Granulomatous growth in group - B_1 animal.

Fig. 12 - Site of administration of the drug posterior to the growth.

Fig. 13 - Site of administration of the drug anterior to the growth.

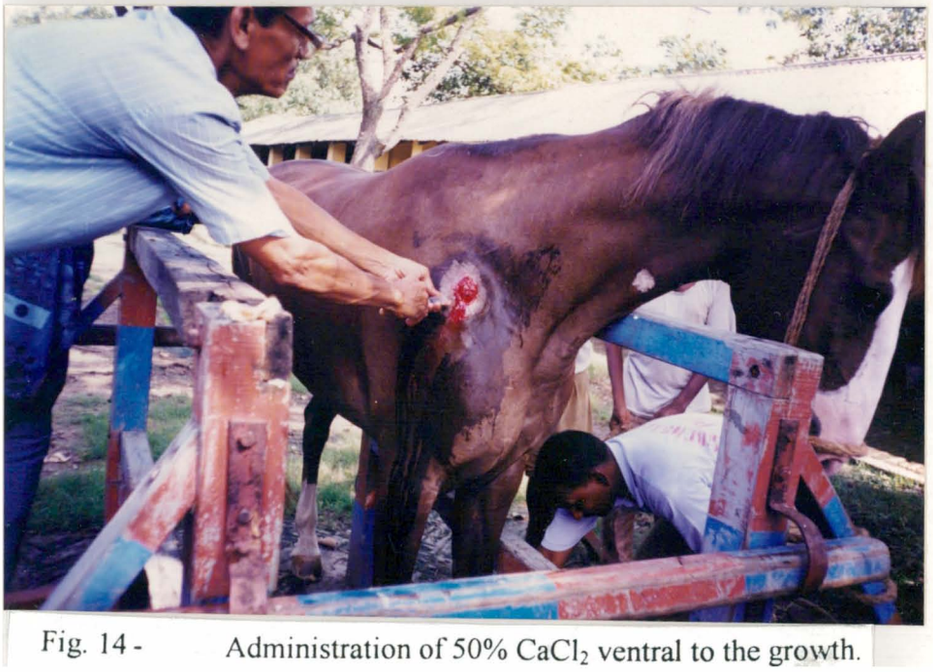


Fig. 14 - Administration of 50% $CaCl_2$ ventral to the growth.

Fig. 15 - The growth at 4th post-operative day.

Fig. 16 - The growth at 6th post-operative day.

Fig. 17 - The growth at 8th post-operative day.

Fig. 18 - A wide area of fibrous tissue was evident following sloughing of the growth.

Fig. 19 - Cosmesis gradually improved in group – B_1 animal.

Fig. 20 - Photograph showing suppurative lesion (sinus tract) in group - B_2 animal.

Fig. 21 - Irrigation of the wound with 10% $CaCl_2$, Placentrex & Floxidin.

Fig. 22 - Delayed primary closure being performed on 6th day following initiation of the treatment.

Fig. 23 - Photograph showing complete healing of the wound following removal of the suture.

Fig. 24 - Photograph revealing suppurative lesion (bilateral anal fistulla) which defied conventional therapy, in group - B_2 animal.

Fig. 25 - Irrigation of the suppurative lesion (bilateral anal fistulla).

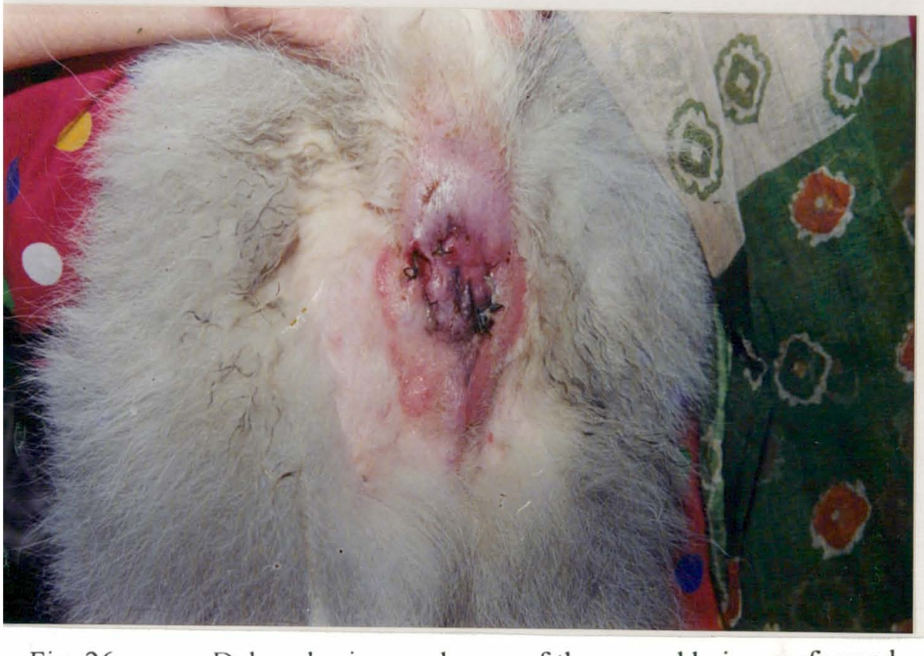


Fig. 26 - Delayed primary closure of the wound being performed on 7th day following initiation of the treatment.

Fig. 27 - Complete healing of the wound following removal of the sutures.

Fig. 28 - Photograph revealing suppurative lesion (Capped Elbow) in group - B_2 animal.

Fig. 29 - Irrigation of the lesion with the drug combination.

Fig. 30 - Antibiotic cream being applied following delayed primary closure.

Fig. 31 - Scar tissues & darkening of the area were observed following resolution for the wound.

Fig. 32 - Photograph showing suppurative lesion (sinus tract) in group - B_2 animal.

Fig. 33 - Irrigation of the wound with the drug combination in group – B_2 animal.

Fig. 34 - Complete healing of the wound following removal of the sutures.

Chapter IV

Results

RESULTS

Results and observations of all the experimental animals have been presented in this chapter. The results of different parameters have been discussed accordingly.

4.1. CLINICAL CHARACTERISTICS :

The clinical characteristics of all the animals i.e, temperature, respiration rate & heart rate of the five group of animals at pre and different days of post - operative state have been presented in table nos 6-8. The analysis of variance between different days of all the groups and between different groups of calves e.i, A_1, A_2 & A_3 have been presented in table nos. 17 & 18 respectively. Apart from this, the results of other clinical studies like appetite & water intake, micturition, defecation etc. of all the groups have been briefed seperately.

4.1.1. Temperature :

Temperature of different groups of animals at pre and different days of postoperative state were presented in table no.6. The following facts were evidenced from table nos. 6 & 17 →

In group A_1, there was increase in temperature in post operative days, when compared to preoperative state, but this increase was insignificant statistically.

In group A_2 & group B_1, temperature varies significantly ($P<0.01$) between pre and post operative state.

In group A_2, temperature increased steadily upto 3rd postoperative day, whereas the increase was upto 5th postoperative day for group B_1.

In group A_3 & group B_2, temperature variation was insignificant at different days of pre & post operative state.

From table no. - 18, it was evidenced that the temperature varied significantly ($P<0.01$) between groups A_1, A_2 & A_3 at 1st, 3rd, 5th, 7th & 15th postoperative days. The temperature being significant ($P<0.05$) at 30th & 45th postoperative day between groups A_1, A_2 & A_3.

4.1.2. Respiration rate :

Respiration rate of different groups of animals at pre and different days of post operative state were presented in table no. 7. The following facts were evidenced from table nos. 7 & 17. →

In group A_1 & group A_2, respiration rate increased significantly ($P<0.01$) between pre & post operative state. In both the groups A_1 & A_2, respiration rate increased upto 3rd post - operative day, thereafter a steady decline to base level was observed.

Group A_3, which was the control group, variation of respiration rate was insignificant.

In group B_1 , respiration rate increased significantly ($P<0.05$), highest rate being observed at 3rd postoperative day.

In group B_2 , respiration rate decreased significantly ($P<0.05$) between pre and postoperative state & this decrease was steady at all the postoperative days.

From table no. 18, it was evidenced that respiration rate varied significantly ($P<0.01$) between

Groups A_1, A_2 & A_3 upto 7th post - operative day. Thereafter the variation was insignificant.

4.1.3. Heart rate :

Heart rate of different groups of animals at pre and different days of post operative state were presented in table no. 8. The table nos. 8 & 17, reveals the following facts. →

In group A_1 & A_2 , heart rate increased significantly ($P<0.01$) when compared between pre & post operative state.

In groups B_2 & A_3 , variation of heart rate was insignificant between pre & post operative days.

In group B_1 , heart rate varied significantly ($P<0.01$) between pre & post operative state.

From table no. 18, it was evidenced that the heart rate varied signifi-

cantly ($P<0.01$) between groups A_1, A_2 & A_3 at 1st, 3rd, 5th & 7th post operative day. The heart rate being significant ($P<0.05$) at 15th, 30th & 45th post - operative day, between the said three groups.

In group A_1, appetite, micturition, defecation was more or less normal in all the calves, Water & milk intake in all the calves were less than normal for a few post - operative day.

In group A_2, Calf no. 8, 9, 10 & 11 refused feed upto 2nd post operative day, whereas the remaining calves resumed normal feeding from the evening of the 1st post operative day. Water & milk intake in all the calves were less than normal for a few post - operative day. Micturition & defecation were scanty for the first 2-3 days in all the calves.

In group A_3, the control group, appetite, micturition, defecation was normal in all the post operative days of study.

In group B_1 , the animals refused feed & water on the 1st post operative day. However, appetite & water intake gradually reversed to normal state within a few days following administration of $CaCl_2$. Defecation & urination was more or less normal in all the horses of this group, whereas in group B_2 , appetite, defecation & urination was normal in all the dogs throughout the course of the study.

4.2. HAEMATOLOGICAL CHARACTERISTICS :

The haematological characteristics of all the animals i.e, total count of erythrocyte & leucocyte & differential count of leucocyte of the five group of animals at pre and different days of post operative state were given in table nos. 9 - 14. The ANOVA between different days and between different groups of bull calves i.e, A_1, A_2 & A_3 at pre & post operative days have been presented in table nos. 15 & 16 respectively.

4.2.1. Erythrocyte :

The means of total count of erythrocyte at pre & different days of post operative state using different concentrations of $CaCl_2$ along with normal saline in chemical debudding and different concentration of $CaCl_2$ in chemo-surgery were presented in table no. 9. Table nos. 9 & 19 reveals the following facts →

In groups A_1, A_2 & B_1 , total count of erythrocyte reduced significantly

($P<0.01$) between pre and post operative state.

Group A_3, which was the control group, variation in total count of erythrocyte was insignificant.

In group B_2 total count of erythrocyte steadily increased in all the post operative days, but statistical analysis failed to show this increase as a significant increase.

From the result of different days in post operative state it was evidenced that the highest values were observed at 45th day & the lowest value at 3rd day in groups A_1, A_2 & B_1.

It was also evidenced from table no. 20 that erythrocyte count varied significantly ($P<0.01$) between groups A_1, A_2 & A_3 at 1st, 3rd, 5th & 7th post operative days. The count being significant ($P<0.05$) at 15th post operative day. Thereafter the count was insignificant at the remaining 30th & 45th post operative days.

4.2.2. Leucocyte :

The means of total count of leucocyte at pre and different days of post operative state using different concentration of $CaCl_2$ solution in both chemical debudding & chemosurgery were presented in table no 10. The following informations were evidenced from table nos. 10 & 19 →

In group A_1 & A_2, the total count of leucocyte decreased upto 5th day, then gradually increased upto 45th day. The change being significant ($P<0.01$).

In group A_3, variation in total count of leucocyte was insignificant.

In group B_1, total count decreased upto 7th post operative day, thereby gradually increased upto 45th day, the variation being statistically significant ($P<0.01$).

