EFFICACY EVALUATION OF ETHANOLIC AND AQUEOUS EXTRACTS OF *Tamarindus indica* L. LEAVES IN SEPTIC ARTHRITIS MODEL OF RABBIT

A Thesis

Submitted to the

West Bengal University of Animal and Fishery Sciences In partial fulfillment of the requirement for the Degree of

Master of Veterinary Science

In

VETERINARY PHARMACOLOGY AND TOXICOLOGY

By

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CERTIFICATE

This is to certify that the work recorded in the thesis entitled ***EFFICACY EVALUATION OF ETHANOLIC AND AQUEOUS EXTRACTS OF Tamarindus indica L. LEAVES AS POSSIBLE THERAPY IN SEPTIC ARTHRITIS MODEL OF RABBIT*** *submitted by* ***Dr. Bishnu Prasad Sinha*** *in the partial fulfillment of the requirement for the award of the Degree of Master of Veterinary Science in Veterinary Pharmacology and Toxicology of the West Bengal University of Animal and Fishery Science, 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 for been submitted for any other Degree or Diploma. The assistance and help received during the course of investigation have been duly acknowledged.*

Sar 23/9/2016

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Dedicated To My Beloved Parents

APPROVAL OF EXAMINERS FOR THE AWARD OF THE DEGREE OF MASTER OF VETERINARY SCIENCE IN VETERINARY PHARMACOLOGY AND TOXICOLOGY

We, the undersigned, having been satisfied with the performance of **Bishnu Prasad Sinha** in the VIVA-VOCE Examination, conducted today, the 14th Dec 2016, recommended that the thesis be accepted for the award of the Degree of Master of Veterinary Science in Veterinary Pharmacology and Toxicology .

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ACKNOWLEDGEMENT

It cannot be argued with that the most influential person in my post graduate program has been the chairman of my advisory committee, ***Dr. Tapas Kumar Sar****. I am especially grateful to him for his devotion to his students' education and success. His dynamism, vision, sincerity and motivation have deeply inspired me. He has taught me the methodology to carry out the research and to present the research works as clearly as possible. It was a great privilege and honor to work and study under his guidance.*

I owe deepest sense of gratitude and indebtedness to ***Dr. Tapan Kumar Mandal*** *for the support to make this thesis possible with his valuable suggestions and unconditional help. His passion, guidance and discipline have been indispensable to my growth as a student and as a person over the past three years.*

I express my honorable thanks and gratitude to ***Dr. S. N. Joardar****,* ***Dr. I. Samanta,Dr. P. Das, Dr. A. K. Maji, Dr. S. Choudhury, Dr. P. Mukherjee, Late Prof. (Dr.) J. D. Ghosh, Dr. U. Sarkar, Dr. S Batabyal, Dr. P. R. Ghosh, Dr. S. Pradhan, Dr. S. Mukhopadhyay, Dr. C. Debnath*** *for their suggestions and help which became a backbone behind my efforts from the inception to the end of the work.*

I would like to extend my solemn regards and sincere thanks to ***Dr.S. Baidya*** *for his valuable suggestions, guidance and encouragement whenever I was in need.*

I am very much thankful to ***Dr. Anirban Mallick Thakur****,Group Lead at* ***Cipla Ltd.*** *for his immense help for providing two valuable standards for my research.*

I am very much grateful to ***Dr. U. Biswas*** *Assistant Professor, Department of Preventive Veterinary Medicine, W.B.U.A.F.S., for full hand help for collection of samples.*

I am obliged to ***Vice-Chancellor***, ***Registrar*** *and* ***Controller of examinations***, *W.B.U.A.F.S., for providing scope and opportunities to carry out work.*

I am also obliged to ***Dean***, *Faculty of Veterinary and Animal Sciences for providing necessary facilities to conduct the research work.*

Only words are not appropriate to thank ***Dr. Achintya Mahanti*** *for full hand help, valuable suggestions and unlimited encouragement to carry out the research.*

I cannot forget kind co-operation and consistent encouragement of my seniors ***Dr. Akhilesh Mishra, Dr. Binita Angom, Dr. Amit Kumar Pandey, Dr. Raja L., Dr. Rahul Nanotkar, Dr. Prabhakar Maurya, Dr. Mithin U. C., Dr. (Md.) Amirul Islam, Dr. Krishnendu Mondal,*** *I am thankful to my colleagues* ***Ekta Singh, Souvik Chatterjee, Sudipta Roy, Praloy Shankar Layek, Piyali Mukherjee*** *for immense help during my research work.*

With deep sense of obligation from the core of my heart, I place on record my sincere regards to my juniors ***Dr. Swaraj Biswas, Dr. Rinku Buraguhan, Dr. Shabnam Akhtar, Dr. Arkaprava Shee, Dr. Satyaki Chakraborty, Dr. Sagun Soren, Dr. Vanujyoti Sarker, Dr. Aniruddha Banerjee, Dr. Mofizul Islam, Dr. Debobrata Khamrai, Dr. Amal Sarkar and many others*** *for their inspiration which are highly appreciated for the accomplished work.*

I shall remain thankful to my very own (department staffs), ***Mr. Tapas Podder (Lal Da), Mr. Nitai Chandra Bera, Mr. Abhishek Chattopadhyay, Mr. Dasarath Mandal, Mrs. Anjali Mandal, Mr. T. N. Dubey, Mr. Biswanath Daftari, Mr. Kailash Yadav And Ms. Ruma majumder*** *for their kind hearted support during the course of work.*

I also extend my thanks to the library staffs, security guards, and all working staffs of Mohanpur Campus, West Bengal University of Animal and Fishery Sciences for their immense help.

Though, this thesis is a result of patient survey and persistent effort of my past, it could have not been possible without a few who have ungrudgingly took pains and shared both time and energy for it. So I extremely thankful to all of my friends, especially ***Ajit Singh, Pranoy Bhar, Sohini Sen, Jyotirmay Adhikary*** *because of their unexpected cooperation.*

Finally but most important, I cannot formulate any word that would express my full respect and whole hearted gratitude to my ***Parents***, ***Sisters, Brothers, Brothers in Law, Uncles and my two little nephews*** *for their love, inspiration and continuous support both spiritually and materially for my success.*

Finally I would like to acknowledge the ***innocent animals (Rat & Rabbit)****, whose great sacrifice led to the completion of my work.*

A formal statement of acknowledgement will hardly meet the ends of justice in the matter of expression of my deeply felt sincere and allegiant gratitude to all who encouraged and helped me during my study. Thanking to each and every person soliciting their good wishes for my future.

Date: Bishnu Prasad Sinha

Place: Mohanpur ***Bishnu Prasad Sinha***

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%		Percentage
@		at the rate of
Alp		Alkaline phosphatase
b.w		body weight
c^0		Centigrade
ca-p		calcium phosphate
DLC		Differential leucocytic count
EDTA		Ethylene diamine tetraacetic acid
et al		et alli, and others
Fig		Figure
g/dl		gram per decilitre
Gr		Group
Hb		Haemoglobin
H&E		Haematoxylin & eosin
HNO_3		Nitric acid
hr		hours
HPLC		High Performance Liquid Chromatography
IM		Intramuscular
Inj		Injection
IV		Intravenous
IU/L		International unit per liter
Kg		Kilogram
LDH	:	Lactate dehydrogenase

mins		minutes
ml		millilitre
mm		millimetre
mm^3		millimetre cube
PCV		Packed cell volume
P^H		Negative logarithm of hydrogen ion concentration
rpm		Rotation per minute
S.E		Standard error
TC		Total count
μ		micron
μg		microgram
μm		micrometer
hr		Hour
PMN	:	Polymorphonuclear Leukocytes
L		Lymphocyte,
HPLC		High performance liquid chromatograph
K12 compartment.	:	First order rate constant for transfer of drug from central compartment to peripheral
K21 compartment.	:	First order rate constant for transfer of drug from peripheral compartment to central
Ka		Absorption half-life
Kel		First order rate constant for drug elimination from central compartment.
Kg	:	Kilogram
L		Litre
lbs		Pounds
m mole		Millimole
M	:	Molar

mg		Milligram
ng ml^{-1}	:	Nanogram per millilitre
MIC		Minimum inhibitory concentration
min		Minute
ml		Millilitre
CRP		C – Reactive Protein
PCT		Procalcitonin
mM		Millimolar
MRT		Mean residence time
PBS		Phosphate buffer saline
PDA		Photo Diode-Array
$_{P}H$		Negative logarithm of hydrogen ion concentration
ppm		Parts per million
rpm		Rotation per minute
RP		Reversed phase
Rt		Retention time
SD		Standard deviation
SE		Standard error
T ~ P		Tissue to plasma ratio
t1/2 α		Biological half-life(distribution phase)
t1/2 β		Biological half-life(elimination phase)
UV/Vis		Ultraviolet/Visible
v/v		volume/volume
Vdarea		Apparent volume of distribution (area method)
VdC		Apparent volume of distribution in central compartment
VdSS	:	Steady state volume of distribution

α	Distribution rate constant
β	Elimination rate constant
JSW	joint space width
TP	Total protein
EE	ETHANOLIC EXTRACT
AE	AQUEOUS EXTRACT
AB	ANTIBIOTIC
AB + STER :	ANTIBIOTIC + STEROID

INTRODUCTION

Introduction

Septic arthritis is inflammation of joint caused by bacterial infection. It is also known as infectious or bacterial arthritis. Acute bacterial arthritis, or "septic arthritis", is a rheumatologic emergency. Bacterial replication in the joint and the ensuing inflammatory process can lead to rapid local joint destruction, and may be accompanied by systemic infection. The clinician's prompt recognition of the infected joint and implementation of appropriately targeted therapy is therefore critical to limit the morbidity and mortality associated with these infections (Sharff *et al.* 2013).

The condition is most commonly caused by staphylococcal or streptococcal bacteria. These bacteria may have entered via wound and travelled through the bloodstream to the affected joint, or may have infected joint directly following an injury or during surgery.

Any joint can be affected by septic arthritis and more than one joint can also be affected at a time but the condition is most common in the knees and hips.

The term "suppurative arthritis" is a near synonym for septic arthritis."Suppurative" refers to the production of pus, without necessarily implying sepsis.

ICD-10 (International Classification of Diseases) uses the term "pyogenic arthritis". Pyogenic also refers to the production of pus.

In cattle 47% to 72.2% of all lameness other from the foot is located to the joint and ligament. The most common lesions affecting the joint are of traumatic origin, developmental (Ostochondrosis), and septic. In Israel, arthritis accounts for 13.8% of lameness cases. In American feedlots, swollen joints are linked to 12% of lameness. The most common joints infected are front fetlock, hock and elbow. A Canadian survey in feedlot calves from Saskatchewan showed that 1.3% become chronic, 39% of these calves had a diagnosis of polyarthritis. In Sweden, the incidence rate of arthritis in dairy calves was reported to be 0.002 cases per calf months at risk. In a study in veal calves in Belgium, the incidence rate of septic arthritis was 0.11 cases per 1000 calf days at risk (Andŕe Desrochers 2014).

A retrospective study of horses affected with septic arthritis showed that the most common causes of infection are traumatic articular injuries (24%), iatrogenic infections associated with intra-articular injections (22%), infections related to surgery (13%), hematogenous infections (17-34%) and idiopathic causes (6%) (Schneider *et al.* 1992).In horses, the iatrogenic infections are mostly caused by staphylococcal infections (Bertone *et al.* 1999; Lapointe *et al.* 1992)

The result regarding the prevalence of septic arthritis in broilers caused by *S. aureus* was 68%(64% in a farm and 72% in another broiler farm) and the findings about number and percentage prevalence of septic arthritis in layers caused by *S. aureus* was 64%(72% in a farm and 56% in another layer farm) in farms at Tandojam in Pakistan (Nazia *et al.* 2015).

The incidence of septic arthritis has been estimated at 2 to 10 cases per 100,000 in the general population and as high as 30 to 70 cases per 100,000 in patients with rheumatoid arthritis. The most common mode of spread is haematogenous, with predisposing factors including intravenous drug use, presence of indwelling catheters, and underlying immune-compromised states. Other potential predisposing conditions include preexisting arthritis such as rheumatoid arthritis, gout, or osteoarthritis. The knee is the most commonly involved joint, accounting for about 50% of the cases (Abby Abelson 2010)

Use of medicinal plants in the treatment of various human and veterinary diseases is an ancient idea. The recent and rigorous advancement in arthritis research has endorsed the use of medicinal plants in the disease treatment. Arthritis is a degenerative joint disease affecting socio-economic life of middle-aged population of human as well as of animals. Recent survey by the World Health Organization reports that 10-15% of world and 15% of Indian population is arthritic and expected to be double by 2030.

Tamarind Seed (*Tamarindus indica* L.) Extract (TSE) along with its composition were found to inhibit cartilage and bone degrading factors (enzymatic and non-enzymatic). Furthermore, it also mitigated the augmented state of inflammation and oxidative stress by blocking over production of pro-inflammatory mediators and maintaining the homeostasis of endogenous antioxidant system (Sundaram *et al.* 2015).

A hydroethanolic extract of *Tamarindus indica* L. leaves was found to have potential anti-inflammatory as well as anti-nociceptive actions against carrageenan-induced hind paw oedema in male Wistar albino rats (Bhadoriya *et al.*2012).

The current preferred therapeutic drug treatments (Antibiotics and corticosteroids), often result in severe secondary ill effects in prolonged use drew our attention to use of medicinal plant in the management of septic arthritis.

phytochemical studies of *Tamarindus indica* L. leaves extracts revealed the presence of tannins, saponins, sesquiterpenes, alkaloids and phlobatamins. The extracts were found to be active against both gram positive and gram negative bacteria. The activity of the plant extracts were not affected when treated at different temperature ranges (4°C, 30°C, 60°C and 100°C), but reduced at alkaline pH (JH Doughari 2006).

Tamarindus indica has broad spectrum antibacterial activity and a potential source of new classes of antibiotics that could be useful for infectious disease chemotherapy and control (JH Doughari 2006).

Studies on the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the *Tamarindus indica L.* leaves extracts on some organisms showed lowest MIC and MBC against *Klebsiella pneumoniae* and *Micrococcus luteus*, but the highest MIC and MBC was exhibited against *Pseudomonas aeruginosa* and methicillin resistant *Staphyllococcus aureus* (MRSA)(Gumgumjee *et al.*2012).

From various literature it was found that *Tamarindus indica L.* leaves extracts are having good antimicrobial property as well as anti-inflammatory, antioxidant and anti-nociceptive property. But the study regarding antimicrobial property of *Tamarindus indica L.* leaves extracts have been performed so far were *in vitro*. No infectious disease model study have been done yet to check the efficacy of *Tamarindus indica L.* leaves extracts against sepsis and inflammation together in a living system. Several earlier studies reported better efficacy of antibiotic and corticosteroid therapy compared to only antibiotic therapy in treatment of septic arthritis in different animal species (Wysenbeek *et al.*, 1998; Jaberi *et al*, 2003). Linezolid was found to be one of the most effective antibiotics against *S. aureus* (including MRSA) infection particularly in septic arthritis (Schroeder *et al.*, 2011). The conventional dosage regimen of Linezolid in septic

arthritis in rabbits was reported to be 75 mg kg^{-1} for 10 days (Schroeder *et al*, 2011).So, an attempt has been made to evaluate pharmacokinetics of Linezolid in healthy rabbits following single oral dosing @75 mg kg^{-1} and to monitor its persistence and level in synovial fluid.

The aim of the present study was to evaluate the efficacy of Linezolid only, Linezolid and Betamethasone and Aqueous and Ethanolic extracts of *Tamarindus indica* L. leaves for treatment of septic arthritis by monitoring haematological, biochemical, immunological (C-Reactive protein, Procalcitonin), X-ray and histomorphological study. In consideration of the above facts, the present research work was undertaken with following objectives –

- To establish septic arthritis model in rabbits
- To study safety of aqueous and ethanolic extracts of *Tamarindus indica* L. leaves
- To evaluate and compare efficacy of Linezolid only, Linezolid and Betamethasone and Aqueous and Ethanolic extract of *Tamarindus indica* L. leaves for treatment of septic arthritis
- To study pharmacokinetics of Linezolid following single oral dosing in rabbits with monitoring of drug concentration in synovial fluid.

REVIEW OF LITERATURE

Review of Literature:

Septic arthritis animal model

Linhart *et al.* (1990) presented an experimental animal model for bacterial joint inflammation. Using 16 rabbits divided into 4 groups, they injected knee joints of two groups with *Staphylococcus aureus* and the other 2 with NaCl. They checked Leucocyte and differential blood cell counts at 12 hour intervals.

Bremell *et al.* (1991) presented a mouse model of *S. aureus* arthritis in which the intravenous administration of 10^7 cells of *S. aureus* LS-1 induced arthritis or osteitis or both within 3 weeks in 80 to 90% of the mice. Signs of arthritis emerged within the first few days after the injection. An interesting finding was that the *S. aureus* strain used in this study binds bone sialoprotein, a glycoprotein known to be specifically localized to bone tissue.

Septic arthritis model in rabbits

Maren *et al.* (1986) developed an antigen-induced arthritis model of rabbit to examine the histopathologic differences between normal and arthritic joints in the same animal infected by intra-articular injections of *Staphyllococcus aureus*. Microscopic examination of whole joint sections and a quantitative histopathologic scale were used to compare changes in all the articular components of 17 normal and 17 arthritic joints infected for less than 2 weeks. The histological changes were more severe in infected arthritic joints than in normal joints (mean ± SD totol histology score, 13.8±2.4 and 9.3±4.0, respectively; $P < .001$). In infected arthritic joints, subsynovial abscesses extended into subchondral bone via the pannus of chronic synovitis at articular margins and intra-articular attachments of cruciate ligaments, rather than by initial cartilage destruction and direct extension into subchondral bone.

Wysenbeek *et al.* (1996) induced arthritis by the intra-articular injection of *Staphylococcus epidermidis* in rabbits. The experimental scheme included three groups of animals: animals that were infected but not treated (group 1); animals treated with systemic antibiotics (group 2); and animals treated with systemic antibiotics and intra-articular steroids (group 3). Nine days later the animals were sacrificed and joint histopathological-histochemical indices were calculated. Animals from groups 2 and 3 had a smaller pannus, reduced proteoglycan loss, no loss of

cartilage height and diminished synovial inflammation in comparison to the animals from group 1. The animals from groups 2 and 3 were identical in terms of cartilage cellularity, surface erosion, chondrocyte cloning, pannus formation and proteoglycan loss. Synovial inflammation appeared to be less pronounced in group 3 animals when compared to animals of group 2. Concomitant antibiotic-steroid treatment of septic arthritis seems to be harmless in that experimental setting.

Smith *et al.* (1997) studied that the effects of combining antibiotic therapy with the application of a non- steroidal anti-inflammatory drug on degradation of articular cartilage for a rabbit model of staphylococcal septic arthritis. Rabbits were infected intra-articularly by *Staphyllococcus aureus*.

Wysenbeek *et al.* (1998) reported that the effects of intra-articular corticosteroids added to systemic antibiotics in experimental septic arthritis. In brief, rabbits were injected with *Staphylococcus epidermidis* intrarticularly. Rabbits were divided into three groups i.e. treated with a. systemic antibiotics, b. systemic antibiotics and intra articular corticosteroids c. without treatment (control). Since Histopathological and histochemical evidences of the group received systemic antibiotics and intra articular corticosteroids showed the lowest scores compared to that of the other two groups and control group showed the highest score. Histopathological observations revealed least damage in clustering of chondrocytes, pannus formation, proteoglycan depletion, synovitis in case of group received both the systemic antibiotics and intra articular corticosteroids.

Jaberi *et al.* (2003) showed the effect of intra-articular corticosteroids added to systemic antibiotics in the treatment of experimental staphylococcal knee joint infection in rabbits. Thirty rabbits were injected in their knees by *Staphylococcus aureus*. The rabbits were divided into 3 equal groups. In group A, rabbits received no treatment. In group B, rabbits were treated with systemic antibiotics alone. Group C, received systemic antibiotics and intra-articular corticosteroids. After 16 days animals were killed and knee joint X-Ray as well as histopathological- histochemical parameters were assessed. All rabbits survived the experiment; the treated groups (B, C) had better histological-histochemical scores in comparison with the untreated group (A). Group C had significantly better scores in joint sections in comparison with group B (mean SD = 6.7 ± 2.3 v 4.0 ± 2.4; P= 0.019). Lower damage in the former group was

expressed in lesser clustering of chondrocytes, proteoglycan depletion, and severity of synovitis. Radiological soft tissue scoring was significantly better in group C in comparison with group B. Three peri-articular abcesses were observed in group C but none in group B.

Nielsen *et al.*(2009) established rabbit model of septic arthritis. Eighty-five rabbits were injected in one knee with *Staphlycoccus aureus* in order to study the time-related changes in untreated septic arthritis up to 3 months. In the synovial membrane a severe release of lysosomal enzymes was observed. The activity was mainly located in and around lining cells and leucocytes in the pannus demonstrating increasing destructive characteristics. This resulted in marginal erosion and undermining of the cartilage border visible from Day 5 continuing gradually to total joint destruction after 5 weeks. The glycosaminoglycan depletion was observed at the surface of the cartilage at Day 2 and was total after 2 weeks.

Schroeder *et al.* (2011) investigated the efficacy of Linezolid versus Vancomycin in experimental implant infections and the influence on implant stability in a rabbit model. Thirty-six female New Zealand white rabbits received surgical insertion of titanium implants into their distal femurs and were randomly assigned to six groups (A: infected, no treatment; B: infected, vancomycin; C: infected, linezolid; D: no infection, no treatment; E/F: no infection, vancomycin or linezolid, respectively) . Antibiotics were administered, and plasma levels determined. Bone implant specimens were tested for mechanical stability of fixation. Quantitative histomorphometry of bone and soft tissue was performed using computerized image analysis. Plasma levels of linezolid and vancomycin were within the respective therapeutic ranges. Microbiological analysis of specimens from infected rabbits showed MRSA tissue colonization in all untreated animals, in 2 of 6 vancomycin-treted animals, and in none of the linezolid-treted animals. Antibiotic treatment improved mechanical stability significantly ($p = 0.004$) with both vancomycin and linezolid. Mechanical testing correlated with histomorphometry result. A significant negative correlation was found between displacement of the implant and the percentage of the calcified tissue around the implant, and the significant positive correlation was found between displacement of the implant and the amount of non- calcified tissue. Their data indicated that the both treatment regimens improved implant stability.

Desando *et al.* (2013) aimed to investigate the efficacy of intra-articular adipose-derived stromal cell (ASC) injection in the healing process on cartilage, synovial membrane and menisci in an

experimental rabbit model. The induction of osteoarthritis was performed surgically through bilateral anterior cruciate ligament transection (ACLT) to achieve eight week from ACLT a mild grade of osteoarthritis. A total of 2×10^6 and 6×10^6 autologous ASCs isolated from inguinal fat, expanded *in vitro* and suspended in 4% rabbit serum albumin (RSA) were delivered in the hind limbs, 4% RSA was used as the control. Local bio-distribution of the cell was verified by injecting chloro-methyl-benzamido- 1, 1′-dioctadecyl - 3, 3, 3′, 3′- tetra-methyl-indo-carbocyanine perchlorate (CM- Dil) labeled ASCs in the hind limbs. Cartilage and synovial histological sections were scored by Laverty's scoring system to assess the severity of the pathology. Protein expression of some extra cellular matrix molecules (Collagen I & II), catabolic (metalloproteinase-1&-3) and inflammatory (TNF-α) markers were detected by immunohistochemistry. Assessments were carried out at 16 and 24 weeks. Labeled ASCs were detected unexpectedly in the synovial membrane and medial meniscus but not in cartilage tissue at 3-20 days from ASC treatment. Intra-articular ASC administration decreased osteoarthritis progression and exerted a healing contribution in the treated animals in comparison to osteoarthritis and 4% RSA groups. Their data revealed a healing capacity of ASCs in promoting cartilage and menisci repair and attenuating inflammatory events in synovial membrane inhibiting osteoarthritis progression. On the basis of the local bio-distribution findings, the benefits obtained by ASC treatment could be due to a trophic mechanism of action by the release of growth factors and cytokines.

Septic arthritis confirmation with some markers

Faryna *et al.* (1990) described Joint fluid in normal and non-inflammatory conditions usually maintains a glucose level close (within 10 mg dl^{-1}) to the serum concentration because glucose enters the synovia from blood by facilitated diffusion with equilibration. The synovial–serum difference is most reliable only in the fasting state because equilibration is slow and unpredictable after a meal. In inflammatory and infectious conditions, synovial glucose is often reduced as a result of its utilization by the metabolic activity of the neutrophils and bacteria. In the presence of bacterial infection, synovial fluid glucose may be at least 25 mg dl^{-1} lower than a simultaneous blood glucose, providing the patient is fasting. Joint fluid in normal and non-inflammatory conditions contains about one-fourth the total protein present in blood. Coagulation proteins are absent, hence normal joint fluid does not clot. Smaller molecules, such

as albumin, are usually present in greater concentrations than larger molecules, such as most of the globulins. In inflammatory conditions, however, the concentration of these components may be equal to the plasma concentrations because of increased synovial blood flow. Synovial fluid protein levels greater than 2.5 g dl^{-1} are abnormal, and those greater than 4.5 g dl^{-1} indicate significant inflammation.

Simon *et al.* (2004) performed a meta-analysis to evaluate the accuracy of determination of procalcitonin (PCT) and C-reactive protein (CRP) levels for the diagnosis of bacterial infection. The analysis included published studies that evaluated these markers for the diagnosis of bacterial infections in hospitalized patients. PCT level was more sensitive (88% [95% confidence interval {CI}, 80%–93%] vs. 75% [95% CI, 62%–84%]) and more specific (81% [95% CI, 67%–90%] vs. 67% [95% CI, 56%–77%]) than CRP level for differentiating bacterial from noninfective causes of inflammation. The Q value for PCT markers was higher (0.82 vs. 0.73). The sensitivity for differentiating bacterial from viral infections was also higher for PCT markers (92% [95% CI, 86%–95%] vs. 86% [95% CI, 65%–95%]); the specificities were comparable (73% [95% CI, 42%–91%] vs. 70% [95% CI, 19%–96%]). The Q value was higher for PCT markers (0.89 vs. 0.83). PCT markers also had a higher positive likelihood ratio and lower negative likelihood ratio than did CRP markers in both groups. On the basis of this analysis, the diagnostic accuracy of PCT markers was higher than that of CRP markers among patients hospitalized for suspected bacterial infections.

Sato *et al.* (2012) assessed serum procalcitonin (PCT) levels to distinguish bacterial infection from other complications in patients with Rheumatoid arthritis (RA).One hundred eighteen patients experiencing an RA flare, noninfectious complication of RA or its treatment, nonbacterial infection, or bacterial infection were studied. Serum PCT concentrations were determined with a chemiluminescent enzyme immunoassay.All patients experiencing an RA flare showed negative PCT levels ($\leq$ 0.1 ng ml^{-1}; n = 18). The PCT level was higher in the bacterial infection group (25.8% had levels $\geq$ 0.5 ng ml^{-1}) than in the other 3 groups (0.0-4.3% had levels $\geq$ 0.5 ng ml^{-1}) and the difference was significant among groups (p = 0.003). Conversely, no statistically significant difference was observed among the groups with C-reactive protein (CRP) concentration $\geq$ 0.3 mg/dl (p = 0.513), white blood cell (WBC) count > 8500/mm^3 (p = 0.053), or erythrocyte sedimentation rate (ESR) > 15 mm/h (p = 0.328). The OR

of high PCT level (≥ 0.5 ng ml^{-1}) for detection of bacterial infection was 19.13 (95% CI 2.44-149.78, p = 0.005). Specificity and positive likelihood ratio of PCT ≥ 0.5 ng ml^{-1} were highest (98.2% and 14.33, respectively) for detection of bacterial infection, although the sensitivity was low (25.8%).

Maharajan *et al.* (2013) reported that early diagnosis of Acute Osteomyelitis (OM) and Septic Arthritis (SA) is of vital importance to avoid devastating complications. There is no single laboratory marker which is sensitive and specific in diagnosing these infections accurately. Total Count, ESR and CRP are not specific as they can also be elevated in non-pyogenic causes of inflammation. Pus Culture and sensitivity is not a true gold standard due to its varied positivity rates (40 - 70%). Serum Procalcitonin (PCT), at 0.5 ng ml^{-1} is found to be an accurate marker for pyogenic infections. The objectives of this study were to show that PCT is an accurate marker in differentiating Acute Osteomyelitis and Septic Arthritis from viral and non-infective inflammatory bone and joint conditions. Patients of all age groups (n = 82) with suspected Acute Osteomyelitis and Septic Arthritis were prospectively included in their study. All patients were subjected to TC, CRP, PCT, IgM Dengue, IgM Chikungunya, pus and blood culture and sensitivity. At the end of the study, patients were classified into 3 groups: Group 1 = Confirmed Pyogenic (n = 27); Group 2 = Presumed Pyogenic (n = 21); Group 3 = Non - infective inflammatory (n = 34).Group 1 had higher mean PCT levels than Group 2 and 3 ($p < 0.05$). PCT, at 0.4 ng ml^{-1}, was 85.2% sensitive and 87.3% specific in diagnosing Septic Arthritis and Acute Osteomyelitis. In comparison, PCT at conventional cut - off of 0.5 ng ml^{-1} is 66.7% sensitive and 91% specific.Thus they concluded serum Procalcitonin, at a cut - off of 0.4 ng ml^{-1}, as a sensitive and specific marker in the diagnosis of Septic Arthritis and Acute Osteomyelitis.

Pharmacological effects of various parts of *Tamarindus indica* L.

Ghelardia *et al.* (2000) reported that the efficacy of a novel muco-adhesive polymer, the tamarind seed polysaccharide, as a delivery system for the ocular administration of hydrophilic and hydrophobic antibiotics. Healthy rabbits were subjected to repeated ocular instillations with either conventional gentamicin or ofloxacin or these agents viscosified with the tamarind seed polysaccharide. Administration of viscosified preparations produced antibiotic concentrations both in the aqueous humour and cornea that were significantly higher than those achieved with the drugs alone. The increased drug absorption and the

prolonged drug elimination phase obtained with the viscosified formulations indicate the usefulness of the tamarind seed polysaccharide as an ophthalmic delivery system for topical administration of antibiotics.

Meléndez *et al.* (2006) reported that tamarind leaves possessed a strong *in vitro* antibacterial activity against more than 13(81%) common gram positive and gram negative bacteria that were tested. They also reported that tamarind leaf extract was very effective against *E. coli.*

Caluwé *et al.* (2010) reported that Tamarind leaves were a fair source of vitamin C and α-carotene. Mineral contents were found in very high amount, particularly P, K, Ca and Mg. Anti-oxidant, anti-inflammatory, anti-microbial and anti-fungal activity had been documented from several plant parts.

Bhadoriya *et al.* (2012) aimed to investigate the anti-inflammatory and anti-nociceptive potential of a hydroethanolic extract of *Tamarindus indica* L. leaves (HTI) along with its possible mode of action. The anti-inflammatory activity of HTI was estimated by carrageenan-induced hind paw oedema in male Wistar albino rats. Furthermore, HTI was assessed to determine its effects on membrane stabilization. The antinociceptive action was determined by acetic acid-induced writhing, tail-flick, and the hot plate model. Oral administration of HTI at the dose of 500, 750, and 1000 mg/kg body weight produced significant ($P< 0.01$) anti-inflammatory as well as anti-nociceptive actions in a dose-dependent manner. Among all tested doses, 1000 mg/kg, *p. o.* reduced carrageenan-induced rat paw oedema at 1, 2, 3, and 4 h. Moreover, the 1000 mg/kg dose exhibited maximum percentage inhibition of acetic acid-induced writhing (48.9%), whereas standard drug diclofenac (25 mg kg^{-1}, *p. o.*) showed maximum inhibition (50.9%) of writhing. In the hot plate model, HTI (1000 mg kg^{-1}, orally) increased mean basal reaction time after 120 min (7.12±0.05 sec). In the tail flick model, HTI increased the maximum percentage of latency (36.06%), whereas the standard drug pethidine (4 mg kg^{-1}, intra-peritoneally) showed maximum percentage of latency (43.85%) after 60 min.

Gumgumjee *et al.* (2012) investigated the antimicrobial activities of the leaves extract of *Tamarindus indica* L. against Gram negative and Gram positive bacteria. The results were supported by scanning electron microscopy images. The phytochemical constituents of the dried powder leaves were extracted using aqueous and organic solvents. The antimicrobial activity of

this extract was evaluated by using inhibition zone diameter, of both Gram negative and positive bacteria and fungi using agar well diffusion method. The most pronouns effect was shown by ethanol extract. Studies on the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the extracts on the tested organisms showed that the lowest MIC and MBC were demonstrated against *Klebsiella pneumonia* and *Micrococcus luteus*, but the highest MIC and MBC was exhibited against *Pseudomonas aeruginosa* and methicillin resistant *Staphyllococcus aureus* (MRSA). The phytochemical analysis revealed the presence of four major compounds, identified as flavanoidal glycosides. Using the total ion chromatography (TIC) two major compounds were identified as Orientin and Vitexin.

Sundaram *et al.* (2014) depicted continuous generation of free radicals like reactive oxygen and nitrogen species is considered as a key culprit in the initiation and propagation of oxidative damage. In addition, activation of T and B cells, macrophages, inflammatory mediators such as TNF-α, IL-1β and IL-6 aggravates the oxidative damage of the vital organs, particularly the liver. They demonstrated oxidative stress in the liver of arthritic rats and its amelioration by the procyanidin-rich tamarind seed extract (TSE). The arthritic liver homogenate, mitochondrial and cytosolic fractions were found with increased levels of oxidative stress markers including free radicals. As a consequence, depletion in the levels of glutathione, total thiols, glutathione peroxidase and reductase was evident. Furthermore, the activities of endogenous antioxidant enzymes like superoxide dismutase, catalase and glutathione-S-transferase were found to be significantly altered. The increased and decreased activity of transaminases respectively in serum and liver, along with histological observations, further confirms the liver damage. Unfortunately, the commonly used drugs like NSAIDs and DMARDs have failed to prevent oxidative damage, rather they were found to be the inducers themselves. Interestingly, TSE supplementation was found to significantly inhibit oxidative burst in the liver and maintain homeostasis. Thus, the study clearly demonstrated the protective efficacy of TSE against arthritis-associated oxidative liver damage, including mitochondrial oxidative burst and its associated secondary complications.

Sundaram *et* al. (2015) demonstrated the anti-arthritic efficacy of tamarind seed extract (TSE). TSE exhibited cartilage and bone protecting nature by inhibiting the elevated activities of MMPs, HAase, exoglycosidases, cathepsins and TRAP. It also mitigated the augmented levels

of inflammatory mediators like interleukin (IL)-1β, tumor necrosis factor-α, IL-6, IL-23 and cyclooxygenase-2. Further, TSE administration alleviated increased levels of ROS and hydroperoxides and sustained the endogenous antioxidant homeostasis by balancing altered levels of endogenous antioxidant markers. Overall, TSE was observed as a potent agent abrogating arthritis-mediated cartilage/bone degradation, inflammation and associated stress *in vivo*.

Yeasmen *et* al. (2015) investigated the influence of two extraction solvents (ethanol and acetone) and two extraction techniques i.e., hot extraction at 40 °C and cold extraction at 26°C on the phenolic content and antioxidant activity of extracts from Tamarindus indica seed. The antioxidant activity of T. indica was determined by evaluating 1,1-Diphenyl-2-picrylhydrazyl (DPPH) scavenging activity, ferric reducing power assay (FRAP) and ascorbic acid equivalent content (AAC). The tested sample showed appreciable amounts of total phenolic contents (51.45-71.68 mg GAE/gm of dry extract), DPPH scavenging capacity (61.18- 71.17%), IC50 values (98.30-248.60), reducing power (0.6377-0.7702) and total antioxidant capacity (22.75- 43.80 AAE/gm) at different solvents and techniques. Current study data shown higher extract yields, phenolic contents, scavenging activity, reducing power and antioxidant activity using ethanol solvent compared to the respective acetone solvent. In addition, higher extract yields and other properties were obtained by hot extraction at 40°C compared to the cold extraction at 26°C. Their study suggested that ethanol as a solvent and hot extraction technique could be better to preserve the antioxidant properties of tamarind seed.

Linezolid

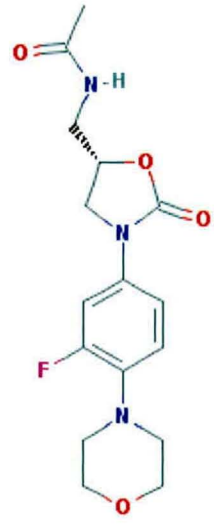

Figure i: Linezolid molecule

Table 1: Properties of Linezolid

Property	Information	Source
I.U.P.A.C name	N-[[(5S)-3-(3-fluoro-4-morpholin-4-ylphenyl)-2-oxo-1,3-oxazolidin-5-yl]methyl]acetamide	PubChem
Chemical formula	$C_{16}H_{20}FN_3O_4$	PubChem
Melting point	177 °C	Biosynth
pKa value	1.Strongest acidic: 14.45 2.Strongest basic: - 0.66	ChemAxon
Molecular weight	337.346103 g/mol	PubChem
Solubility	1.in Water : 3 mg/mL, 2.in DMSO: 100 mM 3.in ethanol: 25 mM	1. Pubchem, 2. & 3.Tocris Bioscience
LD_{50}	>5000 mg/kg (Rat oral)	Pfizer

Betamethasone

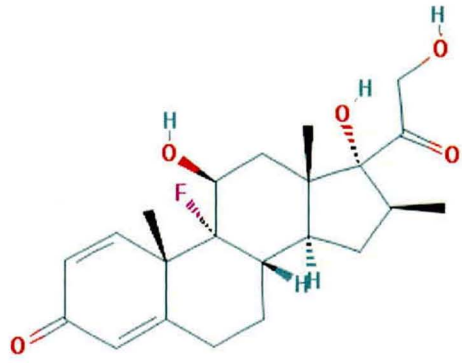

Figure ii: Betamethasone molecule

Table 2: properties of betamethasone

Property	Value	Source
Melting point	232 °C	U.S. Patent 3,164,618
Water solubility	66.5 mg/L (at 25 °C)	EPA
I.U.P.A.C name	(11b,16b)-9-fluoro-11,17,21-trihydroxy-16-methylpregna-1,4-diene-3,20-dione	Chemspider
Chemical formula	C_{22} H_{29} FO_5	Chemspider
LD_{50}	LD50 >5000 mg/kg (rat)	MSD
pKa	1.Strongest acidic: 12.42 2.Strongest basic: - 3.3	ChemAxon
Molecular weight	392.461	Chemspider

Pharmacokinetics of Linezolid by High Performance Liquid Chromatography

Peng *et al.* (1999) developed an HPLC–UV method for assay of linezolid in dog, rat, mouse, and rabbit plasma. Linezolid and the internal standard were extracted on a solid phase cartridge (SPE) and separated on a reversed-phase column (C8, 4.6×150 mm and 5 μm) with 20% acetonitrile in water as mobile phase. The SPE quantitatively recovered linezolid and the internal standard from plasma samples. The chromatographic peak height ratio or peak area ratio based on UV absorbency at 251 nm was used for quantitative analysis. The assay procedures were simple and the assay was specific and had adequate precision and accuracy. Calibration standards with concentrations over the range of 0.01–20 μg ml^{-1} were validated for routine sample analysis to support the pharmacokinetic and toxicology studies with linezolid in dog, rat, mouse, and rabbit. Analysis of quality control samples showed the coefficients of variation were usually <10% and the measured and theoretical concentrations differed by <10% in most assays. Linezolid in the plasma samples was stable when stored at below −20°C for at least 63 days, at room temperature (22–23°C) for up to 24 h, and after three freeze–thaw cycles. This HPLC method has been successfully used in multiple laboratories to assay plasma samples from pharmacokinetic and toxicology studies with linezolid in the animal species.

Rana *et al.* (2002) studied Penetration of linezolid into osteo-articular tissue and fluid in 10 patients undergoing primary total knee replacement. Linezolid 600 mg 12 hourly was given orally over the 48 h before operation and intravenously 1 h before induction of anaesthesia. Mean concentrations of linezolid at 90 min after the final dose, in serum, synovial fluid, synovium, muscle and cancellous bone, assayed by HPLC, were at least twice the MIC_{90} for staphylococci and streptococci. The concentrations obtained indicate good penetration of this antibiotic and support its use in the management of multidrug-resistant Gram-positive bone, joint and deep-seated soft-tissue infections.

Boak *et al.* (2005) developed a simple high-performance liquid chromatographic (HPLC) method and validated for rapid quantification of linezolid in human plasma. Protein precipitation using a mixture of 5% trichloroacetic acid and methanol (3:1, v/v) provided a straightforward method of sample preparation and the internal standard eperezolid was employed. A concentration range from 0.20 to 40.0 mg L^{-1} was utilized to construct calibration curves, and analysis of low- (0.40 mg L^{-1}), medium- (7.50 mg L^{-1}) and high-quality (25.0 mg L^{-1}) control samples revealed

excellent reproducibility (≤ 3.88%) and accuracy (≤ 4.20%). The recovery of both linezolid and eperezolid was approximately 100%. No interference was observed at the retention times of linezolid and eperezolid from blank plasma or eight commonly used antibiotics. Tests confirmed the stability of linezolid in plasma during three freeze–thaw cycles, on the bench during 24 hr and during long-term storage of frozen plasma for up to 12 weeks; in extracts it was stable in the HPLC autosampler over 12 hr. Overall, this assay offers a highly simplistic approach to quantifying linezolid in plasma, and would be well suited to clinical pharmacokinetic, pharmacodynamics and toxicodynamics analyses.

MATERIAL AND METHODS

MATERIAL AND METHODS

Chemicals

All chemicals and kits used in this study were obtained from Bengaluru Genei(India), Cogent(India), Promega(USA), Elabscience Biotechnology(China), Rankem (India), E. Merk(India), Bio Vision(USA) and Sigma chemicals(USA).

Experimental animals

Rabbit

Clinically healthy adult (6 to 8 months of age) Rabbits weighing 1.5 – 2 Kg were housed individually in custom-made stainless steel metabolic cages. Animals were maintained in controlled environment where room temperature was 26° ± 3° C and provided with artificial lighting facilities.

Rat

Healthy female Albino rats (*Rattus norvegicus*), of approximately 8 weeks of age and weighing between 180 and 200 gm were used in the experiment. The animals were placed in galvanized rat cages throughout the period of the study. Animals were maintained in controlled environment where room temperature was 22° ± 3° C and provided with artificial lighting facilities.

Laboratory animals were fed with concentrated pellet food and provided with aquaguard filtered water *ad libitum* throughout the study period.

Before using, each cage was disinfected with hot water and phenol. Each day, floor of the experiment room was cleaned, disinfected and mopped with phenyl solution. The animals were identified using aqueous solution of picric acid (1:1000 w/v) and cages were labelled with tags indicating sex, group and animal number.

Experimental Design

Experiment I

1. Isolation and identification of bacteria from synovial fluid of goat suffering from septic arthritis.
2. Antibiogram for checking resistivity.
3. Determination of Minimum Inhibitory Concentration (MIC) of Linezolid against the isolated organism.

Experiment II

Preperation of ethanolic and aqueous extract from *Tamarindus indica* L. leaf powder.

Experiment III

Toxicity study –

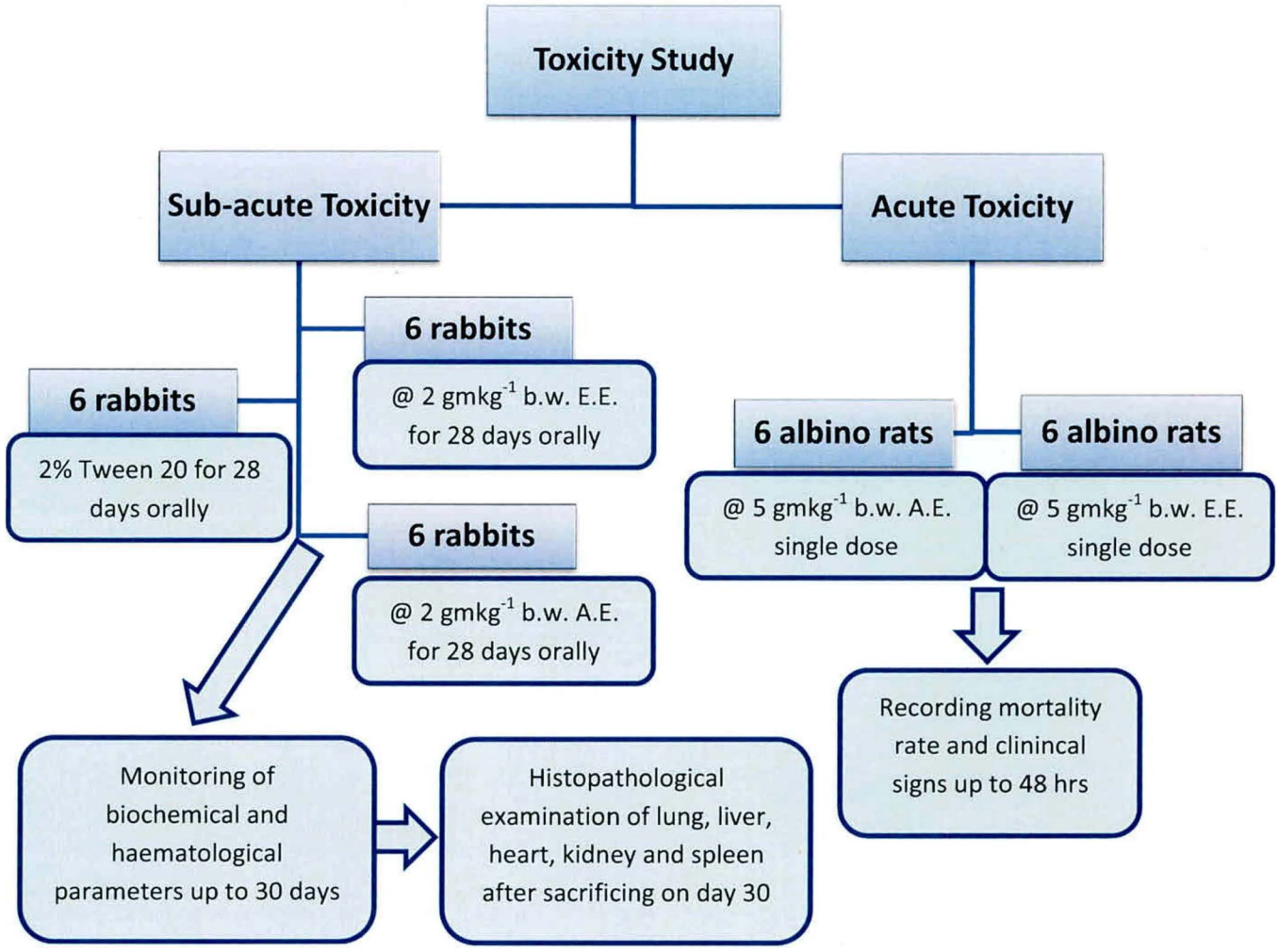

Figure iii: schematic representation of acute and sub-acute toxicity study

Experiment IV

1. Induction of septic arthritis in rabbits by intra-articular inoculation of isolated organism (10^4cfu ml^{-1}).
2. Confirmation of septic arthritis by biochemical, microbiological, haematological and histopathological findings.

Experiment V

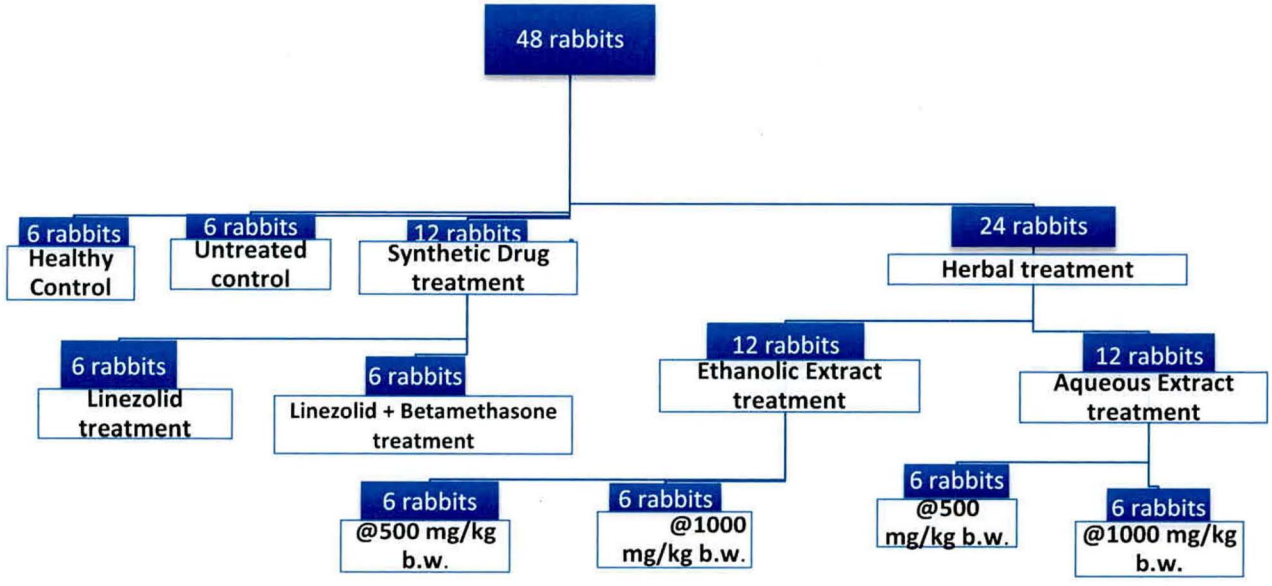

Figure iv: schematic representation of distribution of animals in different study groups

Groups:

Gr- I- Untreated arthritic control

Gr – II - oral Linezolid @ 75 mg kg^{-1} for 10 days

Gr – III -oral Linezolid @ 75 mg kg^{-1} for 10 days with a single intra-articular injection of Betamethasone @ 0.5 mg kg^{-1}

Gr – IV - Ethanolic Extract of *Tamarindus indica* L. leaves treatment @ 500 mg kg^{-1} for 14 days

Gr – V - Ethanolic Extract of *Tamarindus indica* L. leaves treatment @ 1000 mg kg^{-1} for 14 days

Gr – VI - Aqueous Extract of *Tamarindus indica* L. leaves treatment @ 500 mg kg^{-1} for 14 days and

Gr – VII - Aqueous Extract of *Tamarindus indica* L. leaves treatment @ 1000 mg kg^{-1} for 14 days

2. Study of biochemical (Lactate dehydrogenase, Glucose, Total protein), hematological [Hemoglobin (Hb), Total Count (TC), Differential Count (DC), Erythrocyte Sedimentation Rate (ESR), Packed Cell Volume (PCV), Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin Concentration (MCHC), Mean Corpuscular Hemoglobin (MCH)] and microbiological [Bacterial colony count from synovial fluid, ELISA for Rabbit's specific C-Reactive Protein (CRP) and Procalcitonin (PCT)] parameters from collected serum and or synovial fluid at regular intervals (0,2,7,14 days).
3. Monitoring of body weight, body temperature, measurement of joint radius (externally) at regular intervals.
4. Euthanasia of all animals for collection of menisci for histomorphological scoring

Experiment VI

1. Pharmacokinetics study of Linezolid in healthy rabbits

Experiment -I

Isolation and identification of bacteria

Isolation and identification of *Staphylococcus aureus* from goats suffering from arthritis

The synovial fluid and exudate from lesion was collected aseptically in sterile vial from a black Bengal kid aged 3 months suffering with septic arthritis. The clinical sample was inoculated into nutrient broth (HiMedia, India) and incubated at 37°C for overnight. The growth on the next day was transferred into Mannitol salt agar (HiMedia, India) and incubated at 37°C for 24 hours. Characteristic colonies surrounded by bright yellow zone (Figure 2) were selected for confirmation. The selected single colonies were transferred into nutrient agar slant. The colonies were preliminarily identified by Gram's staining, standard biochemical tests such as catalase, oxidase, urease, carbohydrate fermentation with glucose, sucrose, maltose, mannitol (Quinn *et al.*, 1994).

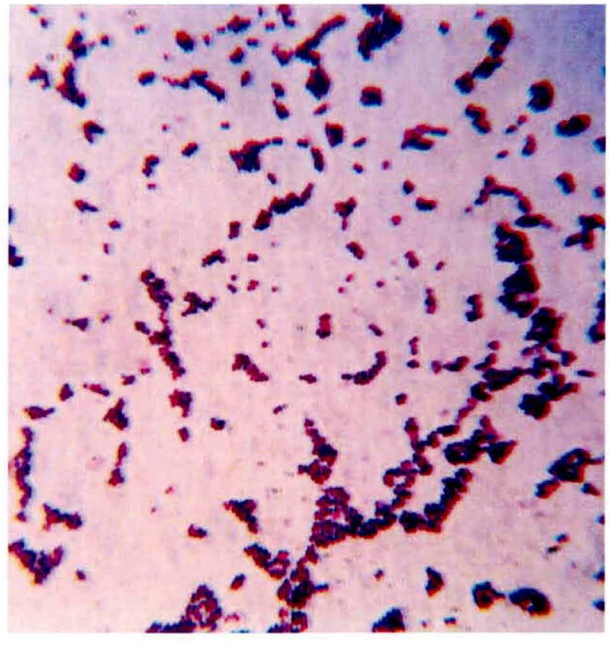

Figure v: *Staphylococcus aureus* colony

PCR based confirmation of *Staphylococcus aureus* isolates

S. aureus isolates identification based on biochemical tests were confirmed by possession of *nuc* gene in PCR. The primers and cycle condition for *nuc* detection, PCR was adopted from earlier work (Brakstad *et al.*, 1992).

Detection of phenotypical antibiotic resistance of *Staphylococcus aureus* isolates

The confirmed *S. aureus* isolates were subjected to antibiotic sensitivity test with ceftriaxone, cefotaxime, ampicillin, gentamycin, amoxyclav, Linezolid (Bio-Rad), ampicillin & sulbactum, ceftizoxime, piperacillin and tazobactum, ticarcillin and clavulanic acid, imipenem-EDTA, enrofloxacin, methicillin, ciprofloxacin, ceftriaxone and tazobactum, vancomycin etc. antibiotic discs procured from HiMedia, India following CLSI guidelines (CLSI, 2008).

Detection of *mecA* gene in *Staphylococcus aureus* isolates

The *mecA* gene is used for rapid identification of methicillin-resistant *S. aureus* (MRSA) in bacterial subcultures on solid media. PCR for *mec A* gene was conducted using the primers and cycle conditions as described earlier (Shrestha *et al.*, 2002).

MIC of linezolid

MIC of linezolid had been performed as per protocol using market strip [Linezolid Ezy MICTM Strip (LNZ) (0.016-256 mcg ml^{-1}), HIMEDIA]

Experiment -II

Identification of *Tamarindus indica* L.

The plant was identified by Botanical Survey of India, Howrah (Specimen no. - WBUAFS/LJ 03). The leaves were collected from the plant during the month of December and January.

Figure vi: *Tamarindus indica* L. leaves

Preparation of leaf extracts of *Tamarindus indica* L.

Ethanolic extraction of *Tamarindus indica* L. leaves

- The plant materials were dried in shade, and powdered in a mechanical grinder.
- The powder of leaves of *Tamarindus indica* L. plant was initially defatted with petroleum benzene (60-80°C) followed by 1000 ml of ethanol by using a Soxhlet extractor for 72 hr at a temperature not exceeding the boiling point of the solvent [Lin et al., 1999].
- The extract was filtered using Whattman filter paper (No. 1) and then concentrated in vacuum and dried at 45°C for ethanol removal, and the extracts were kept in sterile bottles under refrigerated conditions until use.
- The dry weight of the plant extracts was obtained by the solvent evaporation and used to determine concentration in mg ml^{-1}.

Figure vii: Ethanolic extract of *Tamarindus indica* L. leaves

Aqueous Leaf Extract of *Tamarindus indica* L.

Dried leaves were submitted to decoction (10% w/v, Leaf powder: water) for 15 min at a temperature of around 100°C to obtain the aqueous leaf extract of leaves of *Tamarindus indica* L. (yield: 17% relative to dry plant). The aqueous extract obtained after vacuum filtration was freeze-dried.

Experiment -III

Toxicity Study

Acute toxicity study

A total of 12 rats were divided into two groups each containing 6 rats. One group of 6 rats were orally administered single dose of 5 gm of ethanolic extract of *Tamarindus indica* L. leaves. Another group of 6 rats were also orally given a single dose of 5 gm of aqueous extract of *Tamarindus indica* L. leaves. Rats of both the groups were observed up to 48 hrs for recording the clinincal signs.

Sub-acute toxicity study

A total of 18 rabbits were divided into 3 groups each containing 6 animals. Ethanolic extract of *Tamarindus indica* L. leaves @2 $gmkg^{-1}$ b.w., aqueous extract of *Tamarindus indica* L. leaves @2 $gmkg^{-1}$ bw and 2% tween 20 in distilled water were orally administered for 28 consecutive days to three different groups. Biochemical parameters like Aspartate Aminotransferase (AST), Alanine Transaminase (ALT) level , Alkaline Phosphatase (ALP) activity, Blood Urea Nitrogen (BUN) and Creatinine and haematological parameters like White Blood Corpuscles (WBC), Red Blood Corpuscles (RBC), ESR, Hb, PLATELET, PCV were monitored on different days.

Estimation of Biochemical parameters:

Estimation of Aspartate aminotransferase (AST)

Aspartate aminotransferase activity of the prepared extract was estimated by using diagnostic kit (M/S Span Diagnostics Ltd. Plot No. 336, 338, 340, Road No. 3, G.I.D.C., SACHIN 394 230 (Surat) INDIA) as per 2, 4- DNPH method described by Reitman and Frankel (1957).

Principle:

Aspartate aminotransferase catalyses the transamination of L- Aspartate and α- Ketoglutarate to form Oxaloacetate and L- Glutamate. Oxaloacetate so formed is coupled with 2, 4- Dinitrophenyl hydrazine (2, 4- DNPH) to form corresponding hydrazone, a brown coloured complex in alkaline medium.

α- KG + L-Aspartate ⟶ Pyruvate + L-Glutamate

Oxaloacetate +2, 4- DNPH ⟶ Corresponding Hydrazone (brown colour)

Reagents:

Reagent 1- Buffered Aspartate- α-KG Substrate, pH-7.4

Reagent 2- 2, 4- DNPH Colour Reagent

Reagent 3- Sodium Hydroxide

Reagent 4- Working Pyruvate Standard

Working Reagent Preparation: Reagent 1, 2, and 4 were ready to use

Solution 1: 1 ml of Reagent 3 was diluted to 10 ml with purified Water

Procedure:

(a) Reagent 1(0.25 ml) was pipetted into all the tubes marked as Blank, Standard,

(b) Test and Control respectively.

(c) Serum (0.05 ml) was added to the tube marked as Test.

(d) Standard (0.05 ml) was added to Standard tube.

(e) It was mixed well and incubated at 37°C for 60 minutes.

(f) Now 0.25 ml of Reagent2 was added to all the tubes.

(g) Deionised Water (0.05 ml) was added to the Blank.

(h) Serum (0.05 ml) was added to the Control tube.

(i) It was mixed well and allowed to stand at room temperature (15-30°C) for 20 minutes.

(j) Solution 1 (2.5 ml) was then pipetted to all the tubes.

(k) It was then mixed well and absorbance was read against Purified Water at 505 nm within 15 minutes.

Calculation:

$$\text{AST activity } (IUL^{-1}) = \frac{\text{Absorbance of Test} - \text{Absorbance of Control}}{\text{Absorbance of Standard} - \text{Absorbance of Blank}} \times \text{Conc. Of Standard}$$

Estimation of Alanine aminotransferase (ALT)

Alanine aminotransferase activity of the prepared extract was estimated by using diagnostic kit (M/S Span Diagnostics Ltd. Plot No. 336, 338, 340, Road No. 3, G.I.D.C., SACHIN 394 230 (Surat) INDIA) as per 2,4- DNPH method described by Reitman and Frankel (1957).

Principle:

Alanine aminotransferase (ALT) catalyses the transamination of L-Alanine and α-Ketoglutarate (α-KG) to form Pyruvate and L- Glutamate. Pyruvate so formed is coupled with 2,4-Dinitrophenyl hydrazine (2,4- DNPH) to form a corresponding hydrazone, a brown coloured complex in alkaline medium.

α- KG + L-Alanine ⟶ Pyruvate + L-Glutamate

Pyruvate +2, 4- DNPH ⟶ Corresponding Hydrazone (brown colour)

Reagents:

Reagent 1- Buffered Alanine.

Reagent 2- 2,4- DNPH Colour Reagent.

Reagent 3- Sodium Hydroxide.

Reagent 4- Working Pyruvate Standard.

Working Reagent Preparation: Reagent 1, 2, and 4 were ready to use.

Solution 1: 1 ml of Reagent 3 was diluted to 10 ml with purified Water.

Procedure:

(a) Reagent 1(0.25 ml) was pipette into all the tubes marked as Blank, Standard, Test and Control respectively.

(b) Serum (0.05 ml) was added to the tube marked as Test.

(c) Standard (0.05 ml) was added to Standard tube.

(d) It was then mixed well and incubated at 37°C for 30 minutes.

(e) Reagent2 (0.25 ml) was pipetted to all the tubes.

(f) Deionised Water (0.05 ml) was added to the Blank.

(g) Serum (0.05 ml) was added to the Control tube.

(h) It was mixed well and allowed to stand at room temperature (15-30°C) for 20 minutes.

(i) Solution 1(2.5 ml) was pipetted to all the tubes.

(j) It was mixed well and absorbance was measured against Purified Water at 505 nm within 15 minutes.

Calculation:

$$\text{ALT activity } (IUL^{-1}) = \frac{\text{Absorbance of Test} - \text{Absorbance of Control}}{\text{Absorbance of Standard} - \text{Absorbance of Blank}} \times \text{Conc. Of Standard}$$

Estimation of Alkaline Phosphatase

Alkaline Phosphatase of the prepared extract was estimated by using diagnostic kit (M/S Span Diagnostics Ltd. Plot No. 336, 338, 340, Road No. 3, G.I.D.C., SACHIN 394 230 (Surat) INDIA) as per Kind and King's method described by Kind *et al.* (1954) and King *et al.* (1959).

Principle:

Alkaline Phosphatase from serum converts Phenyl Phosphate to Inorganic Phosphate and Phenol at pH 10.0. Phenol so formed reacts in alkaline medium with 4- Aminoantipyrine in presence of the oxidizing agent Potassium Ferricyanide and forms a Orange- Red coloured complex. The colour intensity is proportional to the enzyme activity.

Phenyl Phosphate $\xrightarrow{\text{Alkaline Phosphatase (pH- 10.0)}}$ Phenol + Phosphate

Phenyl + 4-Aminoantipyrine $\xrightarrow{\text{Pot- Ferricyanide (OH)}}$ Orange- Red complex (λ max = 510-520 nm)

Reagents:

Reagent 1- Buffered substrate pH- 10.0.

Reagent 2- Chromogen Reagent. Alkaline Phosphatase

Reagent 3- Phenol Reagent, 10 mg %.

Preparation of Working Solution: One vial of Reagent 1, Buffered Substrate was reconstituted with 2.2 ml of Purified Water. Reagents 2 and 3 were ready to use.

Procedure:

(a) Working Buffered (0.5 ml) Substrate was pipetted out to the four tubes labeled as Blank, Standard, Control and Test respectively.

(b) Purified Water (1.5 ml) was added to all the tubes.

(c) It was mixed well and incubated at 37°C for 3 minutes.

(d) Serum (0.05 ml) was added to the tube marked as Test.

(e) Reagent 3 (0.05 ml) was added to the tube marked as Standard.

(f) It was mixed well and incubated at 37°C for 15 minutes.

(g) Reagent 2 (1.0 ml) was pipetted out to all the tubes.

(h) Serum (0.05 ml) was added to the Control tube.

(i) It was then mixed well after addition of each reagent and absorbance was measured of Blank, Standard, Control and Test against Purified Water at 510 nm.

Calculations:

$$\text{Serum Alkaline Phosphatase (KA Units)} = \frac{\text{Absorbance of Test} - \text{Absorbance of Control}}{\text{Absorbance of Standard} - \text{Absorbance of Blank}} \times 30$$

Estimation of BUN

Urea of the prepared extract was estimated by using diagnostic kit (M/S Span Diagnostics Ltd. Plot No. 336, 338, 340, Road No. 3, G.I.D.C., SACHIN 394 230 (Surat) INDIA).

Principle:

Urea reacts with hot acidic Diacetylmonoxime in presence of Thiosemicarbazide and produces a rose- purple coloured complex.

Reagents:

Reagent 1- Urea Reagent.

Reagent 2- Diacetylmonoxime (DAM).

Reagent 3- Working Reagent Standard, 30 mg %.

Preparation of Solution 1: 1 ml of Reagent 1 was diluted to 5 ml with purified water. Reagent 2 and Reagent 3 (Standard) were ready for use.

Procedure:

(a) Solution 1 (2.5 ml) was pipetted to the three tubes marked as Blank, Test and Standard respectively.

(b) Sample (0.01 ml) was added to the tube marked as Test.

(c) Reagent 3 (0.01 ml) was added to the tube marked as Standard.

(d) It was mixed well.

(e) Reagent 2 (0.25 ml- Diacetylmonoxime) was pipetted to all the tubes.

(f) It was mixed well and the tubes were kept in the boiling water exactly for 10 minutes. It was then cooled immediately under running tap water for 5 minutes, mixed by inversion and absorbance was measured at 525 nm.

Calculation:

$$\text{Urea (mg/100 ml)} = \frac{\text{O. D. of test}}{\text{O. D. of standard}} \times 30$$

$$\text{BUN (mg dl}^{-1}\text{)} = \text{Urea (mg dl}^{-1}\text{)} / 2.1428$$

Estimation of Creatinine level

Creatinine level of the prepared extract was estimated by using diagnostic kit (M/S Span Diagnostics Ltd. Plot No. 336, 338, 340, Road No. 3, G.I.D.C., SACHIN 394 230 (Surat) INDIA) as per Alkaline Picrate method described by Bonses *et al.* (1945).

Principle:

Creatinine in a protein free solution reacts with Alkaline Picrate and produces a red coloured complex.

Reagents:

Reagent 1- Picric Acid.

Reagent 2- Sodium Hydroxide, 0.75 N.

Reagent 3- Stock Creatinine Standard, 150 mg %.

Preparation of Working Standard Solution- 0.1 ml of Reagent 3 (Stock Creatinine Standard) was diluted to 10 ml wit Purified water and mixed well. All other reagents were ready to use.

Procedure:

STEP 1:

Deproteinization of test sample:

(a) Sample (0.5 ml) was added with 0.5 ml of Purified Water and 3.0 ml of Picric acid (Reagent 1).

(b) It was mixed well and kept in a boiling water bath exactly for 1 minute. It was then cooled immediately under running tap water and centrifuged or filtered.

STEP 2:

Colour Development:

(a) Filtrate (2.0 ml) from Step 1 was added to the tube marked as Test.

(b) Working Standard (0.5 ml) was added to the Standard tube.

(c) Purified Water (0.5 ml) was added to Blank tube.

(d) Reagent 1 (1.5 ml -Picric Acid) was pipetted out to Blank and Standard tubes.

(e) Reagent 2 (0.5 ml -Sodium Hydroxide, 0.75 N) was added to all the tubes.

(f) It was mixed well and allowed to stand at room temperature exactly for 20 minutes and absorbance was measured against Purified Water at 520 nm.

Calculations:

$$\text{Serum Creatinine (mg/100 ml)} = \frac{\text{O. D. of Test} - \text{O. D. of Blank}}{\text{O. D. of Standard} - \text{O. D. of Blank}} \times 3.0$$

Estimation of haematological parameters:

Haematological parameters had been estimated as per standardized protocol.

Fixation of dose rates for the experiment

Fixation of dose of extracts

Prepared *Tamarindus indica* L. leaf extracts (ethanolic and aqueous) were administered orally once daily at 2 gm/kg body weight mixing with 2% tween 20 (for ethanolic extract) and distilled water (for aqueous extract) for consecutive 28 days to 6 healthy rabbits and the animals were kept under close observation for 30 days. The animals were closely observed for any untoward/unpleasant symptoms. Haemobiogram (Hb, WBC, RBC, ESR, PCV, Platelet), Biochemical parameters like ALT, AST, ALP, BUN and Creatinine level were also monitored

during 28 days treatment and 2 days observation period. Histomorphological study of liver and kidney were performed on day 30 after humane slaughtering of the animals of each groups.

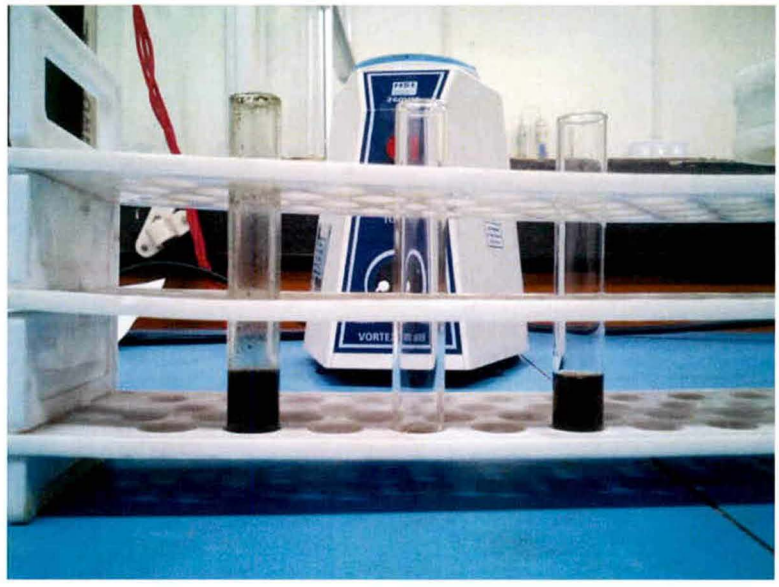

Figure viii: after preparation of dose of both extract. ethanolic (left), aqueous (right)

Fixation of dose rates for linezolid and betamethasone

Dosage regimen for Linezolid was fixed @ 75 mg kg-1 body weight p.o. twice daily for 10 days (Schroeder *et al.*,2011) and the Betamethasone dose rate for single intra-articular administration was fixed @ 0.5 mg kg^{-1} (Jaberi *et al.*,2003)

Treatment of septic arthritis:

Following induction of septic arthritis in rabbits treatment was started after 48 hours with oral Linezolid @ 75 mg kg^{-1} for 10 days twice daily (Gr – II), oral Linezolid @75 mg kg^{-1} for 10 days twice daily with a single intra-articular injection of Betamethasone @0.5 mg kg^{-1} (Gr – III), Ethanolic Extract of *Tamarindus indica* L. leaves @ 500 mg kg^{-1} for 14 days(Gr – IV), Ethanolic Extract of *Tamarindus indica* L. leaves @ 1000 mg kg^{-1} for 14 days(Gr – V), Aqueous Extract of *Tamarindus indica* L. leaves @ 500 mg kg^{-1} for 14 days (Gr – VI) and Aqueous Extract of *Tamarindus indica* L. leaves @ 1000 mg kg^{-1} for 14 days (Gr – VII) while Gr- I rabbits were kept untreated. Efficacy of different treatments was compared by monitoring haematological, biochemical, immunological (C-Reactive protein, Procalcitonin), X-ray and histomorphological study.

Experiment –IV & V

Inoculation of experimental rabbits with *Staphylococcus aureus* isolates

The experimental rabbits were inoculated with 18 hour old *Staphylococcus aureus* culture possessing 10^4cfu ml^{-1} concentration in the stifle joints of rabbits, intra-articularly.

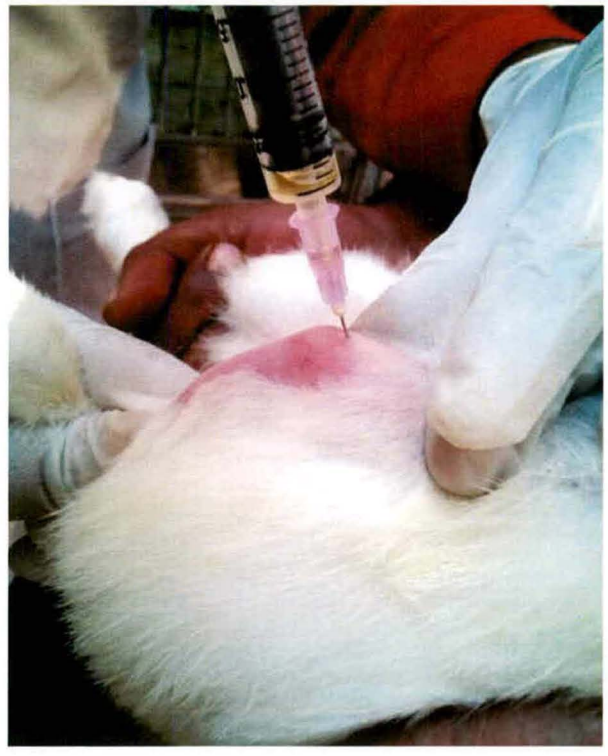

Figure ix: Inoculation of 10^4 cfu ml^{-1} *Staphylococcus aureus* in the left stifle joint of a rabbit

Medication to the animal:

With the help of syringe (without needle) oral medication was performed (depicted in figure x)

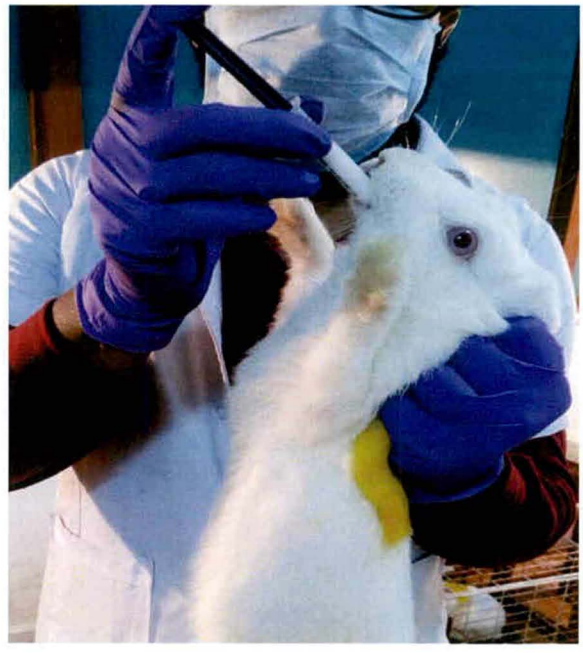

Figure x: Oral medication to the rabbit

Collection of blood

- The animal was placed in a restrainer.
- Ear was cleaned with 95% v/v alcohol and local anaesthetic cream was applied on the collection site 10 min prior to sampling. Ortho-Xylene was applied topically on the collection site to dilate blood vessels.
- Tuberculin syringes were used to collect blood from animal marginal vein.
- After collecting blood, clean sterile cotton was kept on the collection site and finger pressure was applied to stop the bleeding.

Figure xi: Collection of blood from ear vein of rabbit

Arthrocentesis

Knee arthrocentesis of the rabbit was performed via a parapatellar approach. The skin was prepared with sterile solution before collection.

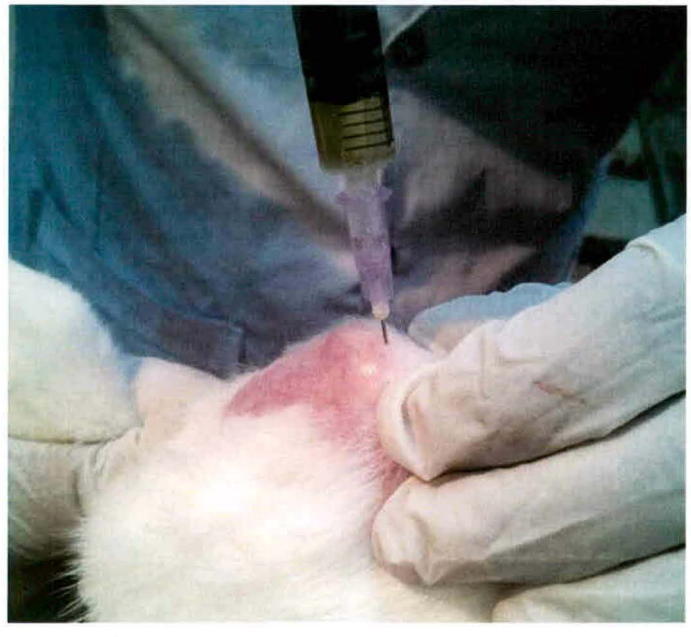

Figure xii: collection of synovial fluid from left stifle joint of rabbit

Methods of various parameters

Bacterial colony count

Bacterial colony count was conducted as per the standard protocol by Quinn *et al.* (1994).

Reagents

1. Synovial fluid samples
2. Micro centrifuge tube (1ml) (Tarson, India)
3. Sterile L-spreader (Hi Media, India)
4. Autoclaved distilled water
5. Micropipette (Tarson, India)
6. Petridish plates (Borosil)
7. Mannitol salt agar media (Hi media, India)
8. Autoclaved tips for micropipette (100µL, 1000µL) (Tarson, India)

Composition of Mannitol Salt Agar

1. Nacl – 7.5%
2. Peptic digest of animal tissue –5 gm/L
3. Pancreatic digest of casein –5 gm/L
4. Beef extract – 1 gm/L
5. D- manitol 10 gm/L
6. Phenol - 0.025 gm/L
7. Agar – 15 gm/L
8. pH after sterilization – 7.4 ± 0.2

Preparation of agar

MSA (Mannitol Salt Agar) (Himedia, India) (111.02) was suspended in 1000ml of distilled water and heated to boiling to dissolve the media completely. The media was sterilized by autoclaving at 15 lbs pressure 121°C for 15 min.

Procedure

With 2 µL of synovial fluid, 10 µL distilled water was added. From this mixture 10 µL was spread in Mannitol salt agar plate (for *S. aureus*) and incubated at 37°C for overnight. After 18 – 24 h of incubation colonies were counted.

Calculations

Bacterial count in the sample = X × c.f.uml^{-1}

Where X = Numbers of colonies seen in the plate

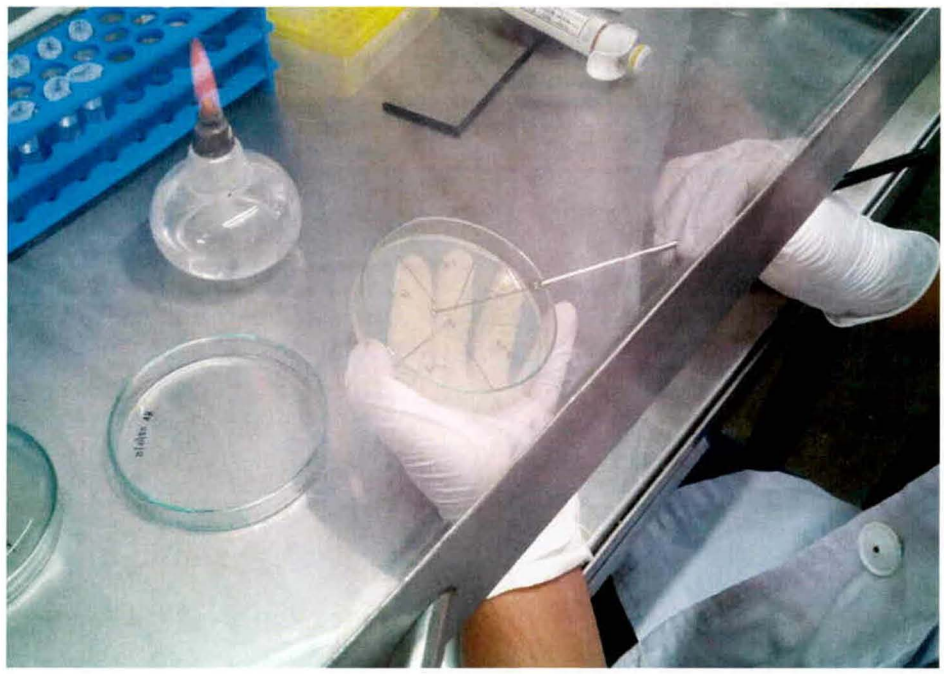

Figure xiii: bacterial colony counting

Haematological parameters

Haematological parameters like T.C., D.C., Hb, E.S.R., MCV, MCHC,MCH, Platelet count were done as per standard methods.

W.B.C. count from Synovial fluid

W.B.C. count from Synovial fluid was done as per standard method.

Measurement of joint radius

With a non-elastic measuring thread, the joints were measured unbiasedly. From the circumference the radius was measured.

Measurement of body weight

With a standard balance, the body weights of animals were taken.

Measurement of body temperature

With the help of a standard thermometer, rectal temperature was recorded.

Joint space (lateral and medial) measurement

Lateral and medial joint space width between distal end of femur and proximal end of tibia-fibula were measured by digital radiograph with the help of a software on different days in each animal of all the groups before and after induction of septic arthritis as well as during and after the treatment. The angle between tibia-fibula and femur were maintained at a more or less similar degree during radiography.

Surgical removal of Menisci

Surgical removal of menisci had been performed as per standardized surgical protocol (Figure xiv).

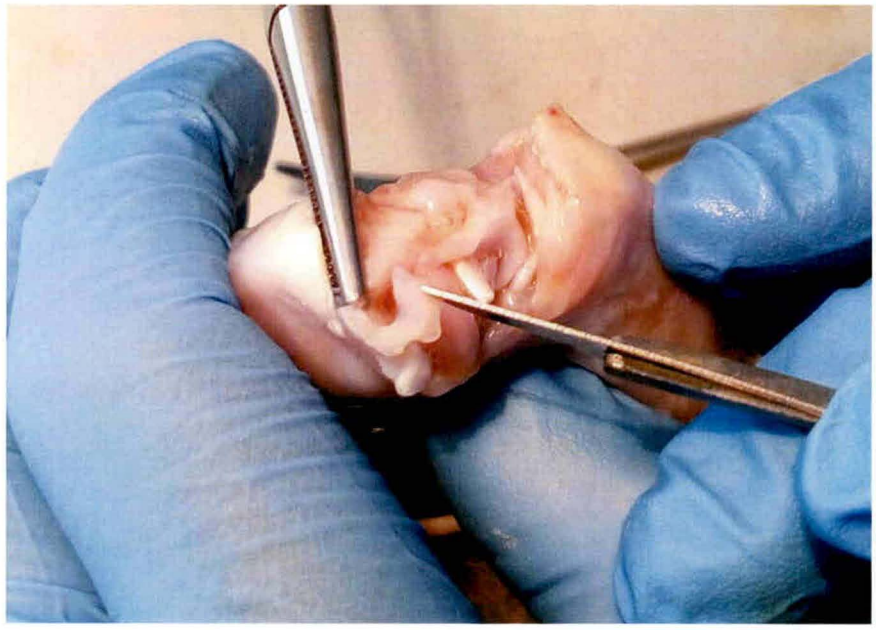

Figure xiv : surgical removal of meniscus from infected stifle joint

Biochemical parameters

Estimation of Glucose

Glucose of the prepared extract was estimated by using diagnostic kit (M/S Span Diagnostics Ltd. Plot No. 336, 338, 340, Road No. 3, G.I.D.C., SACHIN 394 230 (Surat) INDIA) as per End Point Assay method described by Kaplan *et al.* (1984).

Principle:

Glucose Oxidase (GOD) oxidizes Glucose to Gluconic Acid and Hydrogen Peroxide. In presence of enzyme Peroxidase, released Hydrogen Peroxide is coupled with Phenol and 4-Aminoantipyrine (4-AAP) to form colored Quinoeimine dye. Absorbance of colored dye is measured at 505 nm and is directly proportional to Glucose concentration in the Sample.

$$\text{Glucose} + O_2 + H_2O \xrightarrow{\text{Glucose Oxidase}} \text{Gluconic Acid} + H_2O_2$$

$$H_2O_2 + \text{Phenol} + \text{4- AAP} \xrightarrow{\text{Peroxidase}} \text{Quinoeimine dye} + H_2O$$

Reagents:

Reagent 1: Glucose Reagent.

Reagent 2: Glucose Diluent.

Reagent 3: Glucose Standard (100 mg/dl).

Reagent 4: Glucose Standard (100 mg/dl).

Preparation of Working Reagent:

One vial of Reagent 1 was mixed with 50 ml of Reagent 2. The powder was dissolved completely. The working Reagent was light sensitive, thus it was stored at 2- 8°C in dark colored bottle to protect it from light.

Preparation of synovial fluid:

Synovial fluid ⟶ centrifugation at 3,500 r.pm. for 15 min ⟶ Collection of supernatant ⟶ keep at 20° c upto analysis (Doha Yahia *et al.* 2013)

Procedure:

a) Three test tubes were taken and marked as Blank, Standard and Test respectively.
b) Sample (20µl) was pipetted out into the tube marked as Test and 20 µl of Reagent 3 into Standard tube.
c) Working Glucose Reagent (500 µl) was added into all the three tubes.
d) It was mixed well and incubate at 37°C for 10 minutes at room temperature (15- 30°C)
e) Purified water (1500 µl) was added into all the tubes and their absorbance were measured at 490-550 nm and results were calculated accordingly.

Calculation:

$$\text{Glucose (mg/dl)} = \frac{\text{O.D. of test}}{\text{O.D. of standard}} \times 100$$

Principle

Lactate dehydrogenase catalyzes the reduction of pyruvate with NADH to form NAD. The rate of oxidation of NADH to NAD is measured as a decrease in absorbance, which is proportional to the LDH activity in the sample.

Pyruvate + NADH + H^+ $\xrightarrow{LDH}$ Lactate + NA

Estimation of LDH (pyruvate to lactate)

Reagents:

Reagent 1 : Buffer Reagent (Tris Buffer 100 mM; pH 6.8; Pyruvate 1.2 mM; NaCl 200 mM; NADH 0.18 mM)

Reagent 2 : Starter Reagent

Preparation of synovial fluid:

Synovial fluid ⟶ centrifugation at 3,500 rpm for 15 min ⟶ Collection of supernatant ⟶ keep at 20° c up to analysis (Doha Yahia *et al.* 2013).

Preparation of working reagent:

The content of 1 bottle of L2 (Starter Reagent) was mixed with 1 bottle of L1 (Buffer Reagent).

Procedure:

Working reagent 1 ml + sample 20 µl (at 37° C) Mixed it well and took reading of the initial absorbance after 1 minute and repeat the absorbance reading after every 1, 2, & 3 minutes. Calculate the mean absorbance change per minute (δA/ min).

Calculation:

LDH activity in U/L (at 37° C) = δA/ min. x 8095

Estimation of Total Protein

Total Protein of the prepared extract was estimated by using diagnostic kit (M/S Span Diagnostics Ltd. Plot No. 336, 338, 340, Road No. 3, G.I.D.C., SACHIN 394 230 (Surat) INDIA) as per Biuret, End Point Assay method described by Kaplan *et al.* (1983).

Principle:

The peptide bonds of Proteins react with Cupric ions in alkaline solution to form a colored chelate, the absorbance of which is measured at 578 nm. The Biuret Reagent contains Sodium-Potassium Tartarate, which helps in maintaining solubility of this complex at alkaline pH. The absorbance of final colour is proportional to the concentration of Total Protein in the Sample.

$$\text{Protein} + Cu^{++} \xrightarrow{\text{Alkaline pH}} \text{Cu- Protein Complex}$$

Reagents:

Reagent 1- Biuret Reagent.

Reagent 2- Protein Standard.

Preparation of synovial fluid:

Synovial fluid ⟶ centrifugation at 3,500 r.pm. for 15 min ⟶ Collection of supernatant ⟶ keep at 20° c up to analysis (Doha Yahia *et al.* 2013).

Procedure:

a) Three tubes were taken and marked as Control, Standard and Test.
b) Sample (10 µl) was pipetted into the tube marked as Test.
c) Reagent 2 (10 µl) was pipetted into the Standard tube.
d) Reagent 1 (1000 µl) was added into all the three tubes.
e) It was mixed well and incubated at 37°C for 10 minutes and absorbance was taken at 578 nm.

Calculation:

$$\text{Total Protein concentration (g/dl)} = \frac{\text{Absorbance of test}}{\text{Absorbance of standard}} \text{ X } 6.5$$

CRP estimation

C-reactive protein levels from rabbit serum were estimated by the Rabbit CRP (C-reactive protein) ELISA kit prepared by Elabscience Biotechnology Co., Ltd (Building 4, Room 401, Guandong Science and Technology Industry Park, WuHan, P.R.C.).

Test principle

The micro ELISA plate provided in this kit had been pre-coated with an antibody specific to CRP. Standards or samples were added to the appropriate micro ELISA plate wells and combined with the specific antibody. Then a biotinylated detection antibody specific for CRP and Avidin-Horseradish Peroxidase (HRP) conjugate was added to each micro plate well successively and incubated. Free components were washed away. The substrate solution was added to each well. Only those wells that contain CRP, biotinylated detection antibody and Avidin-HRP conjugate were appeared blue in color. The enzyme-substrate reaction was terminated by the addition of a sulphuric acid solution and the color turns yellow. The optical density (OD) were measured spectrophotometrically at a wavelength of 450 nm ± 2 nm. The OD value is proportional to the concentration of CRP. Concentration of CRP in the samples were determined by comparing the OD of the samples to the standard curve.

Detection Range

0.781-50 ng ml^{-1}

Table 3: Reagents for rabbit specific CRP (Kit components)

Item	Specifications	Storage
Reference Standard	2 vials	4°C/-20°C#
Reference Standard & Sample Diluent	1vial 20mL	4°C
Concentrated Biotinylated Detection Ab	1vial 120µL	4°C/-20°C#
Biotinylated Detection Ab Diluent	1vial 10mL	4°C
Concentrated HRP Conjugate	1vial 120µL	4°C(shading light)
HRP Conjugate Diluent	1vial 10mL	4°C
Concentrated Wash Buffer (25×)	1vial 30mL	4°C
Substrate Reagent	1vial 10mL	4°C(shading light)
Stop Solution	1vial 10mL	4°C

for longer storage.

Sample collection and storage

Serum - samples were kept to clot for 2 hours at room temperature or overnight at 4°C before centrifugation for 15 minutes at 1000 × g. Supernatants were collected and stored at -22 °C. Blood collection tubes were disposable, non-pyrogenic, and non-endotoxin.

Reagent preparation

All the reagents were brought to room temperature (18-25°C) before use.

Wash Buffer - 30 mL of Concentrated Wash Buffer was diluted into 750 mL of Wash Buffer with deionized or distilled water. Unused solution was put back at 4°C. The solution was taken to room temperature before use.

Standard - Standards were prepared within 15 minutes before use, centrifuged at 10,000×g for 1 minute, and reconstituted the Standard with 1.0mL of Reference Standard & Sample Diluent. The lid was tightened and kept standing for 10 minutes and turned it upside down for several times. After it dissolved fully, solution was mixed thoroughly with a pipette. This reconstitution produced a stock solution of 50 ng ml^{-1}. Then serial dilutions were made as needed. The recommended concentrations were as follows: 50、25、12.5、6.25、3.125、1.563、0.781、0 ng ml^{-1}. The undiluted standard served as the highest standard (50ng ml^{-1}). The Reference Standard & Sample Diluent served as the zero (0 ng ml^{-1}).