In group B_2, total count of leucocyte decreased throughout the course of the study but statistical analysis failed to show this variation, a significant one.

From table no. 20, it was evidenced that significant ($P<0.01$) difference was present among different post operative days with the exception of 45th day, between the groups A_1, A_2 & A_3.

4.2.3. Neutrophil :

The means of neutrophil number at pre and different days of post operative state using different concentrations of $CaCl_2$ both for chemical debudding and chemosurgery, were presented in table no. 11. It was evidenced from table nos. 11 & 19 that the neutrophil count varies significantly ($P<0.01$) between pre and different post operative days in group A_1 ,A_2 & B_1 .

In group B_2 , the count decreased in all the postoperative days, the decrease being significant ($P<0.05$).

Variation of neutrophil count was insignificant for the control group A_3, between pre & different post operative days.

It was evidenced that highest neutrophil count was observed at 5th post operative day in groups A_1 & A_2 and for group B_1, highest value was observed at 7th post operative day. The lowest value of all these 3 groups were observed at 45th post operative day.

From table no. 20, it was evidenced that there was significant ($P<0.01$) change of neutrophil count in all the three groups of A_1 ,A_2 & A_3 at 1st, 3rd, 5th, 7th & 15th post operative days. Thereafter the change was insignificant.

4.2.4. Eosinophil :

The means of eosinophil count at pre and different days of post operative state of the different groups of animals were presented in table no. 13. The following facts were evidenced from table nos. 13 & 19.

Eosinophil count increased significantly ($P<0.05$) in groups A_1 & B_1.

A similar increasing trend could be observed in groups A_2 & B_2 but analysis failed to show this increase a significant increase.

In group A_3, the control group, eosinophil count varied insignificantly between pre & post operative days.

It was also evidenced from table no. 20 that eosinophil count varied significantly ($P<0.05$) between groups A_1 ,A_2 & A_3 at 3rd. 5th & 7th postoperative days. The count being insignificant at 1st, 15th, 30th & 45th postoperative days.

4.2.5. Monocyte :

The means of monocyte count at pre and different days of post operative state was shown in table no. 14 It was observed from table nos. 14 & 19 monocyte count increased in groups A_1 & A_2 . However this change was insignificant. The highest count in this two groups were recorded at 7th post operative day.

Monocyte count varied insignificantly between pre & post operative days in group A_3.

Monocyte count increased significantly ($P<0.05$) in group B_1 , highest count being observed at 7th post operative day, & the count decreased steadily thereafter.

In contrast to the above facts, monocyte count in group B_2 ,decreased significantly ($P<0.01$) from elevated levels to normal levels.

From table no. 20, it was observed that significant ($P<0.05$) difference was present among groups A_1 ,A_2 & A_3 at 7th post operative day. The changes were insignificant at the remaining post operative days.

4.2.6. Lymphocyte :

The means of lymphocyte count at pre and different days of post operative state using different concentrations of $CaCl_2$ in chemical debudding & chemosurgery were presented in table no. - 12.

The following facts were evidenced from table nos. 12 &19 →

Lymphocyte count decreased upto 5th postoperative day in groups A_1 & A_2 .Thereafter the count increased towards base level upto 45th post operative day. This change proved significant ($P<0.01$) statistically.

A similar trend was observed for group B_1, with the difference that the count decreased upto 7th post operative day. This change also proved significant ($P<0.01$).

In contrast, lymphocyte count in group B_2 increased throughout the course of the study, the increase being insignificant.

Lymphocyte count varied insignificantly in the control group A_3 between pre & post operative days.

From table no. 20, it was observed that there was significant ($P<0.01$) change of lymphocyte in all the three groups i.e, A_1 ,A_2 & A_3 at each post operative day excepting the 3rd & 45th day.

4.3. BIOCHEMICAL CHARACTERISTICS :

The biochemical characteristics of all the animals i.e, blood urea nitrogen & blood creatinine of all the five groups of animals at pre and different days of post operative state have been presented in table nos. 15 & 16. The analysis of variance between different days of all the groups and between different groups of calves e.i, A_1 ,A_2 & A_3 have been presented in table nos. 17 & 18 respectively.

4.3.1. Blood Urea Nitrogen :

The means of blood urea nitrogen of different groups of animals at pre and different days of post operative state were presented in table no. 15. The following informations were revealed from table nos. 15 & 21 →

Blood Urea nitrogen level increased upto 5th postoperative day in groups A_1 ,A_2 & B_1 , but statistical analysis failed to show this increase, a significant variation, between pre & post operative days.

Blood urea nitrogen did not varry significantly in group A_3, between pre & post operative days.

From table no. 22, it was observed that the changes occuring at all the post operative days were insignificant between the groups of bull calves e.i, A_1 ,A_2 & A_3 .

4.3.2. Blood Creatinine :

The means of blood creatinine at pre and different days of post operative state using different concentration of $CaCl_2$ solution in both chemical debudding & chemosurgery were presented in table no. 16.

It was evidenced from table nos. 16 & 21 that the levels of blood creatinine in all the groups did not varry significantly between pre and different post operative days.

From table no. 22 it was evidenced that no significant change occurred at all the post operative days between the groups A_1 ,A_2 & A_3 .

4.4. STUDIES ON INFLAMMATORY CHANGES & MORPHOLOGICAL FEATURES.

Group A_1

Pain was evident in all the animals when the needle was driven under the hornbuds during administration of 50% $CaCl_2$.(Fig 2) Following the injection, some pain was evident upto 3rd post operative day as the animals reacted on touching the horn buds.

In calves no. 1, 2, 4, 5, 6 & 7, within seconds, following injection a circle of erythematous slight swelling of 2-5 cm in diameter surrounded the horn buds (Fig - 3) & it marked the boundary of subsequent necrosis (fig.4) A circle of dry gangrene can be palpated by post injection day 3 or 4 and seperation begins approximately on day 10. (fig 5) Healing was completed in 6 - 10 weeks, result resembled a natural poll. (fig 6).

In contrast to the above results, in one bull calf (calf no. 3) failure was evident after 10 days as the horn buds in that calf appeared vital & growing (fig. 7).

Group A_2

Comparitively more pain was evident in all the animals during & following administration of the drug (70% $CaCl_2$) (Fig.8) upto a period of 5th post operaive day & during that period the animals reacted reacted severely on touching.

The sequence of inflammatory reactions observed in this group were more or less same as that of group I. However the extent of inflammation was more severe in all the calves of this group.

Failure was observed in calf No. 14 as continued growth of horn buds were evident after 10 days.

Group A_3

Pain was evident when the needle was driven under the horn bud. However, pain sensation could not be detected in the post operative days following administration of normal saline.

Following the initial redness due to hyperaemia at the site of administra-

tion of the normal saline, no other sequence of inflammatory reactions were evident in all the calves of this group.

Horn buds were seen to grow in the usual manner, in all the calves, throughout the course of the study.

Group B_1

Severe pain was evident in all the animals as the needle was pricked at specific sites surrounding the growth. (figs 12,13 &14)

Pain sensation & tenderness could also be observed in this group, upto 5th postoperative day.

Severe inflammatory reactions followed by marked necrosis of both the affected & normal tissues surrounding the growth upto a maximum diameter of 5 cm was evident during the course of the study.

The growths gradually atroplied (figs, 15,16 & 17) reduced in size & ultimately stoughed off within 8 - 10 days (fig 18) following administration of the drug.

Group B_2

Pain reactions were not evident while irrigating the wound with the drug combination. (Figs. 21,25, 29, 33).

Amount of pus & inflammatory exudates in all the dogs gradually reduced and healthy granulation tissues could be observed within 5th to 8th post operative days.

Post operative complications following suturing of wounds & till removal of the sutures were negligible and healing progressed in its normal sequence in all the animals of this group. However, The Golden Retriever suffering from capped elbow developed some scar tissues & darkening of the area (fig. 31) during the course of the healing.

Table - NO. 6

Least Square Means with standard error (S.E.) of Temperature of different groups of animals in pre & post operative states.

Group	Pre - operative	Post - operative						
		0	1	3	5	7	15	30	45
A_1	Mean	101.94	102.17	102.14	102.11	102.057	102.0	101.88	101.8
	± SE	0.0719	0.0918	0.0719	0.085	0.084	0.097	0.0961	0.097
A_2	Mean	101.97	102.42	102.42	102.31	102.17	101.97	101.97	101.77
	± SE	0.08	0.0680	0.068	0.0105	0.08	0.08	0.08	0.08
A_3	Mean	101.65	101.65	101.65	101.65	101.65	101.6	101.6	101.51
	± SE	0.121	0.120	0.121	0.121	0.121	0.115	0.115	0.085
B_1	Mean	100.34	100.74	100.74	100.74	100.65	100.42	100.37	100.31
	± SE	0.071	0.057	0.057	0.057	0.057	0.068	0.068	0.073
B_2	Mean	101.77	101.77	101.8	101.77	101.71	101.68	101.65	101.64
	± SE	0.125	0.125	0.100	0.103	0.106	0.08	0.113	0.115

Table - NO. 7

Least Square Means with standard error (S.E.) of Respiration of different groups of animals in pre & post operative states.

Group	Pre - operative		Post - operative						
		0	1	3	5	7	15	30	45
A_1	Mean	28.28	31.00	30.71	30.57	30.0	28.28	28.14	28.0
	± SE	0.563	0.786	0.808	0.718	0.654	0.565	0.633	0.577
A_2	Mean	28.57	33.0	32.71	31.85	31.28	28.85	28.57	28.28
	± SE	0.528	0.72	0.86	0.884	0.606	0.404	0.528	0.42
A_3	Mean	28.0	28.0	28.0	28.0	28.0	28.0	27.57	27.28
	± SE	0.617	0.617	0.617	0.617	0.617	0.617	0.571	0.644
B_1	Mean	12.85	15.85	15.85	15.42	15.57	13.28	12.85	12.57
	± SE	0.936	0.986	0.986	0.922	0.966	0.892	0.737	0.685
B_2	Mean	24.28	23.71	23.42	22.42	22.14	22.14	21.85	21.42
	± SE	0.644	0.473	0.428	0.428	0.508	0.508	0.508	0.368

Table - NO. 8

Least Square Means with standard error (S.E.) of Heart Rate of different groups of animals in pre & post operative states.

Group	Pre - operative		Post - operative						
		0	1	3	5	7	15	30	45
A_1	Mean	60.42	70.0	67.28	64.57	61.28	59.71	59.57	58.85
	± SE	1.231	1.046	0.993	1.109	1.189	1.285	1.192	1.37
A_2	Mean	59.57	70.57	69.57	66.42	63.42	62.0	61.0	60.85
	± SE	0.685	1.250	0.947	0.868	0.922	0.755	0.786	0.769
A_3	Mean	58.14	58.14	58.14	58.14	58.14	57.71	57.42	57.28
	± SE	0.799	0.799	0.799	0.799	0.799	0.68	0.528	0.606
B_1	Mean	36.71	46.14	44.0	41.0	39.28	36.41	36.28	36.28
	± SE	0.680	0.459	0.577	0.487	0.808	0.746	0.719	0.714
B_2	Mean	82.0	82.28	82.0	81.857	81.285	81.0	81.0	81.142
	± SE	0.872	0.968	0.899	0.911	0.865	0.816	0.816	0.799

Table - NO. 9

Least Square Means with standard error (S.E.) of Erythrocyte (x10^6/cumm) of different groups of animals in pre & post operative states.

Group	Pre - operative		Post - operative						
		0	1	3	5	7	15	30	45
A_1	Mean	4.245	4.032	3.79	3.86	3.98	4.16	4.27	4.31
	± SE	0.071	0.061	0.055	0.063	0.075	0.058	0.046	0.036
A_2	Mean	4.151	3.90	3.57	3.68	3.74	3.96	4.17	4.27
	± SE	0.095	0.809	0.432	0.650	0.566	0.638	0.264	0.264
A_3	Mean	4.22	4.22	4.22	4.22	4.22	4.22	4.22	4.22
	± SE	0.041	0.041	0.041	0.040	0.041	0.039	0.039	0.039
B_1	Mean	7.12	6.22	6.09	6.18	6.23	6.45	6.64	6.81
	± SE	0.062	0.073	0.08	0.067	0.066	0.077	0.067	0.075
B_2	Mean	5.17	5.17	5.26	5.46	5.60	5.64	5.74	5.75
	± SE	0.166	0.166	0.147	0.142	0.139	0.146	0.145	0.138

Table - NO. 10

Least Square Means with standard error (S.E.) of Leucocyte (x10^3/cumm) of different groups of animals in pre & post operative states.

Group	Pre - operative		Post - operative						
		0	1	3	5	7	15	30	45
A_1	Mean	8.17	7.78	7.60	7.51	7.58	7.80	7.95	8.08
	± SE	0.078	0.028	0.045	0.059	0.061	0.034	0.018	0.054
A_2	Mean	8.20	7.67	7.49	7.40	7.44	7.73	7.87	8.04
	± SE	0.101	0.024	0.052	0.068	0.059	0.024	0.021	0.079
A_3	Mean	8.19	8.19	8.19	8.19	8.19	8.19	8.19	8.19
	± SE	0.084	0.084	0.084	0.083	0.083	0.085	0.085	0.085
B_1	Mean	7.52	6.58	6.46	6.41	6.35	6.56	6.90	7.20
	± SE	0.112	0.12	0.102	0.086	0.089	0.073	0.09	0.093
B_2	Mean	13.9	13.81	13.40	12.74	12.19	11.05	9.89	9.40
	± SE	2.02	1.935	1.77	1.557	1.366	0.859	0.391	0.273

Table - NO. 11

Least Square Means with standard error (S.E.) of Neutrophil in different groups of animals in pre & post operative states.

Group	Pre - operative	Post - operative						
		0	1	3	5	7	15	30	45
A_1	Mean	38.42	47.71	50.57	49.14	48.0	45.285	40.714	37.857
	± SE	0.996	1.189	1.231	1.078	0.872	1.209	1.084	0.961
A_2	Mean	38.85	49.42	52.71	50.71	49.57	46.85	42.14	40.14
	± SE	1.261	1.461	1.554	1.322	1.192	1.502	1.203	1.121
A_3	Mean	39.71	40.42	39.85	39.71	39.71	39.71	39.42	39.14
	± SE	0.644	0.685	0.633	0.644	0.644	0.644	0.571	0.553
B_1	Mean	54.42	63.0	66.0	68.0	68.57	65.71	61.85	58.42
	± SE	1.020	1.362	1.215	0.755	0.685	0.714	0.854	0.840
B_2	Mean	57.57	56.14	54.0	52.57	51.57	50.85	50.28	50.0
	± SE	0.782	0.986	1.215	1.151	1.377	1.387	1.491	1.234

Table - NO. 12

Least Square Means with standard error (S.E.) of Lymphocyte in different groups of animals in pre & post operative states.

Group	Pre - operative		Post - operative						
		0	1	3	5	7	15	30	45
A_1	Mean	58.71	50.57	47.57	46.57	47.42	50.71	54.42	58.0
	± SE	0.808	0.611	0.649	0.718	0.719	1.106	0.841	0.975
A_2	Mean	58.28	50.42	47.85	46.57	47.57	50.57	54.28	57.0
	± SE	0.968	0.719	0.828	0.895	0.782	1.250	1.084	1.327
A_3	Mean	57.14	56.85	56.71	57.14	57.14	57.14	57.0	57.0
	± SE	0.828	0.737	0.746	0.828	0.828	0.828	0.755	0.755
B_1	Mean	35.28	27.71	21.71	21.0	19.71	25.14	28.42	32.57
	± SE	0.837	0.778	0.680	0.899	0.968	0.961	1.151	0.841
B_2	Mean	21.71	21.71	22.28	22.42	23.28	23.85	24.42	24.85
	± SE	1.106	1.106	1.106	0.996	0.808	1.633	0.480	0.553

Table - NO. 13

Least Square Means with standard error (S.E.) of Eosinophil in different groups of animals in pre & post operative states.

Group	Pre - operative	Post - operative						
		0	1	3	5	7	15	30	45
A_1	Mean	1.57	1.42	2.42	2.57	2.42	2.42	2.28	2.00
	± SE	0.789	0.202	0.202	0.202	0.202	0.297	0.285	0.218
A_2	Mean	1.85	1.42	2.42	2.42	2.42	2.14	2.14	2.14
	± SE	0.142	0.202	0.200	0.202	0.202	0.34	0.34	0.34
A_3	Mean	1.71	1.71	1.57	1.71	1.71	1.57	1.57	1.57
	± SE	0.184	0.184	0.202	0.184	0.184	0.202	0.202	0.202
B_1	Mean	4.28	4.42	5.0	5.85	6.42	7.14	6.0	5.28
	± SE	0.521	0.571	0.487	0.459	0.480	0.508	0.487	0.359
B_2	Mean	3.42	3.57	3.71	3.71	3.85	3.85	4.28	4.28
	± SE	0.297	0.202	1.184	0.184	0.142	0.142	0.285	0.285

Table - NO. 14

Least Square Means with standard error (S.E.) of Monocyte in different groups of animals in pre & post operative state.

Group	Pre - operative		Post - operative						
		0	1	3	5	7	15	30	45
A_1	Mean ± SE	1.28 0.184	1.28 0.184	1.28 0.184	1.71 0.285	1.85 0.26	1.85 0.26	1.42 0.199	1.42 0.199
A_2	Mean ± SE	1.28 0.184	1.28 0.184	1.28 0.184	2.0 0.0	2.14 0.260	1.85 0.34	1.57 0.20	1.57 0.20
A_3	Mean ± SE	1.28 0.184	1.28 0.184	1.28 0.184	1.28 0.184	1.28 0.184	1.57 0.202	1.57 0.202	1.14 0.142
B_1	Mean ± SE	4.28 0.519	4.71 0.521	4.85 0.508	5.71 0.473	7.14 0.594	7.14 0.594	6.85 0.633	6.42 0.649
B_2	Mean ± SE	14.0 1.086	14.0 1.086	13.42 0.972	12.57 1.109	11.85 0.799	10.0 0.654	9.57 0.48	8.42 0.528

Table - NO. 15

Least Squae Means with standard error (S.E.) of Blood Urea Nitrogen (mgm/dl) in different groups of animals in pre & post operative states.

Group	Pre - operative		Post - operative						
		0	1	3	5	7	15	30	45
A_1	Mean ± SE	5.17 0.189	5.18 0.179	5.25 0.201	5.27 0.214	5.27 0.212	5.21 0.212	5.15 0.191	5.15 0.191
A_2	Mean ± SE	5.44 0.273	5.47 0.257	5.51 0.264	5.58 0.271	5.51 0.264	5.44 02.73	5.44 0.273	5.44 0.273
A_3	Mean ± SE	5.12 0.183	5.12 0.183	5.1 0.185	5.11 0.188	5.11 0.188	5.11 0.188	5.11 0.188	5.11 0.188
B_1	Mean ± SE	10.14 0.363	10.14 0.363	10.22 0.340	10.24 0.368	10.24 0.368	10.15 0.361	10.14 0.363	10.12 0.37
B_2	Mean ± SE	14.85 0.799	14.85 0.799	14.85 0.799	14.14 0.737	13.57 0.685	12.28 0.68	11.0 0.654	10.428 0.423

Table - NO. 16

Least Square Means with standard error (S.E.) of Blood Creatinine (mgm/dl) in different groups of animals in pre & post operative state.

Group	Pre - operative		Post - operative						
		0	1	3	5	7	15	30	45
A_1	Mean	1.31	1.31	1.32	1.35	1.35	1.31	1.31	1.31
	± SE	0.026	0.026	0.028	0.020	0.020	0.026	0.026	0.026
A_2	Mean	1.32	1.32	1.34	1.37	1.35	1.32	1.32	1.32
	± SE	0.018	0.020	0.020	0.018	0.020	0.018	0.018	0.018
A_3	Mean	1.31	1.31	1.35	1.35	1.32	1.31	1.31	1.31
	± SE	0.014	0.014	0.02	0.02	0.018	0.014	0.014	0.014
B_1	Mean	1.31	1.31	1.38	1.38	1.37	1.34	1.31	1.31
	± SE	0.040	0.040	0.040	0.040	0.028	0.036	0.04	0.04
B_2	Mean	1.70	1.70	1.71	1.64	1.58	1.47	1.37	1.31
	± SE	0.089	0.089	0.086	0.086	0.073	0.056	0.042	0.014

Table - NO. 17

ANOVA of different Clinical Characteristics of the different groups of animals between different days following Chemosurgery.

CHARACTERISTICS	SOURCE OF VARIANCE	DEGREE OF FREEDOM	Mean - Sum - Squares				
			A_1	A_2	A_3	B_1	B_2
Temperature	Between days	7	0.1216	0.3100 **	0.017	0.266 **	0.023
	Error	48	0.0536	0.046	0.094	0.028	0.101
Heart rate	Between days	7	118.44 **	122.68 **	0.928	102.834 **	1.8775
	Error	48	9.797	5.529	3.767	3.041	5.3035
Respiration rate	Between days	7	12.03 **	28.56 **	0.530	20.059 *	7.091 *
	Error	48	3.142	2.904	2.648	5.666	1.678

** $P < 0.01$ * $P < 0.05$

Table - NO. 18

ANOVA of different Clinical Characteristics between different groups of animals (A_1,A_2 & A_3) at pre & post operative days.

Characteristics	Source of variation	Degree of freedom	Mean - Sum - Squares (day)							
			Preoperative	Post operative						
			0	1	3	5	7	15	30	45
Temperature	Between days	2	0.211	1.08 **	1.065 **	0.794 **	0.510 **	0.348 *	0.264 *	0.373 *
	Error	18	0.061	0.064	0.057	0.077	0.066	0.068	0.067	0.054
Heart rate	Between days	2	9.33	344.61**	256.0 **	132.33 **	49.47 **	32.19 *	22.619 *	22.428 *
	Error	18	6.126	7.69	5.88	6.127	6.77	6.26	5.412	6.619
Respiration rate	Between days	2	0.571	44.333**	39.1904**	27.0 **	19.1904**	1.333	1.761	1.856
	Error	18	2.285	3.555	4.158	3.92	2.746	2.0158	2.349	2.158

** P< 0.01 *P<0.05

Table - NO. 19

ANOVA of different Haematological Characteristics of the different groups of animals between different days following Chemosurgery.

CHARACTERISTICS	SOURCE OF VARIANCE	DEGREE OF FREEDOM	Mean - Sum - Squares				
			A_1	A_2	A_3	B_1	B_2
Erythrocyte	Between days	7	0.2655 **	0.446 **	0.00008	0.923 **	0.422
	Error	48	0.023	0.028	0.0101	0.034	0.155
Leucocyte	Between days	7	0.41 **	0.58 **	0.00004	1.232 **	21.477
	Error	48	0.017	0.025	0.049	0.065	14.21
Neutrophil	Between days	7	176.57 **	192.017 **	0.938	168.397 **	54.834 *
	Error	48	8.863	12.494	2.767	6.327	10.46
Eosinophil	Between days	7	1.3061 *	0.834	0.04	6.834 *	0.67
	Error	48	0.369	0.464	0.261	1.666	0.351
Monocyte	Between days	7	0.446	0.813	0.16	9.418 *	32.46 **
	Error	48	0.351	0.321	0.238	2.238	5.369
Lymphocyte	Between days	7	160.153 **	139.02 **	0.181	220.262 **	10.408
	Error	48	4.696	7.053	4.369	5.666	5.309

** $P < 0.01$ * $P < 0.05$

Table - NO. 20

ANOVA of different Haematological Characteristics between different groups (A_1,A_2 & A_3) at pre & post operative days.