Biotinylated Detection Ab - The required amount was calculated before experiment (100μL/well). The stock tubes were centrifuged before use, the concentrated Biotinylated Detection Ab was diluted to the working concentration using Biotinylated Detection Ab Diluent (1:100).

Concentrated HRP Conjugate - The required amount was calculated before experiment (100μL/well). The Concentrated HRP Conjugate was diluted to the working concentration using Concentrated HRP Conjugate Diluent (1:100).

Substrate Reagent - The needed dosage of the reagent was aspirated with sterilized tips and the unused residual reagent wasn't dumped back into the vial again.

Washing Procedure

Washing procedure was achieved by using a automated washer.

Assay Procedure

1. 100µL standard or sample was added to each well and incubated for 90 min at 37°C

2. The liquid was removed. 100µL Biotinylated Detection Ab was added and incubated for 1 hour at 37°C

3. Aspirated and washed 3 times

4. 100µL HRP Conjugate was added and incubated for 30 minutes at 37°C

5. Aspirated and washed 5 times

6. 90µL Substrate Reagent was added and incubated for 15 minutes at 37°C

7. 50µL Stop Solution was added and read at 450nm immediately

8. Calculations were done .

PCT Estimation

Test principle

The micro ELISA plate provided in this kit had been pre-coated with an antibody specific to PCT. Standards or samples were added to the appropriate micro ELISA plate wells and combined with the specific antibody. Then a biotinylated detection antibody specific for PCT and Avidin-Horseradish Peroxidase (HRP) conjugate was added to each micro plate well successively and incubated. Free components were washed away. The substrate solution was added to each well. Only those wells that contain PCT, biotinylated detection antibody and Avidin-HRP conjugate were appeared blue in color. The enzyme-substrate reaction was terminated by the addition of a sulphuric acid solution and the color turnd to yellow. The optical density (OD) was measured spectrophotometrically at a wavelength of 450 nm ± 2 nm. The OD value was proportional to the concentration of PCT. The concentration of PCT in the samples were calculated by comparing the OD of the samples to the standard curve.

Table 4: Reagents for rabbit specific PCT (Kit components)

Item	Specifications	Storage
Reference Standard	2 vials	4°C/-20°C#
Reference Standard & Sample Diluent	1vial 20mL	4°C
Concentrated Biotinylated Detection Ab	1vial 120μL	4°C/-20°C#
Biotinylated Detection Ab Diluent	1vial 10mL	4°C
Concentrated HRP Conjugate	1vial 120μL	4°C(shading light)
HRP Conjugate Diluent	1vial 10mL	4°C
Concentrated Wash Buffer (25×)	1vial 30mL	4°C
Substrate Reagent	1vial 10mL	4°C(shading light)
Stop Solution	1vial 10mL	4°C

for longer storage.

Sample collection and storage

Serum - samples were kept to clot for 2 hours at room temperature or overnight at 4°C before centrifugation for 15 minutes at 1000 × g. Supernatants were collected and stored at -22 °C. Blood collection tubes were disposable, non-pyrogenic, and non-endotoxin.

Reagent preparation

All the reagents were brought to room temperature (18-25°C) before use.

Wash Buffer - 30 mL of Concentrated Wash Buffer was diluted into 750 mL of Wash Buffer with deionized or distilled water. Unused solution was put back at 4°C. The solution was taken to room temperature before use.

Standard - Standards were prepared within 15 minutes before use, centrifuged at 10,000×g for 1 minute, and reconstituted the Standard with 1.0mL of Reference Standard & Sample Diluent. The lid was tightened and kept standing for 10 minutes and turned it upside down for several times. After it dissolved fully, the solution was mixed it thoroughly with a pipette. A stock solution of 2000 pg ml^{-1} was produced. Then serial dilutions were made as needed. The

recommended concentrations were as follows: 2000、1000、500、250、125、62.5、31.25、0 pg ml^{-1}. The undiluted standard served as the highest standard (2000 pg ml^{-1}). The Reference Standard & Sample Diluent served as the zero (0 $pgml^{-1}$).

Biotinylated Detection Ab - The required amount was calculated before experiment (100μL/well). The stock tube was centrifuged before use and diluted the concentrated Biotinylated Detection Ab to the working concentration using Biotinylated Detection Ab Diluent (1:100).

Concentrated HRP Conjugate - The required amount was calculated before experiment (100μL/well). The Concentrated HRP Conjugate was diluted to the working concentration using Concentrated HRP Conjugate Diluent (1:100).

Substrate Reagent - The needed dosage of the reagent was aspirated with sterilized tips and the unused residual reagent wasn't dumped back into the vial again.

Washing Procedure

Washing procedure was achieved by using a automated washer.

Assay procedure:

1. 100μL standard or sample was added to each well and incubated for 90min at 37°C
2. The liquid was removed. 100 μL Biotinylated Detection Ab was added and incubated for 1 hour at 37°C
3. Aspirated and washed 3 times
4. Add 100μL was added HRP Conjugate and incubated 30 minutes at 37°C
5. Aspirated and washed 5 times
6. 90 μL Substrate Reagent was added and incubated 15 minutes at 37°C
7. 50 μL Stop Solution was added.
8. Absorbance was read at 450 nm immediately.
9. Calculation was done.

Histomorphological Analysis

After treatment, the animals were weighed, anesthetized with ketamine (50 mg/Kg) and xylazine (10 mg/kg) and guillotined. The knees were dissected and fixed in 10% formalin for 24 hours, and then they were decalcified in trichloroacetic acid (TCA) to 5% for approximately 5 days. The samples were dehydrated in alcohols 70%, 80%, and 90% for 1 hour each and stayed in 95% alcohol overnight. Then, the samples were passed through four baths of 100% alcohol for 1 hour each and processed for paraffin embedding. Cuts of 7 μm were obtained in Olympus CUT 4055 microtome, and the slides were stained with hematoxylin and eosin for tissue morphological analysis and safranin-o for measuring the optical density of proteoglycans.

Table 5: Histologic-histochemical scoring (Salter *et* al. 1981)

Parameters		Scoring
1. Cellularity of cartilage	Normal	0
	<10%	1
	10% - 25%	2
	>25%	3
2. Loss of matrix (erosions)	Normal	0
	<10%	1
	10% - 25%	2
	>25%	3
3. Cloning of chondrocytes	Normal	0
	<10%	1
	10% - 25%	2
	>25%	3
4. Adhesions (pannus)	No Adhesions	0
	Covering only margin of cartilage	1
	Covering < 50%	2
	Covering >50%	3
5. Grey reads(Red value) * with Safranin-O	Values more than 160	0
	Values within 140-160	1
	Values within 120-140	2
	Values within 100-120	3
	Values within 80-100	4

*Grey reads had been taken instead of orthochromasia in Salter's study.

Image Analysis

The various parameters were scored with the assistance of an image analysis software LEICA QWIN under LEICA DM 2000 microscope. The slides were interpreted in one session and in same lighting condition. The session did not last for more than four hours, as heating of the camera and light may cause changes in densitometric study.

The morphological parameters were measured. Cellularity of cartilages were determined as a function of nuclei density. Nuclei density was defined as number of nuclei per square area of cartilage matrix. A density that was three standard deviations less than the normal cartilage was defined as acellular cartilage. The percentage of eroded joint surface was calculated as length of eroded surface divided by total joint surface length in a photo-micrograph. Clustering of chondrocytes was determined as number of clones divided by total number of chondrocytes. Pannus formation was measured as length of joint surface covered by pannus divided by total length of joint surface in a photo-micrograph.

Instead of orthochromasia in Salter's study, proteoglycans (appeared red) optical density were measured over green background from safranin-o stained field. Two controls were measured - that is, normal cartilage (containing a large amount of proteoglycans and showing a higher value of optical density) and an untreated infection control cartilage (containing very less amount of proteoglycans and showing a lower value of optical density). The values were measured in a scale where maximum red value was 255 and minimum red value was 0

Experiment -VI

Estimation of Linezolid in plasma and synovial fluid

To 0.5 ml of plasma, 5ml acetonitrile (HPLC grade) was added for deprotienisation, vortexed and shaken vigorously followed by centrifugation at 5000 rpm for 10 min. The supernatant was collected and filtered through 0.2 µm membrane filter and 20 µl of sample was injected into HPLC. The concentration of Linezolid in plasma and synovial fluid was calculated using following equations:

$$\text{Concentration of drug in plasma (µgml1)} = \frac{a2 \times v2 \times C}{a1 \times v1}$$

a_1= Area of standard chromatogram

a_2= Area of sample chromatogram

v_2= Final volume of sample after processing (ml)

v_1= Volume of plasma taken for processing (ml)

C= Concentration of standard

Instrumental condition

SHIMANDZU LC-20 AT liquid chromatograph coupled with Photo Diode-Array (PDA) detector attached with computer SPD-MXA 10 software was used for analysis of the drug.

Mobile phase

A. Phosphate buffer, 1% Diethyl amine; $_pH = 2.5$

B. Acetonitrile : HPLC grade water = 90:10

Column - Thermo Hypersil ODS C_{18}; 250 × 4.6 mm, 5µ

Flow rate - 1 ml min^{-1}

Wave length - 256 nm

Injection - Standard and sample (20 µl) were injected by Hamilton Syringe into the injector port of HPLC.

Extraction of linezolid from plasma

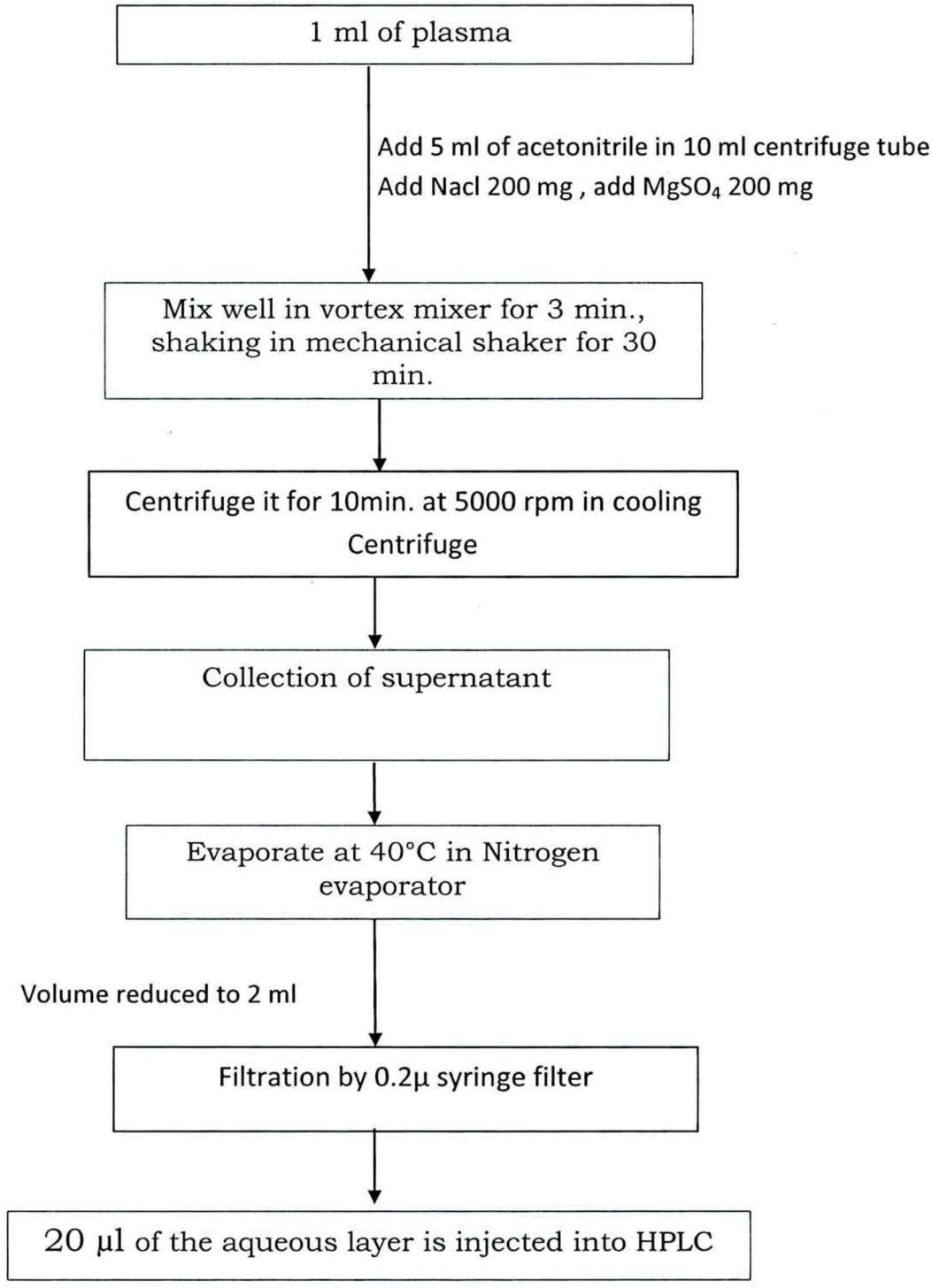

Figure xv: schematic presentation of extraction of linezolid from plasma

Extraction of linezolid from synovial fluid

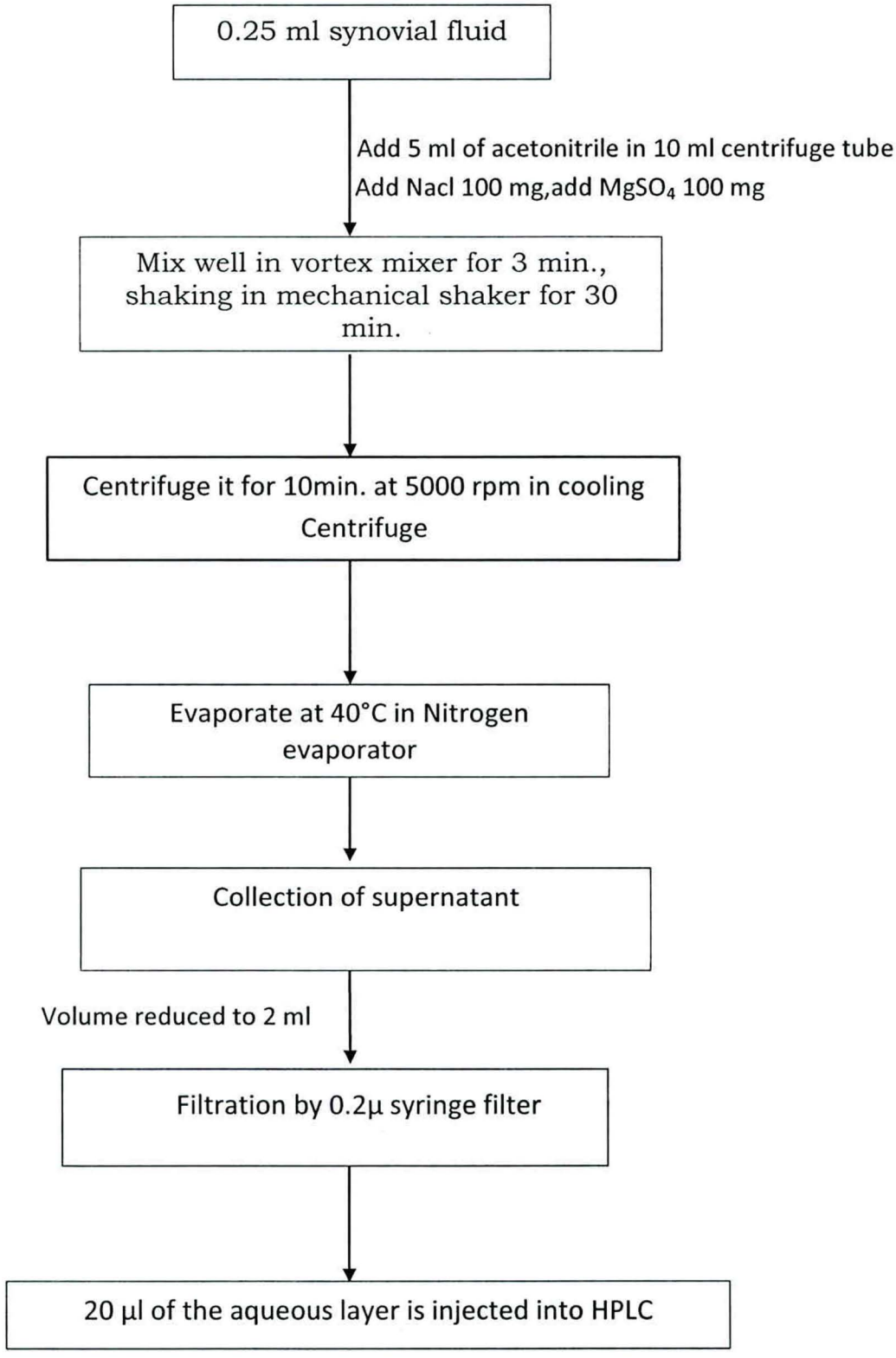

Figure xvi: schematic presentation of extraction of linezolid from synovial fluid

Pharmacokinetic Parameters

Pharmacokinetic parameters of Linezolid were determined from computerized curve fitting programme "PHARMKIT" supplied by the Department of Pharmacology, JIPMER, Puducherry, India. The data obtained from this programme in healthy rabbits were analysed for deriving some of the pharmacokinetics parameters as per standard formulae (Baggot, 1977).

A, B: Zero time plasma drug concentration intercepts of biphasic intravenous disposition curve. The co-efficient A is the point of intercept of residuals and co-efficient B is based on the terminal elimination phase. These are expressed in $\mu g\ ml^{-1}$.

C^0_P: The theoretical zero time plasma drug concentration.

$C^0_P = A + B\ \mu g\ ml^{-1}$

α and β: The hybrid rate constants of disposition curve. Values of α and β are related to the slope of distribution and elimination curve. These rate constants are obtained from the terminal slope of semi-logarithmic plot of plasma drug concentrations versus time are expressed as h^{-1}.

K_a : Absorption rate constant expressed as h^{-1}.

$t_{½}K_a$: Absorption half-life expressed as h.

$t_{½}\ \alpha$ & $t_{½}\ \beta$: The half-lives of drug in distribution and elimination phase respectively. They are expressed in h (Baggot, 1977).

$$t_{½}\ \alpha = \frac{0.693}{\alpha}$$

$$t_{½}\ \beta = \frac{0.693}{\beta}$$

K_{12} : The first order rate constant for transfer of drug from central to peripheral compartment and expressed as h^{-1}.

$K_{12} = \alpha + \beta - k21 - kel$

K_{21} : The first order rate constant for transfer of drug from peripheral to central compartment and expressed as h^{-1}.

$K_{21} = \frac{A\beta + B\alpha}{A+B \text{ or } C^{*}P}$

K_{el} : First order rate constant for drug elimination from the central compartment.

$$K_{el} = \frac{\alpha\beta}{k21}$$

f_C : The fraction of the amount of drug in the body that is contained in the central compartment.

$$fc = \frac{\beta}{Kel}$$

Vdc : The apparent volume of distribution of drug in the central compartment and expressed as L kg^{-1}.

$$Vdc = \frac{D}{A+B}$$

Where, D is the dose (mg kg^{-1})

Vd_{area} : The apparent volume of drug distribution based on total area under plasma drug concentration versus time curve (area method) and expressed as L kg^{-1}.

$$Vdarea = \frac{D}{AUC \times \beta}$$

Vd_{ss} : The apparent volume of drug distribution at steady state and expressed as L kg^{-1}.

$$Vdss = \frac{K12+K21}{K21} \times Vdc$$

AUC : The total area under the plasma drug concentration versus time curve from 't_0' to 't_∞' after administration. The unit of measurement is µg h ml^{-1}.

$$AUC = \frac{A}{\alpha} + \frac{B}{\beta}$$

Cl_B : Total body clearance of a drug representing the sum of all clearance process in the body and expressed as L kg^{-1} h^{-1}.

$$Cl_B = Vd_{area} \times \beta$$

MRT : Mean residence time expressed as h.

T~P : Tissue- plasma ratio which is obtained from the equation;

$$T \sim P = \frac{K12}{K21 - \beta}$$

Pharmacokinetic parameters were determined for each rabbits individually and then mean and SE of each parameters was calculated from the sum up values of six rabbits.

RESULTS AND DISCUSSION

RESULTS AND DISCUSSION

Isolation and identification of *Staphylococcus aureus* from goats suffering with arthritis

Staphylococcus aureus was isolated and identified by standard biochemical tests from goats suffering with arthritis. All the isolates possessed *nuc* gene in PCR.

Detection of phenotypical antibiotic resistance of *Staphylococcus aureus* isolates

S. aureus isolates were found resistant against ampicillin, gentamycin, amoxicillin + clavulinic acid, ampicillin + sulbactum, ceftizoxime, ticarcillin + clavulanic acid, methicillin, Cefotaxime. However, the isolates did not possess *mecA* gene in PCR.

Antimicrobial Susceptibility Test (AST)

AST report for the isolated *S. aureus* has been depicted in the table 6.

Table 6: showing the result of AST. CTR - Ceftriaxone, CTX - Cefotaxime, AMP- Ampicillin, HLG - Gentamicin, LZD - linezolid, AMC - Amoxicillin + Clavulanic Acid, A/S - Ampicillin + Sulbactam, CZX - Ceftizoxime, PIT - Piperacillin + Tazobactam, TCC - Ticarcillin + Clavulanic Acid, MET - Methicillin, CIP - Ciprofloxacin, VA - Vancomycin, EX - Enrofloxacin I – Intermediate Sensitive, R – Resistant, S –Sensitive.

CTR	CTX	AMP	HLG	LZD	AMC	A/S	CZX	PIT	TCC	MET	CIP	VA	EX
I	R	R	R	S	R	R	R	S	R	R (No zone)	S	I	S

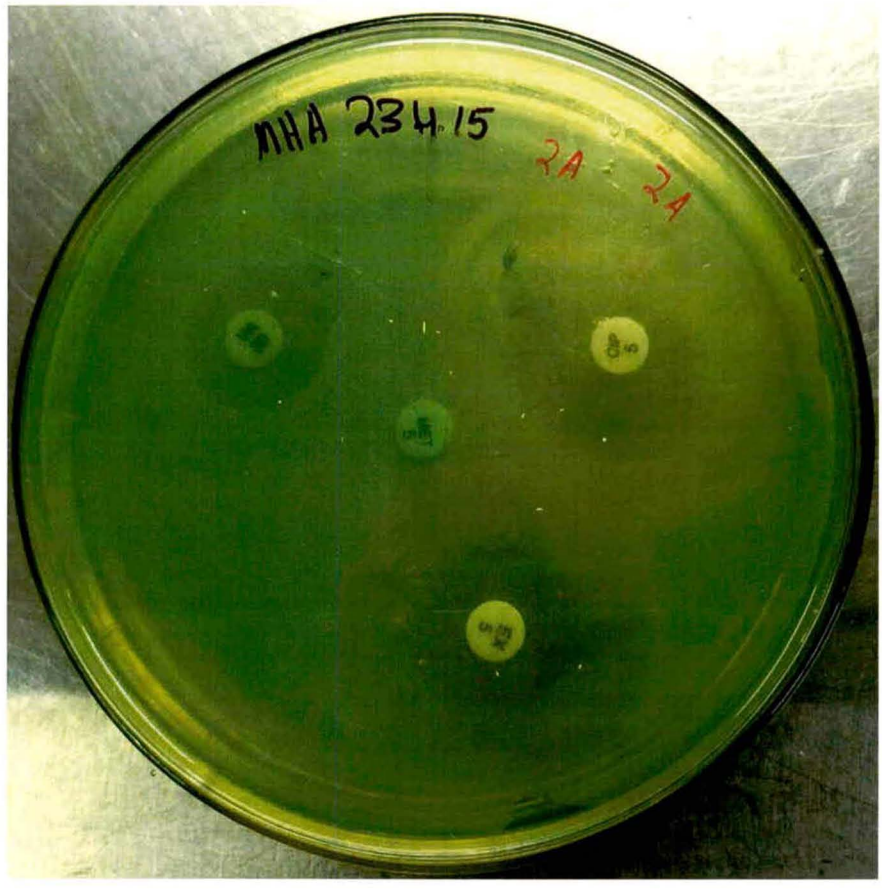

Fig xvii: Showing AST plate for four antimicrobial discs. In the centre of the plate MET (Methicillin) is showing no zone of inhibition.

Safety evaluation of ethanolic and aqueous extract of *Tamarindus indica* L. leaves at higher dose rate

Total 12 rats were divided into two groups each containing 6 rats. 6 rats of one group were orally administered single dose of 5 gm of ethanolic extract of *Tamarindus indica* L. leaves while another group of 6 rats were orally given a single dose of 5 gm of aqueous extract of *Tamarindus indica* L. leaves. Rats of both the groups did not show any adverse clinical sign and they also consumed normal quantity of feed and water during the observation period of 48 hours. So, both the ethanolic and aqueous extract of *Tamarindus indica* L. leaves were found to be practically non-toxic.

Safety evaluation of *Tamarindus indica* L. leaf extracts in rabbits

Biochemical parameters

Mean values with S.E. of Serum ALT (Alanine Aminotransferase) level (IU/L) on different days following oral dosing of ethanolic extract in tween 20 (2%) and aqueous extract of Tamarindus indica L. leaves @2gm kg^{-1} for consecutive 28 days and oral dosing of Tween 20(2%) for 28 days in rabbits have been depicted in table 7. Mean ±S.E. values for ALT level in healthy rabbits ranged from 33.44 ± 2.88 to 46.98 ± 8.43 IU/L. Mean ALT level did not differ significantly ($p<0.05$) on day 0, 7, 14, 21 and 30 following oral administration of ethanolic and aqueous extracts of *Tamarindus indica* L. leaves for @2gm kg^{-1} body weight for consecutive 28 days. Mean ALT level also did not differ significantly ($p<0.05$) on day 0, 7, 14, 21 and 30 following oral administration of 2% tween 20 (as vehicle) for consecutive 28 days.

Table 7: Mean values with S.E. of Serum ALT level (IUL^{-1}) on different days following oral dosing of ethanolic extract in tween 20(2%), aqueous extract of *Tamarindus indica* L. leaves @2 $gmkg^{-1}$ for and oral dosing of Tween 20(2%) for consecutive 28 days in rabbits [n=6]

DAY / GROUP	0	7	14	21	30
E.E. (2 gm Kg^{-1})	43.41^{NS} ± 4.22	44.60^{NS} ± 5.29	45.00^{NS} ± 4.90	43.34^{NS} ± 5.07	44.45^{NS} ± 3.95
A.E. (2 gm Kg^{-1})	46.98^{NS} ± 8.43	49.53^{NS} ± 8.83	50.03^{NS} ± 9.54	47.70^{NS} ± 7.05	49.07^{NS} ± 8.63
Vehicle Control (2% tween 20)	42.21^{NS} ± 1.82	43.20^{NS} ± 1.79	45.08^{NS} ± 1.74	45.80^{NS} ± 1.72	44.74^{NS} ± 1.63

Table 7: Values are Mean ±S.E., where n =6 in each group;
NS = non significant($p<0.05$)

Mean values with S.E. of Serum AST(Aspartate aminotransferase) level (IU/L) on different days following oral dosing of ethanolic extract in tween 20 (2%) and aqueous extract of *Tamarindus indica* L. leaves for @2gm/kg for consecutive 28 days and oral dosing of Tween 20(2%) for 28 days in rabbits have been depicted in table 8. Mean ±S.E. values for AST level in healthy rabbits ranged from 43.91 ± 9.78 to 48.98 ± 6.39 IU/L. Mean AST level did not differ significantly ($p<0.05$) on day 0, 7, 14, 21 and 30 following oral administration of ethanolic and aqueous extracts of *Tamarindus indica* L. leaves @2 $gmkg^{-1}$ body weight for consecutive 28 days. Mean AST level did not differ significantly ($p<0.05$) on day 0, 7, 14, 21 and 30 following oral administration of 2% tween 20 (as vehicle) for consecutive 28 days.

Table 8: Mean values with S.E. of serum AST level (IUL^{-1}) on different days following oral dosing of ethanolic extract in tween 20(2%), aqueous extract of *Tamarindus indica* L. leaves @2 gmkg-1 for and oral dosing of Tween 20(2%) for consecutive 28 days in rabbits [n=6]

DAY / GROUP	0	7	14	21	30
E.E. (2 gm Kg^{-1})	43.91^{NS} ± 9.78	46.05^{NS} ± 8.08	46.62^{NS} ± 7.77	46.45^{NS} ± 8.73	47.24^{NS} ± 11.03
A.E. (2 gm Kg^{-1})	48.98^{NS} ± 6.39	50.95^{NS} ± 5.94	55.66^{NS} ± 7.06	56.91^{NS} ± 5.12	51.94^{NS} ± 6.91
Vehicle Control (2% tween 20)	44.21^{NS} ± 4.16	46.15^{NS} ± 4.45	48.15^{NS} ± 3.91	47.14^{NS} ± 4.04	46.04^{NS} ± 3.80

Table 8: Values are Mean ± S.E., where n =6 in each group;
NS = Non significant($p<0.05$)

Mean values with S.E. of BUN (Blood Urea Nitrogen) level ($mgdl^{-1}$) on different days following oral dosing of ethanolic extract in tween 20 (2%) and aqueous extract of *Tamarindus indica* L. leaves @2 $gmkg^{-1}$ for consecutive 28 days and oral dosing of Tween 20(2%) for 28 days in rabbits have been depicted in table 9. Mean ±S.E. values for BUN level in healthy rabbits ranged from 16.55 ± 2.17 to 18.42 ± 2.46. Mean BUN level did not differ significantly ($p<0.05$) on day 0, 7, 14, 21 and 30 following oral administration of ethanolic and aqueous extracts of *Tamarindus indica* L. leaves @2 $gmkg^{-1}$ body weight for consecutive 28 days. Mean BUN level did not differ significantly ($p<0.05$) on day 0, 7, 14, 21 and 30 following oral administration of 2% tween 20 (as vehicle) for consecutive 28 days.

TABLE 9: Mean values with S.E. of BUN level ($mgdl^{-1}$) on different days following oral dosing of ethanolic extract in tween 20(2%), aqueous extract of *Tamarindus indica* L. leaves @2 gmkg-1 for and oral dosing of Tween 20(2%) for consecutive 28 days in rabbits [n=6]

DAY / GROUP	0	7	14	21	30
E.E. (2 gm Kg^{-1})	16.55^{NS} ± 2.17	16.59^{NS} ± 1.42	19.72^{NS} ± 2.10	20.64^{NS} ± 2.41	17.77^{NS} ± 1.95
A.E. (2 gm Kg^{-1})	18.42^{NS} ± 2.46	20.06^{NS} ± 2.69	21.58^{NS} ± 2.63	22.32^{NS} ± 1.89	20.19^{NS} ± 1.12
Vehicle Control (2% tween 20)	18.19^{NS} ± 1.38	18.76^{NS} ± 1.18	19.8^{NS} ± 1.38	21.31^{NS} ± 1.62	21.00^{NS} ± 1.21

Table 9: Values are Mean ±S.E., where n =6 in each group;
NS = Non significant($p<0.05$)

Mean values with S.E. of serum Creatinine level (mg/dl) on different days following oral dosing of ethanolic extract in tween 20(2%) and aqueous extract of *Tamarindus indica* L. leaves for @2 gm kg^{-1} for consecutive 28 days and oral dosing of Tween 20(2%) for 28 days in rabbits have been presented in table 10. Mean ±S.E. values for Creatinine level in healthy rabbits ranged from 1.48 ± 0.18 to 1.54 ± 0.17. Mean Creatinine level did not differ significantly ($p<0.05$) on day 0, 7, 14, 21 and 30 following oral administration of ethanolic and aqueous extracts of *Tamarindus indica* L. leaves for @2 gm kg^{-1} body weight for consecutive 28 days. Mean Creatinine level did not differ significantly ($p<0.05$) on day 0, 7, 14, 21 and 30 following oral administration of 2% tween 20 (as vehicle) for consecutive 28 days.

TABLE 10: Mean values with S.E. of Serum Creatinine level (mg dl^{-1}) on different days following oral dosing of ethanolic extract in tween 20(2%) and aqueous extract of *Tamarindus indica* L. leaves @2gm kg^{-1} for consecutive 28 days and oral dosing of Tween 20(2%) for 28 days in rabbits [n=6]

DAY / GROUP	0	7	14	21	30
E.E. (2 gm Kg^{-1})	1.48^{NS} ± 0.18	1.70^{NS} ± 0.17	1.77^{NS} ± 0.13	1.83^{NS} ± 0.19	1.80^{NS} ± 0.15
A.E. (2 gm Kg^{-1})	1.54^{NS} ± 0.17	1.63^{NS} ± 0.16	1.68^{NS} ± 0.16	1.85^{NS} ± 0.15	1.85^{NS} ± 0.15
Vehicle Control (2% tween 20)	1.52^{NS} ± 0.18	1.56^{NS} ± 0.15	1.70^{NS} ± 0.17	1.57^{NS} ± 0.16	1.50^{NS} ± 0.14

Table 10: Values are Mean ±S.E., where n =6 in each group;
NS = Non significant($p<0.05$)

Mean values with S.E. of serum ALP (Alkaline phosphatase) activity (IU/L) on different days following oral dosing of ethanolic extract in tween 20(2%) and aqueous extract of *Tamarindus indica* L. leaves for @2 gm kg^{-1} for consecutive 28 days and oral dosing of Tween 20(2%) for 28 days in rabbits have been depicted in table 11. Mean ±S.E. values for ALP in healthy rabbits ranged from 6.72 ± 0.79 to 7.01 ± 1.10. Mean ALP level did not differ significantly ($p<0.05$) on day 0, 7, 14, 21 and 30 following oral administration of ethanolic and aqueous extracts of *Tamarindus indica* L. leaves for @2gm/kg body weight for consecutive 28 days. Mean ALP level did not differ significantly ($p<0.05$) on day 0, 7, 14, 21 and 30 following oral administration of 2% tween 20 (as vehicle) for consecutive 30 days.

TABLE 11: Mean values with S.E. of serum ALP level (IU/L) on different days following oral dosing of ethanolic extract in tween 20(2%) and aqueous extract of *Tamarindus indica* L. leaves @2 gm kg^{-1} for consecutive 28 days and oral dosing of Tween 20(2%) for 28 days in rabbits [n=6]

DAY / GROUP	0	7	14	21	30
E.E. (3gm Kg^{-1} b.w.)	6.98^{NS} ± 0.75	7.08^{NS} ± 0.33	6.89^{NS} ± 0.72	5.90^{NS} ± 1.20	6.30^{NS} ± 1.28
A.E. (3gm Kg^{-1} b.w.)	7.01^{NS} ± 1.10	7.78^{NS} ± 1.27	8.63^{NS} ± 1.83	7.90^{NS} ± 1.50	7.54^{NS} ± 1.34
Vehicle control (2% tween 20)	6.72^{NS} ± 0.79	6.88^{NS} ± 0.76	7.25^{NS} ± 0.81	7.55^{NS} ± 0.86	7.25^{NS} ± 0.82

Table 11: Values are Mean ± S.E., where n =6 in each group;
NS = non significant($p<0.05$)

Haemobiogram

Haemobiograms following oral administration of ethanolic extract of *Tamarindus indica* L. leaves (@2 gm kg^{-1} b.w.) for consecutive 28 days in healthy rabbits have been depicted in the table 12. No blood parameters were found to be altered significantly.

TABLE 12: Haemobiograms following oral administration of ethanolic extract of *Tamarindus indica* L. leaves (@2gm kg^{-1} b.w.) for consecutive 28 days in healthy rabbits [n = 6]

Day / parameters	DAY 0	DAY 7	DAY 14	DAY 21	DAY 30
WBC (10^9 lit^{-1})	$7.22^{NS} \pm$ 0.13	$7.24^{NS} \pm$ 0.12	$7.29^{NS} \pm$ 0.10	$7.33^{NS} \pm$ 0.10	$7.28^{NS} \pm$ 0.07
RBC (10^6 mm^{-3})	$4.67^{NS} \pm$ 0.07	$4.65^{NS} \pm$ 0.09	$4.69^{NS} \pm$ 0.09	$4.72^{NS} \pm$ 0.10	$4.70^{NS} \pm$ 0.11
ESR (mm hr^{-1})	$1.67^{NS} \pm$ 0.33	$1.75^{NS} \pm$ 0.25	$1.50^{NS} \pm$ 0.22	$1.83^{NS} \pm$ 0.31	$1.58^{NS} \pm$ 0.20
Hb (gm dl^{-1})	$13.58^{NS} \pm$ 0.42	$13.67^{NS} \pm$ 0.40	$13.75^{NS} \pm$ 0.36	$13.42^{NS} \pm$ 0.52	$13.92^{NS} \pm$ 0.47
PLATELET (10^3 mm^{-3})	$225.83^{NS} \pm$ 9.08	$229.33^{NS} \pm$ 9.82	$226.17^{NS} \pm$ 5.57	$227.33^{NS} \pm$ 9.02	$224.33^{NS} \pm$ 7.61
PCV (%)	$37.67^{NS} \pm$ 0.61	$38.00^{NS} \pm$ 0.68	$38.67^{NS} \pm$ 0.67	$37.50^{NS} \pm$ 0.67	$37.00^{NS} \pm$ 0.52

Table 12: Values are Mean ±S.E., where n =6 in each group;
NS = non significant($p<0.05$)

Haemobiograms following oral administration of aqueous extract of *Tamarindus indica* L. leaves (@2 gm kg^{-1} b.w.) for consecutive 28 days in healthy rabbits have been given in the table 13. Any of the blood parameters did not change significantly.

TABLE 13: Haemobiograms following oral administration of aqueous extract of *Tamarindus indica* L. leaves (@2gm kg^{-1} b.w.) for consecutive 28 days in healthy rabbits [n = 6]

Day / Parameters	DAY 0	DAY 7	DAY 14	DAY 21	DAY 30
WBC (10^9 lit^{-1})	$7.07^{NS} \pm$ 0.08	$7.12^{NS} \pm$ 0.10	$7.09^{NS} \pm$ 0.12	$7.13^{NS} \pm$ 0.12	$7.06^{NS} \pm$ 0.09
RBC (10^6 mm^{-3})	$4.68^{NS} \pm$ 0.06	$4.71^{NS} \pm$ 0.05	$4.75^{NS} \pm$ 0.06	$4.70^{NS} \pm$ 0.06	$4.65^{NS} \pm$ 0.08
ESR (mm hr^{-1})	$1.83^{NS} \pm$ 0.33	$2.00^{NS} \pm$ 0.29	$1.92^{NS} \pm$ 0.35	$2.08^{NS} \pm$ 0.37	$1.75^{NS} \pm$ 0.36
Hb (gm dl^{-1})	$14.00^{NS} \pm$ 0.29	$14.25^{NS} \pm$ 0.50	$14.17^{NS} \pm$ 0.38	$14.33^{NS} \pm$ 0.31	$14.08^{NS} \pm$ 0.24
PLATELET (10^3 mm^{-3})	$233.33^{NS} \pm$ 9.28	$237.50^{NS} \pm$ 7.50	$236.67^{NS} \pm$ 8.63	$239.17^{NS} \pm$ 9.87	$235.83^{NS} \pm$ 8.00
PCV (%)	$36.83^{NS} \pm$ 1.22	$35.67^{NS} \pm$ 1.38	$37.17^{NS} \pm$ 1.14	$36.67^{NS} \pm$ 0.80	$36.50^{NS} \pm$ 0.99

Table13:Values are Mean ±S.E., where n =6 in each group;
NS = non significant($p<0.05$)

Haemobiograms following oral administration of 2% Tween 20 (vehicle for ethanolic extract) for consecutive 28 days in healthy rabbits have been displayed in the table no 14. Blood parameters did not show any significant changes.

TABLE 14: Haemobiograms following oral administration of 2% Tween 20 (vehicle for ethanolic extract) for consecutive 28 days in healthy rabbits [n = 6]

Day / Group	DAY 0	DAY 7	DAY 14	DAY 21	DAY 30
WBC (10^9 lit^{-1})	$7.13^{NS} \pm$ 0.07	$7.1^{NS} \pm$ 0.10	$7.14^{NS} \pm$ 0.11	$7.07^{NS} \pm$ 0.06	$7.15^{NS} \pm$ 0.04
RBC (10^6 mm^{-3})	$4.75^{NS} \pm$ 0.09	$4.79^{NS} \pm$ 0.06	$4.72^{NS} \pm$ 0.05	$4.78^{NS} \pm$ 0.08	$4.73^{NS} \pm$ 0.08
ESR (mm hr^{-1})	$2.17^{NS} \pm$ 0.28	$2.25^{NS} \pm$ 0.25	$2.08^{NS} \pm$ 0.15	$2.33^{NS} \pm$ 0.31	$2.25^{NS} \pm$ 0.11
Hb (gm dl^{-1})	$13.92^{NS} \pm$ 0.33	$13.58^{NS} \pm$ 0.35	$13.33^{NS} \pm$ 0.36	$13.42^{NS} \pm$ 0.27	$13.25^{NS} \pm$ 0.51
PLATELET (10^3 mm^{-3})	$235.83^{NS} \pm$ 6.11	$234.17^{NS} \pm$ 4.90	$240.00^{NS} \pm$ 7.30	$238.33^{NS} \pm$ 5.87	$241.67^{NS} \pm$ 5.58
PCV (%)	$36.17^{NS} \pm$ 1.19	$37.00^{NS} \pm$ 1.37	$36.67^{NS} \pm$ 1.52	$36.83^{NS} \pm$ 1.30	$36.33^{NS} \pm$ 1.09

Table 14: Values are Mean ±S.E., where n =6 in each group;
NS = non significant($p<0.05$)

Body weight

Body weight in healthy rabbits following oral administration of ethanolic and aqueous extract of *Tamarindus indica* L. at 2 gm kg^{-1} body weight for consecutive 28 days have been depicted in table 15. Mean ±S.E. values for body weight in healthy rabbits ranged from 6.72 ± 0.79 to 7.01 ± 1.10. Mean ALP level did not differ significantly ($p<0.05$) on day 0, 7, 14, 21 and 30 following oral administration of ethanolic and aqueous extracts of *Tamarindus indica* L. leaves for @2 gm kg^{-1} body weight for consecutive 28 days. Mean body weight did not differ significantly ($p<0.05$) on day 0, 7, 14, 21 and 30 following oral administration of 2% tween 20 (as vehicle) for consecutive 28 days.

But mean values showed a regular increment of bodyweight. It was observed that the extracts do not have any negative effect such as causing anorexia or any debilitating changes to the animals.

Table 15: Mean values with S.E. of body weights on different days following oral dosing of ethanolic extract in tween 20(2%) and aqueous extract of *Tamarindus indica* L. leaves for @2 gm kg^{-1} for consecutive 28 days and oral dosing of Tween 20(2%) for 28 days in rabbits [n=6]

DAY / GROUP	DAY 0	DAY 7	DAY 14	DAY 21	DAY 30
E.E. (2gm Kg^{-1} b.w.)	1976.67^{NS} ± 77.78	1991.67^{NS} ± 76.35	2025.83^{NS} ± 72.37	2046.67^{NS} ± 72.15	2068.33^{NS} ± 75.34
A.E. (2gm Kg^{-1} b.w.)	1705.00^{NS} ± 80.29	1723.33^{NS} ± 77.37	1750.83^{NS} ± 72.25	1771.67^{NS} ± 81.47	1782.50^{NS} ± 81.14
Vehicle control (2% tween 20)	1810.67^{NS} ± 67.58	1820.33^{NS} ± 72.37	1834.83^{NS} ± 74.72	1845.33^{NS} ± 70.45	1863.67^{NS} ± 75.35

Table 15: Values are Mean ±S.E., where n =6 in each group;
NS = non significant($p<0.05$)

Once daily oral administration of ethanolic and aqueous extract of *Tamarindus indica* L. leaves @2 gm kg^{-1} bw for 28 consecutive day did not alter AST, ALT level, ALP activity, BUN, Creatinine and haematological parameters like WBC, RBC, ESR, HB, Platelet, PCV which indicated that these two herbal extracts had no adverse effect during 30 days period of study which may be used safely in rabbits.