Characteristics	Source of variation	Degree of freedom	Mean - Sum - Squares (day)							
			Preoperative	Post operative						
			0	1	3	5	7	15	30	45
Erythrocyte	Between days	2	0.017	0.18 **	0.77 **	0.528 **	0.398 **	0.13 **	0.017	0.013
	Error	18	0.036	0.027	0.014	0.022	0.023	0.02	0.012	0.011
Leucocyte	Between days	2	0.0016	0.531 **	1.00 **	1.26 **	1.10 **	0.426 **	0.194 **	0.079
	Error	18	0.05	0.019	0.027	0.035	0.032	0.02	0.018	0.034
Neutrophil	Between days	2	3.0	159.85 **	332.14 **	247.76 **	196.33 **	98.61 **	12.90	9.19
	Error	18	7.0	9.38	10.111	7.761	6.063	9.65	6.88	5.809
Eosinophil	Between days	2	0.142	0.269	1.714 *	1.476 *	1.19 *	1.33	1.00	3.476
	Error	18	0.222	0.190	0.285	0.269	0.269	0.571	0.55	1.714
Monocyte	Between days	2	0.006	0.006	0.006	0.904	1.333 *	0.19	0.476	0.333
	Error	18	0.237	0.237	0.904	0.269	0.39	0.523	0.285	0.238
Lymphocyte	Between days	2	4.61	94.33 **	189.142**	260.76 **	217.00 **	98.61 **	16.33	2.33
	Error	18	8.65	3.349	3.88	4.68	4.238	8.111	5.73	7.66

** P< 0.01 *P<0.05

Table - NO. 21

ANOVA of different Biochemical Characteristics of the different groups of animals between different days following Chemosurgery.

CHARACTERISTICS	SOURCE OF VARIANCE	DEGREE OF FREEDOM	Mean - Sum - Squares				
			A_1	A_2	A_3	B_1	B_2
Blood Urea Nitrogen	Between days	7	0.0174	0.01	0.00005	0.0175	22.602**
	Error	48	0.2835	0.5065	0.244	0.9431	3.5059
Blood Creatinine	Between days	7	0.002	0.001	0.002	0.007	0.004
	Error	48	0.004	0.002	0.001	0.010	0.036

** $P < 0.01$

Table - NO. 22

ANOVA of different Biochemical Characteristics between different groups (A_1,A_2 & A_3) at pre & post operative days.

Characteristics	Source of variation	Degree of freedom	Mean - Sum - Squares (day)							
			Preoperative	Post operative						
			0	1	3	5	7	15	30	45
Blood Urea Nitrogen	Between days	2	0.203	0.236	0.306	0.306	0.284	0.198	0.223	0.223
	Error	18	0.336	0.309	0.340	0.362	0.353	0.365	0.344	0.339
Blood Creatinine	Between days	2	0.0004	0.0004	0.501	0.0004	0.001	0.0004	0.0004	0.0004
	Error	18	0.002	0.002	0.003	0.002	0.002	0.002	0.002	0.002

Fig.35:REPRESENTATION OF AV. COMPERATIVE TEMP. IN ALL GROUPS OF ANIMALS.[vide tab.no.6].

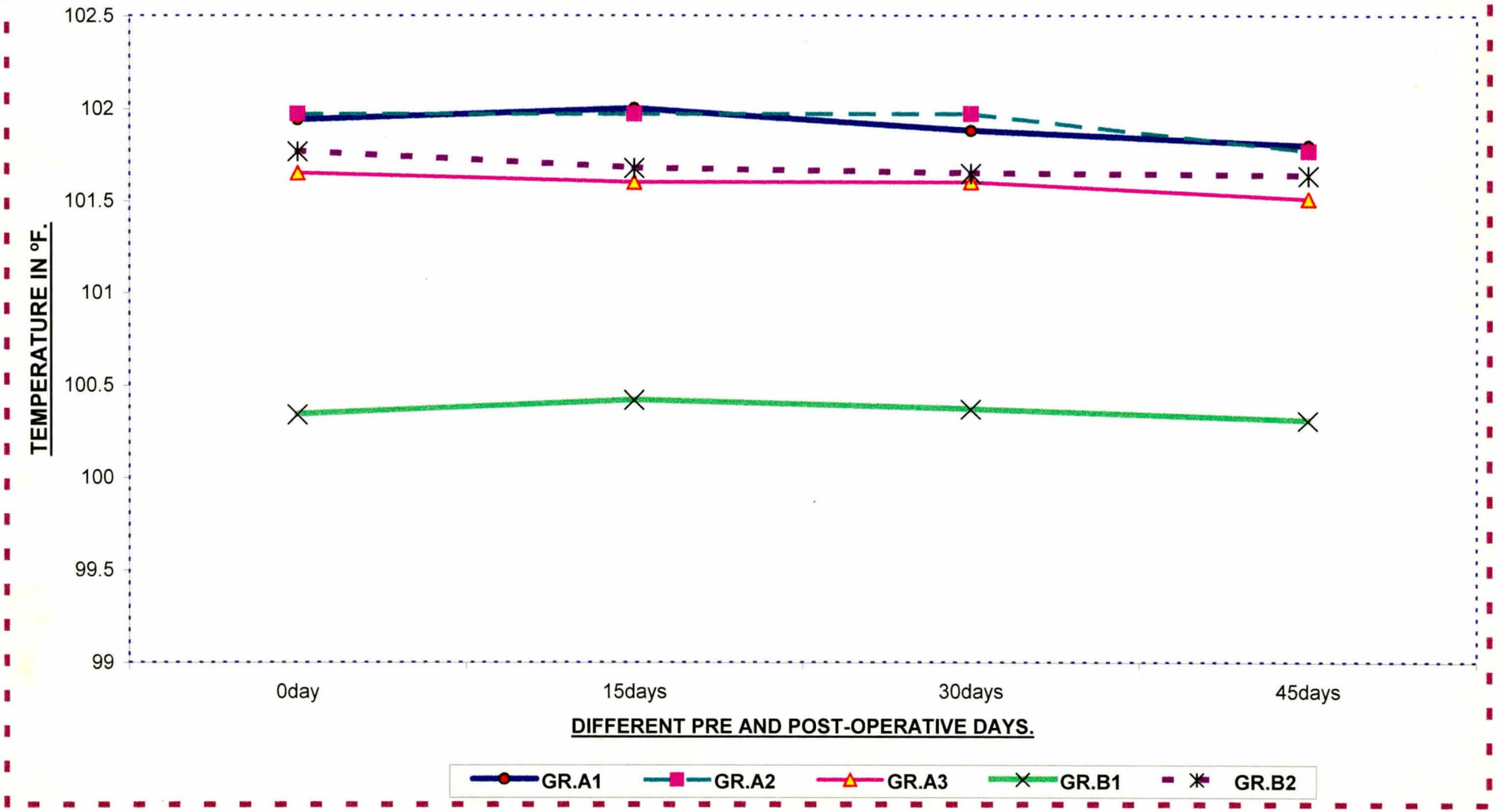


Fig.36:REPRESENTATION OF AV. COMPERATIVE RESPIRATION IN ALL GROUPS OF ANIMALS.[vide tab.no.7].

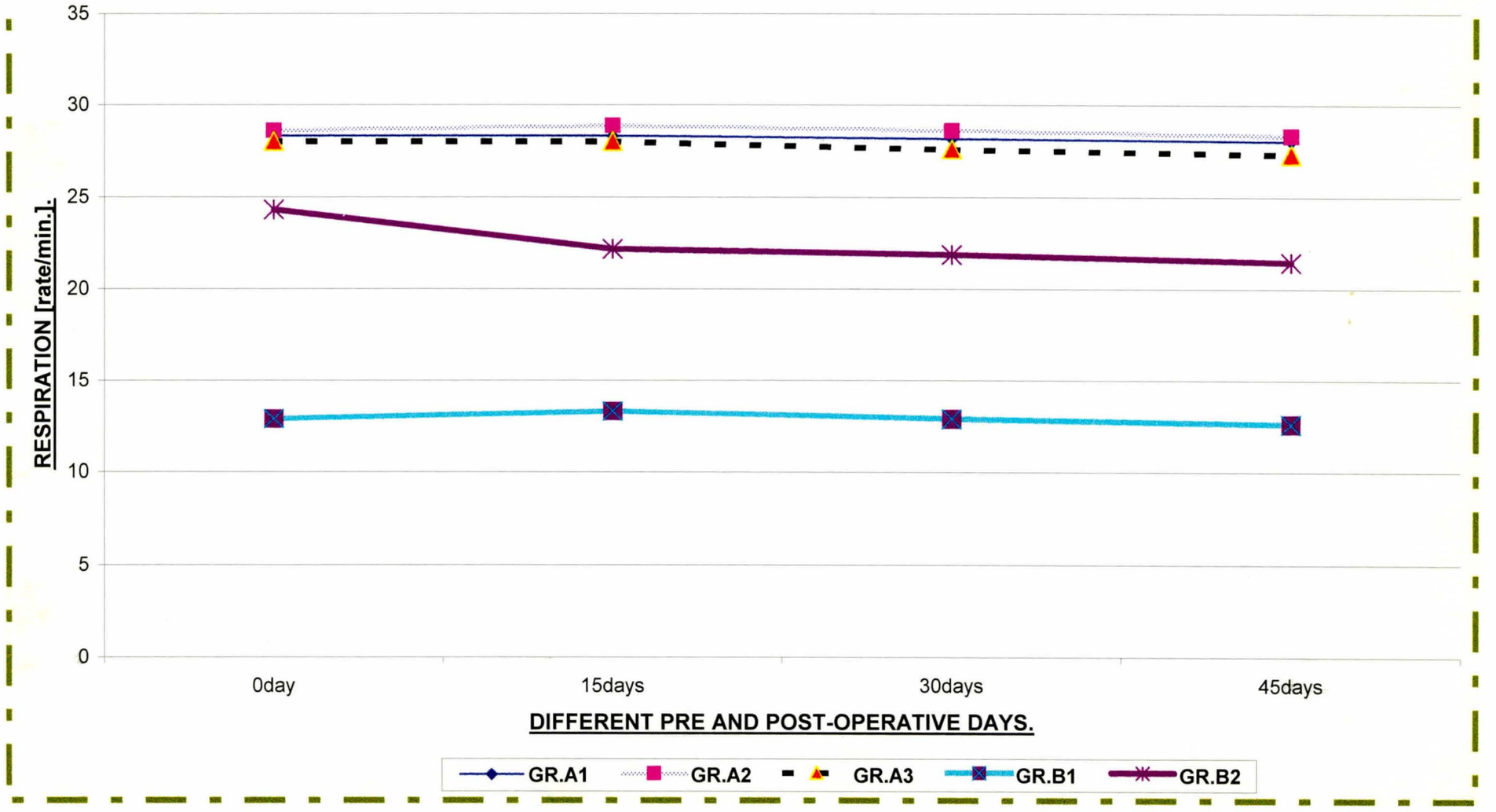


Fig.37:REPRESENTATION OF AV. COMPERATIVE HEART-RATE IN ALL GROUPS OF ANIMALS [vide tab.no.8].

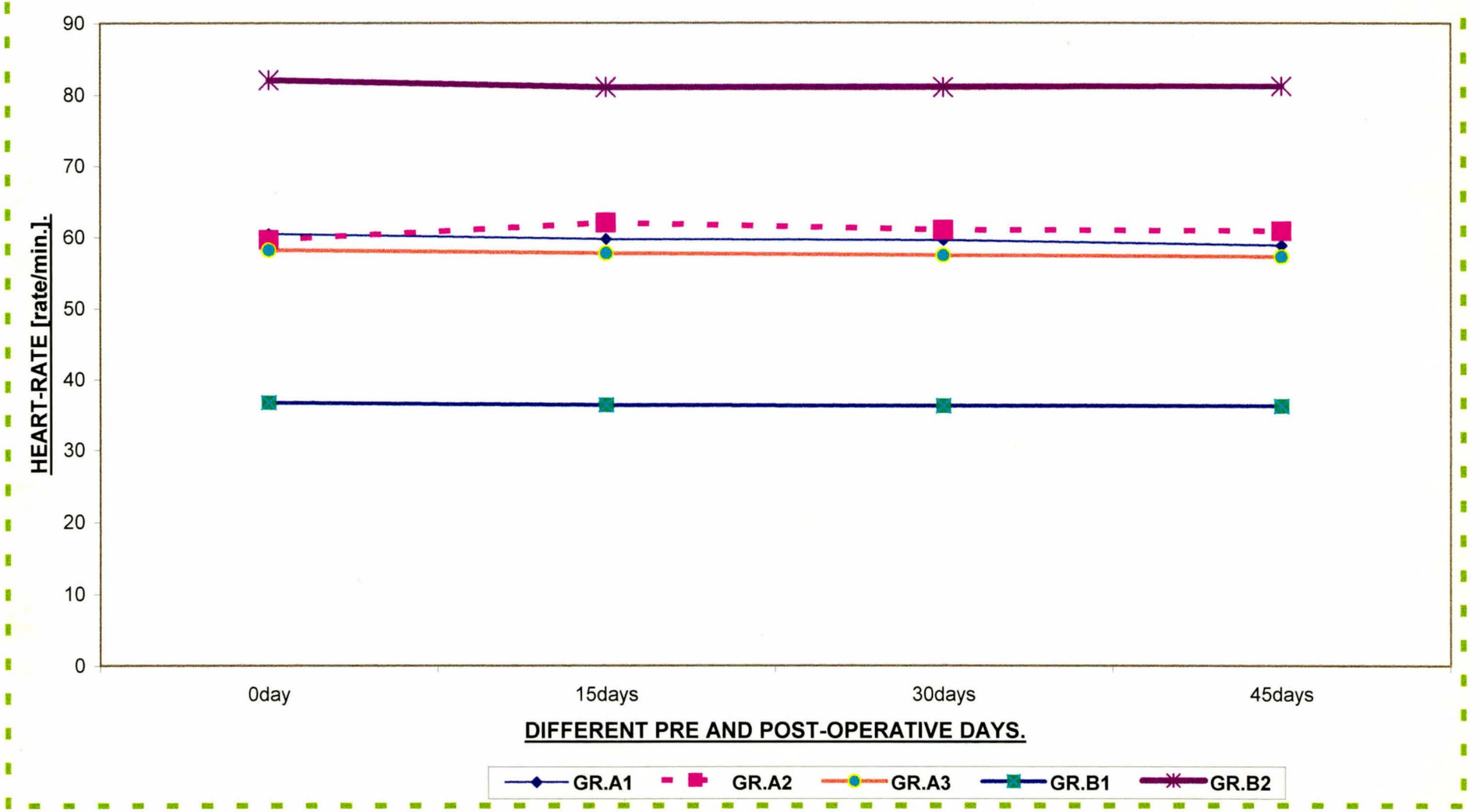


Fig.38:REPRESENTATION OF AV. COMPERATIVE ERYTHROCYTE COUNT IN ALL GROUPS OF ANIMALS [vide tab.no.9].

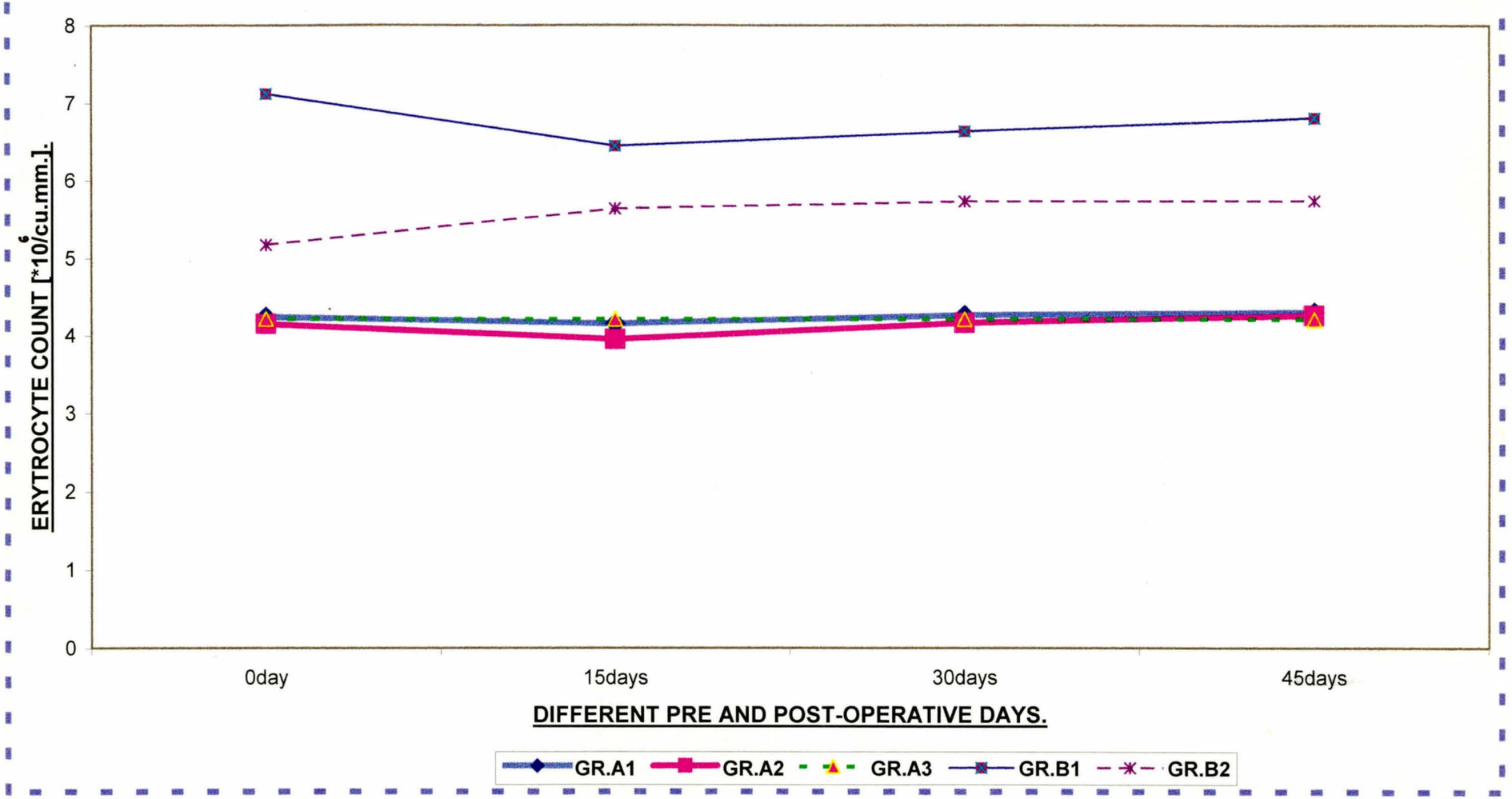


Fig.39:REPRESENTATION OF AV. COMPERATIVE LEUCOCYTE COUNT IN DIFFERENT GROUPS OF ANIMALS [vide tab.no.10].

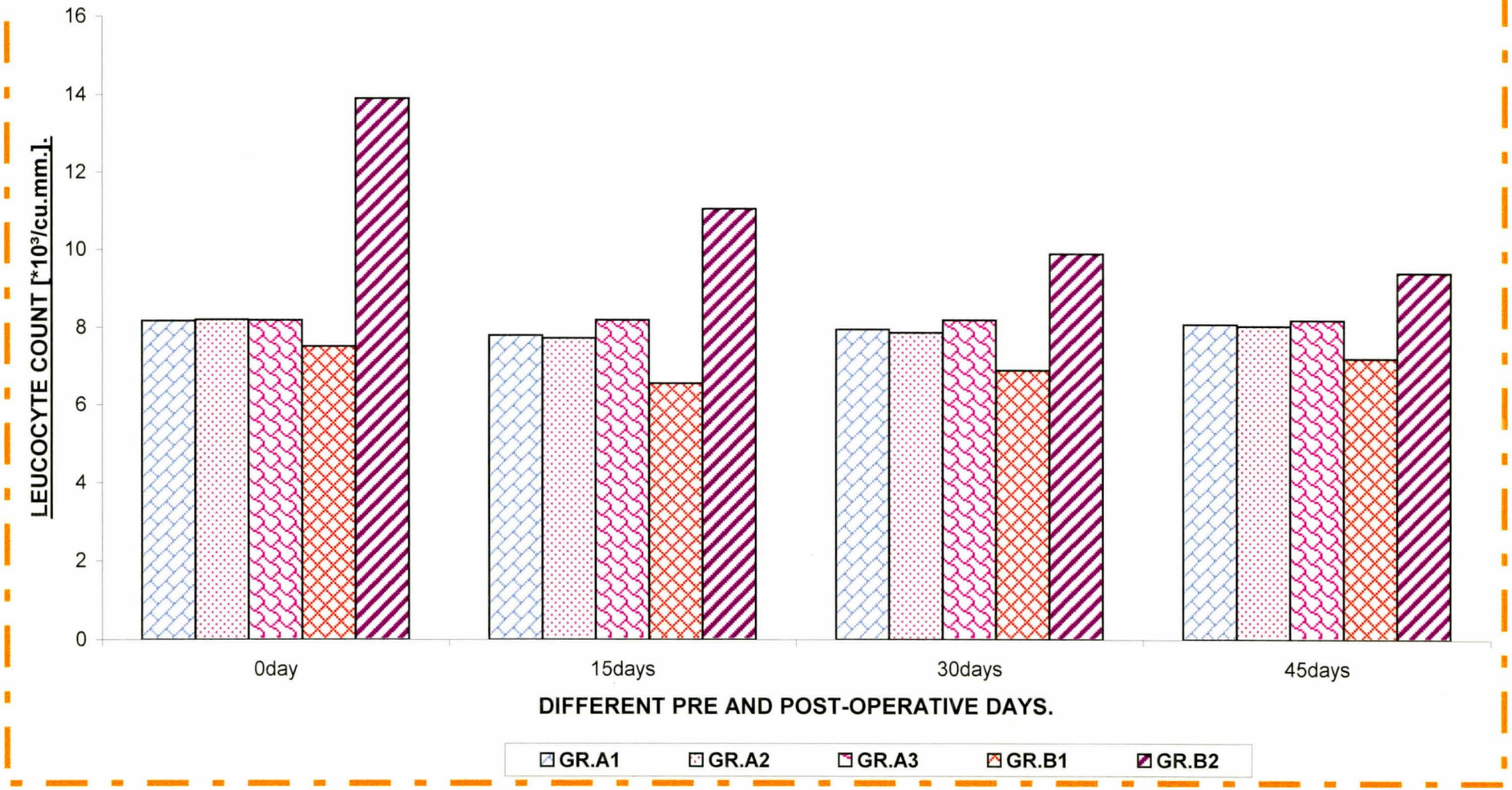


Fig.40:REPRESENTATION OF AV. NEUTROPHIL IN DIFFERENT GROUPS OF ANIMALS[vide tab.no.11].