Histopathology for different extracts treated groups

Histopathology study of liver and kidney did not show any marked change, in ethanolic extract treated group, aqueous extract treated group and vehicle control group in compared to the normal rabbit (figure:)

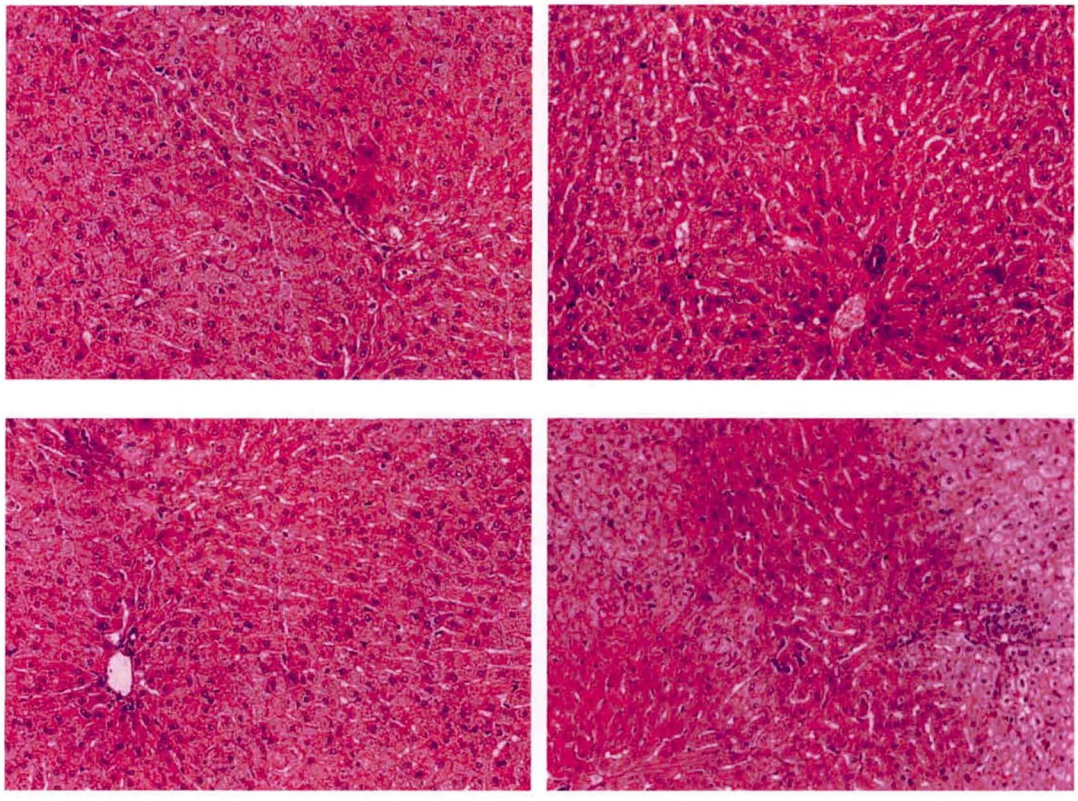

Fig xviii: (a) upper left, (b) upper right, (c) lower left, (d) lower right ; photomicrographs of (a)control liver, (b) ethanolic extract treated group, (c) aqueous extract treated group, (d)vehicle control liver respectively (H.E. stain, 10x)

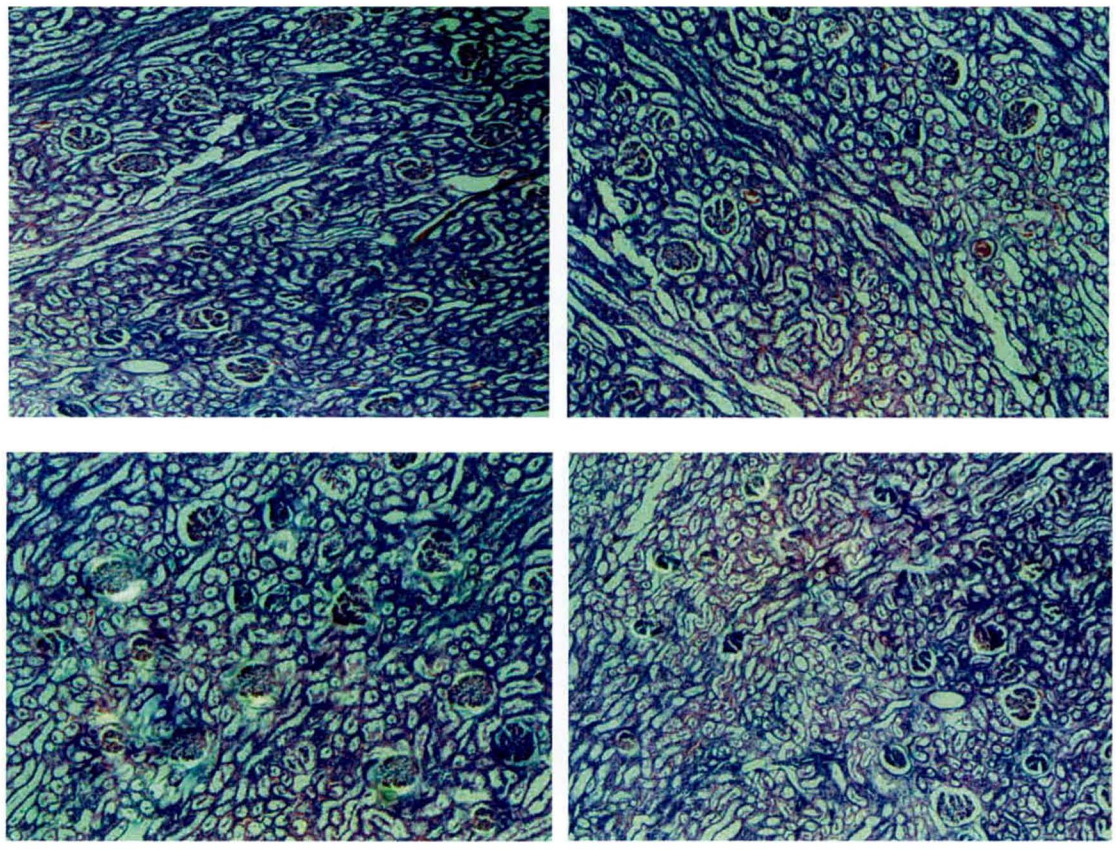

Fig xix: (a) upper left, (b) upper right, (c) lower left, (d) lower right; photomicrographs of (a)control kidney, (b) kidney of ethanolic extract taken group, (c) kidney of aqueous extract taken group, (d)vehicle control kidney respectively (H.E. stain, 10x)

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Fixation of Dose rates of ethanolic and aqueous extracts of *Tamarindus indica* L. leaves

The results of the biochemical parameters did not show any significant changes within the study period. It was found that administration of hydroethanolic extract of *T. indica* L. leaves @500 mg to 1 gm kg^{-1} body weight did not show any untoward effects in albino rats rather it produced remarkable anti-inflammatory as well as anti-nociceptive actions in albino rats (Bhadoriya *et al.*, 2012). Therefore 500 mg kg^{-1} body weight and 1000 mg/kg body weight dose rates were used for efficacy study.

Determination of MIC of linezolid

The MIC of linezolid against the isolated organism was 1.5 µg/mL

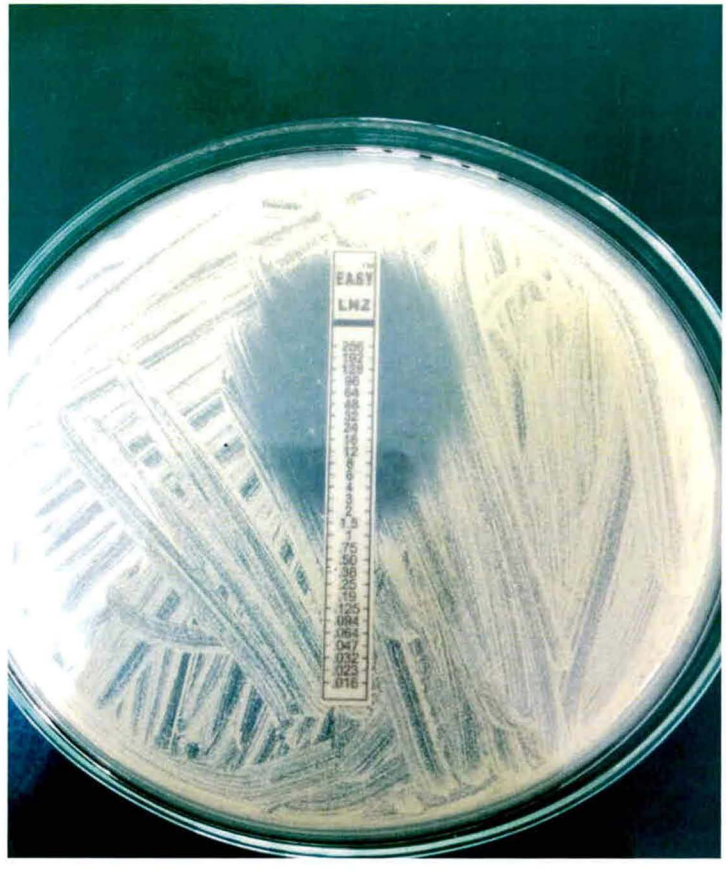


Fig xx: Showing MIC of linezolid against isolated organism.

Confirmation of septic Arthritis

The rabbits inoculated with 10^4 CFU *S. aureus* in the left stifle joint showed marked swelling and redness of the particular joint, lameness and restricted movement, restlessness, elevated body temperature and severe anorexia. Presence of pus was observed in the inoculated joint. Subsequent culturing of the collected pus from the particular joint showed the same organism with which the infection was given which confirmed induction of septic arthritis.

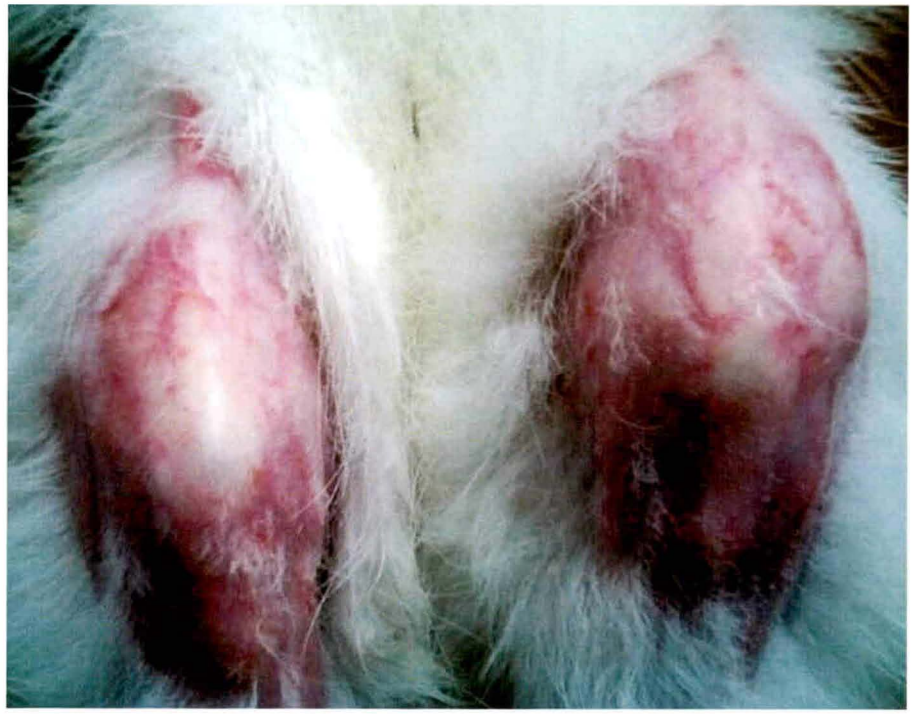


Fig xxi: Swelled left stifle joint with septic arthritis

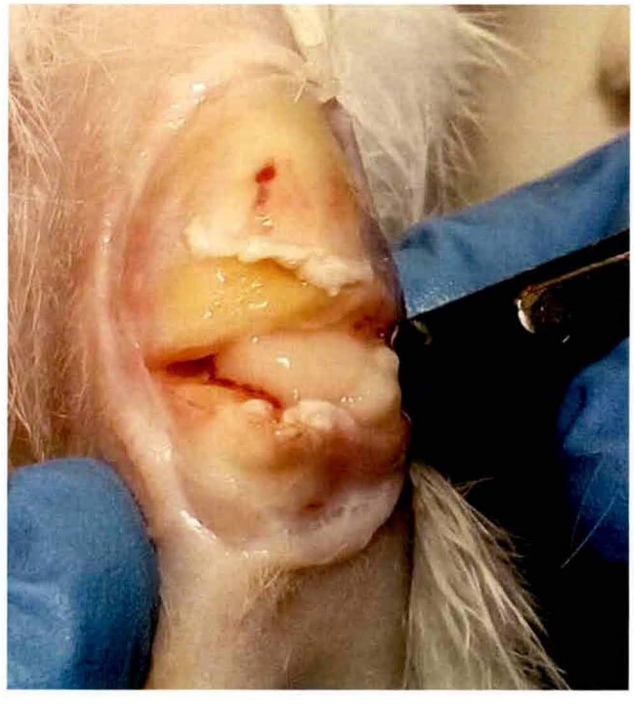


Fig xxii: septic joint showing pus

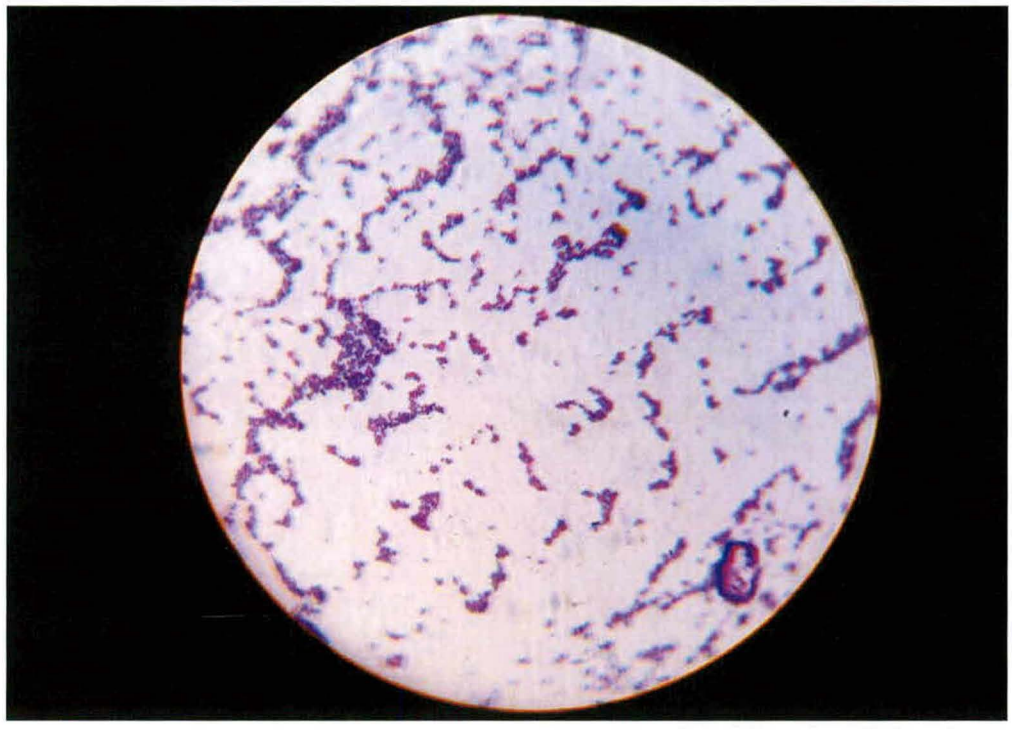
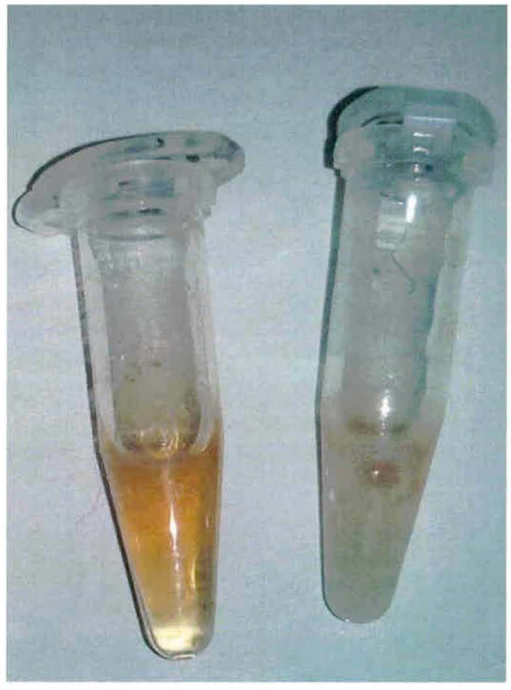


Fig xxiii: Arthritic synovial fluid culture showing same organism (*Staphylococcus aureus*)

Characterization of synovial fluid :

The visual appearance of joint fluids collected in the 2nd day after intra-articular inoculation of 10^4 CFU *Staphylococcus aureus* (phenotypically resistant to Methicilin), were cloudy or purulent; white, grey, yellow or green in colour and very less viscosity was observed.



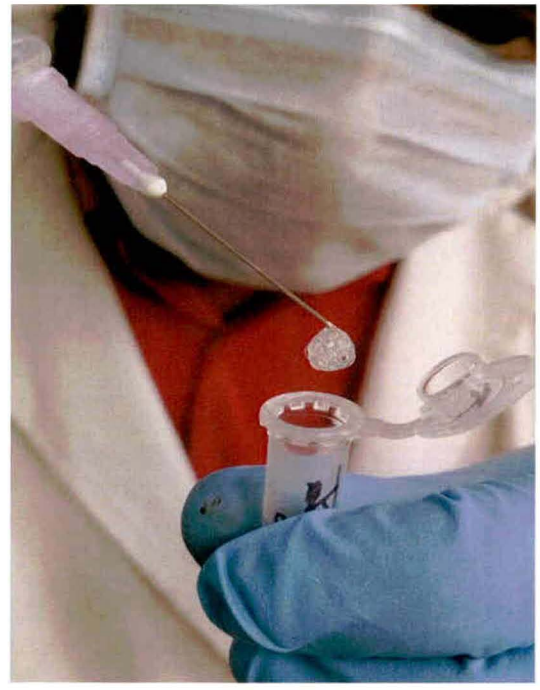


Fig xxiv: (left) Cloudy appearance of synovial fluids collected from same synovial joint after 2 days of intra-articular inoculation of 10^4 CFU *Staphylococcus aureus*

Fig xxv: (right) Showing absence of viscosity in synovial fluid fluid sample collected from same synovial joint after 2 days of intra-articular inoculation of 10^4 CFU *Staphylococcus aureus*

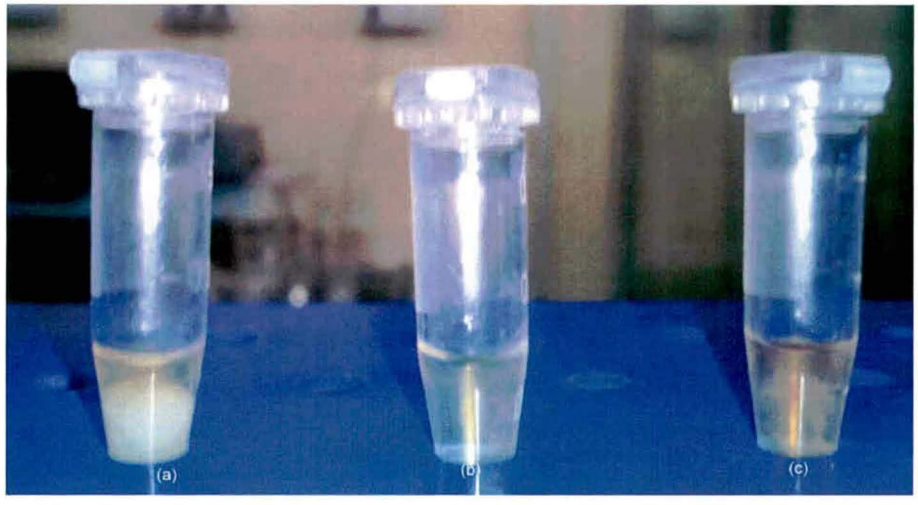


Fig xxvi: (a) Showing white (b) clear normal (c) yellowish coloured of synovial fluid collected from same synovial joint after 2 days of intra-articular inoculation of 10^4 CFU *Staphylococcus aureus*

Confirmation was also done by blood ESR, serum PCT, serum CRP level and synovial WBC count. ESR level for all the animals were gone at least more than 6 mm/hour, PCT level for all the animals were more than 1 ng ml^{-1}, CRP level for all animals were found more than 50 ng ml^{-1} and the synovial WBC count was more than 50,000 mm^{-3}.

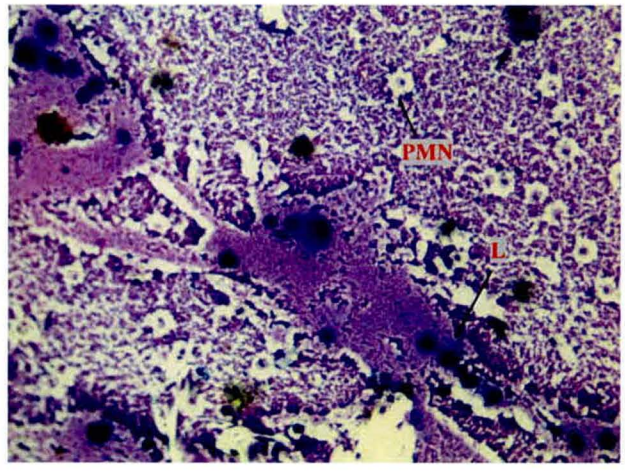


Fig xxvii: Leishman staining of Synovial fluid showing WBC (L - Lymphocyte, PMN – Polymorphonuclear Leukocytes)

Joint radius

Joint Radius(cm) of septic arthritic (Gr- I), oral linezolid @ 75 mg kg^{-1} for 10 days twice daily (Gr – II), oral linezolid @75 mg kg^{-1} for 10 days twice daily with a single intra-articular injection of betamethasone @0.5 mg kg^{-1} (Gr – III), ethanolic extract of *Tamarindus indica* L. leaves treatment @ 500 mg kg^{-1} for 14 days(Gr – IV), ethanolic extract of *Tamarindus indica* L. leaves treatment @ 1000 mg kg^{-1} for 14 days(Gr – V), aqueous extract of *Tamarindus indica* L. leaves treatment @ 500 mg kg^{-1} for 14 days (Gr – VI) and aqueous extract of *Tamarindus indica* L. leaves treatment @ 1000 mg kg^{-1} for 14 days (Gr – VII) have been exhibited in table. Mean value of joint radius of healthy rabbits was ranged from 1.358 ± 0.006 to 1.403 ± 0.006 cm. Swelling of the inoculated stifle joint caused increase of the circumference of the particular joint, leading to increase of joint radius.

Gradual increased joint radius was recorded up to day 8 following intra-articular inoculation of 10^4 CFU *S. aureus* in the left stifle joint of rabbits followed by decrease in joint radius in the same arthritic joint. However, interestingly the joint radius of the inoculated stifle joint was observed to be reduced in all the treatment groups (Gr – II, Gr – III, Gr – V, Gr – VI and Gr – VII) except ethanolic extract of *Tamarindus indica* L. leaves treatment @ 500 mg kg^{-1} for 14 days(Gr – IV) from day 5 (Figure xxviii).

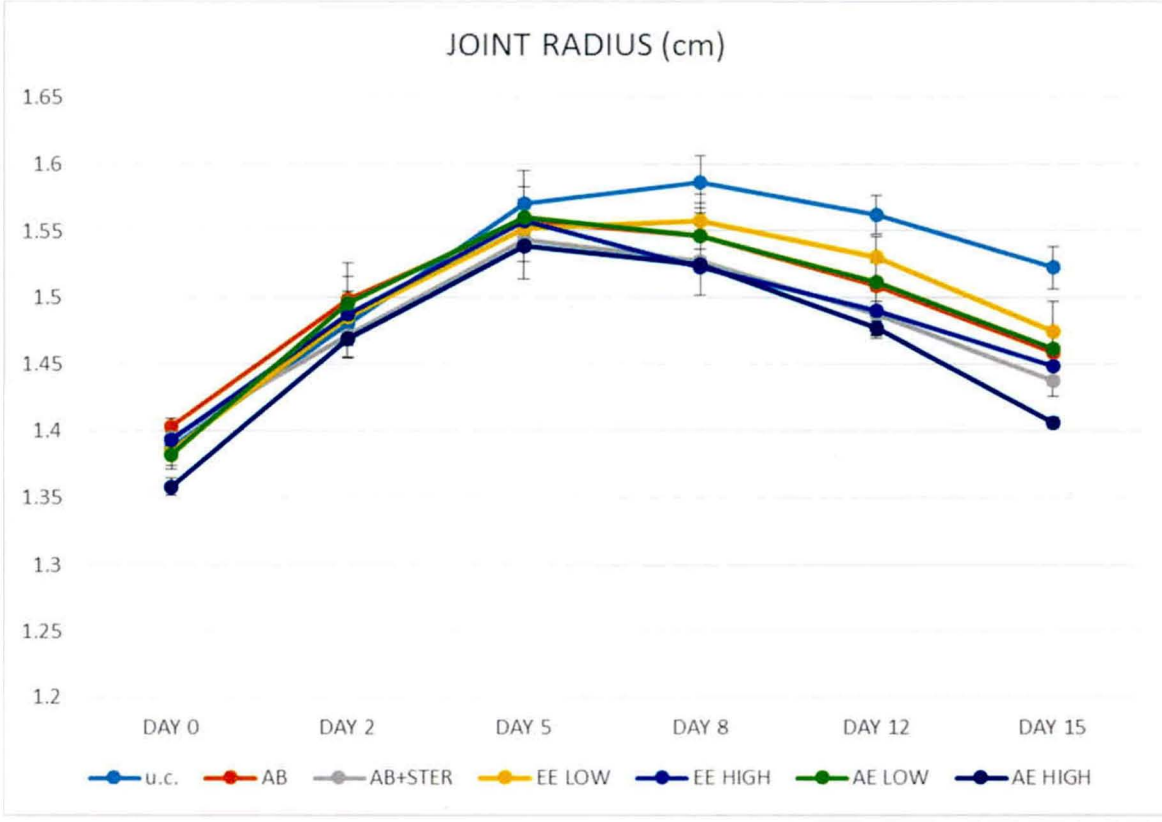


Fig xxviii: mean ±S.E. joint radius of affected knee joint of different groups on different days

Table 16: Joint Radius(cm) of septic arthritic (Gr- I), oral linezolid @ 75 mg kg^{-1} for 10 days twice daily (Gr – II), oral linezolid @75 mg kg^{-1} for 10 days twice daily with a single intra-articular injection of betamethasone @0.5 mg kg^{-1} (Gr – III), ethanolic extract (2% tween 20) of *Tamarindus indica* L. leaves treatment @ 500 mg kg^{-1} for 14 days(Gr – IV), ethanolic extract (2% tween 20) of *Tamarindus indica* L. leaves treatment @ 1000 mg kg^{-1} for 14 days(Gr – V), aqueous extract of *Tamarindus indica* L. leaves treatment @ 500 mg kg^{-1} for 14 days (Gr – VI) and aqueous extract of *Tamarindus indica* L. leaves treatment @ 1000 mg kg^{-1} for 14 days (Gr – VII) [n=6]

	DAY 0	DAY 2	DAY 5	DAY 8	DAY 12	DAY 15
Gr-I	$1.387^{a} \pm 0.016$	$1.480^{bf} \pm 0.024$	$1.570^{cdeg} \pm 0.025$	$1.586^{de} \pm 0.020$	$1.562^{eg} \pm 0.014$	$1.523^{fg} \pm 0.016$
Gr-II	$1.403^{a} \pm 0.006$	$1.499^{bdef} \pm 0.017$	$1.557^{cgh} \pm 0.016$	$1.546^{dgh} \pm 0.024$	$1.509^{ehf} \pm 0.021$	$1.459^{f} \pm 0.017$
Gr-III	$1.395^{a} \pm 0.010$	$1.472^{bdef} \pm 0.018$	$1.544^{cgh} \pm 0.030$	$1.528^{dgh} \pm 0.026$	$1.488^{ehf} \pm 0.017$	$1.438^{af} \pm 0.012$
Gr-IV	$1.385^{a} \pm 0.011$	$1.485^{bef} \pm 0.016$	$1.552^{cgh} \pm 0.017$	$1.557^{dgh} \pm 0.020$	$1.531^{eh} \pm 0.016$	$1.475^{f} \pm 0.023$
Gr-V	$1.393^{a} \pm 0.007$	$1.488^{bdef} \pm 0.009$	$1.557^{eg} \pm 0.010$	$1.523^{dge} \pm 0.020$	$1.491^{e} \pm 0.021$	$1.448^{f} \pm 0.014$
Gr-VI	$1.382^{a} \pm 0.011$	$1.496^{bdef} \pm 0.030$	$1.560^{cgh} \pm 0.023$	$1.546^{dgh} \pm 0.017$	$1.512^{ehf} \pm 0.014$	$1.462^{f} \pm 0.012$
Gr-VII	$1.358^{a} \pm 0.006$	$1.470^{be} \pm 0.005$	$1.538^{cd} \pm 0.004$	$1.525^{d} \pm 0.006$	$1.477^{e} \pm 0.005$	$1.406^{f} \pm 0.004$

Values are Mean ±S.E., n=6 in each groups

Values with dissimilar superscript (abcde) in the row vary significantly (P<0.05)

Blood parameters

Blood parameters of septic arthritic (Gr- I), oral linezolid @ 75 mg kg^{-1} for 10 days twice daily (Gr – II), oral linezolid @ 75 mg kg^{-1} for 10 days twice daily with a single intra-articular injection of betamethasone @ 0.5 mg kg^{-1} (Gr – III), ethanolic extract (in 2% tween 20) of *Tamarindus indica* L. leaves treatment @ 500 mg kg^{-1} for 14 days(Gr – IV), ethanolic extract (in 2% tween 20) of *Tamarindus indica* L. leaves treatment @ 1000 mg kg^{-1} for 14 days(Gr – V), aqueous extract of *Tamarindus indica* L. leaves treatment @ 500 mg kg^{-1} for 14 days (Gr – VI) and aqueous extract of *Tamarindus indica* L. leaves treatment @ 1000 mg kg^{-1} for 14 days(Gr – VII) in rabbits have been depicted in table 17 (a –g) . No significant changes in any parameters were found. Only in Neutrophil % in peripheral blood was found to be non-significantly elevated.

Table 17(a – g): Blood parameters of septic arthritic(Gr- I),oral linezolid @ 75 mg kg^{-1} for 10 days twice daily (Gr – II), oral linezolid @ 75 mg kg^{-1} for 10 days twice daily with a single intra-articular injection of betamethasone @ 0.5 mg kg^{-1} (Gr – III), ethanolic extract of *Tamarindus indica* L. leaves treatment @ 500 mg kg^{-1} for 14 days(Gr – IV), ethanolic extract of *Tamarindus indica* L. leaves treatment @ 1000 mg kg^{-1} for 14 days(Gr – V), aqueous extract of *Tamarindus indica* L. leaves treatment @ 500 mg kg^{-1} for 14 days (Gr – VI) and aqueous extract of *Tamarindus indica* L. leaves treatment @ 1000 mg kg^{-1} for 14 days (Gr – VII) in rabbits [n=6]

Table 17 (a): Gr – I

Day / Parameters	0	2	4	7	14
Hb (g dl^{-1})	$12.33^{NS} \pm$ 0.54	$12.58^{NS} \pm$ 0.24	$12.50^{NS} \pm$ 0.43	$11.92^{NS} \pm$ 0.49	$12.58^{NS} \pm$ 0.44
RBC (10^6 mm^{-3})	$4.54^{NS} \pm$ 0.10	$4.68^{NS} \pm$ 0.12	$4.40^{NS} \pm$ 0.18	$4.67^{NS} \pm$ 0.12	$4.68^{NS} \pm$ 0.12
WBC (10^6 l^{-1})	$6816.67^{NS} \pm$ 152.25	$6716.67^{NS} \pm$ 265.10	$7133.33^{NS} \pm$ 167.55	$7016.67^{NS} \pm$ 94.58	$6933.33^{NS} \pm$ 66.67
Neutrophil (%)	$56.33^{NS} \pm$ 4.48	$61.50^{NS} \pm$ 3.55	$64.17^{NS} \pm$ 4.74	$62.67^{NS} \pm$ 3.62	$63.33^{NS} \pm$ 4.84
Lymphocyte (%)	$42.00^{NS} \pm$ 3.79	$36.67^{NS} \pm$ 2.33	$34.50^{NS} \pm$ 2.43	$35.50^{NS} \pm$ 3.45	$35.00^{NS} \pm$ 3.76
Eosinophil (%)	$1.50^{NS} \pm$ 0.22	$1.50^{NS} \pm$ 0.22	$1.17^{NS} \pm$ 0.17	$1.50^{NS} \pm$ 0.22	$1.33^{NS} \pm$ 0.21
Monocyte (%)	$0.17^{NS} \pm$ 0.17	$0.33^{NS} \pm$ 0.21	$0.17^{NS} \pm$ 0.17	$0.33^{NS} \pm$ 0.21	$0.33^{NS} \pm$ 0.21
Platelet (10^3 mm^{-3})	$198.00^{NS} \pm$ 8.62	$207.50^{NS} \pm$ 6.55	$201.67^{NS} \pm$ 6.41	$214.17^{NS} \pm$ 9.87	$209.17^{NS} \pm$ 6.11
PCV (%)	$37.50^{NS} \pm$ 0.56	$37.67^{NS} \pm$ 1.05	$38.67^{NS} \pm$ 1.20	$36.83^{NS} \pm$ 0.95	$35.83^{NS} \pm$ 1.56
MCV ($mm^{3)}$	$81.69^{NS} \pm$ 0.82	$79.74^{NS} \pm$ 1.67	$81.39^{NS} \pm$ 2.82	$82.43^{NS} \pm$ 1.28	$83.03^{NS} \pm$ 1.75
MCH (pg /cell)	$26.87^{NS} \pm$ 0.54	$27.38^{NS} \pm$ 0.76	$26.94^{NS} \pm$ 0.67	$27.18^{NS} \pm$ 1.04	$27.17^{NS} \pm$ 0.83
MCHC (%)	$33.17^{NS} \pm$ 0.48	$32.43^{NS} \pm$ 0.31	$33.46^{NS} \pm$ 1.23	$32.90^{NS} \pm$ 0.21	$32.85^{NS} \pm$ 0.26

Table 17(a): Values are Mean ±S.E., where n =6 in each group;
NS = non significant ($p<0.05$)

Table 17 (b): Gr – II

Day / Parameters	0	2	4	7	14
Hb (g dl^{-1})	$12.42^{NS} \pm$ 0.58	$12.83^{NS} \pm$ 0.34	$11.92^{NS} \pm$ 0.47	$12.08^{NS} \pm$ 0.42	$12.05^{NS} \pm$ 0.48
RBC (10^6 mm^{-3})	$4.60^{NS} \pm$ 0.13	$4.38^{NS} \pm$ 0.16	$4.51^{NS} \pm$ 0.13	$4.66^{NS} \pm$ 0.14	$4.67^{NS} \pm$ 0.19
WBC (10^6 l^{-1})	$6416.67^{NS} \pm$ 178.25	$6533.67^{NS} \pm$ 265.10	$6781.33^{NS} \pm$ 261.55	$6388.67^{NS} \pm$ 141.65	$6538.33^{NS} \pm$ 97.33
Neutrophil (%)	$57.00^{NS} \pm$ 4.17	$60.50^{NS} \pm$ 4.35	$62.50^{NS} \pm$ 4.74	$63.67^{NS} \pm$ 3.62	$61.00^{NS} \pm$ 4.84
Lymphocyte (%)	$41.33^{NS} \pm$ 3.79	$37.67^{NS} \pm$ 2.33	$36.17^{NS} \pm$ 2.43	$34.50^{NS} \pm$ 3.45	$37.33^{NS} \pm$ 3.76
Eosinophil (%)	$1.50^{NS} \pm$ 0.22	$1.50^{NS} \pm$ 0.22	$1.17^{NS} \pm$ 0.17	$1.50^{NS} \pm$ 0.22	$1.50^{NS} \pm$ 0.21
Monocyte (%)	$0.17^{NS} \pm$ 0.17	$0.33^{NS} \pm$ 0.21	$0.17^{NS} \pm$ 0.17	$0.33^{NS} \pm$ 0.21	$0.17^{NS} \pm$ 0.21
Platelet (10^3 mm^{-3})	$203.00^{NS} \pm$ 7.65	$202.17^{NS} \pm$ 5.569	$204.67^{NS} \pm$ 5.43	$210.00^{NS} \pm$ 8.38	$205.33^{NS} \pm$ 6.71
PCV (%)	$34.50^{NS} \pm$ 0.98	$36.33^{NS} \pm$ 1.12	$33.67^{NS} \pm$ 1.72	$34.83^{NS} \pm$ 1.54	$33.50^{NS} \pm$ 1.32
MCV ($mm^{3)}$	$83.74^{NS} \pm$ 0.93	$81.36^{NS} \pm$ 2.62	$80.77^{NS} \pm$ 2.82	$82.00^{NS} \pm$ 1.28	$83.60^{NS} \pm$ 1.59
MCH (pg /cell)	$25.82^{NS} \pm$ 0.76	$26.88^{NS} \pm$ 0.85	$26.76^{NS} \pm$ 0.73	$27.54^{NS} \pm$ 0.97	$26.87^{NS} \pm$ 0.56
MCHC (%)	$32.65^{NS} \pm$ 0.92	$31.89^{NS} \pm$ 0.61	$33.24^{NS} \pm$ 1.54	$31.39^{NS} \pm$ 0.87	$30.85^{NS} \pm$ 1.03

Table 17(b): Values are Mean ±S.E., where n =6 in each group;
NS = non significant($p<0.05$)

Table 17(c): Gr – III

Day / Parameters	0	2	4	7	14
Hb (g dl^{-1})	$12.33^{NS} \pm$ 0.28	$12.67^{NS} \pm$ 0.31	$12.08^{NS} \pm$ 0.30	$12.25^{NS} \pm$ 0.48	$12.83^{NS} \pm$ 0.40
RBC (10^6 mm^{-3})	$4.51^{NS} \pm$ 0.11	$4.72^{NS} \pm$ 0.18	$4.51^{NS} \pm$ 0.09	$4.42^{NS} \pm$ 0.17	$4.66^{NS} \pm$ 0.18
WBC (10^6 l^{-1})	$6734.33^{NS} \pm$ 194.54	$6652.67^{NS} \pm$ 239.43	$6865.00^{NS} \pm$ 234.59	$6588.33^{NS} \pm$ 156.21	$6533.33^{NS} \pm$ 102.19
Neutrophil (%)	$59.50^{NS} \pm$ 3.67	$57.17^{NS} \pm$ 4.31	$64.00^{NS} \pm$ 4.07	$61.17^{NS} \pm$ 3.44	$63.33^{NS} \pm$ 4.58
Lymphocyte (%)	$38.33^{NS} \pm$ 2.57	$40.67^{NS} \pm$ 2.89	$34.17^{NS} \pm$ 3.69	$36.83^{NS} \pm$ 3.03	$35.33^{NS} \pm$ 3.76
Eosinophil (%)	$1.67^{NS} \pm$ 0.21	$1.83^{NS} \pm$ 0.17	$1.50^{NS} \pm$ 0.22	$1.50^{NS} \pm$ 0.22	$1.17^{NS} \pm$ 0.17
Monocyte (%)	$0.50^{NS} \pm$ 0.22	$0.33^{NS} \pm$ 0.21	$0.33^{NS} \pm$ 0.21	$0.50^{NS} \pm$ 0.22	$0.17^{NS} \pm$ 0.17
Platelet (10^3 mm^{-3})	$196.00^{NS} \pm$ 8.46	$200.33^{NS} \pm$ 6.39	$198.67^{NS} \pm$ 8.03	$204.67^{NS} \pm$ 4.26	$207.00^{NS} \pm$ 8.37
PCV (%)	$34.50^{NS} \pm$ 0.98	$36.33^{NS} \pm$ 1.12	$33.67^{NS} \pm$ 1.72	$34.83^{NS} \pm$ 1.54	$33.50^{NS} \pm$ 1.32
MCV ($mm^{3)}$	$83.74^{NS} \pm$ 0.93	$81.36^{NS} \pm$ 2.62	$80.77^{NS} \pm$ 2.82	$82.00^{NS} \pm$ 1.28	$83.60^{NS} \pm$ 1.59
MCH (pg /cell)	$27.05^{NS} \pm$ 0.27	$27.45^{NS} \pm$ 0.36	$27.08^{NS} \pm$ 0.24	$27.15^{NS} \pm$ 0.27	$26.95^{NS} \pm$ 0.54
MCHC (%)	$32.15^{NS} \pm$ 0.72	$31.43^{NS} \pm$ 0.92	$31.65^{NS} \pm$ 0.98	$31.81^{NS} \pm$ 0.82	$32.96^{NS} \pm$ 0.79

Table 17(c): Values are Mean ±S.E., where n =6 in each group;
NS = non significant($p<0.05$)

Table 17 (d): Gr - IV

Day / Parameters	0	2	4	7	14
Hb (g dl^{-1})	$12.75^{NS} \pm$ 0.17	$12.92^{NS} \pm$ 0.15	$13.17^{NS} \pm$ 0.31	$12.67^{NS} \pm$ 0.33	$13.08^{NS} \pm$ 0.37
RBC (10^6 mm^{-3})	$4.52^{NS} \pm$ 0.12	$4.31^{NS} \pm$ 0.18	$4.63^{NS} \pm$ 0.11	$4.71^{NS} \pm$ 0.11	$4.62^{NS} \pm$ 0.16
WBC (10^6 l^{-1})	$6721.33^{NS} \pm$ 198.72	$6709.67^{NS} \pm$ 202.51	$6929.33^{NS} \pm$ 253.51	$6745.33^{NS} \pm$ 167.35	$6698.67^{NS} \pm$ 103.27
Nutrophil (%)	$62.00^{NS} \pm$ 4.13	$65.00^{NS} \pm$ 4.05	$67.50^{NS} \pm$ 4.81	$64.00^{NS} \pm$ 3.76	$61.00^{NS} \pm$ 4.43
Lymphcyte (%)	$36.17^{NS} \pm$ 3.69	$33.67^{NS} \pm$ 2.74	$30.50^{NS} \pm$ 3.76	$34.67^{NS} \pm$ 2.89	$37.17^{NS} \pm$ 3.21
Eosinophil (%)	$1.50^{NS} \pm$ 0.22	$1.17^{NS} \pm$ 0.22	$1.50^{NS} \pm$ 0.17	$1.17^{NS} \pm$ 0.22	$1.50^{NS} \pm$ 0.21
Monocyte (%)	$0.33^{NS} \pm$ 0.17	$0.17^{NS} \pm$ 0.21	$0.50^{NS} \pm$ 0.17	$0.17^{NS} \pm$ 0.21	$0.33^{NS} \pm$ 0.21
Platelet (10^3 mm^{-3})	$206.00^{NS} \pm$ 6.34	$200.33^{NS} \pm$ 7.22	$198.67^{NS} \pm$ 5.26	$201.00^{NS} \pm$ 7.73	$203.33^{NS} \pm$ 5.90
PCV (%)	$33.50^{NS} \pm$ 1.09	$31.33^{NS} \pm$ 1.45	$31.67^{NS} \pm$ 1.77	$34.33^{NS} \pm$ 1.54	$32.67^{NS} \pm$ 1.43
MCV ($mm^{3)}$	$84.43^{NS} \pm$ 1.03	$81.64^{NS} \pm$ 2.84	$82.31^{NS} \pm$ 2.43	$83.05^{NS} \pm$ 1.35	$83.54^{NS} \pm$ 1.76
MCH (pg /cell)	$26.13^{NS} \pm$ 0.86	$25.78^{NS} \pm$ 0.94	$24.92^{NS} \pm$ 0.93	$25.15^{NS} \pm$ 0.76	$26.66^{NS} \pm$ 0.86
MCHC (%)	$31.32^{NS} \pm$ 0.80	$31.73^{NS} \pm$ 0.91	$32.06^{NS} \pm$ 0.95	$31.76^{NS} \pm$ 1.02	$32.81^{NS} \pm$ 1.12

Table 17(d): Values are Mean ±S.E., where n =6 in each group;
NS = non significant($p<0.05$)

Table 17 (e): Gr – V

Day / Parameters	0	2	4	7	14
Hb (g dl^{-1})	$12.58^{NS} \pm$ 0.20	$13.00^{NS} \pm$ 0.26	$12.83^{NS} \pm$ 0.31	$12.75^{NS} \pm$ 0.38	$12.83^{NS} \pm$ 0.33
RBC (10^6 mm^{-3})	$4.56^{NS} \pm$ 0.25	$4.48^{NS} \pm$ 0.23	$4.545^{NS} \pm$ 0.27	$4.64^{NS} \pm$ 0.19	$4.61^{NS} \pm$ 0.21
WBC (10^6 l^{-1})	$6702.67^{NS} \pm$ 164.35	$6751.67^{NS} \pm$ 218.10	$6733.33^{NS} \pm$ 221.53	$6845.67^{NS} \pm$ 188.08	$6723.33^{NS} \pm$ 167.43
Neutrophil (%)	$57.50^{NS} \pm$ 4.71	$60.50^{NS} \pm$ 3.82	$62.33^{NS} \pm$ 4.06	$61.67^{NS} \pm$ 4.02	$63.00^{NS} \pm$ 3.87
Lymphocyte (%)	$40.67^{NS} \pm$ 3.11	$37.50^{NS} \pm$ 2.98	$36.17^{NS} \pm$ 3.03	$36.50^{NS} \pm$ 3.23	$35.33^{NS} \pm$ 2.00
Eosinophil (%)	$1.50^{NS} \pm$ 0.22	$1.33^{NS} \pm$ 0.21	$1.00^{NS} \pm$ 0.00	$1.50^{NS} \pm$ 0.22	$1.50^{NS} \pm$ 0.22
Monocyte (%)	$0.33^{NS} \pm$ 0.21	$0.67^{NS} \pm$ 0.21	$0.50^{NS} \pm$ 0.22	$0.33^{NS} \pm$ 0.21	$0.17^{NS} \pm$ 0.17
Platelet (10^3 mm^{-3})	$216.00^{NS} \pm$ 8.13	$209.17^{NS} \pm$ 6.93	$209.67^{NS} \pm$ 5.15	$211.00^{NS} \pm$ 7.05	$203.33^{NS} \pm$ 7.12
PCV (%)	$33.50^{NS} \pm$ 1.23	$32.33^{NS} \pm$ 1.12	$31.33^{NS} \pm$ 1.72	$34.50^{NS} \pm$ 1.54	$32.50^{NS} \pm$ 1.37
MCV ($mm^{3)}$	$81.74^{NS} \pm$ 1.17	$82.33^{NS} \pm$ 2.23	$81.37^{NS} \pm$ 1.29	$82.08^{NS} \pm$ 1.21	$82.16^{NS} \pm$ 1.55
MCH (pg /cell)	$25.02^{NS} \pm$ 0.97	$25.83^{NS} \pm$ 0.87	$26.02^{NS} \pm$ 0.67	$26.89^{NS} \pm$ 0.93	$25.81^{NS} \pm$ 0.72
MCHC (%)	$32.05^{NS} \pm$ 0.81	$32.88^{NS} \pm$ 0.45	$33.08^{NS} \pm$ 0.73	$32.52^{NS} \pm$ 0.82	$31.39^{NS} \pm$ 1.12