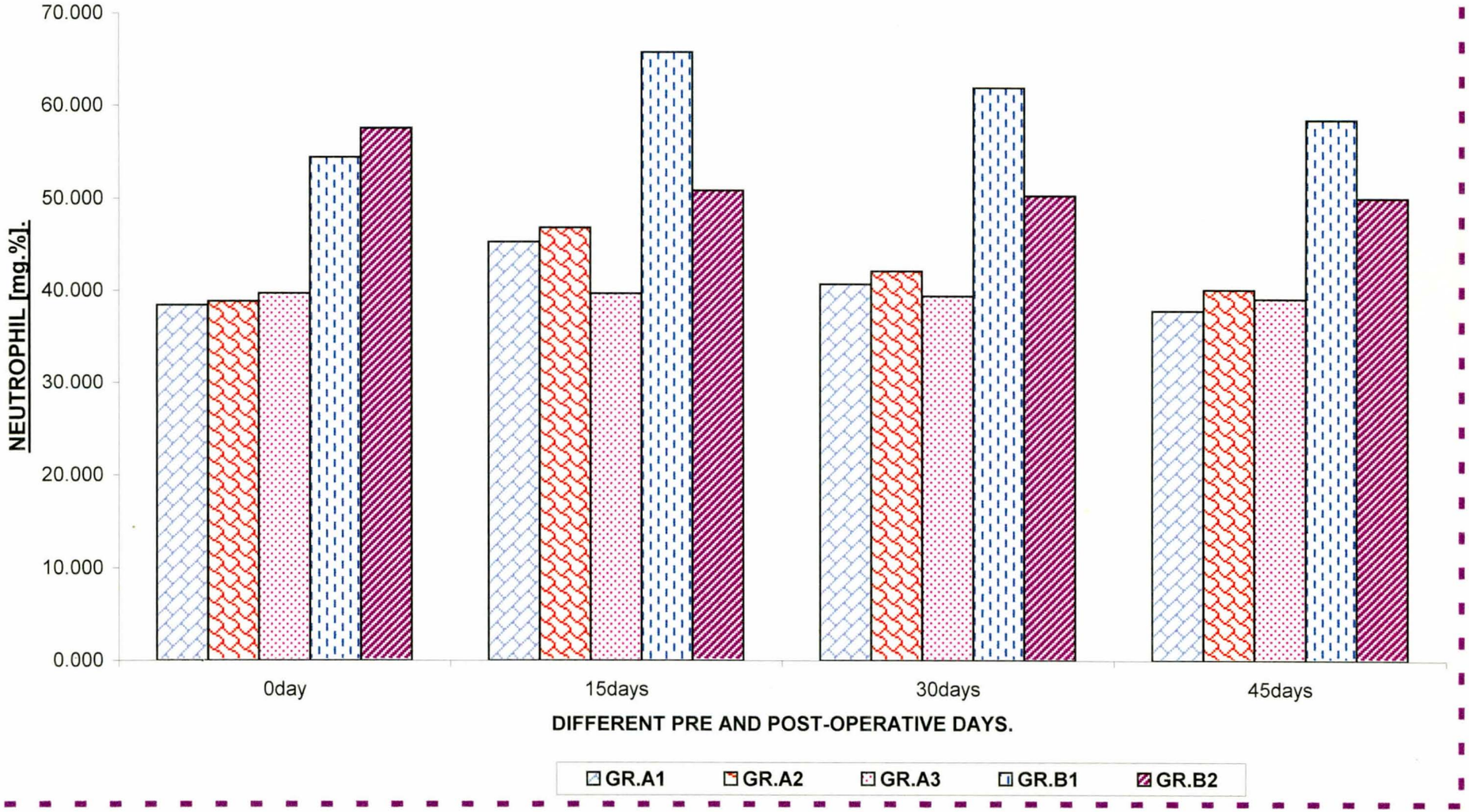
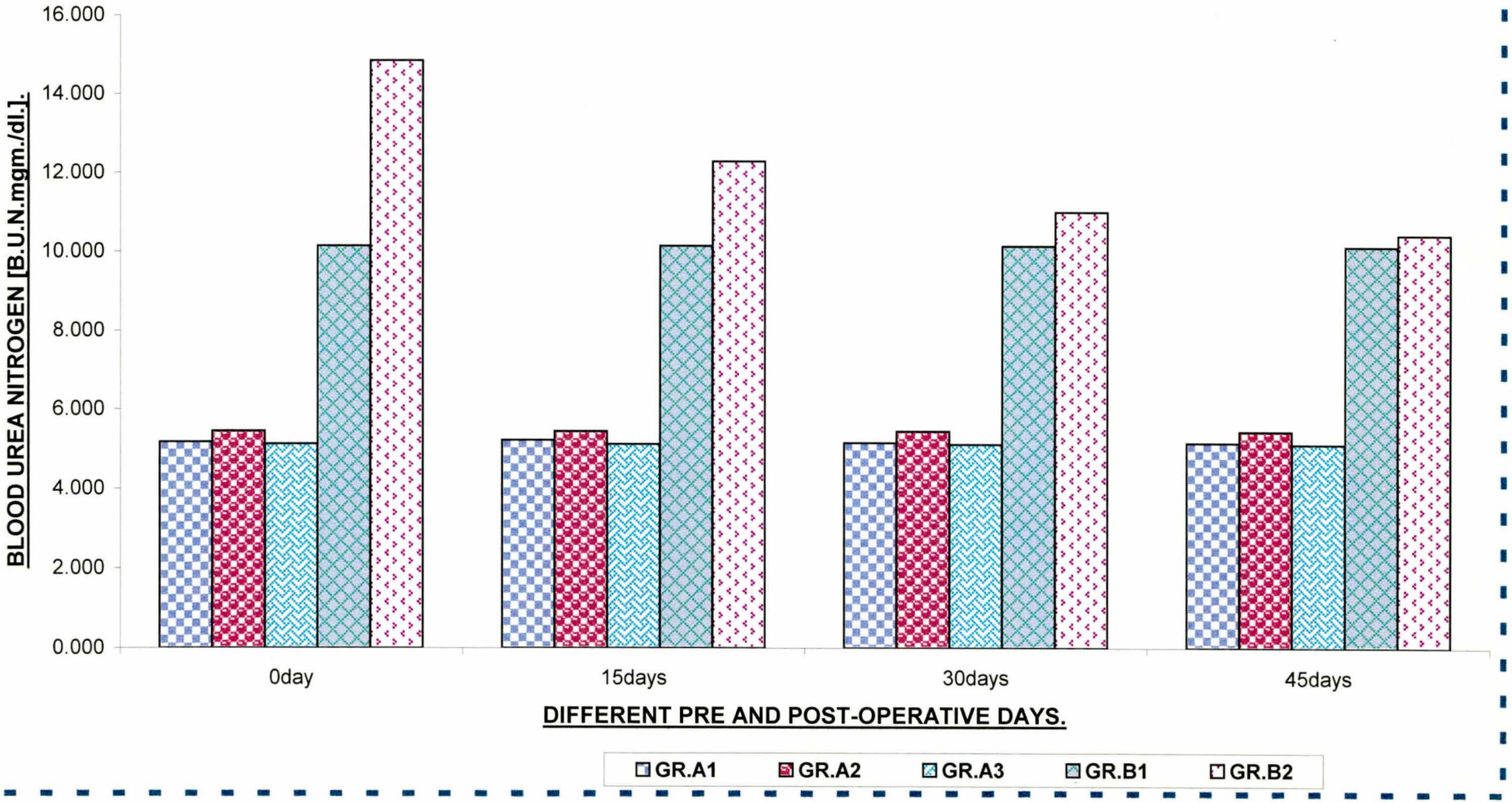


Fig.41:REPRESENTATION OF AV. COMPARATIVE "B.U.N." LEVEL IN DIFFERENT GROUPS OF ANIMALS [vide tab.no.15].



Chapter V

Discussion

DISCUSSION.

In the present study, an attempt has been made to device a modified, simple & inexpensive technique of chemosurgery, without causing much undesirable side effects. This specialised method of surgery is such that it could be conducted in widely diversified directions, in a variety of species of animals.

In chemosurgery, the use of $CaCl_2$ for the treatment of granulomatous growths and for chemical debriding suppurative lesions, is claimed to be a new approach over the conventional methods of therapy. The search of literature till date reveals the authenticity of these claim, as made in the present study. The use of $CaCl_2$ in bull calves as a chemical debudding agent in 50% & 70% concentrations under the present study merits special consideration. In addition to the use of $CaCl_2$, the adjunctive therapy of Placentrex, at the dosage and duration employed in this work, showed desirable effects in animal subjects of different species.

A total number of 35 animals were utilized for the present study which consisted of 21 bull calves, divided equally in 3 groups (A_1, A_2 & A_3), 7 horses (e.i. group B_1) & 7 dogs (e.i. group B_2).

In A_1 group, 1 ml of 50% solution of $CaCl_2$ was injected under the centre of horn bud. In group A_2, 1 ml of 70% solution of $CaCl_2$ was injected under the centre of horn bud & in group A_3, which was the control group, 1 ml of normal saline was injected under the centre of developing horn buds. 1 ml of Placentrex was injected I/M daily for 7 days in all the groups of bull calves.

In group B_1, 2-4 ml of 50% solution of $CaCl_2$ was injected at specific sites surrounding the granulomatous growths, to reach the base of the growth from different directions. 10 ml Placentrex was injected I/M to each animal on alternate days. In group B_2, the lesions were irrigated with 2-5 ml of 10% solution of $CaCl_2$, along with 1-2 ml of Placentrex and 1 ml of Floxidin to attain desirable debridement & subsequent development of healthy granulation tissues. Placentrex was injected I/M, the dose rate being 1 ml below 10 kg & 2 ml above 10 kg body weight.

In chemical debudding, the view behind injecting different concentrations of $CaCl_2$ under the centre of horn bud was to destroy the entire germinal

epithelium of horn buds, utilizing the powerful nectrotizing effect of $CaCl_2$.

The objective of administering 50% $CaCl_2$ at the base of granulomatous growths was to utilize again the necrotizing property of $CaCl_2$. If the base along with the surrounding tissues are necrotized, circulation to the growth, which is maintained via the base would be cut off. As circulation is interrupted, supply of nutrition, O_2 and other vital elements to the growth would be restricted along with continued accumulation of excretory products within the cells of the growth. This would result in gradual necrosis of the tissues of the granuloma. Ultimately, the growth would be sloughed off.

The view behind irrigating the chronic suppurative lesions with 10% $CaCl_2$ was also to utilize the necrotizing property of $CaCl_2$ when administered locally along with placentrex & antibiotics, would necrotize dead & devitalized tissues, promote healing by subsiding infection and by utilizing the biogenous stimulation of Placentrex.

The view of the necrotizing property of $CaCl_2$ was supported by the fact that small amounts of $CaCl_2$ solution resulted in massive tissue destruction when injected into the mass of a draining fibromatous lesion on a ram's jaw, the work being done by Whitford (1976). Likewise, the work of Koger & Pullman (1976) also gave much indication about the necrotizing effect of $CaCl_2$ when injected into the tissues. They observed that experimental intradermal injection of solution of $CaCl_2$ in concentrations ranging from 12%-70% in cattle, horse, sheep & dogs in volumes of 0.05 to 1 ml resulted in sharply defined concentric areas of necrosis upto about 5 cm in diameter. Nectrotizing property of $CaCl_2$ was also evidenced from the works of Mitra (1993) & Samanta (1998). They injected $CaCl_2$ intratesticularly to the testes of adult bulls & stray dogs respectively. This induced degenerative changes resulting in necrosis of total gland parenchyma. Personal communication with Maity (1999), who is conducting researches on chemosterilization on male goats by administering $CaCl_2$, also revealed the same facts as with Mitra (1993) & Samanta (1998).

In the present study, as already mentioned, $CaCl_2$ has been utilized to necrotize the germinal epithelium of horn buds. This view has been supported by Koger & Pullman (1976) & Koger (1976) Koger utilized 12%, 25% & 50% concentrations of $CaCl_2$. With 50% solution of $CaCl_2$, he obtained 82% success in calves aged 3-7 days, 48% in calves aged 8-14 days & 75% in calves belonging to the age group 15-51 days. Observing the limited success of 50% $CaCl_2$ in calves of the larger age group, the present study has been

designed accordingly. In this study, 50% & 70% $CaCl_2$ has been administered in calves of the age group of 3-11 days & 12-30 days respectively. Normal saline was administered in the control groups.

The findings of the present study, agreed with Koger (1976) that the technique of injection was an important factor of success & the failure which occurred in both groups A_1 & A_2, was most probably associated with faulty injections. If the point of injection is near the edge of the horn bud, the solution tends to force out under the less resistant subcutaneous tissues, leaving part of the periphery of the horn bud unaffected. More than 1 penetration in the skin resulted in leakage. However, if the needle penetrated the skin at an ablique angle and firm digital pressure is applied over the puncture site, as the needle is with drawn, leakage did not occur. The bevel of the needle should be oriented towards the periosteum, and a fanwise movement of the tip (approximately 15 to 30 degrees) facilitated the injection. Loading the syringe with only one dose and driving the needle to the hub were safeguards of success & it is in agreement with the view of Koger (1976). Most aqueous solution passes easily through 25 gauze or 23 gauze needles & 21 gauze or 16 gauze needles may be necessary for more viscous solutions or suspension (Mitra, 1993). In this regard, 18 gauze or 16 gauze needle with a length of ¾th inch served the purpose well & may be used as a routine practice. This is in accordance with the technique followed by Koger (1976). However, depending on the toughness of the tissues, the size & diameter of the needle may be suitably adjusted.

During administration of the drug, it is felt that careful control & restraint of the calves were of prime necessity for administering the drug correctly as pain reaction were severe, while the needles were being driven under the horn buds.

For the treatment of granulomatous growths, a 10 cm 21 gauze needle served the purpose best, keeping the facts in mind that the skin of horse is comparitively thin & smooth textured, easy administration of the drug & length of penetration through the tissues in order to reach the base of the growth.

It was felt that the dose of 50% reconstituted solution of $CaCl_2$ should be carefully calculated in order to get desired results. The points which should be considered in this regard are:-

(i) If the amount of drug administrered is more than required will result in a

greater amount of normal tissue destruction surrounding & beneath the growth, thereby complicating healing.

(ii) Greater the amount of drug, greater will be the pain & stress experienced by the animal.

(iii) Amount of drug to be administered depends upon the size of growth. However the main basic point, to be kept in mind, to calculate the amount of dose, is that 1 ml of 50% solution of $CaCl_2$ results in sharply defined concentric areas of necrosis upto about 5 cm in diameter as observed by Koger & Pullman, 1976. Keeping the above mentioned point in mind it can be said that, the diffusion of fluid will cover a diameter from its point of deposition upto a expected length of 5 cm, thereby bringing a circular area of necrosis having a diameter of 5 cm. Surrounding to it there will be a stupefied zone (threatened to death). Circling this stupefied zone will be the zone of reaction indicating the boundary between healthy & unhealthy tissues.

Careful controlling & restraint of the horse during administration were very important & requires trained para-veterinary personnel for doing so.

For the purpose of irrigating the suppurative lesions, 5 ml disposable syringes (DISPOVAN) proved to be ideal. Regarding control & restraint of the animal, an assistant is required to hold the animal and expose the affected part.

In order to assess the physical status of the animals, it was necessary to consider the clinical studies of the animals. In the present study, clinical assessment following chemosurgery using different percentage of $CaCl_2$ in different types of situations and lesions were based upon the evaluation of the clinical signs which necessarily included some purely objective & subjective evidences manifested by the animals.

It was evidenced from table nos. 6 & 17, that temperature increased in groups A_1, A_2 & B_1, the increase being significant ($P<0.01$) in groups A_2 & B_1. The increase was evident upto 3rd day following administration of the drug. It was also evidenced from table nos. 7, 8 & 17 that both respiration & heart rate, were significantly increased ($P<0.01$) in groups A_1, A_2 & B_1. Respiration rate increased upto 3rd post operative day, thereafter decreased steadily in the following post operative days. Heart rate was highest in the 3rd post operative day in groups A_1 & A_2, whereas it was recorded on the 1st post operative day

in group B1. There was no significant variation in the temperature, heart rate & respiration in group, A_3, as evidenced from table nos. 6, 7, 8 & 17.

It was assumed that inflammatory reactions, severe pain, excitement, tissue destruction brought about the increase in temperature & heart rate. This assumption coordinates with the usual common features of acute inflammatory reactions & subsequent necrosis.

In $CaCl_2$ treated groups, with the exception of group B_2, respiration rate was higher which indicated metabolic acidosis & reduced level of bicarbonate. This observation had similarity with the findings of Tucker (1992).

In group B_2, no significant change in the temperature & heart rate could be recorded in the post operative days & remained within physiological limits (Chakraborty, 1994). However respiration rate in this group, decreased significantly ($P<0.05$) & this decrease was observed throughout the course of the study. This may be due to a decrease in the BMR following suppression of infection. Biostimulatory effect of placentrex may have an indirect role in this aspect. This is supported by the findings of Rajasimha (1983) who described that Placentrex through its biogenic stimulating effect raises the level of some prostaglandin series known to be having bronchial smooth muscle relaxing properties, resulting in decreased respiratory rate.

Haematological picture corroborated with the clinical findings. It was observed from table no. 11 & 19 that there was significant ($P<0.01$) increase in the number of neutrophils in the circulating blood in all the groups excepting group A_3, the control group for chemical debudding, along with group B_2 where neutrophil count decreased significantly ($P<0.05$).

In groups A_1, A_2 & B_1, the increased number of neutrophil lasted for 7-15 days. Table nos. 13 & 14 showed that eosinophil & monocyte numbers were also increased. All this indicated chronic inflammation.

From table no. 11, it was evidenced that blood levels of neutrophil decreased in group B_2 throughout the study. This down ward trend was probably due to gradual control of infection. This view coordinates with the findings of Chakraborty (1994). Administration of Placentrex both locally & systematically may also play a role in lowering the neutrophil count. This view has been supported by the findings of Nair (1983), who assessed the effects of biogenous stimulatory properties of placentrex to counteract inflammation particularly in cases of pelvic inflammatory diseases, when used along with antibiotics.

This view has also been supported by the findings of Seethamma (1975), Jain (1980) & Shukla (1972). Similarly, monocyte count also decreased significantly ($P<0.01$) in group B_2 which corroborated with the above findings.

In was also evidenced from table no. 9 that total count of erythrocyte varries following chemosurgery with $CaCl_2$. It was obvious from table no. 19, that total count of R.B.C. significantly ($P<0.01$) reduced in groups A_1, A_2 & B_1. However, erythrocyte count increased in group B_2 but that change was insignificant between days. In the control group, following administration of normal saline, erythrocyte count varied insignificantly between days. Oral administration of $CaCl_2$ produced lowering of plasma bicarbonate level (Tucker, 1992) resulting acidosis. Acidosis caused reduction in the erythrocyte population & was supported by Chatterjee (1985). Paradoxically it was observed that there was long lasting change of total count of erythrocyte in groups A_1, A_2 & B_1; though the metabolic acidosis stage was overcome, which was manifested by the increased heart rate & respiratory rate upto 3rd post operative day.

It has been observed that following treatment with different antifertility agents, reduction of total count of erythrocyte was observed & sialidase enzyme have a role in this context. Activity of erythropoietin was regulated by sialidase (Nag *et al.* 1977). Aged tissues like liver, spleen & red cells have a reduced surface charge mostly due to action of sialidase (Bocci, 1976). Though in the present study sialidase enzyme was not studied. Hydrophilic compounds like cyproterone acetate favours the release of lysosomal enzymes, sialidase from lysosomal membrances was observed by Bangham (1965). $CaCl_2$ being a hydrophilic compound may act like cyproterone acetate; but haematological observations after treatment with cyproterone acetate was not investigated by the workers mentioned above. Supporting the above findings, reduced level of erythrocyte count was observed after intratesticular injection of $CaCl_2$ by Mitra (1993) & he presumed that the decrease was due to acidosis, metabolic & endocrine response. Mitra's views were supported by Annis (1961) & Chatterjee (1985) & all this corroborated with the findings of this study.

It was evidenced from table nos. 10 & 19 that total count of leucocyte decreased significantly ($P<0.01$) in groups A_1, A_2 & B_1 upto 7th post operative day. Thereafter there was an increase in the total count, which indicated inflammatory process was going on. Total count decreased throughout the course of the study in group B_2, the decrease being statistically insignificant. The results indicated that both local & systemic administration of Placentrex may play a role in diminishing the leucocyte count in group B_2. This view was

supported by the findings of Bertone *et al* (1982) who mentioned that placentae extract is active in many chronic inflammatory conditions & along with many other anti inflammatory effects, disappearance of leucocyte infiltration was one of the many important clinical datas.

Biochemical studies included estimation of blood urea nitrogen (BUN) & blood creatinine. From table nos. 15 & 21, it was observed that, BUN increased slightly in the different post operative days in groups A_1, A_2 & B_1 & this increase was statistically insignificant. This meant that vital organs particularly the kidney & liver were unaffected from the effects of this drug & there was no derangement of body metabolism. This view has been supported by Chaudhuri (1993). The slight elevation of BUN may be due to water deprivation as there was reduced intake of water for a few post operative days in the groups A_1, A_2 & B_1, following administration of $CaCl_2$. This view has been supported with the findings of Hardie (1986).

In group B_2, BUN decreased significantly ($P<0.01$) throughout the course of the study but these changes were within the physiological limits of dogs. (Singh *et al.* 1994). It was assumed that the decrease was probably due to gradual suppression of infection & increased renal function. This view has been supported by the findings of Beitschwerdt (1986), during management of renal failure in canines.

From table nos. 16 & 21 blood creatinine levels varied insignificantly between the pre and post-operative days in all the groups. This also indicated that the drug had the mildest effect on the urinary system since one of the early signs of renal failure is rise of creatinine concentration of blood (Chaudhuri, 1994).

Study in inflammatory reaction & morphological feature revealed transient pain, which progressively diminished during and following administration of $CaCl_2$ under the horn buds in groups A_1, & A_2. The pain sensation was however more severe with 70% $CaCl_2$ in group A_2, though to counteract any possible pain reaction local anaesthetic solution was given in combination with the drug (Westhus & Fritsch, 1964). In this regard, the results did not tally with the views of Koger, 1976, that aftercare is not necessary following administration of the drug. To counteract pain which persisted following reduction of pharmacological half-life of local anaesthetic, the situation demanded to give injections of Novalgin to minimise pain reaction to all the animals of groups A_1 & A_2, for a few post-operative days. Similar was the case with group B_1 animals. However, in group B_1 whether the effect of drug caus-

ing pain or the irritation in particular animals like horse, being a highly excitable animal resulted the restlessness, needs to be evaluated properly to establish the claim.

In was felt that along with Novalgin, Placentrex also helped in alleviating the pain. This view has been supported by the findings of Dabral (1976), & Dhir (1985) that placentrex is not only safe but also gave significant relief from pain , swelling & stiffness of joints in osteoarthritis, & degenerative arthritis by delaying the degenerative changes along with improving the regeneration of selected tissue structures.

The sequence of inflammation in group A_1 & A_2, already described in chapter IV, of the study, supports the findings of Koger & Pullman, 1976 and Koger, 1976.

Regarding the extent of inflammation, the findings of the study indicated that in group A_2, the extent of inflammation & the subsequent stress was 2-3 fold more than group A_1 animals.

One failure occurred in each of the groups A_1 & A_2 and made the success rate 85.71% for each group. The failure probably occurred due to leakage of the drug, following inaccurate restraint of the calves, which wiggled, bawled during the time of injection. This resulted in more than 1 penetration of the skin, through which probably the drug leaked, leaving part of the periphery of horn bud unaffected & it may be incriminated to the faulty technique of administration of the drug.

In group B_1, the sequence & extent of inflammation & necrosis as already mentioned in chapter IV, supported the findings of Koger & Pullman (1976). Observation of the study reflected that normal tissues surrounding & beneath the growth were affected in all the animals of the group. The affected tissues undergoes necrosis; followed by sloughing of the growth. Then, healthy granulation tissues followed by formation of fibrous tissue & scar tissues at the affected site, for resolution of the wound, were evident. However, cosmesis & stability gradually improved in all the animals.