Table 17(e): Values are Mean ±S.E., where n =6 in each group;
NS = non significant($p<0.05$)

Table 17 (f): Gr – VI

Day / Parameters	0	2	4	7	14
Hb (g dl^{-1})	12.92^{NS} ± 0.20	13.08^{NS} ± 0.24	12.50^{NS} ± 0.34	12.67^{NS} ± 0.33	12.83^{NS} ± 0.33
RBC (10^6 mm^{-3})	4.63^{NS} ± 0.20	4.47^{NS} ± 0.22	4.51^{NS} ± 0.17	4.61^{NS} ± 0.18	4.72^{NS} ± 0.11
WBC (10^6 l^{-1})	6511.33^{NS} ± 182.45	6612.33^{NS} ± 265.10	6702.33^{NS} ± 261.55	6678.67^{NS} ± 141.65	6572.67^{NS} ± 97.33
Neutrophil (%)	57.00^{NS} ± 4.17	60.50^{NS} ± 4.35	62.50^{NS} ± 4.74	63.67^{NS} ± 3.62	61.00^{NS} ± 4.84
Lymphocyte (%)	41.33^{NS} ± 3.79	37.67^{NS} ± 2.33	36.17^{NS} ± 2.43	34.50^{NS} ± 3.45	37.33^{NS} ± 3.76
Eosinophil (%)	1.50^{NS} ± 0.22	1.50^{NS} ± 0.22	1.17^{NS} ± 0.17	1.50^{NS} ± 0.22	1.50^{NS} ± 0.21
Monocyte (%)	0.17^{NS} ± 0.17	0.33^{NS} ± 0.21	0.17^{NS} ± 0.17	0.33^{NS} ± 0.21	0.17^{NS} ± 0.21
Platelet (10^3 mm^{-3})	203.00^{NS} ± 7.65	202.17^{NS} ± 5.569	204.67^{NS} ± 5.43	210.00^{NS} ± 8.38	205.33^{NS} ± 6.71
PCV (%)	34.50^{NS} ± 0.98	36.33^{NS} ± 1.12	33.67^{NS} ± 1.72	34.83^{NS} ± 1.54	33.50^{NS} ± 1.32
MCV ($mm^{3)}$	83.74^{NS} ± 0.93	81.36^{NS} ± 2.62	80.77^{NS} ± 2.82	82.00^{NS} ± 1.28	83.60^{NS} ± 1.59
MCH (pg /cell)	25.82^{NS} ± 0.76	26.88^{NS} ± 0.85	26.76^{NS} ± 0.73	27.54^{NS} ± 0.97	26.87^{NS} ± 0.56
MCHC (%)	32.65^{NS} ± 0.92	31.89^{NS} ± 0.61	33.24^{NS} ± 1.54	31.39^{NS} ± 0.87	30.85^{NS} ± 1.03

Table 17(f): Values are Mean ±S.E., where n =6 in each group;
NS = non significant($p<0.05$)

Table 17(g): Gr – VII

Day / Parameters	0	2	4	7	14
Hb (g dl^{-1})	$12.92^{NS} \pm$ 0.20	$13.00^{NS} \pm$ 0.13	$13.08^{NS} \pm$ 0.27	$12.83^{NS} \pm$ 0.25	$13.00^{NS} \pm$ 0.29
RBC (10^6 mm^{-3})	$4.52^{NS} \pm$ 0.14	$4.72^{NS} \pm$ 0.26	$4.53^{NS} \pm$ 0.20	$4.63^{NS} \pm$ 0.24	$4.58^{NS} \pm$ 0.25
WBC (10^6 l^{-1})	$6518.33^{NS} \pm$ 178.25	$6539.67^{NS} \pm$ 123.10	$6633.33^{NS} \pm$ 265.71	$6531.67^{NS} \pm$ 121.65	$6620.33^{NS} \pm$ 102.39
Neutrophil (%)	$60.00^{NS} \pm$ 4.17	$62.50^{NS} \pm$ 4.35	$61.50^{NS} \pm$ 4.74	$63.67^{NS} \pm$ 3.54	$62.00^{NS} \pm$ 3.94
Lymphocyte (%)	$37.33^{NS} \pm$ 3.79	$36.17^{NS} \pm$ 2.33	$37.17^{NS} \pm$ 2.43	$34.50^{NS} \pm$ 3.45	$36.33^{NS} \pm$ 3.76
Eosinophil (%)	$1.50^{NS} \pm$ 0.22	$1.17^{NS} \pm$ 0.17	$1.17^{NS} \pm$ 0.17	$1.50^{NS} \pm$ 0.22	$1.50^{NS} \pm$ 0.21
Monocyte (%)	$0.50^{NS} \pm$ 0.22	$0.67^{NS} \pm$ 0.21	$0.17^{NS} \pm$ 0.17	$0.33^{NS} \pm$ 0.21	$0.17^{NS} \pm$ 0.21
Platelet (10^3 mm^{-3})	$190.00^{NS} \pm$ 6.62	$195.33^{NS} \pm$ 5.87	$198.67^{NS} \pm$ 6.03	$201.00^{NS} \pm$ 7.03	$196.33^{NS} \pm$ 5.52
PCV (%)	$32.50^{NS} \pm$ 0.75	$32.33^{NS} \pm$ 0.98	$33.67^{NS} \pm$ 1.03	$32.83^{NS} \pm$ 0.90	$33.50^{NS} \pm$ 1.02
MCV ($mm^{3)}$	$82.04^{NS} \pm$ 1.25	$81.66^{NS} \pm$ 1.64	$80.67^{NS} \pm$ 1.78	$82.29^{NS} \pm$ 1.41	$82.69^{NS} \pm$ 1.37
MCH (pg /cell)	$25.73^{NS} \pm$ 0.71	$25.68^{NS} \pm$ 0.83	$26.06^{NS} \pm$ 0.75	$27.01^{NS} \pm$ 0.95	$26.33^{NS} \pm$ 0.67
MCHC (%)	$31.64^{NS} \pm$ 0.85	$32.04^{NS} \pm$ 0.91	$32.44^{NS} \pm$ 0.79	$31.89^{NS} \pm$ 0.82	$31.45^{NS} \pm$ .92

Table 17(g): Values are Mean ±S.E., where n =6 in each group;
NS = non significant($p<0.05$)

Synovial LDH activity (U/L) of septic arthritic (Gr- I),oral linezolid @ 75 mg kg^{-1} for 10 days twice daily (Gr – II), oral linezolid @ 75 mg kg^{-1} for 10 days twice daily with a single intra-articular injection of betamethasone @ 0.5 mg kg^{-1} (Gr – III), ethanolic extract (in 2% tween 20) of *Tamarindus indica* L. leaves treatment @ 500 mg kg^{-1} for 14 days(Gr – IV), ethanolic extract (in 2% tween 20) of *Tamarindus indica* L. leaves treatment @ 1000 mg kg^{-1} for 14 days(Gr – V), aqueous extract of *Tamarindus indica* L. leaves treatment @ 500 mg kg^{-1} for 14 days (Gr – VI) and aqueous extract of *Tamarindus indica* L. leaves treatment @ 1000 mg kg^{-1} for 14 days(Gr – VII) in rabbits have been depicted in table 18. The synovial fluid LDH level was ranged from 49.40 ± 11.79 to 61.00 ± 7.63 in healthy rabbits. The LDH level of synovial fluid was increased gradually in all the groups up to day 7 and subsequently decreased on day 16. The LDH level was reduced more markedly in different treatment groups while it was found less markedly in rabbits of Gr-I. In accordance with the present findings, Alan S. Cohen (1964) also reported samples of synovial fluid with proven septic arthritis showed marked elevations in LDH (mean 1279 units).

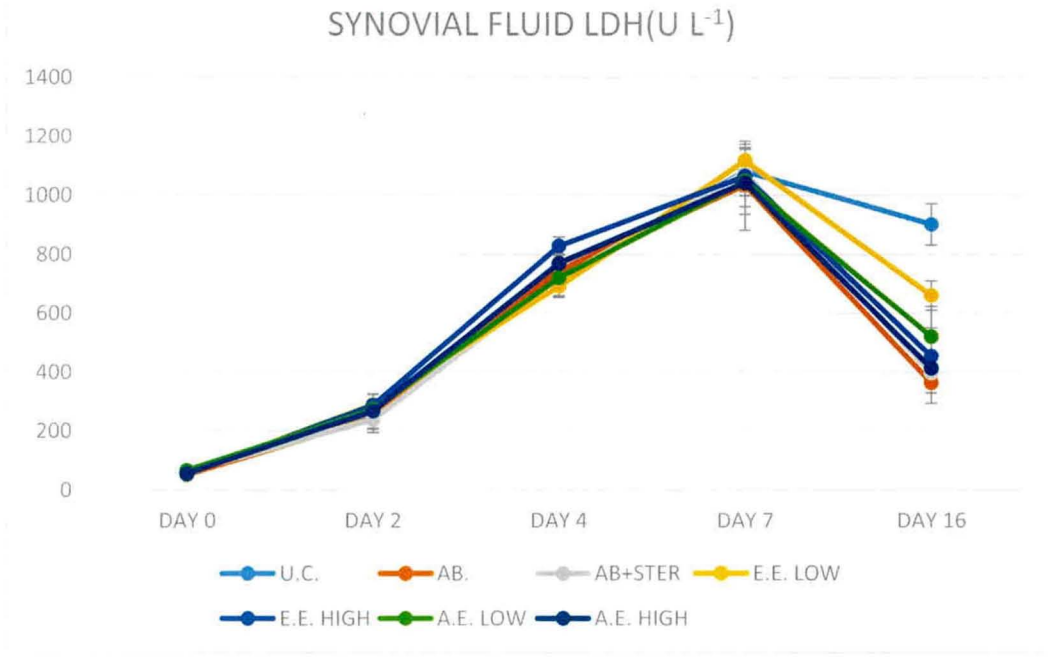


Fig xxix: mean ±S.E. synovial LDH level of different groups on different days

Table 18: Synovial LDH level(U/L) of septic arthritic(Gr- I),oral linezolid @ 75 mg kg^{-1} for 10 days twice daily (Gr – II), oral linezolid @ 75 mg kg^{-1} for 10 days twice daily with a single intra-articular injection of betamethasone @ 0.5 mg kg^{-1} (Gr – III), ethanolic extract (in 2% tween 20) of *Tamarindus indica* L. leaves treatment @ 500 mg kg^{-1} for 14 days(Gr – IV), ethanolic extract (in 2% tween 20) of *Tamarindus indica* L. leaves treatment @ 1000 mg kg^{-1} for 14 days(Gr – V), aqueous extract of *Tamarindus indica* L. leaves treatment @ 500 mg kg^{-1} for 14 days (Gr – VI) and aqueous extract of *Tamarindus indica* L. leaves treatment @ 1000 mg kg^{-1} for 14 days (Gr – VII) in rabbits [n=6]

Day / Group	0	2	4	7	16
Gr-I	$49.40^{a} \pm 11.79$	$252.35^{b} \pm 45.59$	$728.72^{ce} \pm 68.81$	$1081.15^{d} \pm 81.79$	$902.09^{e} \pm 69.66$
Gr-II	$51.22^{a} \pm 10.28$	$241.85^{ad} \pm 48.37$	$745.40^{b} \pm 36.12$	$1034.14^{c} \pm 151.02$	$363.67^{d} \pm 69.68$
Gr-III	$61.00^{a} \pm 7.63$	$237.00^{b} \pm 29.09$	$715.43^{c} \pm 36.20$	$1076.68^{d} \pm 78.07$	$396.92^{e} \pm 67.06$
Gr-IV	$50.75^{a} \pm 8.37$	$289.58^{b} \pm 34.63$	$691.56^{ce} \pm 38.13$	$1116.42^{d} \pm 57.93$	$660.83^{e} \pm 50.34$
Gr-V	$52.31^{a} \pm 8.88$	$286.60^{b} \pm 40.01$	$828.36^{c} \pm 30.76$	$1064.03^{d} \pm 51.02$	$453.97^{e} \pm 97.07$
Gr-VI	$65.62^{a} \pm 6.68$	$272.01^{b} \pm 22.44$	$720.61^{ce} \pm 30.99$	$1047.07^{d} \pm 110.47$	$520.90^{e} \pm 103.37$
Gr-VII	$55.33^{a} \pm 8.68$	$267.13^{b} \pm 29.66$	$770.09^{c} \pm 32.76$	$1042.10^{d} \pm 80.24$	$412.85^{e} \pm 46.30$

Table 18: Values are Mean ±S.E., n=6 in each groups

Values with dissimilar superscript (abcde) in the row vary significantly (P<0.05)

Mean ±S.E. values of serum, Synovial fluid glucose level (mg dl^{-1}) and simultaneous serum, synovial fluid glucose difference of septic arthritic (Gr- I),oral linezolid @ 75 mg kg^{-1} for 10 days twice daily (Gr – II), oral linezolid @ 75 mg kg^{-1} for 10 days twice daily with a single intra-articular injection of betamethasone @ 0.5 mg kg^{-1} (Gr – III), ethanolic extract (in 2% tween 20) of *Tamarindus indica* L. leaves treatment @ 500 mg

kg^{-1} for 14 days(Gr – IV), ethanolic extract (in 2% tween 20) of *Tamarindus indica* L. leaves treatment @ 1000 mg kg^{-1} for 14 days(Gr – V), aqueous extract of *Tamarindus indica* L. leaves treatment @ 500 mg kg^{-1} for 14 days (Gr – VI) and aqueous extract of *Tamarindus indica* L. leaves treatment @ 1000 mg kg^{-1} for 14 days(Gr – VII) in rabbits have been depicted in table 19. The mean Serum glucose level did not alter significantly in any group except in rabbits of Gr-III (oral linezolid @ 75 mg kg^{-1} for 10 days twice daily with a single intra-articular injection of betamethasone @ 0.5 mg kg^{-1}), where it was increased significantly from day 7 to day 16 (Figure xxx). While significant alteration was found in synovial glucose level in all the groups (Figure xxxi). The simultaneous difference in glucose level between Serum and synovial fluid was found to be ranged from 11.38 ± 0.69 to 14.14 ± 1.07 in healthy rabbits. The difference was gradually increased up to day 7 in all the groups but it was subsequently reduced (<25 mg dl^{-1}) significantly in rabbits of Gr - II, Gr – III, Gr – V, Gr – VI and Gr – VII and non-significantly (>25 mg dl^{-1}) in Gr - I and Gr – IV (Figure xxxii).In the presence of bacterial infection, synovial fluid glucose may be at least 25 mg dl^{-1} lower than a simultaneous blood glucose [Joint Fluid (Chapter no.166, Clinical Methods,3rd edition) Alice Faryna and Kim Goldenberg]. So, the findings suggested better recovery in Gr - II, Gr – III, Gr – V, Gr – VI and Gr – VII.

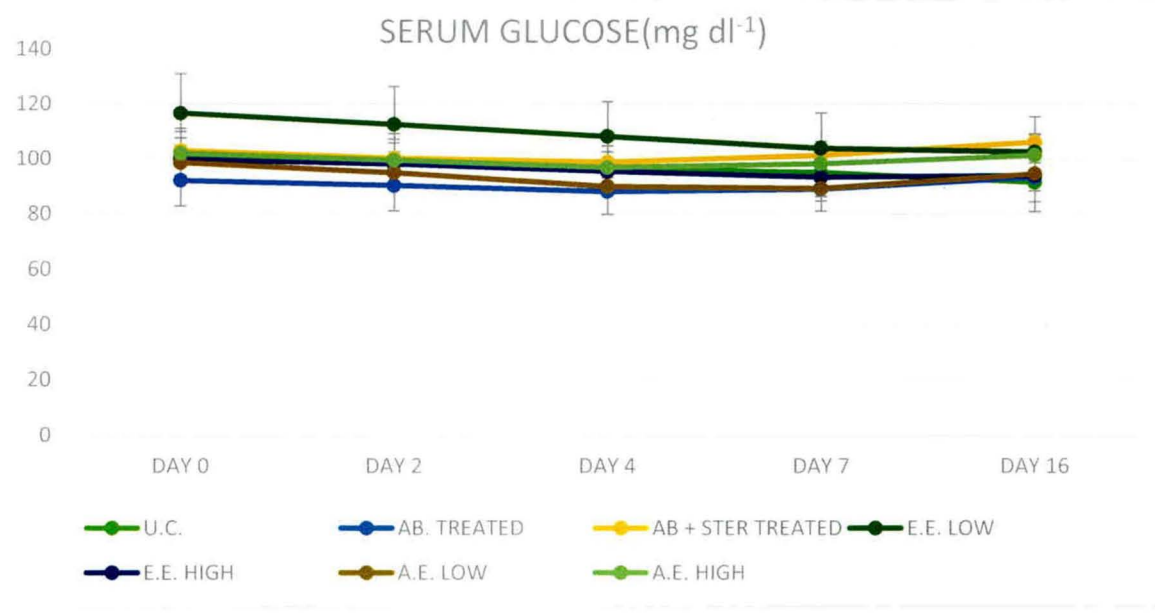


Fig xxx: mean ±S.E. Serum glucose level (mg dl^{-1}) of different groups on different days

Table 19: Mean Glucose level (mg dl^{-1}) in serum and synovial fluid of septic arthritic(Gr- I),oral linezolid @ 75 mg kg^{-1} for 10 days twice daily (Gr – II), oral linezolid @ 75 mg kg^{-1} for 10 days twice daily with a single intra-articular injection of betamethasone @ 0.5 mg kg^{-1} (Gr – III), ethanolic extract (in 2% tween 20) of *Tamarindus indica* L. leaves treatment @ 500 mg kg^{-1} for 14 days(Gr – IV), ethanolic extract (in 2% tween 20) of *Tamarindus indica* L. leaves treatment @ 1000 mg kg^{-1} for 14 days(Gr – V), aqueous extract of *Tamarindus indica* L. leaves treatment @ 500 mg kg^{-1} for 14 days (Gr – VI) and aqueous extract of *Tamarindus indica* L. leaves treatment @ 1000 mg kg^{-1} for 14 days (Gr – VII) in rabbits [n=6]

Day / Group	0			2			4			7			16		
	Serum	S.F.	Diff	Serum	S.F.	Diff	Serum	S.F.	Diff	Serum	S.F.	Diff	Serum	S.F.	Diff
Gr -I	100.90^{a} ± 10.02	87.67^{v} ± 9.89	13.23^{p} ± 0.97	98.82^{a} ± 10.2	78.19^{vw} ± 6.87	20.63^{pr} ± 3.73	96.37^{a} ± 10.13	60.01^{wxy} ± 5.27	36.36^{pqr} ± 6.59	94.93^{a} ± 10.24	47.24^{xy} ± 4.84	47.69^{qr} ± 12.11	91.40^{a} ± 10.51	46.91^{y} ± 6.26	44.49^{r} ± 11.64
Gr -II	91.95^{a} ± 9.24	78.70^{v} ± 7.44	13.25^{p} ± 2.13	90.19^{a} ± 9.12	71.92^{vwx} ± 7.89	18.24^{ps} ± 2.29	88.02^{a} ± 8.22	57.70^{wxy} ± 6.39	30.32^{qr} ± 2.40	88.93^{a} ± 7.94	53.82^{xy} ± 6.25	35.12^{r} ± 3.02	93.22^{a} ± 8.76	71.62^{vy} ± 6.67	21.60^{s} ± 3.58
Gr -III	102.83^{ab} ± 2.12	88.70^{v} ± 2.30	14.14^{p} ± 1.07	100.12^{ab} ± 2.34	81.03^{v} ± 2.72	19.08^{p} ± 2.67	98.72^{a} ± 1.51	69.60^{wx} ± 3.64	29.11^{qr} ± 2.82	101.31^{ab} ± 2.08	66.46^{x} ± 4.53	34.84^{r} ± 3.60	106.05^{b} ± 2.69	85.51^{v} ± 3.48	20.55^{p} ± 2.57
Gr IV	116.43^{a} ± 14.19	102.76^{v} ± 13.8	13.67^{p} ± 1.06	112.37^{a} ± 13.77	94.01^{vwx} ±14.75	18.35^{ps} ± 1.10	108.07^{a} ± 12.72	74.11^{vwx} ± 8.82	33.96^{qrs} ± 5.92	103.83^{a} ± 12.97	64.35^{wx} ± 7.20	39.47^{rs} ± 7.45	102.49^{a} ± 12.91	69.62^{x} ± 8.29	32.88^{s} ± 6.42
Gr -V	99.67^{a} ± 7.79	88.29^{v} ± 7.88	11.38^{p} ± 0.69	97.99^{a} ± 7.75	78.77^{vw} ± 7.76	19.22^{qt} ± 2.79	95.30^{a} ± 7.27	64.58^{wxy} ± 5.25	30.72^{rs} ± 3.65	93.24^{a} ± 6.88	58.91^{xy} ± 5.30	34.34^{s} ± 2.64	93.99^{a} ± 5.62	71.48^{vy} ± 4.51	22.51^{t} ± 1.91
Gr - VI	98.41^{a} ± 4.49	86.17^{v} ± 4.18	12.23^{p} ± 1.02	94.79^{a} ± 4.21	74.82^{vy} ± 4.65	19.96^{q} ± 1.24	89.86^{a} ± 3.20	57.81^{wxy} ± 4.97	32.05^{rst} ± 2.68	89.20^{a} ± 2.87	53.01^{xy} ± 5.44	36.18^{st} ± 3.70	94.53^{a} ± 4.07	64.66^{y} ± 2.94	29.88^{t} ± 3.35
Gr - VII	101.88^{a} ± 7.88	88.30^{vy} ± 7.33	13.58^{p} ± 0.85	99.40^{a} ± 7.75	77.33^{vwxy} ± 5.99	22.08^{qt} ± 2.66	96.77^{a} ± 7.99	66.84^{wxy} ± 8.12	29.93^{rs} ± 3.97	98.20^{a} ± 8.15	67.69^{xy} ± 7.70	30.51^{s} ± 1.96	101.30^{a} ± 7.82	79.53^{y} ± 5.89	21.77^{t} ± 2.44

Values are Mean ±S.E., n=6 in each groups, Serum values with dissimilar superscript (abcde) ; synovial fluid glucose values with dissimilar superscript (vwxyz) and the simultaneous difference of these two values with dissimilar superscript (pqrst) differ significantly (P<0.05)

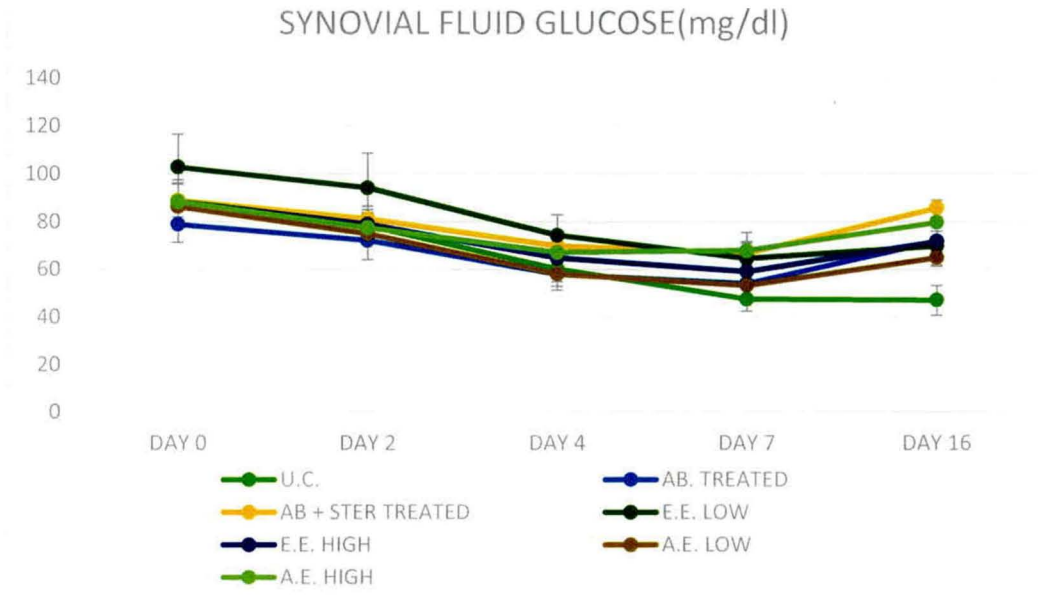


Fig xxxi: mean ±S.E. synovial fluid glucose level (mg dl^{-1}) of different groups on different days

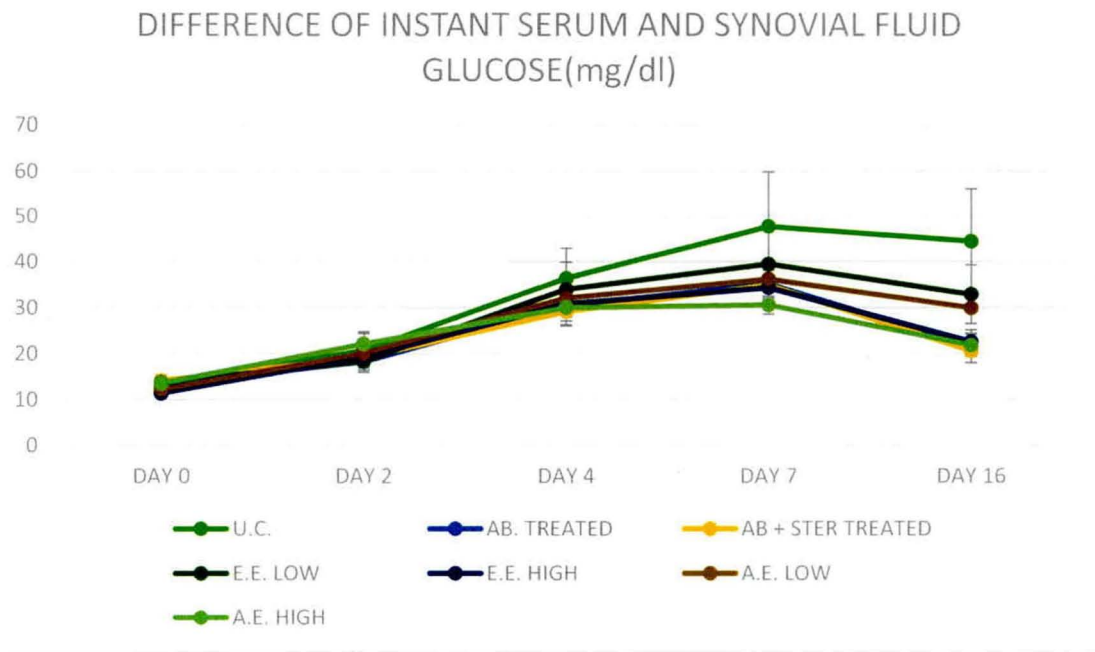


Fig xxxii: mean ±S.E. simultaneous difference in Serum and synovial fluid glucose level (mg dl^{-1}) of different groups on different days

Mean ±S.E. ESR level (mm h^{-1}) in septic arthritic (Gr- I),oral linezolid @ 75 mg kg^{-1} for 10 days twice daily (Gr – II), oral linezolid @ 75 mg kg^{-1} for 10 days twice daily with a single intra-articular injection of betamethasone @ 0.5 mg kg^{-1} (Gr – III), ethanolic extract (in 2% tween 20) of *Tamarindus indica* L. leaves treatment @ 500 mg kg^{-1} for 14 days(Gr – IV), ethanolic extract (in 2% tween 20) of *Tamarindus indica* L. leaves treatment @ 1000 mg kg^{-1} for 14 days(Gr – V), aqueous extract of *Tamarindus indica* L. leaves treatment @ 500 mg kg^{-1} for 14 days (Gr – VI) and aqueous extract of *Tamarindus indica* L. leaves treatment @ 1000 mg kg^{-1} for 14 days(Gr – VII) in rabbits have been depicted in table 20. Mean ESR level was gradually increased up to day 7 following intra-articular inoculation of 10^4 CFU *S. aureus* in the left stifle joint of rabbits and significantly declined on day 16 in all the groups. The intensity of reduction of all the treatment groups was observed to be more compared to untreated arthritic group (Figure xxxiii).

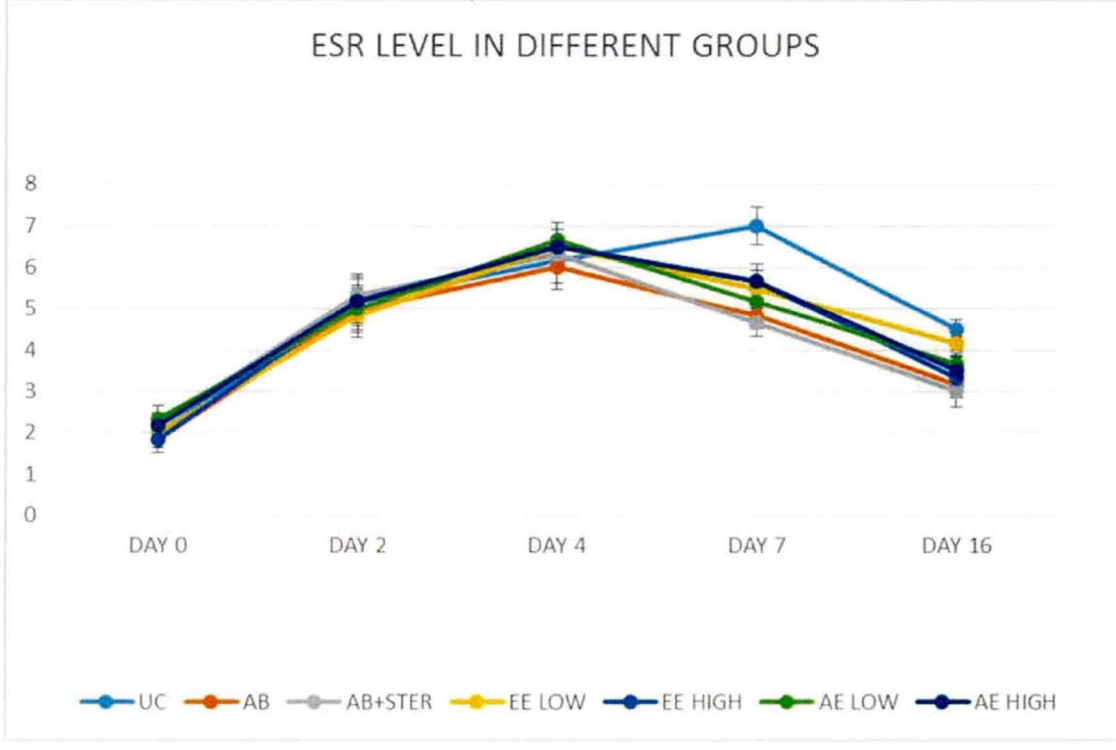


Fig xxxiii: mean ±S.E. synovial fluid ESR level (mm h^{-1}) of different groups on different days

Table 20: Mean ESR level (mm h^{-1}) of septic arthritic(Gr- I),oral linezolid @ 75 mg kg^{-1} for 10 days twice daily (Gr – II), oral linezolid @ 75 mg kg^{-1} for 10 days twice daily with a single intra-articular injection of betamethasone @ 0.5 mg kg^{-1} (Gr – III), ethanolic extract (in 2% tween 20) of *Tamarindus indica* L. leaves treatment @ 500 mg kg^{-1} for 14 days(Gr – IV), ethanolic extract (in 2% tween 20) of *Tamarindus indica* L. leaves treatment @ 1000 mg kg^{-1} for 14 days(Gr – V), aqueous extract of *Tamarindus indica* L. leaves treatment @ 500 mg kg^{-1} for 14 days (Gr – VI) and aqueous extract of *Tamarindus indica* L. leaves treatment @ 1000 mg kg^{-1} for 14 days (Gr – VII) in rabbits [n=6]

DAY / GROUP	0	2	4	7	16
Gr-I	$2.00^{a} \pm 0.26$	$5.17^{bce} \pm 0.60$	$6.17^{cd} \pm 0.54$	$7.00^{d} \pm 0.45$	$4.50^{e} \pm 0.22$
Gr-II	$1.83^{a} \pm 0.31$	$5.00^{bcd} \pm 0.58$	$6.00^{cd} \pm 0.52$	$4.83^{d} \pm 0.31$	$3.17^{e} \pm 0.31$
Gr-III	$2.17^{a} \pm 0.31$	$5.33^{bcd} \pm 0.49$	$6.33^{c} \pm 0.42$	$4.67^{d} \pm 0.33$	$3.00^{a} \pm 0.37$
Gr-IV	$2.00^{a} \pm 0.37$	$4.83^{bde} \pm 0.54$	$6.50^{cf} \pm 0.43$	$5.50^{df} \pm 0.43$	$4.17^{e} \pm 0.17$
Gr-V	$1.83^{a} \pm 0.31$	$5.17^{bd} \pm 0.54$	$6.50^{cf} \pm 0.43$	$5.67^{df} \pm 0.42$	$3.33^{e} \pm 0.49$
Gr-VI	$2.33^{a} \pm 0.33$	$5.00^{bd} \pm 0.52$	$6.67^{c} \pm 0.42$	$5.17^{d} \pm 0.31$	$3.67^{e} \pm 0.21$
Gr-VII	$2.17^{a} \pm 0.31$	$5.17^{bd} \pm 0.54$	$6.50^{cf} \pm 0.43$	$5.67^{df} \pm 0.42$	$3.50^{e} \pm 0.43$

Table 20: Values are Mean ±S.E., n=6 in each groups

Values with dissimilar superscript (abcde) in the row vary significantly (P<0.05)

Mean body temperature (°F) in septic arthritic (Gr- I), oral linezolid @ 75 mg kg^{-1} for 10 days twice daily (Gr – II), oral linezolid @ 75 mg kg^{-1} for 10 days twice daily with a single intra-articular injection of betamethasone @ 0.5 mg kg^{-1} (Gr – III), ethanolic extract (in 2% tween 20) of *Tamarindus indica* L. leaves treatment @ 500 mg kg^{-1} for 14 days(Gr – IV), ethanolic extract (in 2% tween 20) of *Tamarindus indica* L. leaves treatment @ 1000 mg kg^{-1} for 14 days(Gr – V), aqueous extract of *Tamarindus indica* L. leaves treatment @ 500 mg kg^{-1} for 14 days (Gr – VI) and aqueous extract of *Tamarindus indica* L. leaves treatment @ 1000 mg kg^{-1} for 14 days(Gr – VII) in rabbits have been depicted in table 21. Body temperature was significantly increased up to day 5 following intra-articular inoculation of 10^4 CFU *S. aureus* in the left stifle joint of rabbits of all the groups except Gr- VII (Aqueous extract of Tamarindus indica L. leaves treatment @ 1000 mg/kg for 14 days). Thereafter the body temperature was found to be decreased on day 16 in all the groups (figure xxxiv)

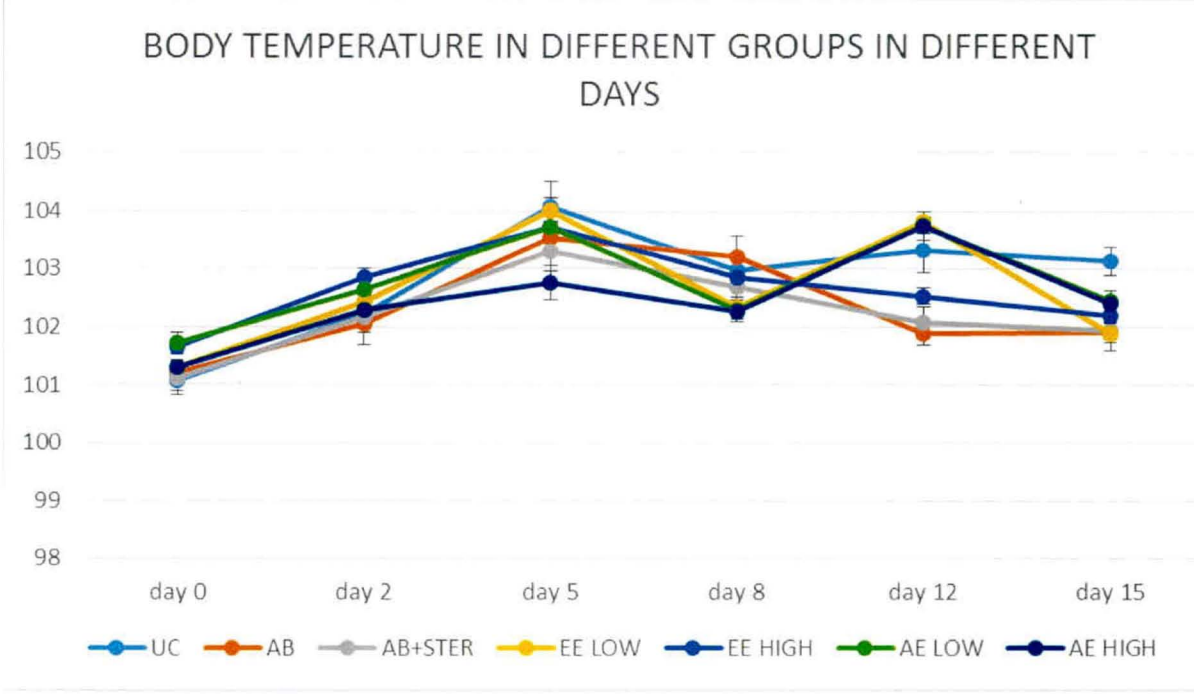


Fig xxxiv: mean ±S.E. of body temperature (°F) of different groups on different days

Table 21: Mean body temperature (°F) in septic arthritic(Gr- I),oral linezolid @ 75 mg kg^{-1} for 10 days twice daily (Gr – II), oral linezolid @ 75 mg kg^{-1} for 10 days twice daily with a single intra-articular injection of betamethasone @ 0.5 mg kg^{-1} (Gr – III), ethanolic extract (in 2% tween 20) of *Tamarindus indica* L. leaves treatment @ 500 mg kg^{-1} for 14 days(Gr – IV), ethanolic extract (in 2% tween 20) of *Tamarindus indica* L. leaves treatment @ 1000 mg kg^{-1} for 14 days(Gr – V), aqueous extract of *Tamarindus indica* L. leaves treatment @ 500 mg kg^{-1} for 14 days (Gr – VI) and aqueous extract of *Tamarindus indica* L. leaves treatment @ 1000 mg kg^{-1} for 14 days (Gr – VII) in rabbits [n=6]

DAY / GROUP	0	2	5	8	12	15
Gr-I	101.07^{a} ± 0.24	102.22^{bd} ± 0.12	104.08^{ce} ± 0.43	102.97^{dgf} ± 0.22	103.32^{egf} ± 0.37	103.13^{f} ± 0.25
Gr-II	101.2^{a} ± 0.15	102.05^{bef} ± 0.35	103.55^{cd} ± 0.2	103.2^{d} ± 0.37	101.88^{ae} ± 0.19	101.91^{af} ± 0.17
Gr-III	101.12^{af} ± 0.23	102.17^{bdeg} ± 0.26	103.3^{ch} ± 0.33	102.68^{dheg} ± 0.27	102.08^{eg} ± 0.27	101.93^{fg} ± 0.33
Gr-IV	101.32^{a} ± 0.11	102.43^{bd} ± 0.1	104.02^{ce} ± 0.21	102.33^{d} ± 0.19	103.82^{e} ± 0.1	101.88^{f} ± 0.09
Gr-V	101.65^{a} ± 0.12	102.85^{bde} ± 0.15	103.72^{c} ± 0.12	102.87^{de} ± 0.08	102.53^{ef} ± 0.15	102.2^{f} ± 0.13
Gr-VI	101.73^{a} ± 0.17	102.65^{bdf} ± 0.11	103.72^{ce} ± 0.22	102.28^{df} ± 0.2	103.75^{e} ± 0.12	102.45^{f} ± 0.19
Gr-VII	101.3^{a} ± 0.11	102.28^{bcdf} ± 0.16	102.77^{cdf} ± 0.3	102.27^{df} ± 0.16	103.75^{e} ± 0.24	102.4^{f} ± 0.11

Table 21: Values are Mean ±S.E., n=6 in each groups

Values with dissimilar superscript (abcde) in the row vary significantly (P<0.05)

Mean ±S.E. values of Serum, Synovial fluid total protein(g/dl) and instant Serum, synovial fluid total protein ratio in arthritic(Gr- I),oral Linezolid @ 75mg/kg for 10 days(Gr – II), oral Linezolid @ 75mg/kg for 10 days with a single intra-articular injection of Betamethasone @ 0.5 mg/kg(Gr – III), Ethanolic Extract(in 2% Tween20) of Tamarindusindica L. leaves treatment @ 500 mg/kg for 14 days(Gr – IV), Ethanolic Extract(in 2% Tween20) of *Tamarindus indica* L. leaves treatment @ 1000 mg/kg for 14 days(Gr – V), Aqueous Extract of *Tamarindus indica* L. leaves treatment @ 500 mg/kg for 14 days (Gr – VI) and Aqueous Extract of *Tamarindus indica* L. leaves treatment @ 1000 mg/kg for 14 days(Gr – VII) have been depicted in table no. . The Serum total protein level did not alter significantly in any group (Figure xxxv). But significant alteration was found in synovial total protein level in all the groups. In all the treatment groups, a trend of declining of synovial fluid total protein level after 4 days post-inoculation of 10^4 CFU *S. aureus* in the left stifle joints of rabbits were noticed. But in untreated group synovial total protein level was found to be increased on different days. Total protein level in synovial fluid was observed to be above 4.5 g dl^{-1} on day 4 following inoculation of 10^4 CFU *S. aureus* in the left stifle joints of rabbits, which indicated significant inflammation in the left stifle joints of rabbits of all the experimental groups. However, the total protein level in synovial fluid of rabbits of Gr-II (oral Linezolid @ 75mg kg^{-1} for 10 days twice daily), Gr-III (oral linezolid @ 75mg kg^{-1} for 10 days twice daily with a single intra-articular injection of betamethasone @ 0.5 mg kg^{-1}) and Gr- VII (Aqueous Extract of *Tamarindus indica* L. leaves treatment @ 1000 mg kg^{-1} for 14 days) were significantly reduced to < 2.5 g dl^{-1} (Figure xxxvi) The findings corroborated with the statement written in Joint Fluid (Chapter no.166, Clinical Methods, 3rd edition) Alice Faryna and Kim Goldenberg where it was mentioned that Synovial fluid protein levels greater 2 .5 g dl^{-1} are abnormal, and those greater than 4.5g/dl indicate significant inflammation. The instant ratio of total protein between Serum and synovial protein in arthritic rabbits approached the normal ratio of healthy rabbits in all the treated groups on day 16 (xxxvii).