In group B_2 animals, pain & discomfort was not evident during irrigation of the wounds with the drug combination. There was dramatical reduction in the amount of pus & exudates from the chronic lesions within 2-3 days, following initiation of the treatment. It suggested that, local & systemic administration of antibiotic to check infection stengthened the action of $CaCl_2$ in

chemical curettage destroying dead & devitalised tissue & thereby favoured to incite the growth of a healthy young vascular connective tissue. Use of Placentrex with its biostimulatory properties accelerated the healing process. The view is supported by the findings of Bertone (1982), Lolli (1982), Moscatelli (1986), Marchegiani (1982), Bigliardi (1982), Jain (1980), Shukla (1972). However in the case of capped elbow excessive scar tissue & darkening of the affected area developed. This could be due to the effect of placentrex as Lolli (1982) observed slow cicatrisation of the wounds following local application of placental extracts after performing gingivectomy operation. Moscatelli et al (1986) described that extracts of human placenta were tested for enhancement of proliferative growth of primary cultures of human epidermal keratinocytes. The factor presumably cames from placental parenchyma.

In both groups B_1 & B_2 success rate was 100%.

In chemical debudding from the viewpoint of success rate, nature & extent of tissue reactions, stress & pain experienced by the animals, reflected that 50% $CaCl_2$ yielded debudding at par with 70% $CaCl_2$ with less untoward side effects. Therefore higher concentration over 50% may not be desirable for debudding because of obvious reasons & with this technique debudding should be conducted preferably in calves within the first 10 days of their age, in order to avoid difficulty in administration of the drug in comparitively tougher tissues which attains faster rate of growth at each successive days. Unlike other caustic agents like KOH & NaOH sticks used for debudding, $CaCl_2$ has been practically found to be non - irritant to the eyes & no special precautions like application of a ring of petroleum jelly surrounding the horn bud need not be taken. No special precautions on the part of technician is necessary with this technique which is of imperative necessity while using NaOH & KOH sticks. Similar findings were also noted by Koger & Pullman (1976) & Koger (1976), Singh (1993), Banerjee (1993), Prasad (1992) & Venugopalan (1994). The technique in addition was simple & easy to perform. It was much more economical, which from the view point of veterinary practice could never be ignored.

In case of granulomatous growths, it was found that the technique was many times simpler & easier than surgical intervention and can be conducted routinely provided the dosage schedule be calculated accurately. The technique required no special attention for sepsis & antisepsis which is of prime importance in surgical interventions.

Additionally it may be claimed that because of the powerful necrotizing role of $CaCl_2$ on cells, it also exerts its similar action on the microbial population, which may be responsible for production of sepsis in the lesion. In this way, $CaCl_2$ also served to some extent the role of an anti - infective therapy. Further that, there are certain conditions where surgical intervention is not feasible & may make the case more complicated. In those cases, this technique may prove a suitable alternative. Finally the cost arising from surgical intervention, post surgical follow - up & medicaments could be easily skipped with this technique.

As a chemical de briding agent $CaCl_2$ also proved to be of great value. Unlike other chemodebriding agents like E.C. lotion, Magsulf, pain reactions were far less. Amount of exudate & pus following treatment diminished remarkably. This technique also proved superior to surgical debridement where uniform depth of tissue can't be sacrificed while curetting the pathological tissues & the tissues are more subject to trauma. On the other hand chemodebridement with this technique resulted in formation of even layer of smooth granulation tissues with minimum trauma to the tissues. From economic & availability point of view the technique proved superior than enzymatic debridement.

From the point of efficacy of the drug, it may be claimed that Placentrex will definitely occupy a place as a necessary adjunctive therapy in chemosurgery in veterinary practice due to the following reasons -

(i) A number of pharmacological effects of the biogenous stimulators present in placental extracts have been determined. These stimulations are taken up by the diseased tissues. These substances stimulate the cellular metabolism and thereby regenerate the disease tissue. Another school of thought is that these biogenous stimulators will give rise in Acetyl - choline production which in turn stimulates neuromuscular excitability & tissue repair, as viewed by Reddy, 1985.

(ii) The human placental extract injection contains significant quantities of DNA & RNA. The nucleotides are known for their tissue regeneration & effect through their unique process of protein synthesis as stated by Lehninger, 1984.

(iii) Placentrex contains hPL (human Placental Lactogen) which has growth promoting & lactogenic activity in humans as observed by Goodman & Gillman, 1985.

(iv) Significant anti-inflammatory effect involving chemical mediators of the immunological response and analgesic effect have been reported with placental extracts, as noted by the study of Bertone, 1982 ; Rosenthal, 1982 ; Purandare, (1969 - 1970).

(v) Placental FSF (fibrin stabilizing factor) is used therapeutically for factor XII deficiency & for the acceleration in wound healing after major surgery. Several placental proteins have been isolated with immunostimulatory & immunodeviatory properties, as observed by Duc *et al* , (1983)

(vi) Placental extracts have distinctive growth promoting properties for the epithelium as stated by Lolli, 1982).

(vii) Easily available & cheap.

In fine, it can be inferred that the combination of $CaCl_2$ & Placentrex proved an ideal combination for use in chemosurgery and can be routinely used under field conditions, without any great risk & undesirable complications.

Chapter VI

Summary

SUMMARY

In the present study, chemosurgery has been conducted in two diversified directions. In one side, chemical debudding has been conducted & in the other side chemosurgery for the treatment of granulomatous growths along with chemical debridement of chronic suppurative lesions have been conducted. $CaCl_2$ has been utilized as the main chemical agent for chemosurgery and in addition to it Placentrex has been used as a therapeutic adjunct.

A comparative evaluation was made among 50% & 70% concentrations of $CaCl_2$ sloution along with normal saline, in chemical debudding in calves 2% Xylocaine was added along with the different concentrations of $CaCl_2$ e.i, 50% & 70%, making the ratio of $CaCl_2$ & Lignocaine 25 : 1 & 35 : 1 respectively. The experiment was carried out on 35 animals, which consisted of 21 bull calves divided equally in 3 groups (A_1,A_2 & A_3), 7 horses (e.i, group B_1) & 7 dogs (e.i, group B_2) . In A_1 group, which consisted of bull calves of the age group 3 - 11 days, 1ml of 50% solution of $CaCl_2$ was injected under the centre of horn bud. In group A_2, which consisted of bull calves of the age group 12 - 30 days, 1ml of 70% solution of $CaCl_2$ was injected under the centre of horn bud. In group A_3, which consisted of calves of the age group 3 days to 1 month, 1ml of normal saline was injected under the developing horn buds. 1 ml of Placentrex was injected I/M daily for 7 days in all the groups of bull calves. Group A_3, was considered as control group. In group B_1, 2 - 4 ml of 50% solution of $CaCl_2$ was injected at specific sites surrounding the granulomatous growths. 10 ml Placentrex was injected I/M to each animal on alternate days. In group B_2, the lesions were irrigated with 2-5ml of 10% solution of $CaCl_2$ along with 1-2ml Placentrex & 1ml of Floxidin. Placentrex was injected I/M, the dose rate being 1ml below 10kg - body weight & 2ml above 10 kg body weight. Pre-treatment values were treated as control.

In groups A_1,A_2 & B_1, there was slight rise in the temperature following administration of $CaCl_2$ & Placentrex & this febrile condition persisted for the next 5 - 7 days. In groups A_3 & B_2 temperature variation was insignificant. Respiration & heart rate were increased in groups A_1,A_2 & B_1, In contrast though no significant change in heart rate could be observed in group B_2, respiration rate decreased significantly ($P<0.05$). In the control group A_3, respiration & heart rate varied insignificantly.

Haematological study revealed that total count of erythrocyte & leuco-

cyte decreased in groups A_1,A_2 & B_1, following chemosurgery. The total count of erythrocyte & leucocyte increased from 5th day in these groups. Erythrocyte count increased in group B_2 accompanied with a decrease in the leucocyte count upto 7th post operative day. No significant variation could be observed in the erythrocyte & leucocyte count in group A_3. From the observations, it was also evident that, there was an increase in the number of neutrophils in groups A_1, A_2 & B_1 & this increase persisted for 7 - 15 days. In group B_2, neutrophil count diminished throughout the course of the study. Neutrophil count varied insignificantly in group A_3.

Biochemical study indicated that variation of BUN was insignificant throughout the course of the study in groups A_1 , A_2, A_3 & B_1. In contrast, BUN level diminished in all the post operative days in group B_2.

Regarding the study on inflammatory changes & morphological features of horn growth, the normal sequences of inflammation, followed by necrosis of the germinal epithlium of horn bud & ultimate seperation of the part was completed within 10 - 15 days following administration of the drug, in groups A_1 & A_2. However, the extent of inflammation was 2 to 3 fold greater in group A_2. The success rate of both the groups were 85.71%. The usual growth of horn bud was evident in group A_3. In group B_1, following the normal sequence of inflammation & necrosis, the growths gradually atrophied & sloughed off within 8 - 10 days following administration of the drug. In group B_2 amount of pus & exudate reduced & healthy granulation tissues were observed within 5th to 8th post operative day. Success rate for both group B_1 & B_2 were 100%.

The changes observed in the study, can be attributed to the powerful necrotizing effects of $CaCl_2$ combined with the biostimulatory properties of human placental extract injection. Their combined effect brought about the desired results.

Chapter VII

Conclusion

CONCLUSION

The following conclusion can be drawn on the basis of the present study .

1. The technique of chemosurgery was easy to perform and inexpensive. Injections or irrigations of $CaCl_2$ into the tissues does not seem to cause undesirable side effects, provided the dosage schedule of the drug combination & post - operative follow up recommended in the study, is carefully followed.

2. Injections of $CaCl_2$ under the centre of horn bud & towards the base of the growths, by the technique followed in the study were found to be highly effective. Failures will generally be observed with faulty technique.

3. The addition of 2% Lignocaine HCl in suitable ratio followed by injections of Novalgin seems to be helpful to control any possible pain reaction during administration of $CaCl_2$ & thereafter for a certain period, in chemical debudding and chemosurgery for granulomatous growths.

4. Addition of antibiotics with $CaCl_2$ & Placentrex in the dosage schedule & duration of employment followed in the study, for chemodebridement of suppurative lessions seems to be ideal.

5. $CaCl_2$ had powerful role in inducing extensive necrosis on the germinal epithelium of horn buds & as well as, dead & devitalised tissues present in chronic suppurative lesions. In case of granulomatous growths it proved an effective substitute for delinking the connections between the healthy & unhealthy granulomatous tissues in place of surgical excision with consequent loss of blood & other vital body components from the lesion.

6. 50% reconstituted solution of $CaCl_2$ proved ideal for both chemical debudding & chemosurgery for granulomatous growths & thereby higher concentrations of $CaCl_2$ over 50% may not be used, unnecessarily.

7. Biostimulatory properties of Placentrex were responsible for stimulating the normal healing process.

Chapter VIII

Future scope of Research

FUTURE SCOPE OF RESEARCH

Elaborate study may be conducted out with regard to the effectiveness of $CaCl_2$ & Placentrex in chemosurgery.

Systemic, metabolic effects & effects of stress following administration of the drug combination should be investigated in detail.

The claim made in the study, on the sequence & extent of inflammation & subsequent necrosis may be substantiated by histomorphological studies.

A comparative study on the effectiveness of $CaCl_2$ with other chemodebriding agents like E.C. lotion, magsulf should be evaluated in detail, considering necessary parameters.

The detail characterization of the atropic changes observed in case of granulomatous growths & MLD of $CaCl_2$ should be evaluated.

To introduce & popularise chemosurgery in a routine manner in Veterinary surgical field in this country, in place of conventional surgical interventions, in certain suppurative, granulomatous & complicated lesions etc. needs a thorough & careful study.

Chapter IX

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