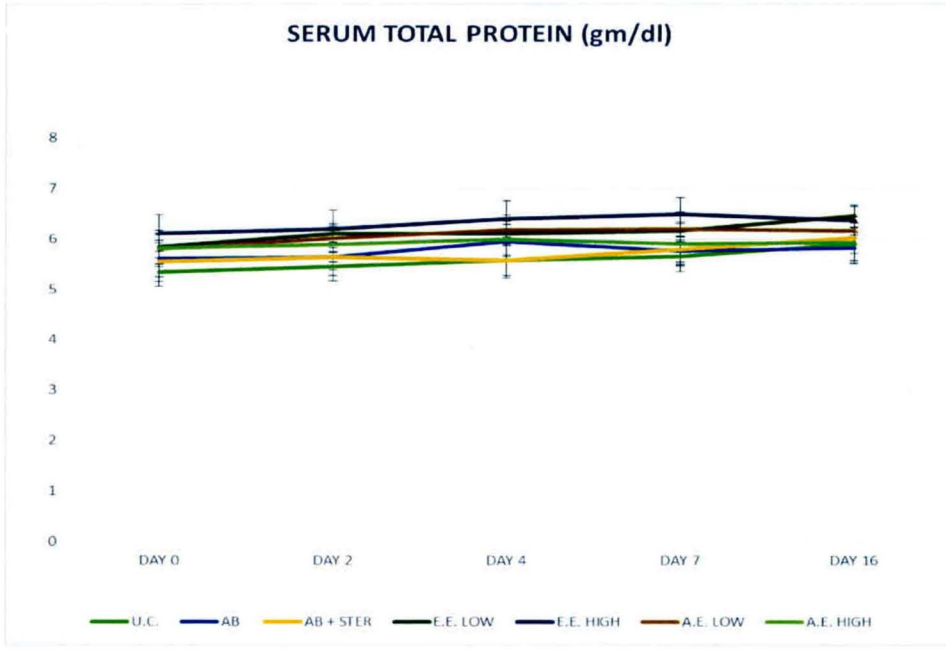


Fig xxxv: mean ±S.E.Serum total protein level (gm dl^{-1}) of different groups of animals on different days

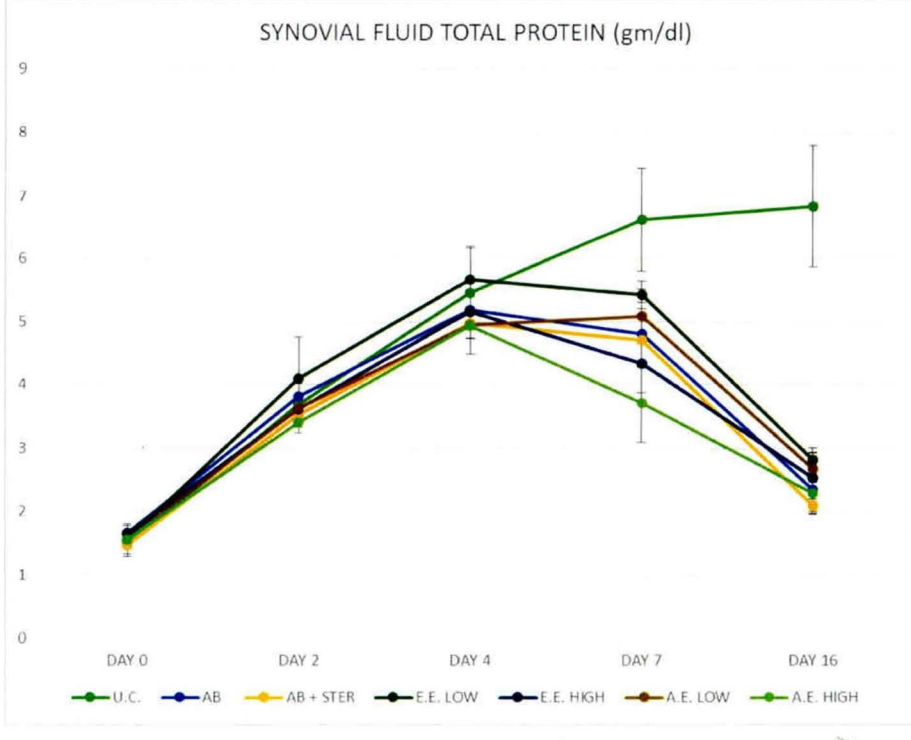


Fig xxxvi: mean ±S.E. synovial protein total protein level (gm dl^{-1}) of different groups of animals on different days

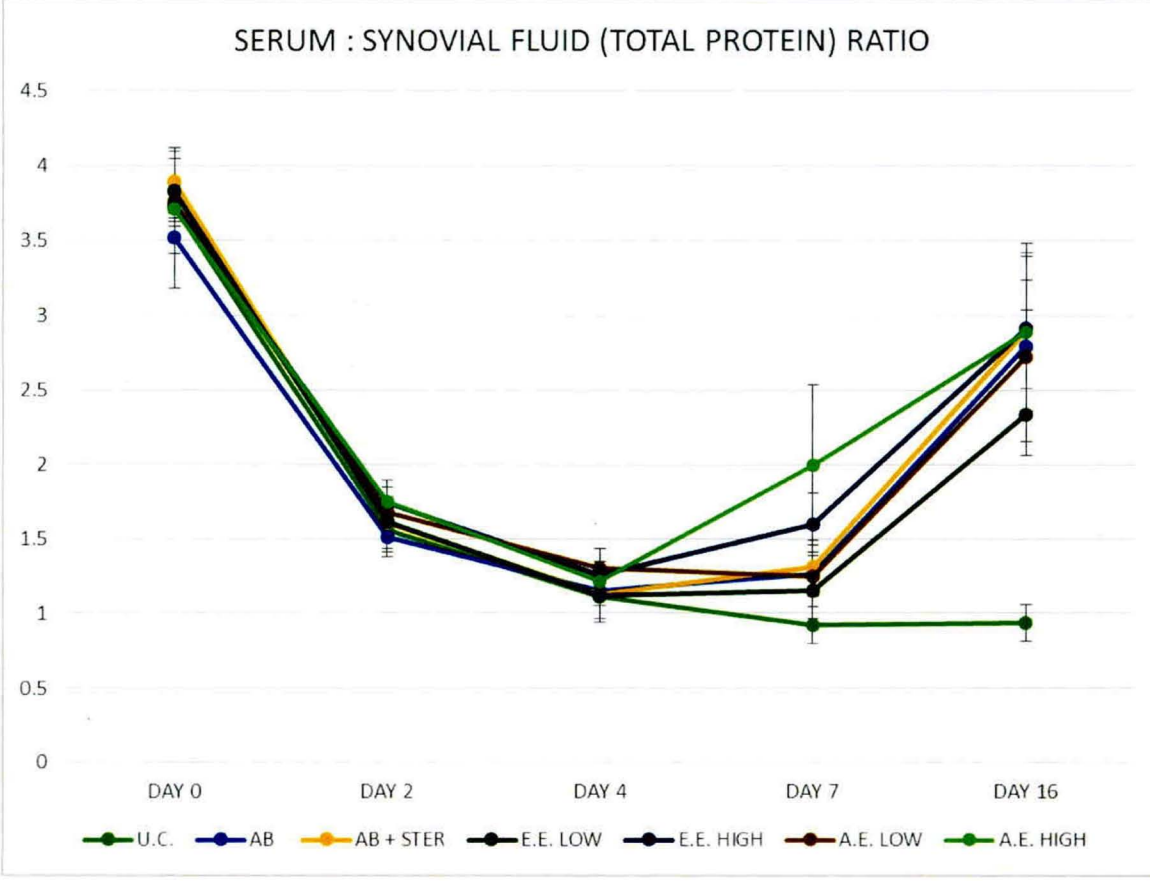


Fig xxxvii: mean ±S.E. synovial protein total protein level (gm dl^{-1}) of different groups of animals on different days

Table 22: Mean total protein level (mg dl^{-1}) in serum and synovial fluid of septic arthritic(Gr- I),oral linezolid @ 75 mg kg^{-1} for 10 days twice daily (Gr – II), oral linezolid @ 75 mg kg^{-1} for 10 days twice daily with a single intra-articular injection of betamethasone @ 0.5 mg kg^{-1} (Gr – III), ethanolic extract (in 2% tween 20) of *Tamarindus indica* L. leaves treatment @ 500 mg kg^{-1} for 14 days(Gr – IV), ethanolic extract (in 2% tween 20) of *Tamarindus indica* L. leaves treatment @ 1000 mg kg^{-1} for 14 days(Gr – V), aqueous extract of *Tamarindus indica* L. leaves treatment @ 500 mg kg^{-1} for 14 days (Gr – VI) and aqueous extract of *Tamarindus indica* L. leaves treatment @ 1000 mg kg^{-1} for 14 days (Gr – VII) in rabbits [n=6]

	Day 0			Day 2			Day 4			Day 7			Day 16		
	Serum	S.F.	Ratio	Serum	S.F.	Ratio	Serum	S.F.	Ratio	Serum	S.F.	Ratio	Serum	S.F.	Ratio
U.C.	5.34^{a} ±0.29	1.49^{v} ± 0.16	3.73^{p} ± 0.32	5.45^{a} ± 0.28	3.68^{wx} ± 0.34	1.56^{qr} ± 0.18	5.58^{a} ± 0.30	5.46^{xyz} ± 0.74	1.11^{rst} ± 0.14	5.65^{a} ± 0.30	6.63^{yz} ± 0.82	0.92^{st} ± 0.12	5.90^{a} ± 0.32	6.84^{z} ± 0.97	0.94^{t} ± 0.12
Ab. treated	5.60^{a} ±0.35	1.65^{v} ± 0.15	3.52^{pt} ± 0.34	5.64^{a} ±0.25	3.81^{wz} ± 0.26	1.51^{qrs} ± 0.10	5.94^{a} ± 0.27	5.19^{xy} ± 0.31	1.15^{rs} ± 0.04	5.75^{a} ± 0.29	4.81^{yz} ± 0.50	1.26^{s} ± 0.14	5.82^{a} ± 0.32	2.35^{v} ± 0.39	2.80^{t} ± 0.44
Ab. + St.treated	5.54^{a} ±0.38	1.46^{vz} ± 0.18	3.89^{p} ± 0.21	5.64^{a} ±0.37	3.53^{w} ± 0.20	1.60^{qs} ± 0.09	5.56^{a} ± 0.33	4.97^{xy} ± 0.23	1.13^{ru} ± 0.07	5.80^{a} ± 0.25	4.70^{y} ± 0.42	1.31^{su} ± 0.18	6.03^{a} ± 0.31	2.09^{z} ± 0.12	2.90^{t} ± 0.14
E.E. low	5.84^{a} ±0.22	1.55^{v} ± 0.10	3.83^{p} ± 0.28	6.10^{ab} ±0.17	4.10^{w} ± 0.65	1.61^{qrs} ± 0.17	6.10^{ab} ± 0.19	5.68^{xy} ± 0.50	1.12^{rs} ± 0.11	6.16^{ab} ± 0.18	5.42^{y} ± 0.22	1.15^{s} ± 0.07	6.46^{b} ± 0.21	2.81^{z} ± 0.13	2.33^{t} ± 0.18
E.E. high	6.11^{a} ±0.37	1.65^{v} ± 0.13	3.73^{p} ± 0.10	6.19^{a} ±0.37	3.61^{wy} ± 0.23	1.74^{qrs} ± 0.15	6.40^{a} ± 0.36	5.16^{xz} ± 0.23	1.25^{rs} ± 0.10	6.50^{a} ± 0.33	4.34^{yz} ± 0.46	1.60^{s} ± 0.21	6.37^{a} ± 0.28	2.54^{v} ± 0.39	2.91^{p} ± 0.57
A.E. Low	5.80^{a} ±0.30	1.56^{v} ± 0.11	3.75^{p} ± 0.13	6.01^{a} ±0.29	3.62^{wz} ± 0.21	1.68^{qrs} ± 0.08	6.17^{a} ± 0.31	4.95^{xy} ± 0.47	1.30^{rs} ± 0.13	6.20^{a} ± 0.33	5.09^{y} ± 0.44	1.24^{s} ± 0.08	6.16^{a} ± 0.28	2.67^{z} ± 0.34	2.73^{t} ± 0.67
A.E. high	5.81^{a} ±0.36	1.56^{v} ± 0.06	3.71^{p} ± 0.11	5.89^{a} ±0.35	3.40^{wy} ± 0.17	1.74^{qrs} ± 0.11	6.00^{a} ± 0.35	4.94^{x} ± 0.21	1.22^{rs} ± 0.06	5.90^{a} ± 0.41	3.71^{y} ± 0.61	1.99^{sp} ± 0.54	5.93^{a} ± 0.40	2.28^{v} ± 0.29	2.89^{p} ± 0.52

Values are Mean ±S.E., n=6 in each groups, Serum values with dissimilar superscript (abcde) ; synovial fluid total protein values with dissimilar superscript (vwxyz) and the ratio of these two values with dissimilar superscript (pqrst) differ significantly (P<0.05)

Mean body weight (gm) in septic arthritic (Gr- I),oral linezolid @ 75 mg kg^{-1} for 10 days twice daily (Gr – II), oral linezolid @ 75 mg kg^{-1} for 10 days twice daily with a single intra-articular injection of betamethasone @ 0.5 mg kg^{-1} (Gr – III), ethanolic extract (in 2% tween 20) of *Tamarindus indica* L. leaves treatment @ 500 mg kg^{-1} for 14 days(Gr – IV), ethanolic extract (in 2% tween 20) of *Tamarindus indica* L. leaves treatment @ 1000 mg kg^{-1} for 14 days(Gr – V), aqueous extract of *Tamarindus indica* L. leaves treatment @ 500 mg kg^{-1} for 14 days (Gr – VI) and aqueous extract of *Tamarindus indica* L. leaves treatment @ 1000 mg kg^{-1} for 14 days(Gr – VII) in rabbits have been displayed in table. . The body weight of the animals was observed to be decreased non-significantly up to day 8 in all the groups while increase in body weight was noticed on day 12 and 16. The body weight of the rabbits treated with aqueous extract of *Tamarindus indica* L. leaves @ 1000 mg kg^{-1} for 14 days (Gr- VII) return to nearly control value on day 16 after completion of the treatment (figure xxxviii)

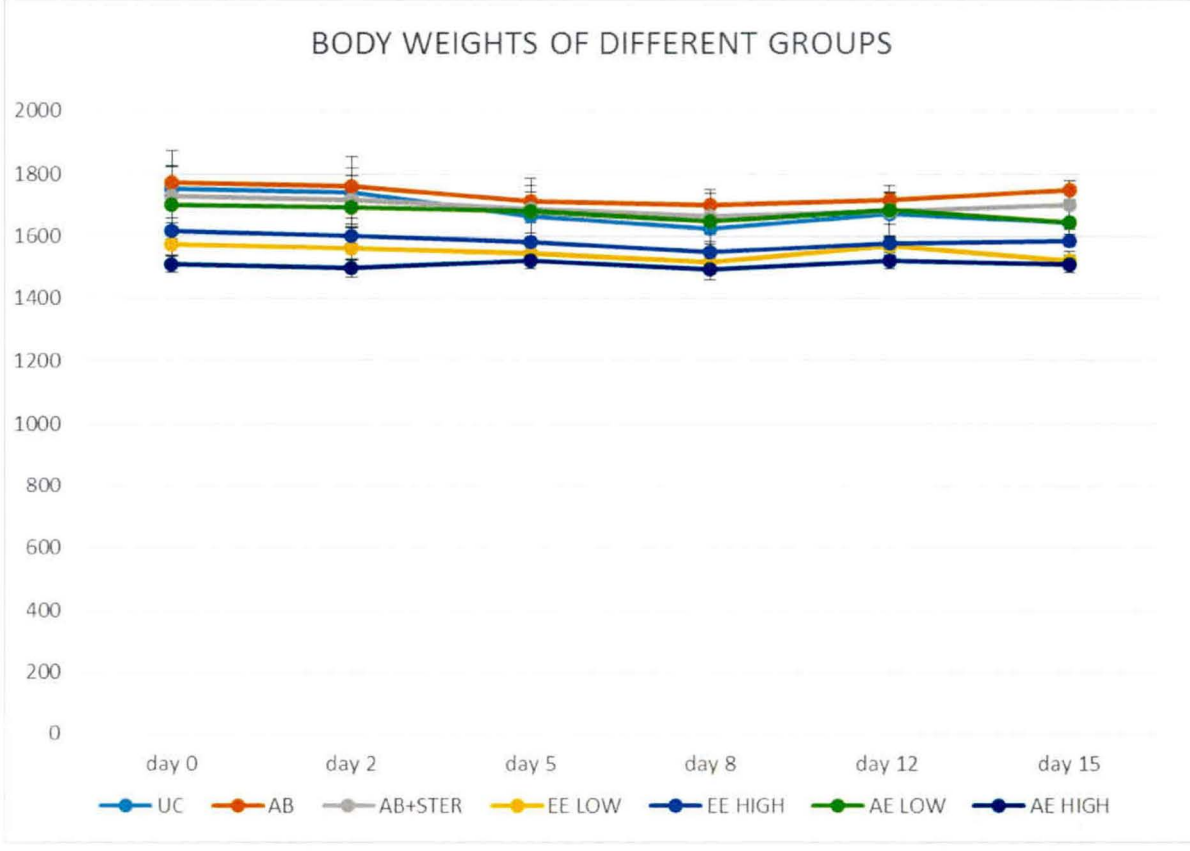


Fig xxxviii: mean ±S.E. body weights of different groups on different days

Table 23: Mean body weight (gm) in septic arthritic(Gr- I),oral linezolid @ 75 mg kg^{-1} for 10 days twice daily (Gr – II), oral linezolid @ 75 mg kg^{-1} for 10 days twice daily with a single intra-articular injection of betamethasone @ 0.5 mg kg^{-1} (Gr – III), ethanolic extract (in 2% tween 20) of *Tamarindus indica* L. leaves treatment @ 500 mg kg^{-1} for 14 days(Gr – IV), ethanolic extract (in 2% tween 20) of *Tamarindus indica* L. leaves treatment @ 1000 mg kg^{-1} for 14 days(Gr – V), aqueous extract of *Tamarindus indica* L. leaves treatment @ 500 mg kg^{-1} for 14 days (Gr – VI) and aqueous extract of *Tamarindus indica* L. leaves treatment @ 1000 mg kg^{-1} for 14 days (Gr – VII) in rabbits [n=6]

day / group	0	2	5	8	12	15
Gr-I	1753.33^{NS} ± 120.04	1742.50^{NS} ± 110.12	1666.67^{NS} ± 75.69	1627.50^{NS} ± 73.41	1673.33^{NS} ± 68.59	1644.17^{NS} ± 46.98
Gr-II	1772.50^{NS} ± 51.08	1762.50^{NS} ± 54.94	1711.67^{NS} ± 52.64	1700.83^{NS} ± 38.89	1715.83^{NS} ± 23.96	1749.17^{NS} ± 28.47
Gr-III	1727.50^{NS} ± 100.35	1718.33^{NS} ± 78.11	1690^{NS} ± 94.84	1665.83^{NS} ± 83.28	1680.83^{NS} ± 81.09	1700.83^{NS} ± 79.01
Gr-IV	1573.33^{NS} ± 33.95	1561.67^{NS} ± 34.32	1544.17^{NS} ± 34.48	1519.17^{NS} ± 31.87	1568.33^{NS} ± 29.12	1523.33^{NS} ± 28.92
Gr-V	1617.50^{NS} ± 25.91	1602.50^{NS} ± 26.86	1580.83^{NS} ± 28.99	1550.83^{NS} ± 25.57	1578.33^{NS} ± 23.15	1587.50^{NS} ± 20.65
Gr-VI	1701.67^{NS} ± 42.10	1692.50^{NS} ± 34.56	1680^{NS} ± 35.71	1648.33^{NS} ± 40.35	1683.33^{NS} ± 45.56	1645.83^{NS} ± 51.94
Gr-VII	1511.67^{NS} ± 26.35	1496.67^{NS} ± 27.86	1521.67^{NS} ± 24.99	1495^{NS} ± 33.07	1523.33^{NS} ± 26.19	1510.83^{NS} ± 28.33

Values are Mean ±S.E., where n =6 in each group;
NS = non significant($p<0.05$)

Synovial fluid WBC mm^{-3} in septic arthritic (Gr- I),oral linezolid @ 75 mg kg^{-1} for 10 days twice daily (Gr – II), oral linezolid @ 75 mg kg^{-1} for 10 days twice daily with a single intra-articular injection of betamethasone @ 0.5 mg kg^{-1} (Gr – III), ethanolic extract (in 2% tween 20) of *Tamarindus indica* L. leaves treatment @ 500 mg kg^{-1} for 14 days(Gr – IV), ethanolic extract (in 2% tween 20) of *Tamarindus indica* L. leaves treatment @ 1000 mg kg^{-1} for 14 days(Gr – V), aqueous extract of *Tamarindus indica* L. leaves treatment @ 500 mg kg^{-1} for 14 days (Gr – VI) and aqueous extract of *Tamarindus indica* L. leaves treatment @ 1000 mg kg^{-1} for 14 days(Gr – VII) in rabbits have been displayed in table no. . WBC count (/mm^3) in normal synovial fluid was ranged from 126.7 ± 14.53 to 180 ± 18.62. The count was raised significantly up to day 7 in all the groups. On day 7 the untreated control group crossed the cut-off of 50000 / mm^3, which confirmed arthritis. On the other hand all the treated groups did not cross the cut-off value of septic arthritis during study period.

Table 24: Synovial fluid WBC /mm^3 in septic arthritic(Gr- I),oral linezolid @ 75 mg kg^{-1} for 10 days twice daily (Gr – II), oral linezolid @ 75 mg kg^{-1} for 10 days twice daily with a single intra-articular injection of betamethasone @ 0.5 mg kg^{-1} (Gr – III), ethanolic extract (in 2% tween 20) of *Tamarindus indica* L. leaves treatment @ 500 mg kg^{-1} for 14 days(Gr – IV), ethanolic extract (in 2% tween 20) of *Tamarindus indica* L. leaves treatment @ 1000 mg kg^{-1} for 14 days(Gr – V), aqueous extract of *Tamarindus indica* L. leaves treatment @ 500 mg kg^{-1} for 14 days (Gr – VI) and aqueous extract of *Tamarindus indica* L. leaves treatment @ 1000 mg kg^{-1} for 14 days (Gr – VII) in rabbits [n=6]

DAY / GR	DAY 0	DAY 2	DAY 7	DAY 16
Gr-I	$145^{a} \pm 15.22$	$16870^{b} \pm 1302.96$	$59016.7^{c} \pm 3363.77$	$39516.7^{d} \pm 3604.76$
Gr-II	$165^{a} \pm 16.07$	$18683.3^{b} \pm 1847.24$	$25550^{c} \pm 1979.35$	$9600^{d} \pm 1035.70$
Gr-III	$161.7^{a} \pm 24.14$	$19200^{b} \pm 1946.79$	$25983.3^{c} \pm 2239.56$	$9833.3^{d} \pm 1693.65$
Gr-IV	$131.7^{a} \pm 13.52$	$18550^{bd} \pm 2261.53$	$42550^{c} \pm 4442.43$	$24616.7^{d} \pm 2598.13$
Gr-V	$126.7^{a} \pm 14.53$	$19200^{bd} \pm 2207.71$	$33166.7^{c} \pm 3145.97$	$13433.3^{d} \pm 1691.28$
Gr-VI	$138.3^{a} \pm 26.26$	$20366.7^{b} \pm 1173.50$	$39933.3^{c} \pm 4808.37$	$29933.3^{d} \pm 1905.55$
Gr-VII	$180^{a} \pm 18.62$	$19933.3^{b} \pm 1864.34$	$29750^{c} \pm 1421.91$	$12766.7^{d} \pm 1657.44$

Values are Mean ±S.E., n=6 in each groups

Values with dissimilar superscript (abcde) in the row vary significantly (P<0.05)

Serum Procalcitonin, at a cut – off of 0.4 ng/ml, is aS.E.nsitive and specific marker in the diagnosis of septic Arthritis in human patients (Maharajan *et al.*2013). Intra-articular inoculation of 10^4 CFU *S. aureus* in the left stifle joint of rabbits produced serum procalcitonin level more than 0.4 ng ml $^{-1}$on day 2,7,16, which confirmed induction of septic arthritis in these animals. But treatment with linezolid, linezolid with betamethasone decreased the values less than 0.4 ng ml^{-1} on day 16. The ethanolic and aqueous extracts (@1000 mg kg^{-1} for 14 days) of *T. indica* L. leaves also reduced serum PCT levels to less than 0.4 ng ml^{-1} on day 16, which indicated probable recovery of septic arthritis in rabbits. However, the lower dosages of ethanolic and aqueous extracts (@500 mg kg^{-1} for 14 days) of *T. indica* L. leaves could reduce Serum PCT level to less than 0.6 and less than 0.5 respectively on day 16. The findings suggested that both the extracts of T. indica L. leaves at higher dosage i.e. @1000 mg/kg for 14 days were found to be more effective compare to lower dosage i.e. @500mg/kg.

Table 25: PCT(procalcitonin) levels(ng ml^{-1}) in septic arthritic(Gr- I),oral linezolid @ 75 mg kg^{-1} for 10 days twice daily (Gr – II), oral linezolid @ 75 mg kg^{-1} for 10 days twice daily with a single intra-articular injection of betamethasone @ 0.5 mg kg^{-1} (Gr – III), ethanolic extract (in 2% tween 20) of *Tamarindus indica* L. leaves treatment @ 500 mg kg^{-1} for 14 days(Gr – IV), ethanolic extract (in 2% tween 20) of *Tamarindus indica* L. leaves treatment @ 1000 mg kg^{-1} for 14 days(Gr – V), aqueous extract of *Tamarindus indica* L. leaves treatment @ 500 mg kg^{-1} for 14 days (Gr – VI) and aqueous extract of *Tamarindus indica* L. leaves treatment @ 1000 mg kg^{-1} for 14 days (Gr – VII) in rabbits [n=6]

DAY / GROUP	0	2	7	16
Gr-I	<0 .2	>1	>0.8	>0.6
Gr-II	<0.2	>1	<0.5	<0.4
Gr-III	<0.2	>1	<0.5	<0.4
Gr-IV	<0.2	>1	<0.7	<0.6
Gr-V	<0.2	>1	<0.6	<0.4
Gr-VI	<0.2	>1	<0.6	<0.5
Gr-VII	<0.2	>1	<0.5	<0.4

The C-Reactive protein in serum was below detectable level in all the groups on day 0. The level was markedly increased (>50 ng ml^{-1}) in all the groups following intra-articular inoculation of 10^4 CFU *S. aureus* in the left stifle joint of rabbits on day 2. However the level of CRP in serum was found to be maintained more than 20 ng ml^{-1} on day 16 in septic arthritis induced rabbits (group-I). On the other hand, rabbits of different treatment groups (Gr- I,II,III,IV,V,VI and VII) showed decreased CRP level on day 7 and 16. The serum CRP level was below 5 ng ml^{-1} in all the treated groups indicating possible recovery of inflammation of joint.

Table 26: C-Reactive Protein Levels (ng ml^{-1}) in septic arthritic(Gr- I),oral linezolid @ 75 mg kg^{-1} for 10 days twice daily (Gr – II), oral linezolid @ 75 mg kg^{-1} for 10 days twice daily with a single intra-articular injection of betamethasone @ 0.5 mg kg^{-1} (Gr – III), ethanolic extract (in 2% tween 20) of *Tamarindus indica* L. leaves treatment @ 500 mg kg^{-1} for 14 days(Gr – IV), ethanolic extract (in 2% tween 20) of *Tamarindus indica* L. leaves treatment @ 1000 mg kg^{-1} for 14 days(Gr – V), aqueous extract of *Tamarindus indica* L. leaves treatment @ 500 mg kg^{-1} for 14 days (Gr – VI) and aqueous extract of *Tamarindus indica* L. leaves treatment @ 1000 mg kg^{-1} for 14 days (Gr – VII) in rabbits [n=6]

Day / Group	0	2	7	16
Gr-I	BDL	>50	>40	>20
Gr-II	BDL	>50	<20	<5
Gr-III	BDL	>50	<20	<5
Gr-IV	BDL	>50	<30	<5
Gr-V	BDL	>50	<25	<5
Gr-VI	BDL	>50	<30	<5
Gr-VII	BDL	>50	<25	<5

Joint Space Width

Table 27(a,b):Joint space width in septic arthritic(Gr- I),oral linezolid @ 75 mg kg^{-1} for 10 days twice daily (Gr – II), oral linezolid @ 75 mg kg^{-1} for 10 days twice daily with a single intra-articular injection of betamethasone @ 0.5 mg kg^{-1} (Gr – III), ethanolic extract (in 2% tween 20) of *Tamarindus indica* L. leaves treatment @ 500 mg kg^{-1} for 14 days(Gr – IV), ethanolic extract (in 2% tween 20) of *Tamarindus indica* L. leaves treatment @ 1000 mg kg^{-1} for 14 days(Gr – V), aqueous extract of *Tamarindus indica* L. leaves treatment @ 500 mg kg^{-1} for 14 days (Gr – VI) and aqueous extract of *Tamarindus indica* L. leaves treatment @ 1000 mg kg^{-1} for 14 days (Gr – VII) in rabbits [n=6]

(a) Medial JSW (mm.)

	DAY 0	DAY 2	DAY 7	DAY 16
Gr-I	0.55 ± 0.022^{a}	0.52 ± 0.031^{ab}	0.4 ± 0.052^{bc}	0.30 ± 0.058^{c}
Gr-II	0.57 ± 0.021^{a}	0.53 ± 0.033^{a}	0.43 ± 0.033^{b}	0.33 ± 0.042^{c}
Gr-III	0.55 ± 0.022^{a}	0.5 ± 0.037^{ab}	0.45 ± 0.034^{bc}	0.38 ± 0.031^{c}
Gr-IV	0.53 ± 0.021^{a}	0.48 ± 0.031^{a}	0.38 ± 0.017^{b}	0.28 ± 0.031^{c}
Gr-V	0.55 ± 0.022^{a}	0.52 ± 0.031^{a}	0.4 ± 0.037^{bc}	0.33 ± 0.021^{c}
Gr-VI	0.55 ± 0.022^{a}	0.5 ± 0.026^{a}	0.38 ± 0.031^{bc}	0.32 ± 0.031^{c}
Gr-VII	0.57 ± 0.021^{a}	0.52 ± 0.030^{a}	0.38 ± 0.017^{bc}	0.33 ± 0.021^{c}

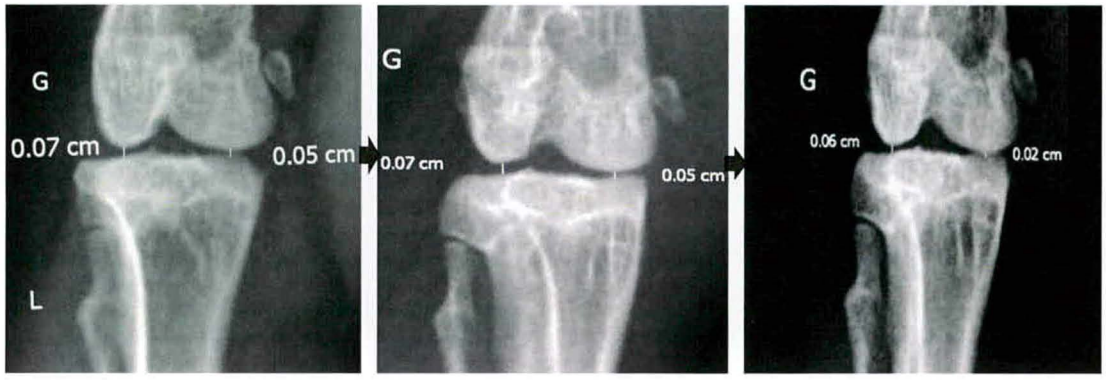
Table 27 (a): Mean ±S.E. value with dissimilar superscript (abc) in a row vary significantly ($P < 0.05$) [n=6]

(b) Lateral JSW (mm.)

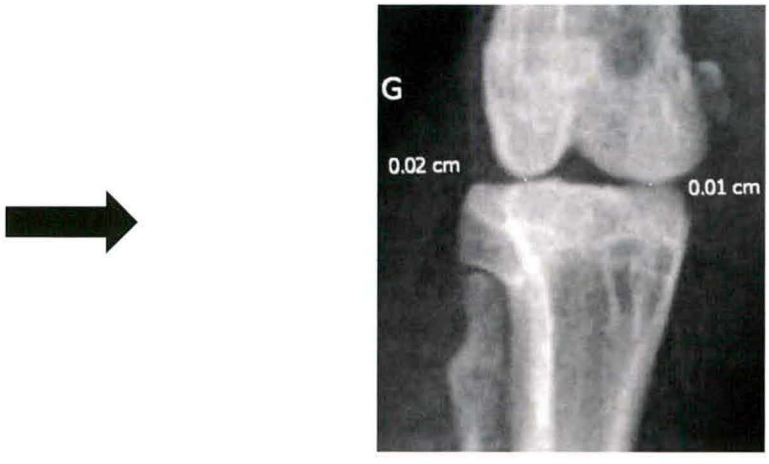
	DAY 0	DAY 2	DAY 7	DAY 16
Gr-I	0.82 ± 0.040^{a}	0.78 ± 0.048^{a}	0.65 ± 0.062^{ab}	0.50 ± 0.058^{b}
Gr-II	0.83 ± 0.033^{a}	0.80 ± 0.026^{ab}	0.72 ± 0.031^{b}	0.62 ± 0.031^{c}
Gr-III	0.87 ± 0.021^{a}	0.82 ± 0.031^{ab}	0.73 ± 0.033^{bc}	0.65 ± 0.034^{c}
Gr-IV	0.85 ± 0.022^{a}	0.82 ± 0.040^{a}	0.68 ± 0.031^{b}	0.57 ± 0.033^{c}
Gr-V	0.85 ± 0.022^{a}	0.83 ± 0.021^{a}	0.73 ± 0.033^{b}	0.63 ± 0.021^{c}
Gr-VI	0.82 ± 0.040^{a}	0.78 ± 0.031^{ab}	0.68 ± 0.031^{b}	0.57 ± 0.033^{c}
Gr-VII	0.80 ± 0.037^{a}	0.77 ± 0.042^{a}	0.70 ± 0.037^{ab}	0.60 ± 0.045^{b}

Table 27(b): Mean ±S.E. value with dissimilar superscript (abc) in a row vary significantly ($P < 0.05$) [n=6]

Digital radiograph of left stifle joint



Day 0 (a) **Day 2 (b)** **Day 7 (c)**

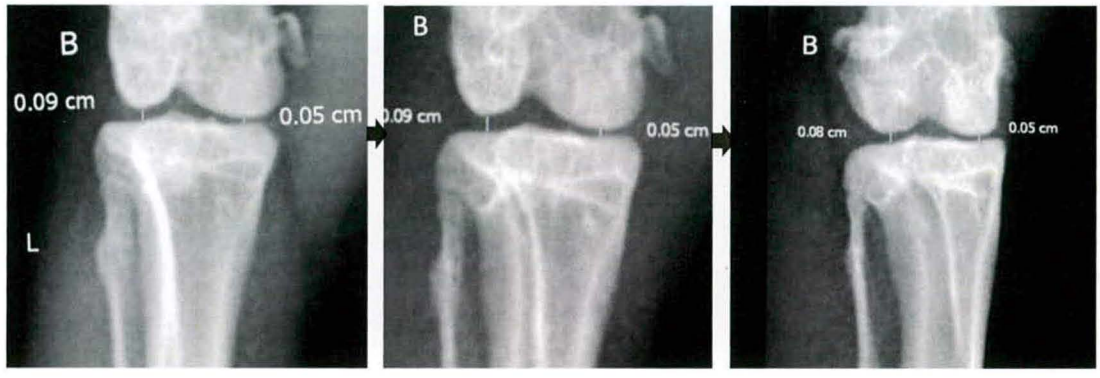


Day 16 (d)

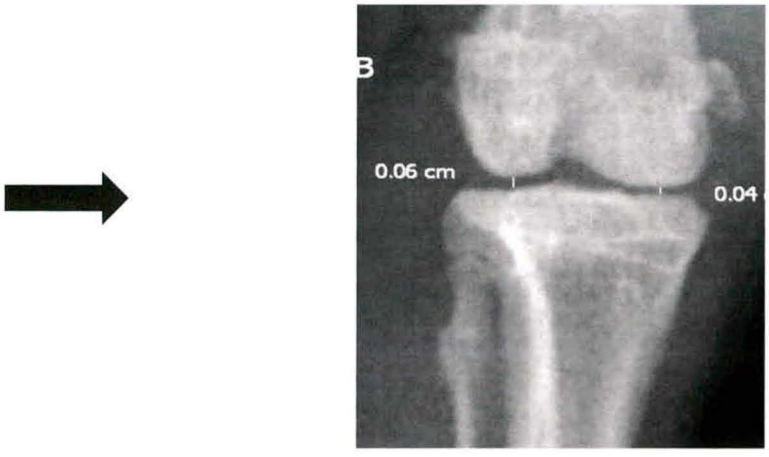
Figure xxxix (a,b,c,d): Digital radiographs are showing the Stifle joint in untreated arthritic control rabbits on different days

Serial digital radiographs of stifle joint at day 0, 2, 7 and 16 were taken in un-treated control group. Radiographs at day '0' and '2' showed presence of uniform joint space, but at day '2' there were evidence of periarticular osteopenia at both distal end of femur and proximal end of tibia-fibula. At day '7' there was marked narrowing of joint space than initial days with more gradual reduction at day '16'. Soft tissue reaction is also evinced at day '16' with irregular bone margin at epiphyseal region. Table 27 (a) shows significant ($P < 0.05$) narrowing of medial joint space width (MJSW) on day '7' and day '16'compared to day '0', whereas insignificant narrowing of lateral joint space width (LJSW) were evident except in day '16' [table 26(b)] that may be due to aggravation of joint infection in untreated control

group. Findings were similar with the findings of Jacobson et al. (2008). Narrowing of joint space occurred due to destruction of sub-chondral bone and cartilage on both sides of joint (Rutten *et al.*, 1998).



Day 0 (a) **Day 2 (b)** **Day 7 (c)**



Day 16 (d)

Figure xxxx(a,b,c,d): Digital radiographs are showing the Stifle joint of linezolid & single intra-articular betamethasone treated rabbits on different days

Serial digital radiographs of stifle joint at day 0, 2, 7 and 16 were taken and measured digitally. Table 27 (a,b) showed insignificant changes in medial (MJSW) and lateral joint space width (LJSW) throughout the study period respectively. That may be due to restriction of infection and cartilage damage due to use of antibiotic with intra-articular corticosteroid. In septic arthritis bacterial antigens causes cytokine proliferation (Saez-Llorens *et al.*, 1990)

inside the joint and activate chondrocyte proteases (Williams *et al.*, 1990) which in turn causes inflammation of joint. Corticosteroid in addition to antibiotics restricts damage of subcondral cartilage (Lane and Merry, 2000) and also acts as anti-inflammatory agent (Rhen, 2005).

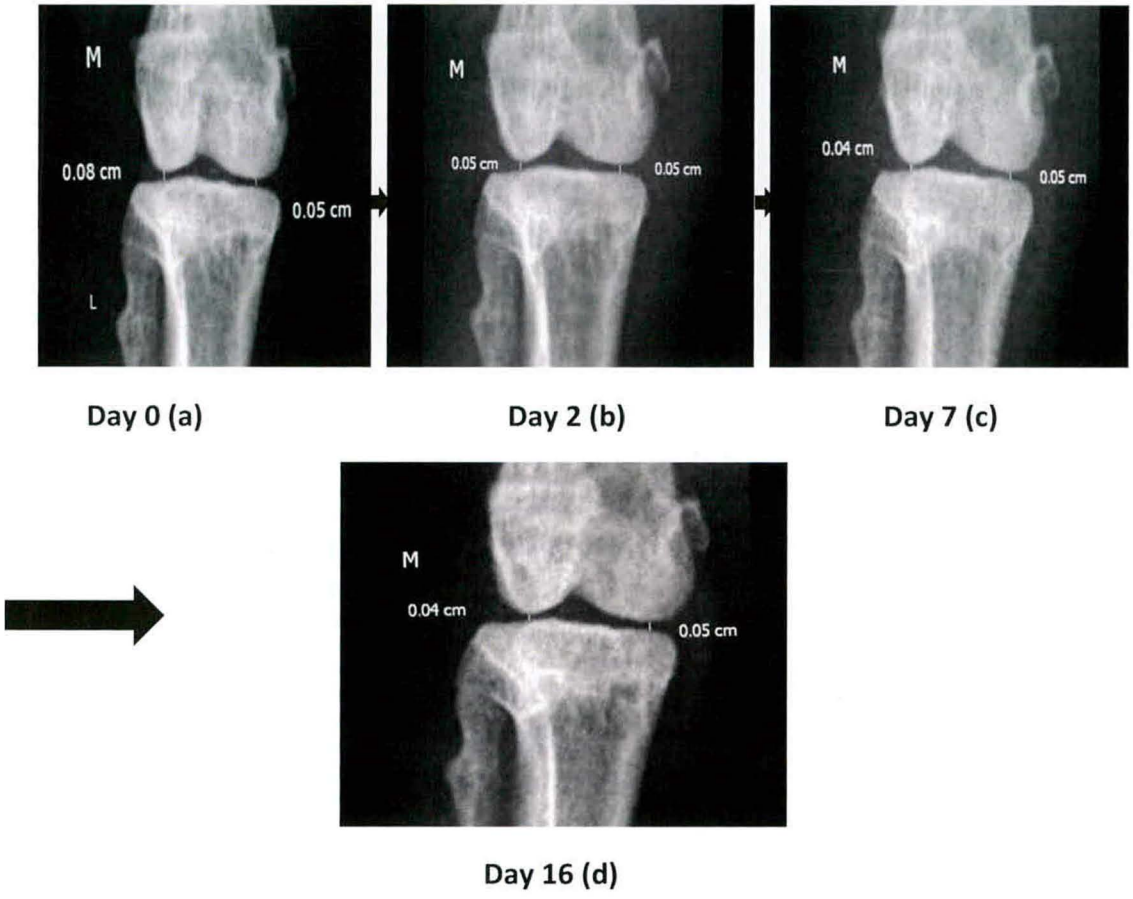


Figure xxxxi (a,b,c,d): Digital radiographs showing stifle joint space of aqueous high dose 1000 mg kg^{-1} treated rabbit on different days

Serial digital radiographs of stifle joint at day 0, 2, 7 and 16 were taken and measured digitally in animals treated with aqueous extract of *Tamarindus indica* L. leaves. @1000 mg kg^{-1} body weight. Table 27 (a,b) shows insignificant changes in medial (MJSW) and lateral joint space width (LJSW) throughout the study period. Restriction of joint space narrowing

may be due to control of joint infection as *Tamarindus indica* L. leaves have bactericidal effect against varieties of gram positive and negative organisms including methicillin resistant *S. aureus* (Meléndez *et al.*, 2006; Gumgumjee *et al.*, 2012). Control of joint space narrowing may have been achieved by the anti-arthritic effect of *Tamarindus indica* L. extract which protect cartilage and bone damage by inhibiting the elevated activities of MMPs, HAaSE, exoglycosidases, cathepsins and TRAP as well as reduce inflammation by alleviating the increased levels of inflammatory mediators like interleukin (IL)-1β, tumor necrosis factor-α, IL-6, IL-23 and cyclooxygenaSE-2 (Sundaram *et al.*, 2015).

Gross pathomorphology of Menisci

Menisci of septic arthritic (Gr- I),oral linezolid @ 75 mg kg^{-1} for 10 days twice daily (Gr – II), oral linezolid @ 75 mg kg^{-1} for 10 days twice daily with a single intra-articular injection of betamethasone @ 0.5 mg kg^{-1} (Gr – III), ethanolic extract (in 2% tween 20) of *Tamarindus indica* L. leaves treatment @ 500 mg kg^{-1} for 14 days(Gr – IV), ethanolic extract (in 2% tween 20) of *Tamarindus indica* L. leaves treatment @ 1000 mg kg^{-1} for 14 days(Gr – V), aqueous extract of *Tamarindus indica* L. leaves treatment @ 500 mg kg^{-1} for 14 days (Gr – VI) and aqueous extract of *Tamarindus indica* L. leaves treatment @ 1000 mg kg^{-1} for 14 days(Gr – VII) in rabbits have been presented in figure xxxxii: X. The menisci were removed after 16 days post inoculation of 10^4 CFU *S. aureus* in the left stifle joint of rabbits. A marked erosion in both of the meniscus were found in septic arthritis group. But the menisci were not found to be badly eroded compared to untreated group after treatment with antibiotic and steroid (group - III), ethanolic extract of *Tamarindus indica* L. leaves @1000 mg kg^{-1} (group - V) and aqueous extract of *Tamarindus indica* L. leaves @1000 mg kg^{-1} (group - VII). The groups treated with only antibiotic (group - II), ethanolic extract of *Tamarindus indica* L. leaves @500 mg/kg (group - IV) and aqueous extract of *Tamarindus indica* L. leaves @500 mg/kg (group - VI) also showed a less erosion compare to the untreated group but not up to group – III, group – V and group – VII.

Figure xxxxii : Menisci of septic arthritic(Gr- I),oral linezolid @ 75 mg kg^{-1} for 10 days twice daily (Gr – II), oral linezolid @ 75 mg kg^{-1} for 10 days twice daily with a single intra-articular injection of betamethasone @ 0.5 mg kg^{-1} (Gr – III), ethanolic extract (in 2% tween 20) of *Tamarindus indica* L. leaves treatment @ 500 mg kg^{-1} for 14 days(Gr – IV), ethanolic extract (in 2% tween 20) of *Tamarindus indica* L. leaves treatment @ 1000 mg kg^{-1} for 14 days(Gr – V), aqueous extract of *Tamarindus indica* L. leaves treatment @ 500 mg kg^{-1} for 14 days (Gr – VI) and aqueous extract of *Tamarindus indica* L. leaves treatment @ 1000 mg kg^{-1} for 14 days (Gr – VII) in rabbits [n=6]

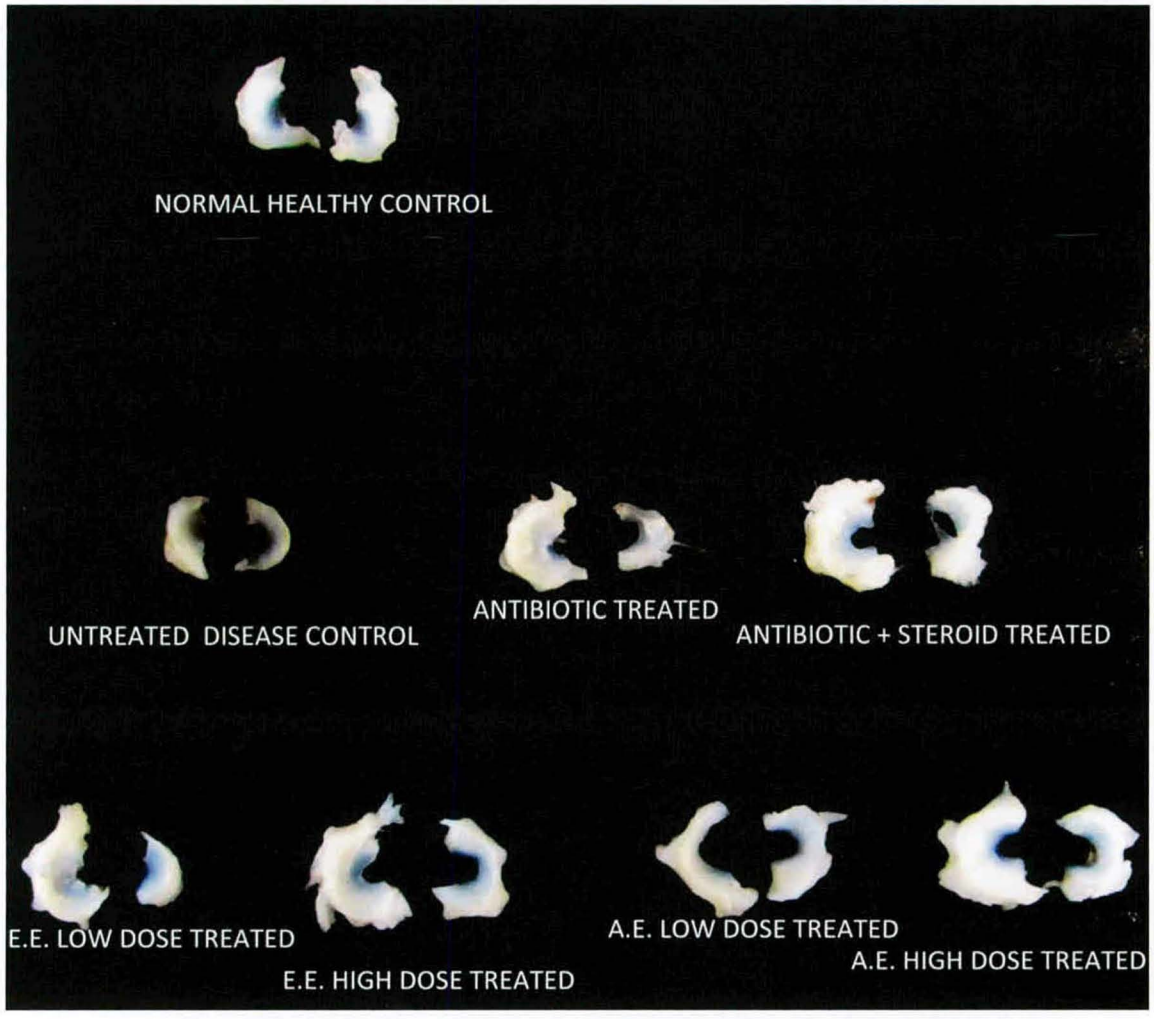


Figure xxxxii : gross pathomorphology of menisci of different groups

Histomorphology

The animals of all the groups under experiment survived up to 16th day of experiment.

Table 28(a – f) revealed the histomorphological observation and scoring of experimental menisci of different groups.

Stifle joint of healthy rabbit showed normal cellularity of meniscus which was scored as 0 (fig xxxxiii) and very less number of chondrocytes which was scored as 2.9 ± 0.08 of septic arthritis induced rabbits [(Gr – I), (figure xxxxiv)].

From the result, it was found that Gr – III (oral Linezolid @ 75 mg kg^{-1} twice daily for 10 days with a single intra-articular injection of betamethasone @ 0.5 mg kg^{-1}) menisci had the lowest score (1.1 ± 0.14) compared to the other groups. The menisci of Gr – VII (Aqueous Extract of *Tamarindus indica* L. leaves treatment @ 1000 mg kg-1 for 14 days) were also found to have a score 1.3 ± 0.19, very close to Gr – III.

Stifle joint of healthy rabbit showed normal matrix with minimal loss of cartilage fibre which was scored as 0 (fig no li) and the maximum loss of matrix, which was scored as 2.8 ±0.07 of septic arthritis induced rabbits [(Gr – I) (figure no lii)].

From the result, it was found that Gr – III (oral Linezolid @ 75mg/kg twice daily for 10 days with a single intra-articular injection of betamethasone @ 0.5 mg/kg) menisci had the lowest score 1.2 ± 0.13 compared to the other groups. The menisci of Gr – V (ethanolic extract of *Tamarindus indica* L. leaves treatment @ 1000 mg kg^{-1} for 14 days) and Gr – VII (aqueous extract of *Tamarindus indica* L. leaves treatment @ 1000 mg kg^{-1} for 14 days) were found to have a score of 1.7 ± 0.11 and 1.3 ± 0.11, respectively.

Stifle joint of healthy rabbit showed normal arrangement of chondrocytes which was scored as 0 (fig no lix.). Chondrocytes are found to be cloned together randomly at few places in case of infectious arthritis. The untreated disease control meniscus was scored as 2.6 ± 0.14. From the photomicrographs, it was found that Gr – III (oral Linezolid @ 75 mg kg^{-1} twice daily for 10 days with a single intra-articular injection of betamethasone @ 0.5 mg kg^{-1}) menisci had the lowest score 1.2 ± 0.11 compared to the other groups. The menisci of Gr- V and Gr – VII were found to have a score of 1.4 ± 0.14 and 1.4 ± 0.07.

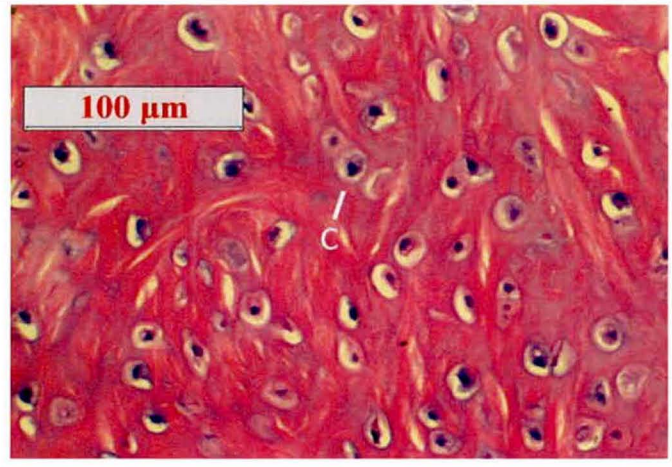
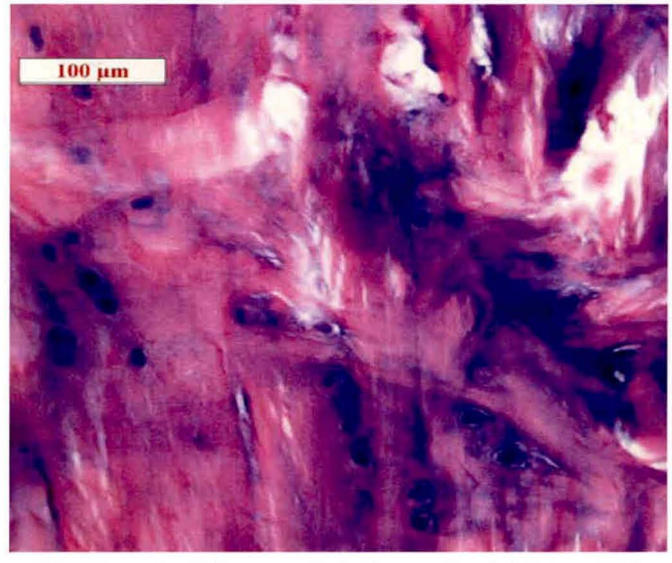
Stifle joint of healthy rabbit showed no adhesion of pannus which was scored as 0 (fig lxvii.). Adhesion of pannus are found in case of septic arthritis and the untreated disease control meniscus was scored as 2.4 ± 0.20. From histopathological study, it was found that Gr – III (oral linezolid @ 75mgkg-1 twice daily for 10 days with a single intra-articular injection of betamethasone @ 0.5 mgkg-1) menisci had the lowest score (1.3 ± 0.14) compared to the

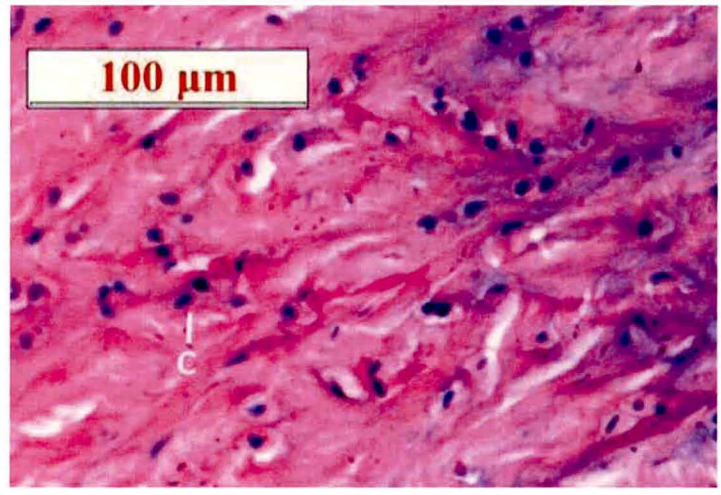
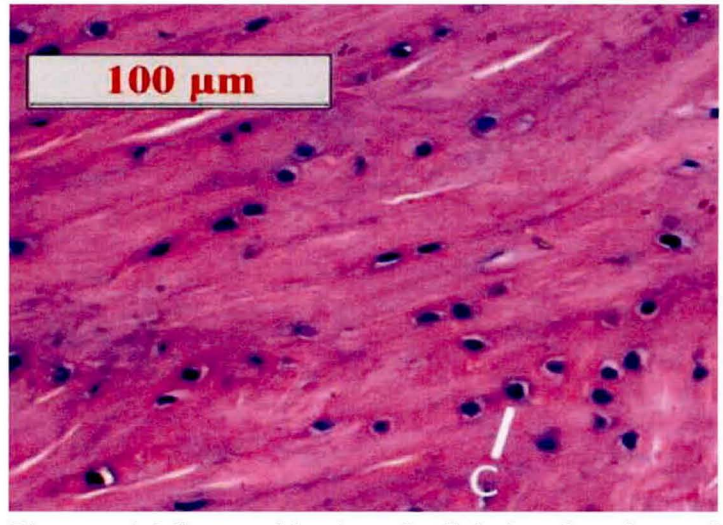
other groups. The menisci of Gr- V and Gr – VII were found to have scores 1.6 ± 0.11 and 1.4 ± 0.09.

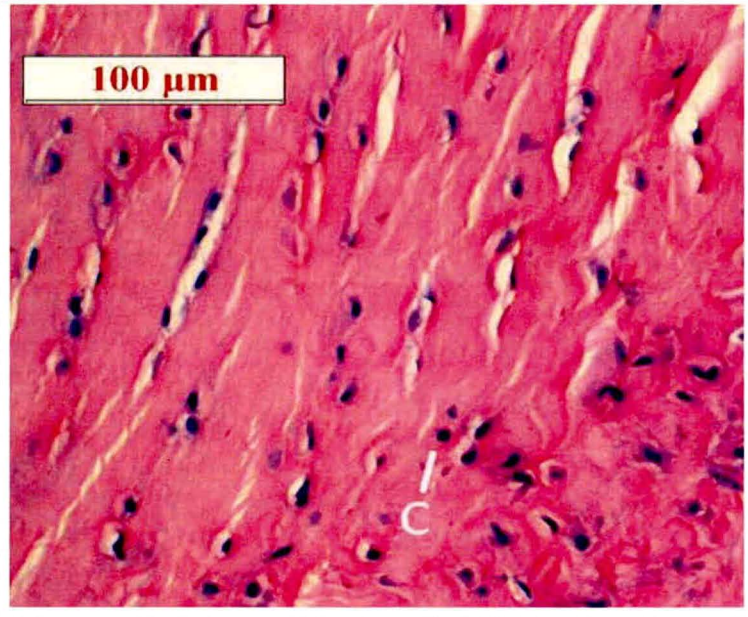
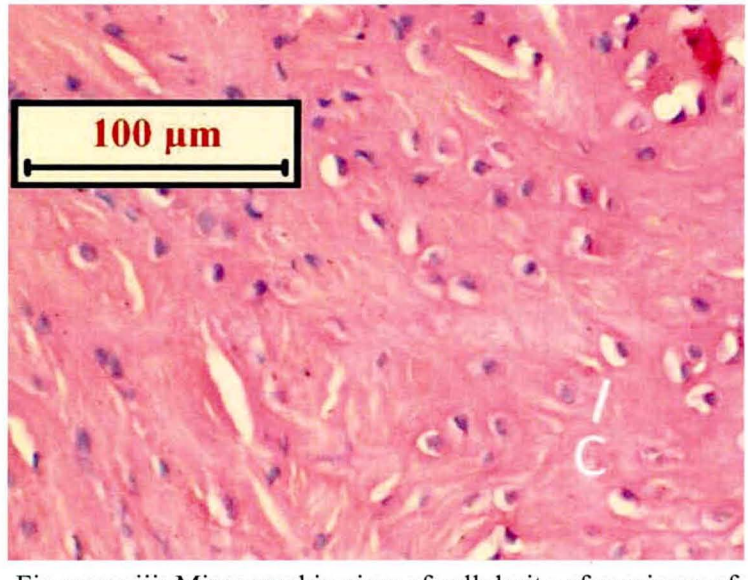
Stifle joint of healthy rabbit showed presence of adequate proteoglycan which appeared red on a greenish background and was scored as 0 (fig no.). Amount of proteoglycans was noticed to be reduced in septic arthritis condition. The untreated control meniscus containing least amount of proteoglycan, has been scored as 3.72 ± 0.10 .From histopathological study, it was found that Gr – III (oral Linezolid @ 75mgkg-1 twice daily for 10 days with a single intra-articular injection of betamethasone @ 0.5 mgkg-1) menisci had the lowest score (1.6 ± 0.10) compared to the other groups. The menisci of Gr- V and Gr – VII were found to have scores 2.08 ± 0.06 and 1.7 ± 0.12.

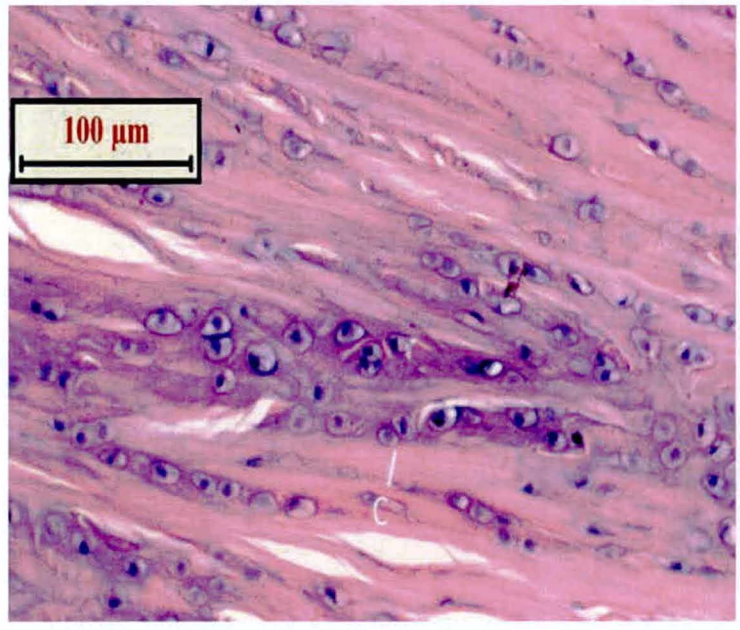
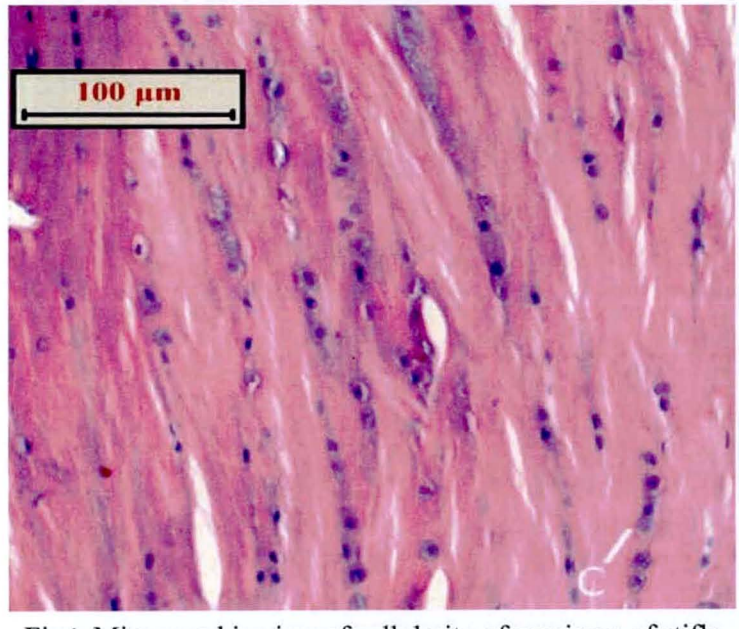
One of the main objectives of the present study was to minimise the damage of menisci in septic arthritis under different combination of drugs including herbal extracts other than the common approach of medication in treating septic arthritis.The findings indicated that both the extracts of T. indica L. (ethanolic and aqueous) at higher dosage regimen have a dramatic chondroprotective effect compared to lower dosage regimen.

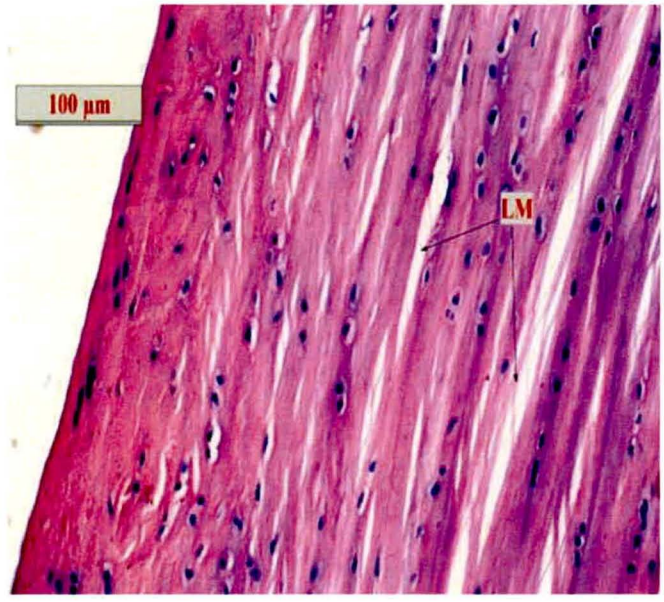
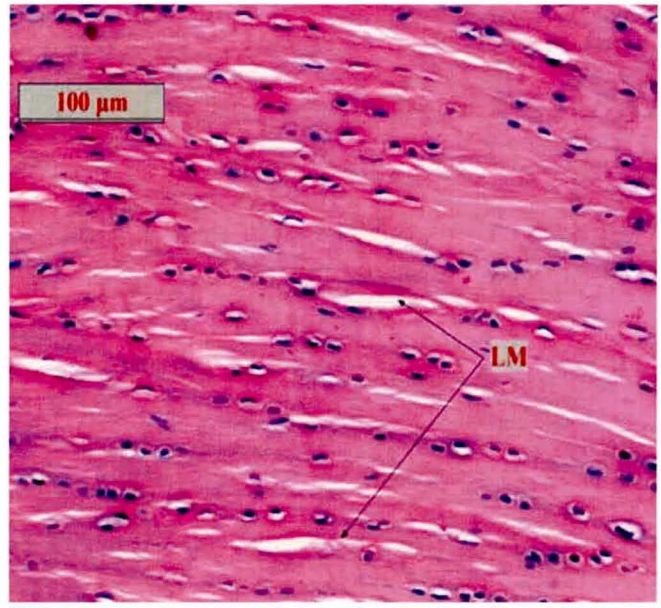
Histopathological and Histochemical overall scoring of of septic arthritic (Gr- I),oral linezolid @ 75 mg kg^{-1} for 10 days twice daily (Gr – II), oral linezolid @ 75 mg kg^{-1} for 10 days twice daily with a single intra-articular injection of betamethasone @ 0.5 mg kg^{-1} (Gr – III), ethanolic extract (in 2% tween 20) of *Tamarindus indica* L. leaves treatment @ 500 mg kg^{-1} for 14 days(Gr – IV), ethanolic extract (in 2% tween 20) of *Tamarindus indica* L. leaves treatment @ 1000 mg kg^{-1} for 14 days(Gr – V), aqueous extract of *Tamarindus indica* L. leaves treatment @ 500 mg kg^{-1} for 14 days (Gr – VI) and aqueous extract of *Tamarindus indica* L. leaves treatment @ 1000 mg kg^{-1} for 14 days(Gr – VII) in rabbits have have been depicted in table .The over-all scoring derived from individual histomorphological parameters suggested that the effects of the treatment groups (Gr –II to VII) were better compare to untreated group with septic arthritis (Gr – I). However, the efficacy of antibiotic and steroid treatment produced most satisfactory improvement followed by high dose of aqueous extract of *Tamarindus indica* L. leaves treatment that also produced comparable effect. Only antibiotic treatment and higher dose of ethanolic extract of *Tamarindus indica* L. also showed promising improvement in septic arthritis. Althoug treatment with oral linezolid for 10 days with single betamethasone injection may produce adverse effects in some animals but aqueous and ethanolic extracts @2 gmkg-1 body weight orally for 28 days did not produce any adverse effect in the safety study which proved that the particular alternate herbal therapy could be not only efficacious but also safer and better tolerable. It is expected that dosage regimen used for two extracts of *T. indica* L. leaves for treatment of septic arthritis which was lesser compared to the dosage regimen employed in safety study, would not produce any undesirable/untoward effect in animals.

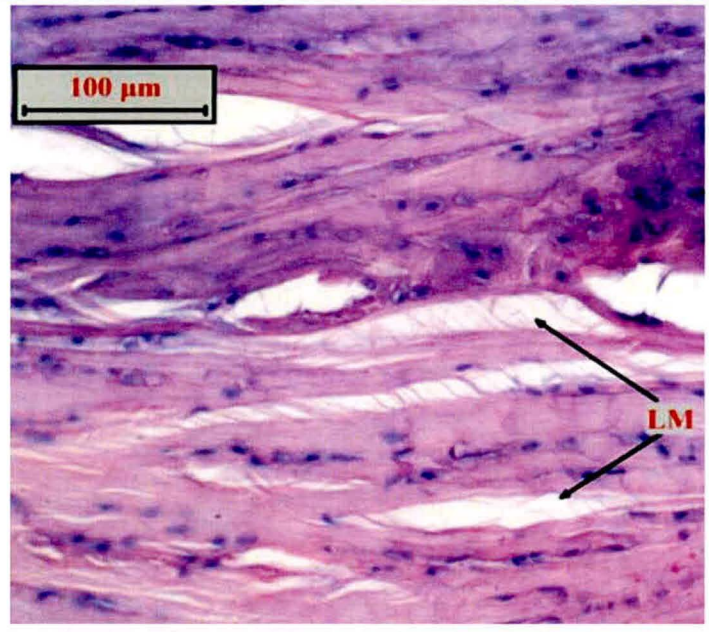
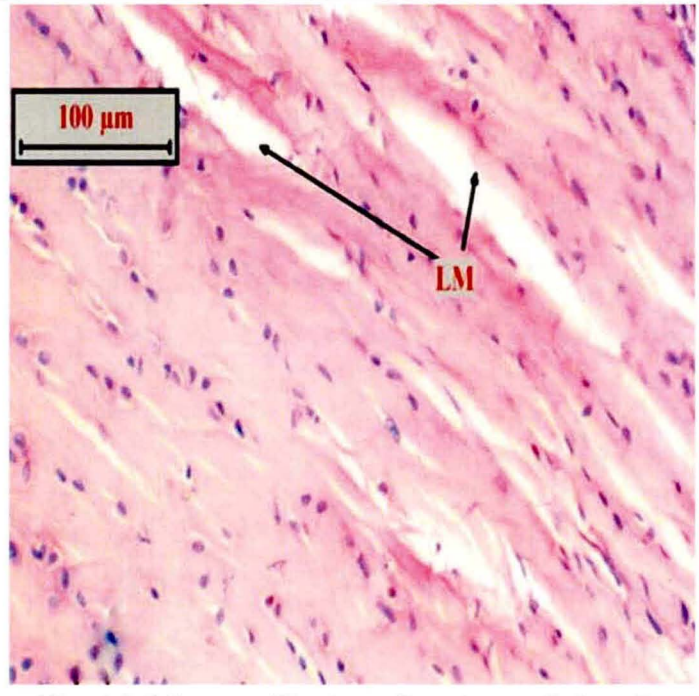
Gr	Parameter	Score (Mean + SE)	Picture
Healthy	Cellularity	0 ± 0.00	 Fig xxxxiii: Micrographic view of normal cellularity of menisci in healthy rabbit (H & E stain)
I	Cellularity	2.9 ± 0.08	 Fig xxxxxiv :Micrographic view of arthritic control meniscus of rabbit with very less cellularity (H & E stain)

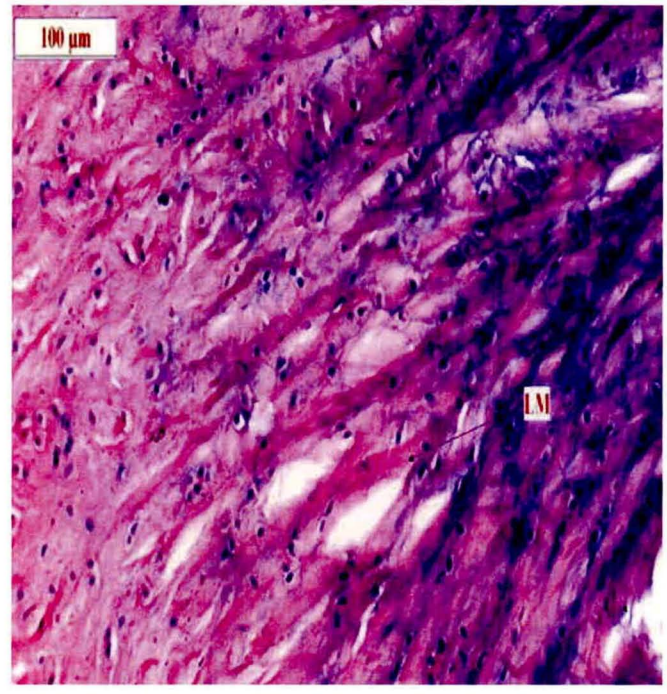
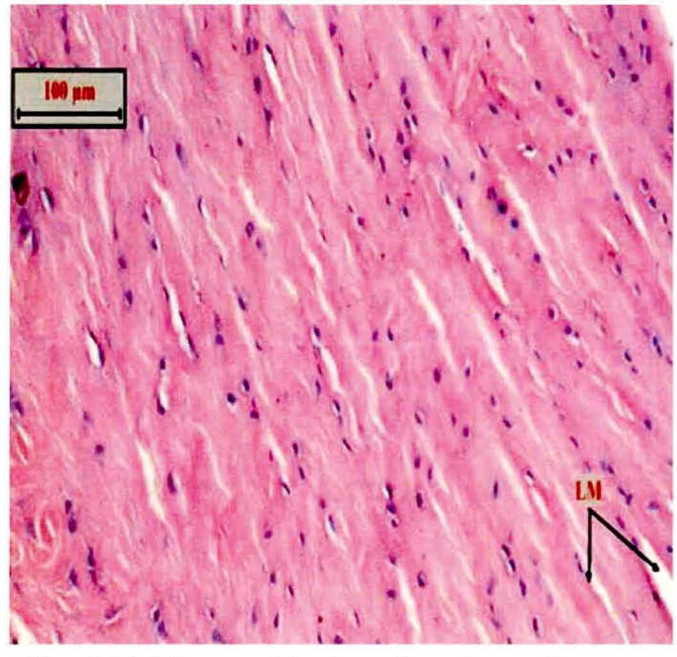
Gr	Parameter	Score (Mean + SE)	Picture
II	**Cellularity**	1.3 ± 0.14	 Fig xxxxv: Micrographic view of cellularity of meniscus of stifle joint in linezolid treated rabbit (H & E stain)
III	**Cellularity**	1.1 ± 0.14	 Fig xxxxvi: Micrographic view of cellularity of meniscus of stifle joint in linezolid and single intra-articular betamethasone treated rabbit (H & E stain)

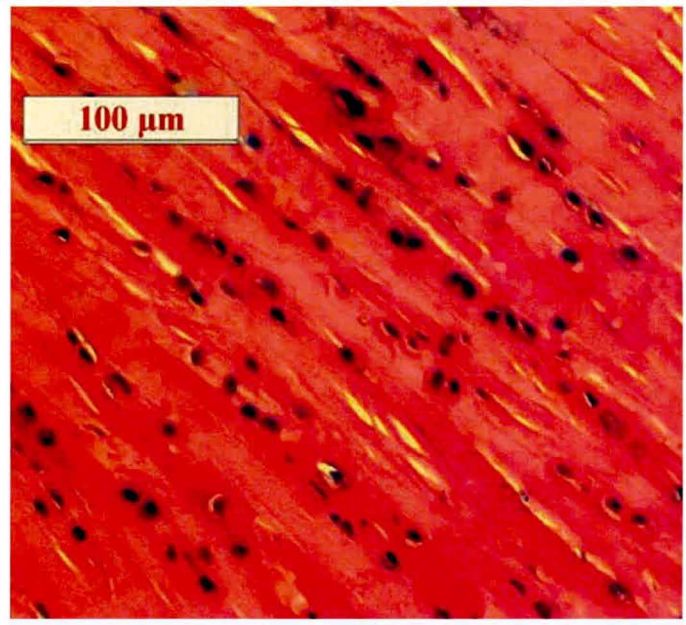
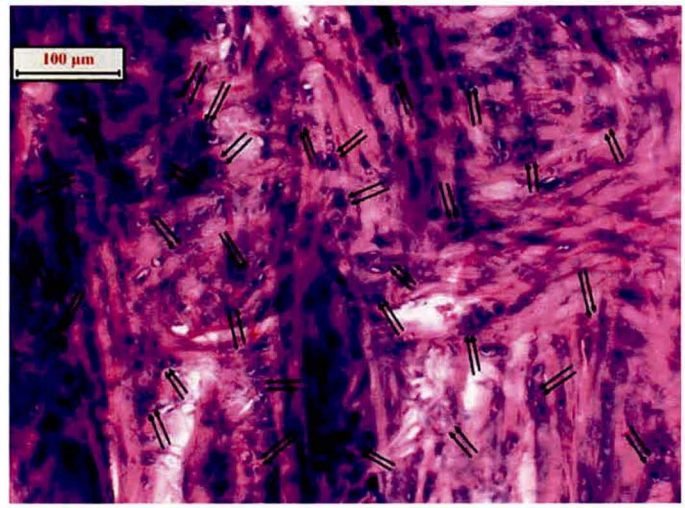
Gr	Parameter	Score (Mean + SE)	Picture
IV	Cellularity	2.3 ± 0.11	 Fig xxxxvii : Micrographic view of cellularity of meniscus of stifle joint in ethanolic extract @500 mg kg^{-1} treated rabbit (H & E stain)
V	Cellularity	1.4 ± 0.18	 Fig xxxxviii: Micrographic view of cellularity of meniscus of stifle joint in ethanolic extract @1000 mg kg^{-1} treated rabbit (H & E stain)

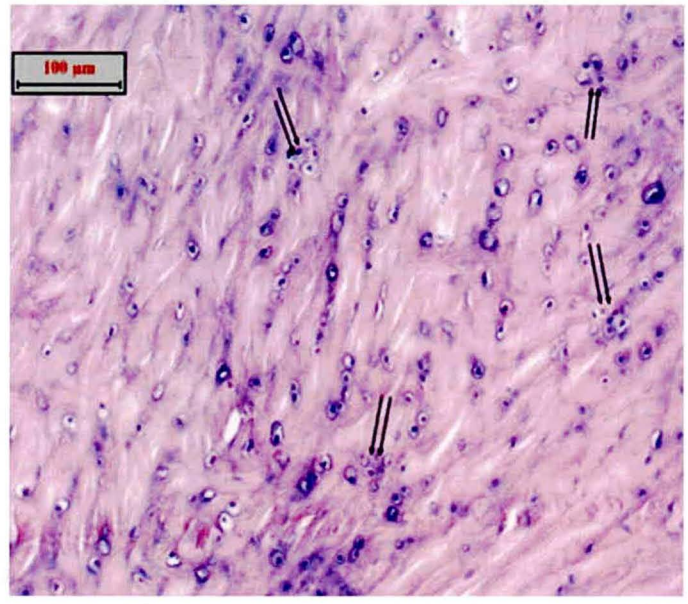
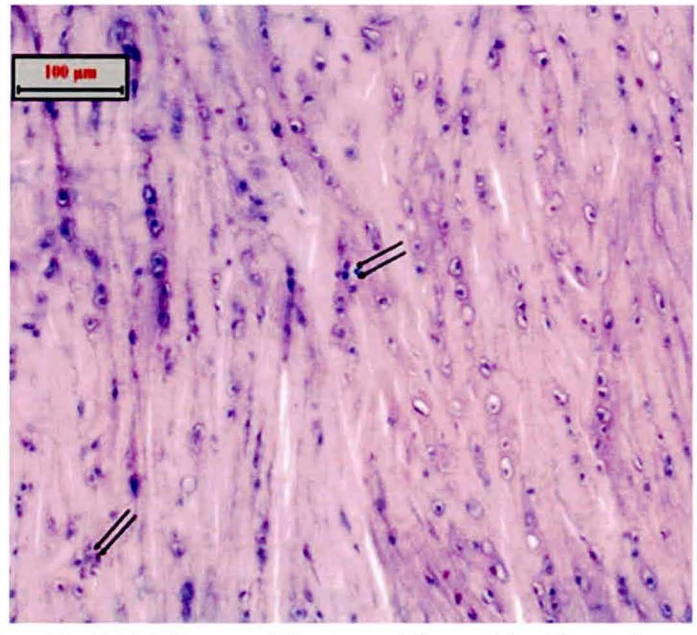
Gr	Parameter	Score (Mean + SE)	Picture
VI	Cellularity	2.1 ± 0.10	 Fig xxxxix: Micrographic view of cellularity of meniscus of stifle joint in aqueous extract @500 mg kg^{-1} treated rabbit (H & E stain)
VII	Cellularity	1.3 ± 0.19	 Fig 1: Micrographic view of cellularity of meniscus of stifle joint in aqueous extract @1000 mg kg^{-1} treated rabbit (H & E stain)

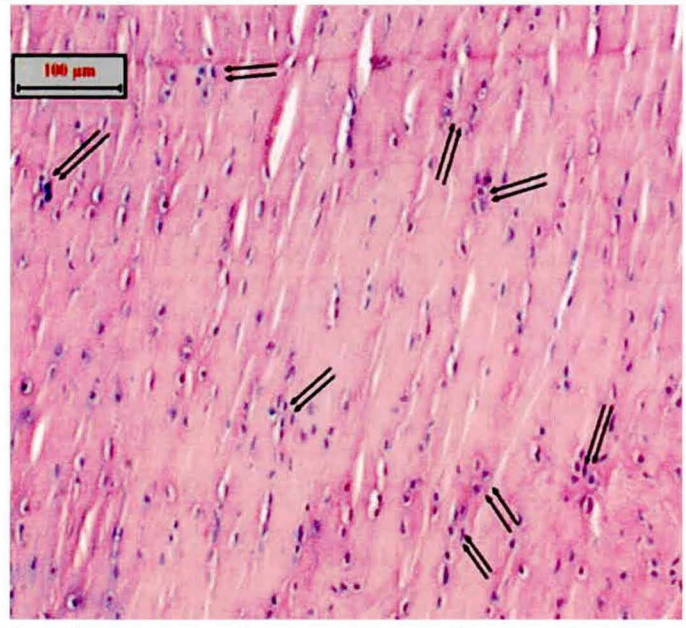
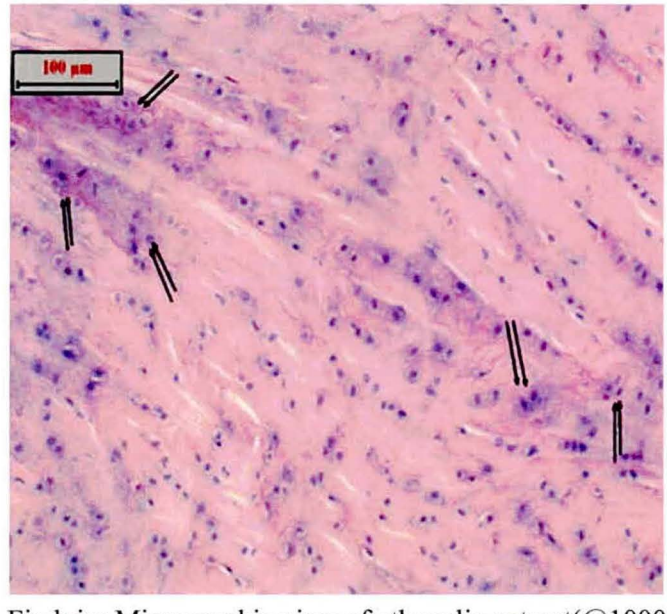
Gr	Parameter	Score (Mean + SE)	Picture
II	Loss of matrix	1.3 ± 0.11	 Fig xliii: Micrographic view of meniscus of linezolid treated rabbit showing little loss of matrix (H & E stain)
III	Loss of matrix	1.1 ± 0.13	 Fig xliv: Micrographic view of meniscus of linezolid with single intra-articular betamethasone treated rabbit showing very little loss of matrix(H & E stain)

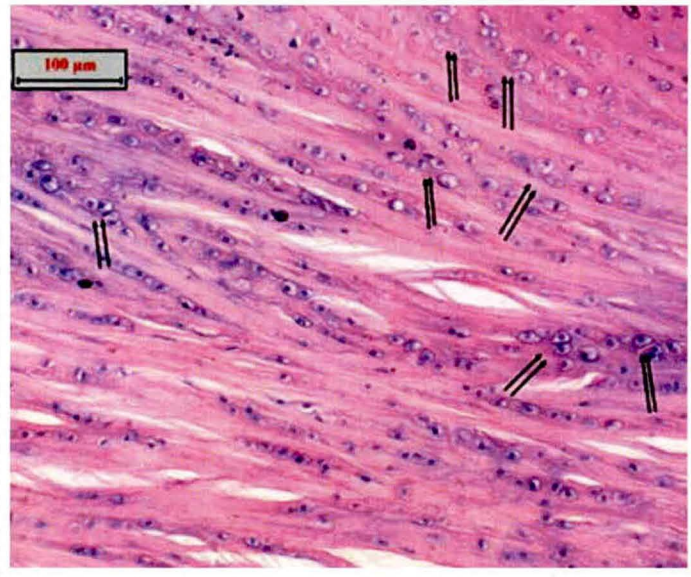
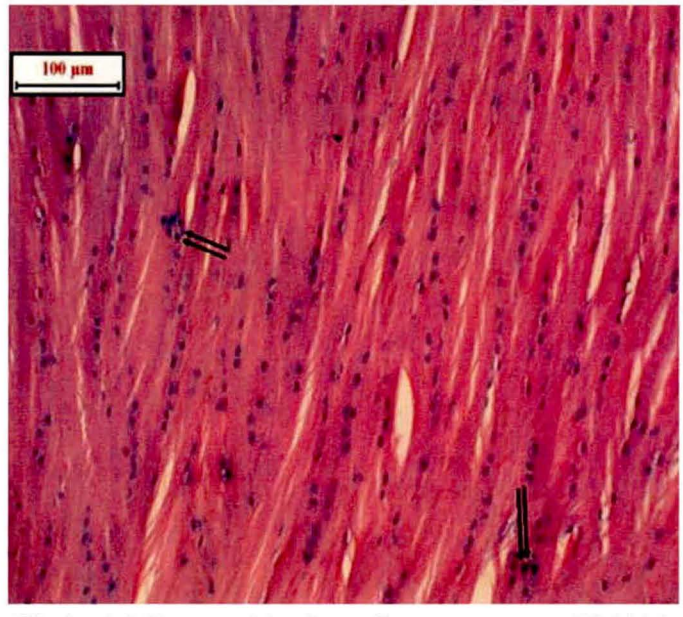
Gr	Parameter	Score (Mean + SE)	Picture
IV	Loss of matrix	1.9 ± 0.07	 Fig xlv: Micrographic view of meniscus of ethanolic extract(@500 mg kg^{-1}) treated rabbit showing loss of matrix (H & E stain)
V	Loss of matrix	1.7 ± 0.10	 Fig xlvi: Micrographic view of meniscus of ethanolic extract(@1000 mg kg^{-1}) treated rabbit showing loss of matrix (H & E stain)

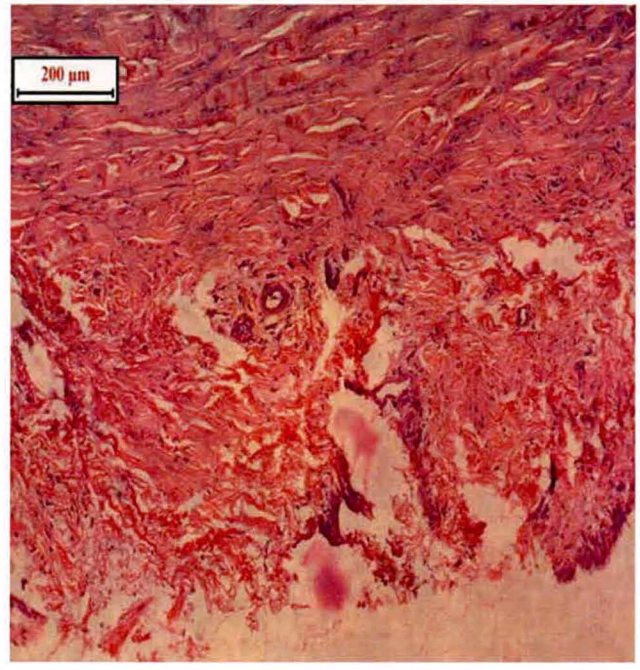
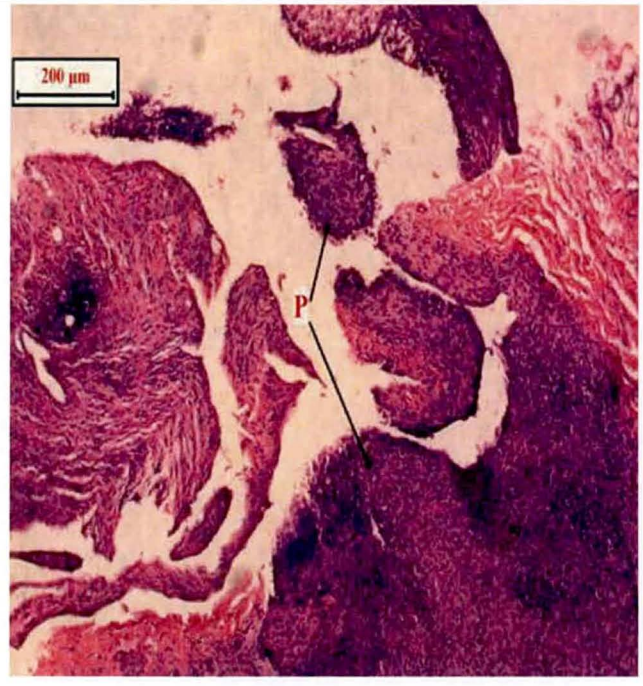
Gr	Parameter	Score (Mean + SE)	Picture
VI	Loss of matrix	2.0 ± 0.08	 Fig xlvii: Micrographic view of meniscus of aqueous extract(@500 mg kg^{-1}) treated rabbit showing loss of matrix(H & E stain)
VII	Loss of matrix	1.3 ± 0.11	 Fig xlviii: Micrographic view of meniscus of aqueous extract(@1000 mg kg^{-1}) treated rabbit showing very little loss of matrix(H & E stain)

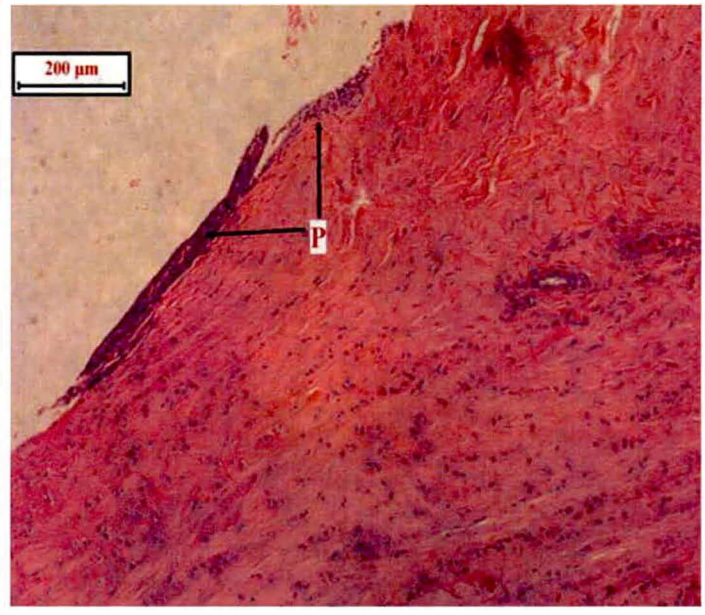
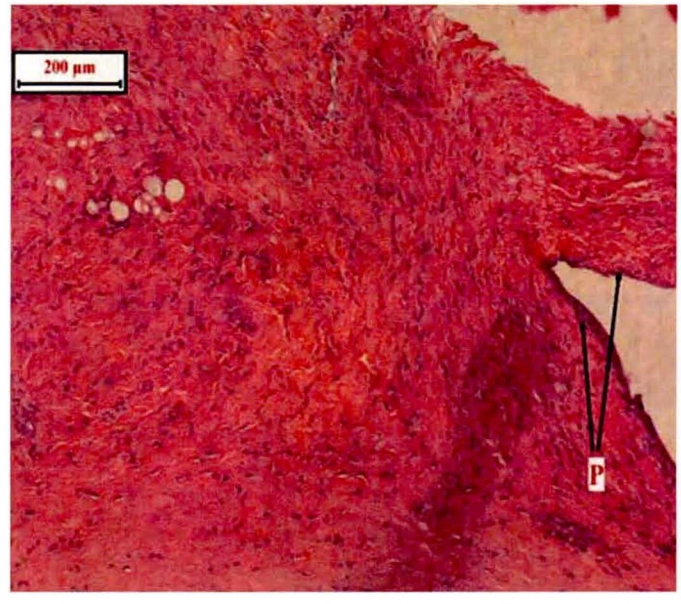
Gr	Parameter	Score (Mean + SE)	Picture
Healthy	Cloning of chondrocyte	0 ± 0.00	 Fig lix: Micrographic view of healthy control rabbit showing normal arrangement of chondrocytes (H & E stain)
I	Cloning of chondrocyte	2.6±0.14	 Fig lx: Micrographic view of septic arthritis induced rabbit showing cloning of chondrocytes (H & E stain)

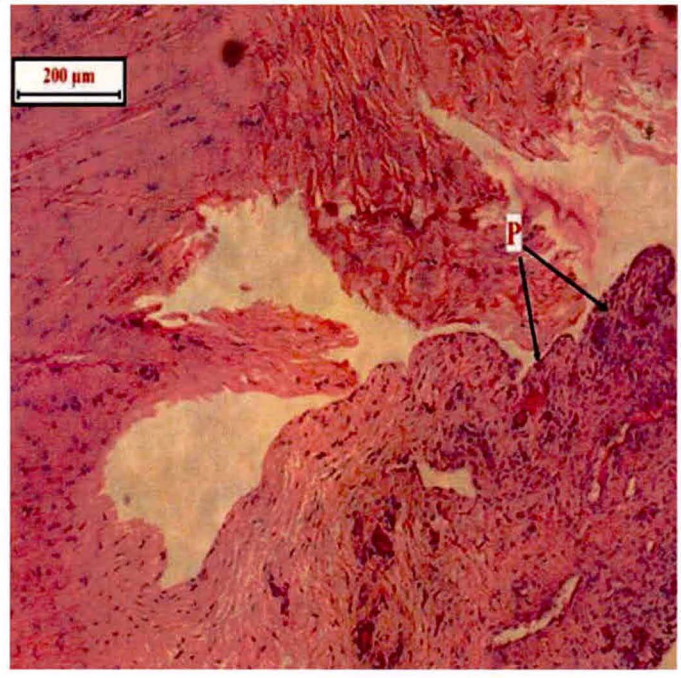
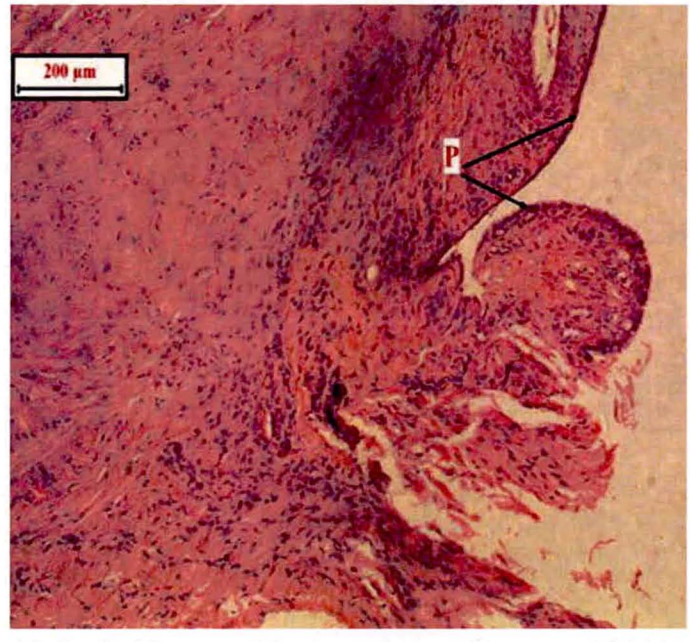
Gr	Parameter	Score (Mean + SE)	Picture
II	Cloning of chondrocyte	1.3±0.11	 Fig lxi: Micrographic view of linezolid treated rabbit showing less number of cloning of chondrocytes (H & E stain)
III	Cloning of chondrocyte	1.2 ± 0.11	 Fig lxii: Micrographic view of linezolid with single betamethasone treated rabbit showing very less number of cloning of chondrocytes (H & E stain)

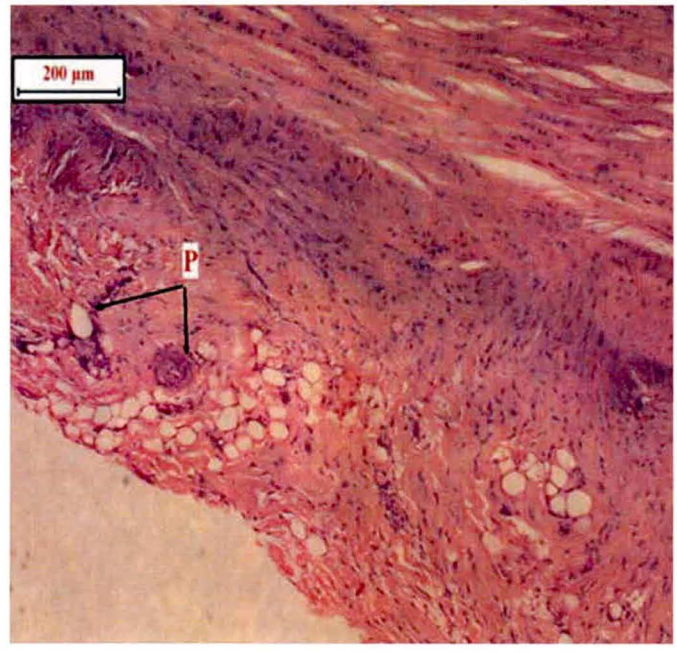
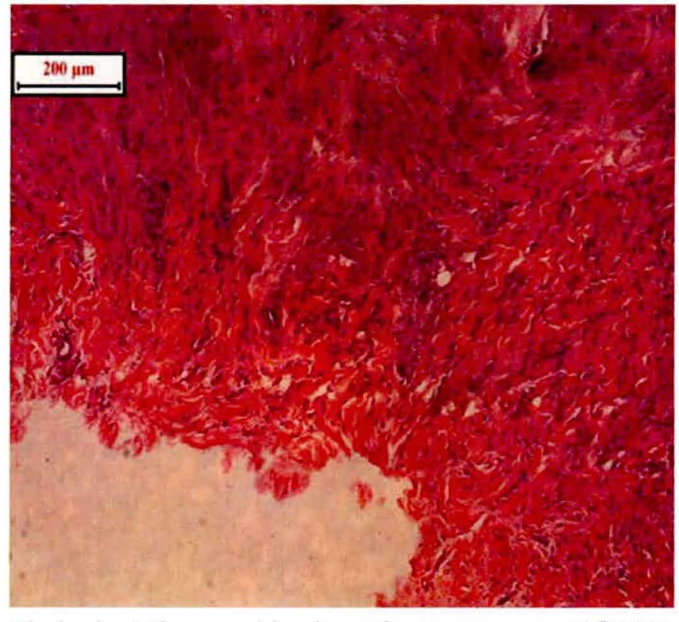
Gr	Parameter	Score (Mean + SE)	Picture
IV	Cloning of chondrocyte	2.0 ± 0.10	 Fig lxiii: Micrographic view of ethanolic extract(@500 mg kg^{-1}) treated rabbit showing cloning of chondrocytes (H & E stain)
V	Cloning of chondrocyte	1.4 ± 0.14	 Fig lxiv: Micrographic view of ethanolic extract(@1000 mg kg^{-1}) treated rabbit showing less number of cloning of chondrocytes (H & E stain)

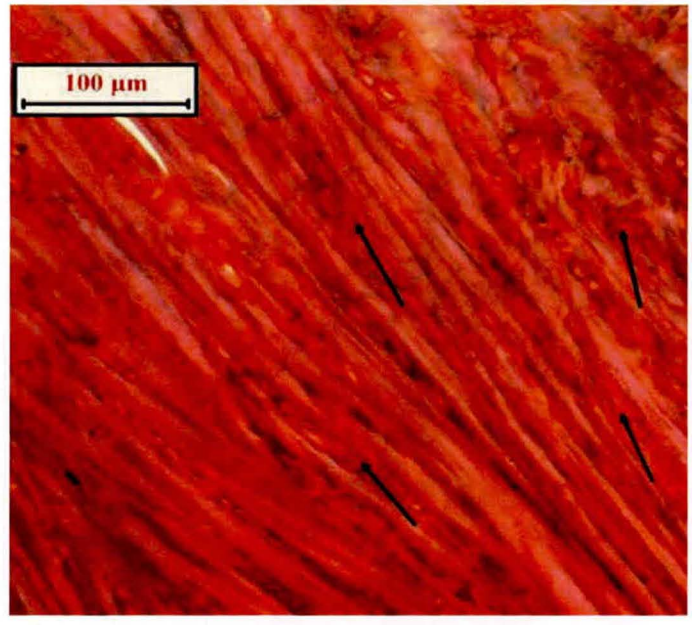
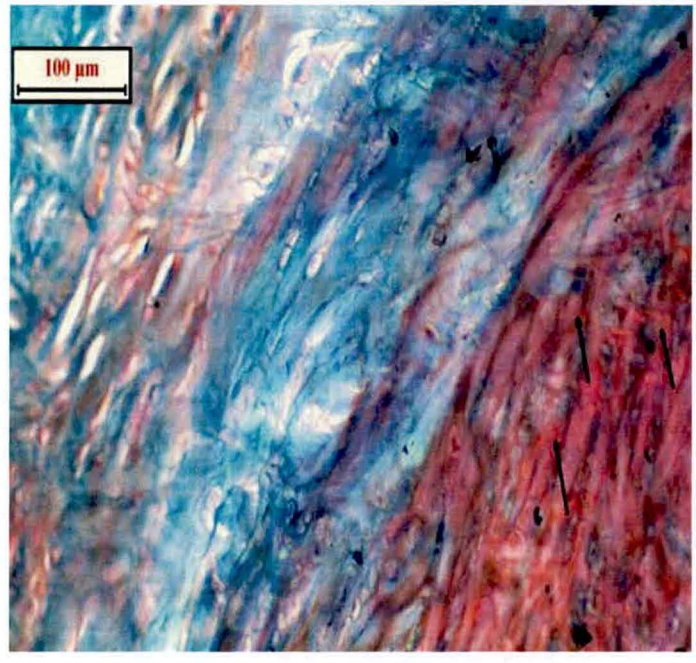
Gr	Parameter	Score (Mean + SE)	Picture
VI	Cloning of chondrocyte	2.0 ± 0.07	 Fig lxv: Micrographic view of aqueous extract(@500 mg kg^{-1}) treated rabbit showing cloning of chondrocytes (H & E stain)
VII	Cloning of chondrocyte	1.3 ± 0.07	 Fig lxvi: Micrographic view of aqueous extract(@1000 mg kg^{-1}) treated very less number of cloning of chondrocytes (H & E stain)

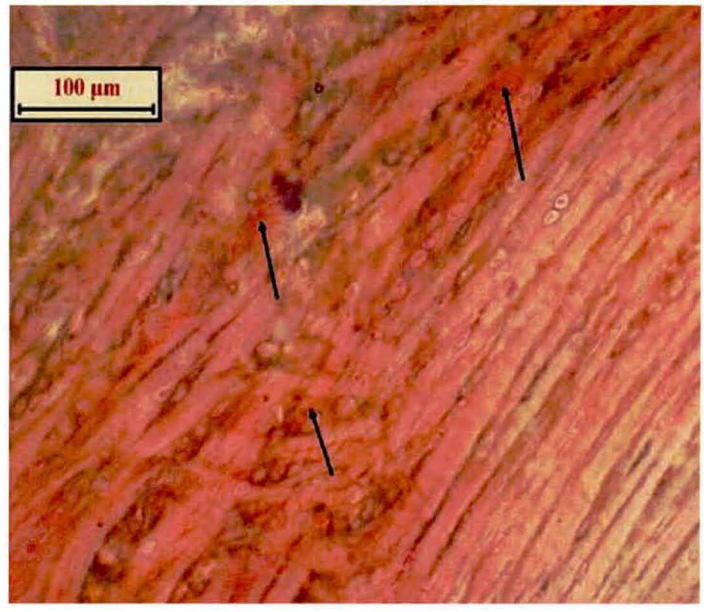
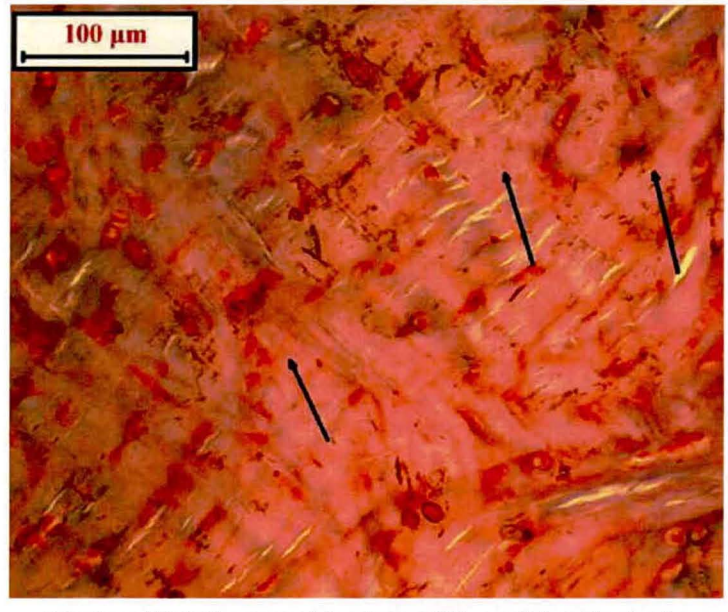
Gr	Parameter	Score (Mean + SE)	Picture
Healthy	Adhesion (pannus)	0 ± 0.00	 Fig lxvii: Micrographic view of healthy control rabbit showing no or least amount of pannus (H & E stain)
I	Adhesion (pannus)	2.4 ± 0.20	 Fig lxviii: Micrographic view of septic arthritis induced rabbit showing highest amount of pannus (H & E stain)

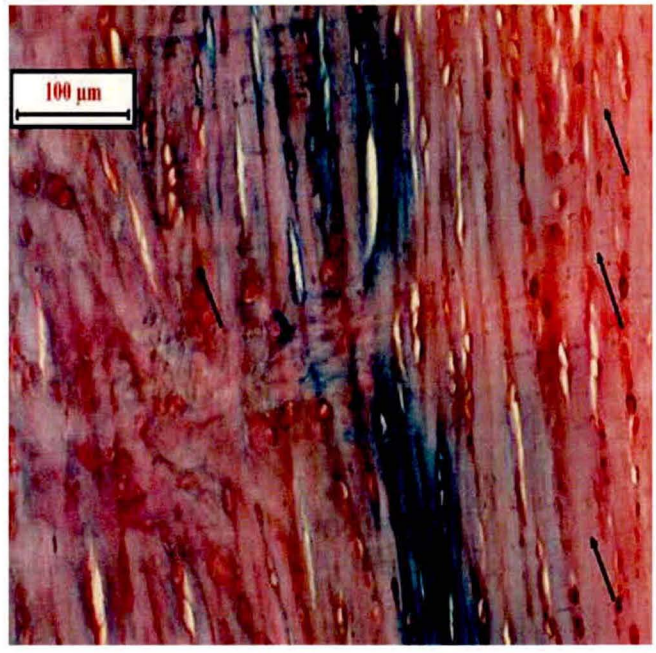
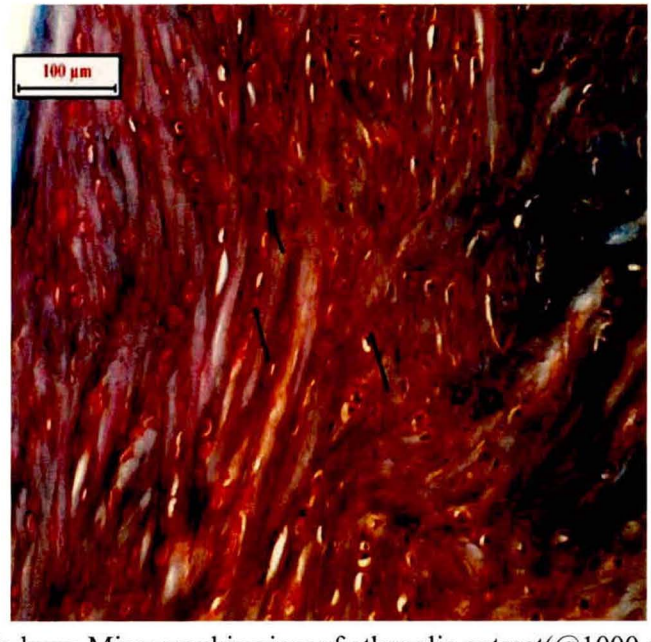
Gr	Parameter	Score (Mean + SE)	Picture
II	Adhesion (pannus)	1.8 ± 0.11	 Fig lxix: Micrographic view of linezolid treated rabbit showing less amount of marginal pannus (H & E stain)
III	Adhesion (pannus)	1.5 ±0.10	 Fig lxx: Micrographic view of linezolid with single betamethasone treated rabbit showing very less amount of pannus (marginal) (H & E stain)

Gr	Parameter	Score (Mean + SE)	Picture
IV	Adhesion (pannus)	2.2 ± 0.07	 Fig lxxi: Micrographic view of ethanolic extract(@500 mg kg^{-1}) treated rabbit showing pannus (H & E stain)
V	Adhesion (pannus)	1.5 ± 0.10	 Fig lxxii: Micrographic view of ethanolic extract(@1000 mg kg^{-1}) treated rabbit showing less amount of pannus (H & E stain)

Gr	Parameter	Score (Mean + SE)	Picture
VI	Adhesion (pannus)	2.1 ± 0.12	 Fig lxxiii: Micrographic view of aqueous extract(@500 mg kg^{-1}) treated rabbit showing pannus (H & E stain)
VII	Adhesion (pannus)	1.3 ± 0.09	 Fig lxxiv: Micrographic view of aqueous extract(@1000 mg kg^{-1}) treated rabbit showing less amount of pannus (H & E stain)

Gr	Parameter	Score (Mean + SE)	Picture
Healthy	Grey reads (Red Value)	0(0.00)	 Fig lxxv: Micrographic view of healthy control rabbit showing highest amount of proteoglycan (Safranin O stain)
I	Grey reads (Red Value)	3.7(0.10)	 Fig lxxvi: Micrographic view of septic arthritis induced rabbit showing least amount of proteoglycan (Safranin O stain)

Gr	Parameter	Score (Mean + SE)	Picture
II	Grey reads (Red Value)	1.8(0.11)	 Fig lxxvii: Micrographic view of linezolid treated rabbit showing good amount of proteoglycan (Safranin O stain)
III	Grey reads (Red Value)	1.5(0.10)	 Fig lxxviii: Micrographic view of linezolid with single betamethasone treated rabbit showing good amount of proteoglycan (Safranin O stain)

Gr	Parameter	Score (Mean + SE)	Picture
IV	Grey reads (Red Value)	2.6(0.23)	 Fig lxxix: Micrographic view of ethanolic extract(@500 mg kg^{-1}) treated rabbit showing less amount of proteoglycan (Safranin O stain)
V	Grey reads (Red Value)	2.0(0.06)	 Fig lxxx: Micrographic view of ethanolic extract(@1000 mg kg^{-1}) treated rabbit showing good amount of proteoglycan (Safranin O stain)

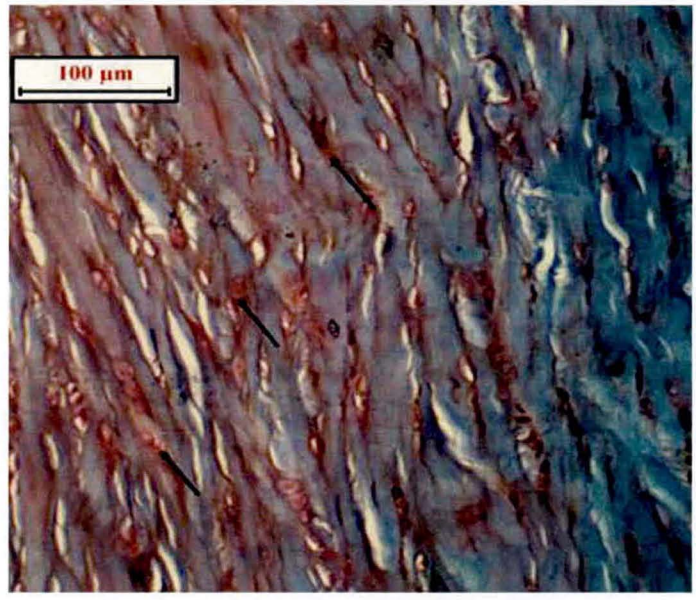
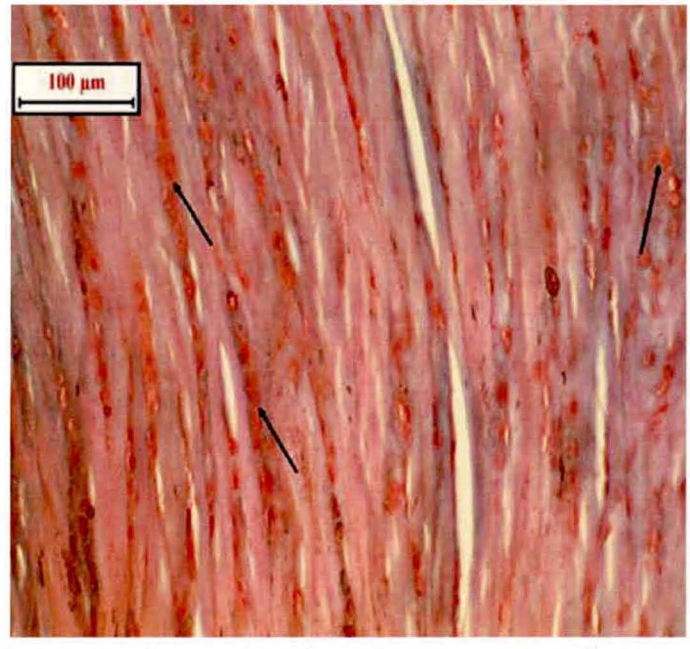
Gr	Parameter	Score (Mean + SE)	Picture
VI	Grey reads (Red Value)	2.5(0.14)	 Fig lxxxi: Micrographic view of aqueous extract(@500 mg kg^{-1}) treated rabbit showing less amount of proteoglycan (Safranin O stain)
VII	Grey reads (Red Value)	1.7(0.11)	 Fig lxxxii: Micrographic view of aqueous extract(@1000 mg kg^{-1}) treated rabbit showing good amount of proteoglycan (Safranin O stain)

Table 28 (a – f): Histopathological and Histochemical scoring of septic arthritic(Gr-I),oral linezolid @ 75 mg kg^{-1} for 10 days twice daily (Gr – II), oral linezolid @ 75 mg kg^{-1} for 10 days twice daily with a single intra-articular injection of betamethasone @ 0.5 mg kg^{-1} (Gr – III), ethanolic extract (in 2% tween 20) of *Tamarindus indica* L. leaves treatment @ 500 mg kg^{-1} for 14 days(Gr – IV), ethanolic extract (in 2% tween 20) of *Tamarindus indica* L. leaves treatment @ 1000 mg kg^{-1} for 14 days(Gr – V), aqueous extract of *Tamarindus indica* L. leaves treatment @ 500 mg kg^{-1} for 14 days (Gr – VI) and aqueous extract of *Tamarindus indica* L. leaves treatment @ 1000 mg kg^{-1} for 14 days (Gr – VII) in rabbits [n=6]

Group	Overall Score
Normal healthy	0
Gr -I	14.4
Gr -II	7.3
Gr -III	6.5
Gr -IV	11.0
Gr -V	8.2
Gr - VI	10.8
Gr - VII	7.1

Table 28(f): over-all histomorphological scoring of different groups

Bacterial colony count ($\times 10^5$) in synovial fluid of left stifle joint on different days septic arthritic (Gr- I),oral linezolid @ 75mg kg^{-1} twice daily for 10 days(Gr – II), oral linezolid @ 75 mg kg^{-1} twice daily for 10 days with a single intra-articular injection of betamethasone @ 0.5 mg kg^{-1} (Gr – III), ethanolic extract (in 2 % tween 20) of *Tamarindus indica* L. leaves treatment @ 500 mg kg^{-1} for 14 days(Gr – IV), ethanolic extract (in 2 % tween 20) of *Tamarindus indica* L. leaves treatment @ 1000 mg kg^{-1} for 14 days(Gr – V), aqueous extract of *Tamarindus indica* L. leaves treatment @ 500 mg kg^{-1} for 14 days (Gr – VI) and aqueous extract of *Tamarindus indica* L. leaves treatment @ 1000 mg kg^{-1} for 14 days(Gr – VII) in rabbits have been presented in table no 29. In untreated group bacterial colony count was increased significantly following intra-articular inoculation of 10^4 CFU *S. aureus* in the left stifle joint of rabbits. Whereas colony count was decreased significantly on different days in linezolid and linezolid with betamethasone treated groups. Both the ethanolic and aqueous extracts of *Tamarindus indica* L. leaves @1000 mg/kg significantly decreased bacterial colony on different days (table 29)

Table 29: Bacterial colony count ($\times 10^5$) in synovial fluid of left stifle joint on different days in septic arthritic(Gr- I),oral linezolid @ 75 mg kg^{-1} for 10 days twice daily (Gr – II), oral linezolid @ 75 mg kg^{-1} for 10 days twice daily with a single intra-articular injection of betamethasone @ 0.5 mg kg^{-1} (Gr – III), ethanolic extract (in 2% tween 20) of *Tamarindus indica* L. leaves treatment @ 500 mg kg^{-1} for 14 days(Gr – IV), ethanolic extract (in 2% tween 20) of *Tamarindus indica* L. leaves treatment @ 1000 mg kg^{-1} for 14 days(Gr – V), aqueous extract of *Tamarindus indica* L. leaves treatment @ 500 mg kg^{-1} for 14 days (Gr – VI) and aqueous extract of *Tamarindus indica* L. leaves treatment @ 1000 mg kg^{-1} for 14 days (Gr – VII) in rabbits [n=6]

DAY / GROUPS	DAY 2	DAY 7	DAY 16
GR-I	1.19 ± 0.49^{a}	10.83 ± 3.2^{b}	UC
GR-II	1.11 ± 0.19^{a}	0.28 ± 0.036^{b}	nil
GR-III	1.13 ± 0.26^{a}	0.26 ± 0.058^{b}	nil
GR-IV	1.08 ± 0.47^{a}	0.79 ± 0.3^{a}	0.37 ± 0.11^{a}
GR-V	1.19 ± 0.49^{a}	0.34 ± 0.043^{ab}	0.057 ± 0.036^{b}
GR-VI	1.16 ± 0.25^{a}	0.62 ± 0.084^{b}	0.14 ± 0.052^{c}
GR-VII	1.28 ± 0.51^{a}	0.31 ± 0.068^{bc}	0.028 ± 0.028^{c}

Table 29: Mean ±S.E. value with dissimilar superscript (abc) in a row vary significantly (P < 0.05) [n=6]

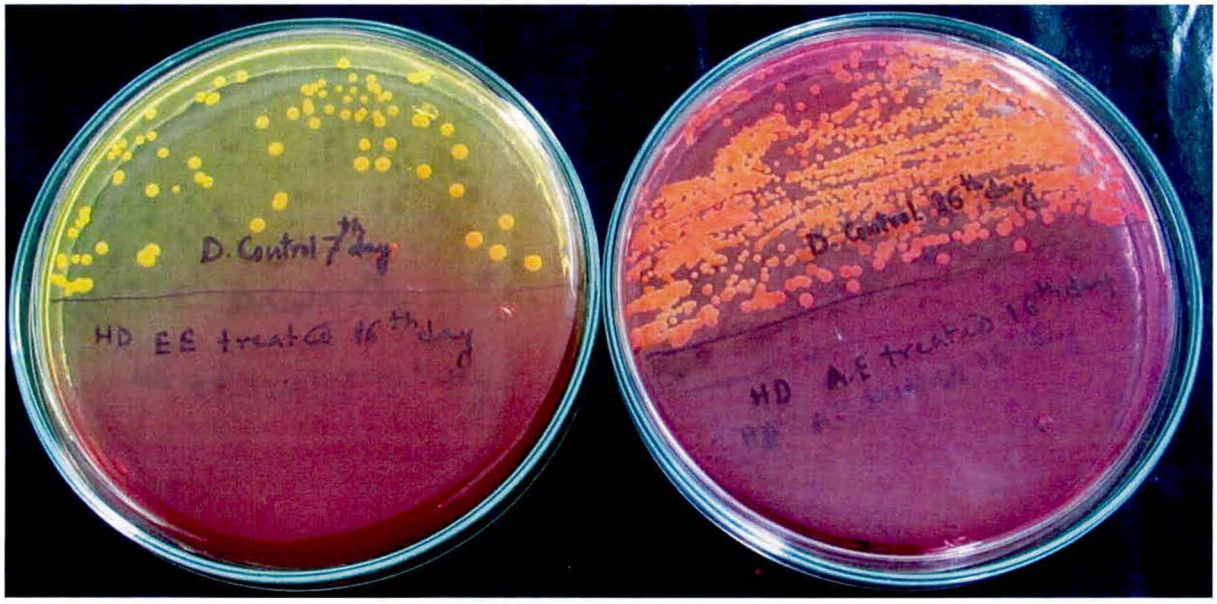


Figure lxxxiii *: S. aureus* colonies isolated from synovial fluid of left stifle joint in septic arthritis induced rabbits on day 7(Upper half of the left plate) and day 16(Upper half of the right plate) following intra-articular inoculation of 10^4 CFU of *S. aureus* isolated from septic arthritis; *S. aureus* colonies isolated from synovial fluid of left stifle joint in septic arthritis induced rabbits on day 16(Lower half of the left plate) following daily oral dosing of ethanolic extract of *T. indica* L. leaves at 1000 mg kg^{-1} for 14 consecutive day; *S. aureus* colonies isolated from synovial fluid of left stifle joint in septic arthritis induced rabbits on day 16(Lower half of the left plate) following daily oral dosing of aqueous extract of *T. indica* L. leaves at 1000 mg kg^{-1} for 14 consecutive day.

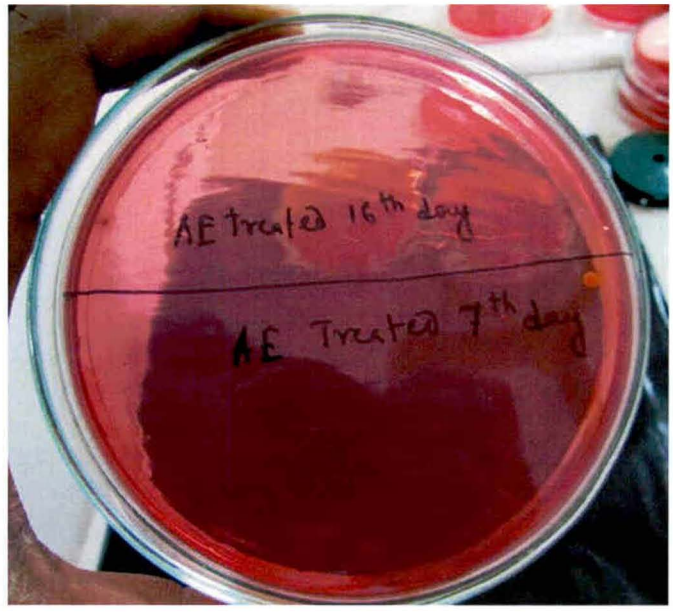


Figure lxxxiv: *S. aureus* colonies isolated from synovial fluid of left stifle joint in septic arthritis induced rabbits on day 7(Lower half of the plate) and day 16(Upper half of the plate) following daily oral dosing of aqueous extract of *T. indica* L. leaves at 1000 mg kg^{-1} for 14 consecutive days

Figure lxxxv*: S. aureus* colonies isolated from synovial fluid of left stifle joint in septic arthritis induced rabbits on day 7(Lower half of the plate) and day 16(Upper half of the plate) following oral dosing of linezolid at 75mg kg^{-1} for 10 consecutive days twice daily

Standardization of the analytical techniques of linezolid

Linezolid from plasma and synovial fluid was estimated by HPLC at 256 nm according to the modified method of Peng *et al.*,1999 under the operating conditions of the chromatograph as stated in 'Material and Methods'.

Recovery of the drug in plasma and synovial fluid

The results of recovery experiments of linezolid from plasma and synovial fluid are summarized in table . The recovery percentage were 88.32 ± 1.45 and 85.72 ± 1.07 respectively in plasma and synovial fluid. The recoveries were found to be satisfactory as the drug was obtained more than 80% in plasma and synovial fluid. Therefore, the methods used for estimation of linezolid was reliable and adopted thereafter. The limit of quantification for the drug in plasma and synovial fluid were 0.08 ppm and 0.1 ppm respectively and limit of detection were 0.05 ppm and 0.075 ppm. The linearity of the calibration curve was checked and linearity was found to be maintained in plasma and synovial fluid, and found to be satisfactory. The linearity of the recovery curves were studied starting from 0.08 to 10 ppm. However the retention time of linezolid showed inter-day variation.

Table 30: Mean ±S.E. recovery percentage of linezolid from plasma and synovial fluid by HPLC estimation method

Substrate	Recovery Percentage
Plasma	88.32 ± 1.45
Synovial fluid	85.72 ± 1.07

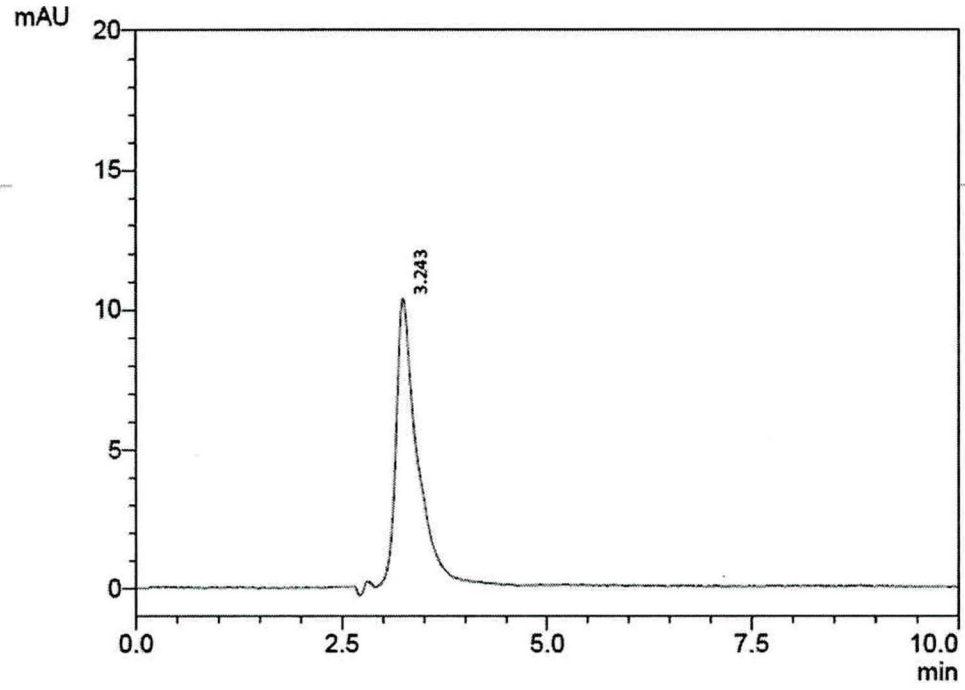


Figure lxxxvi : Chromatogram of standard linezolid 2 ppm (RT – 3.243)

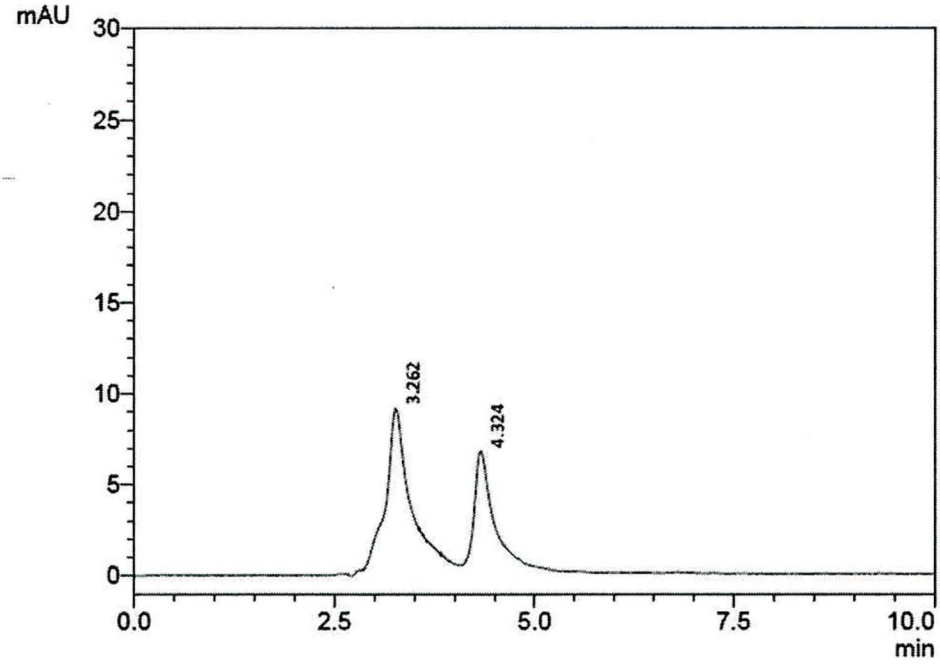
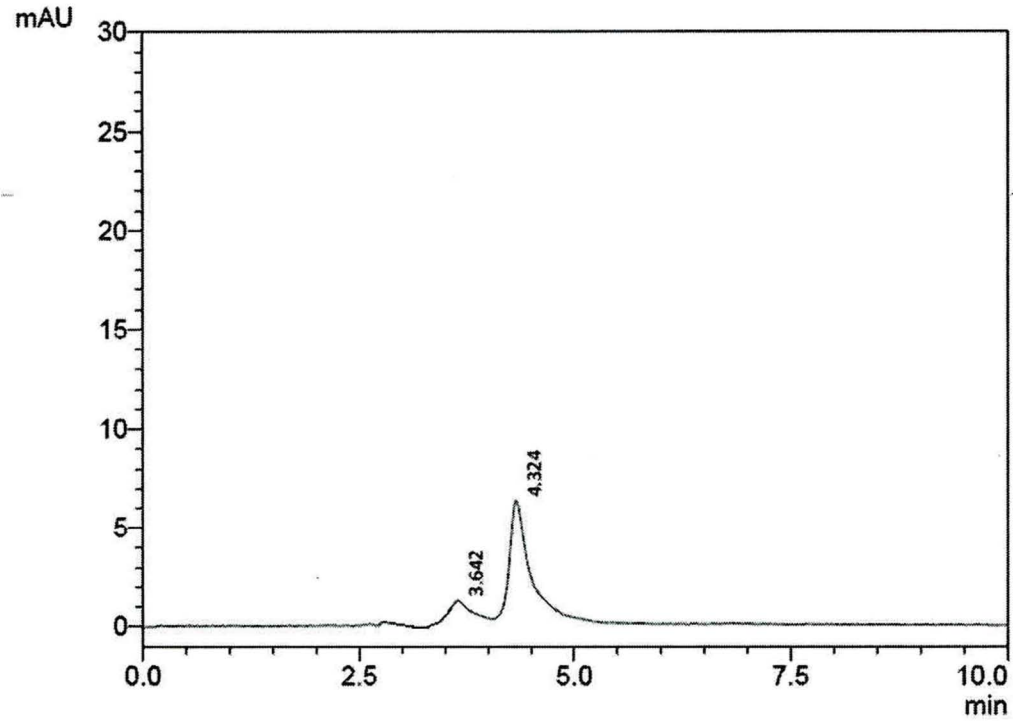


Figure lxxxvii: Chromatogram linezolid detected in plasma sample (Linezolid, RT-3.262)



Figurel xxxviii : Chromatogram of blank plasma

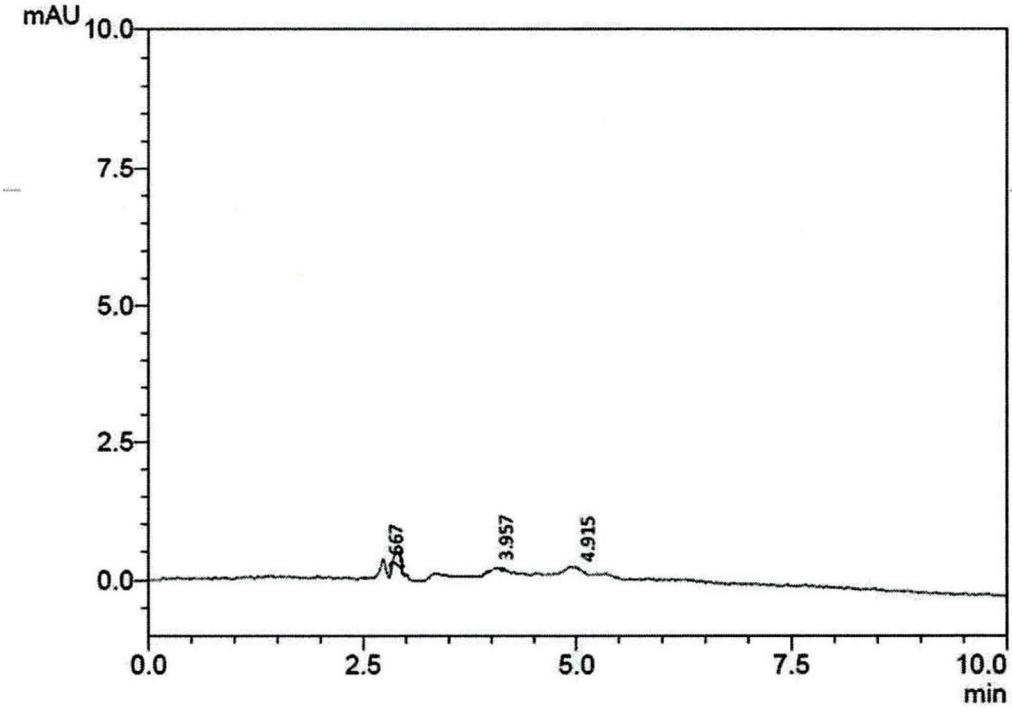


Figure lxxxix: Chromatogram of blank synovial fluid

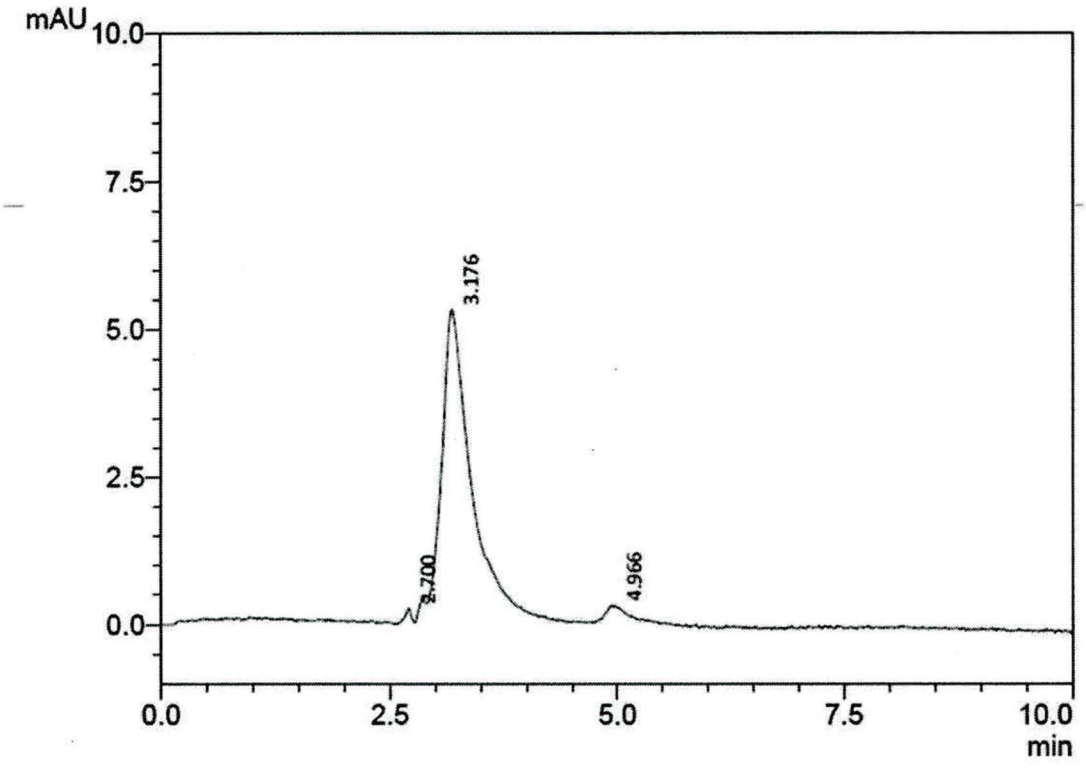


Fig lxxxx: Chromatogram of linezolid detected in synovial fluid (Linezolid, RT- 3.176)

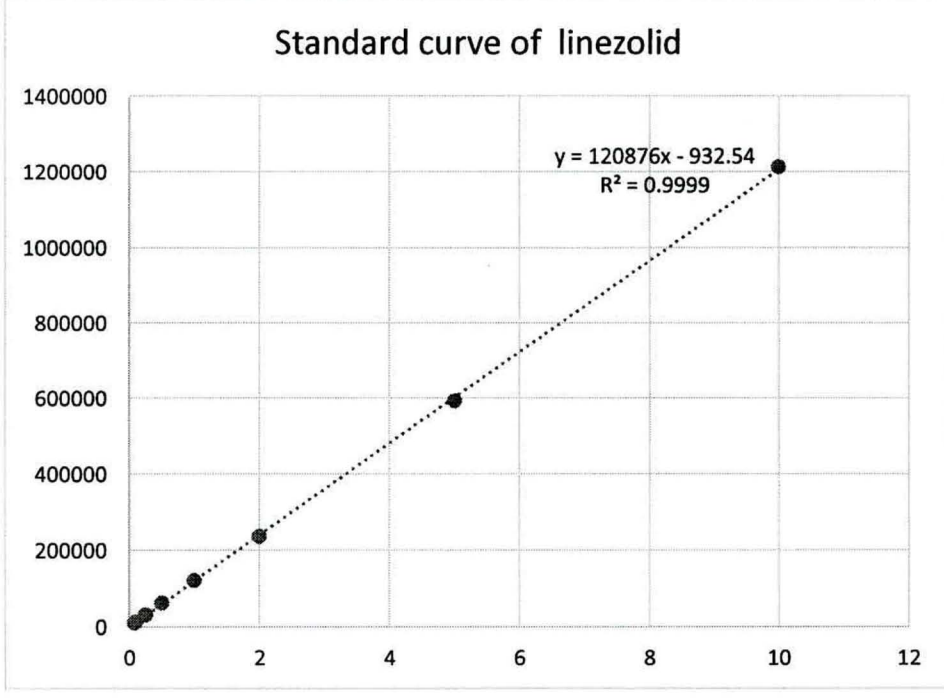


Figure lxxxxi: Standard curve of linezolid

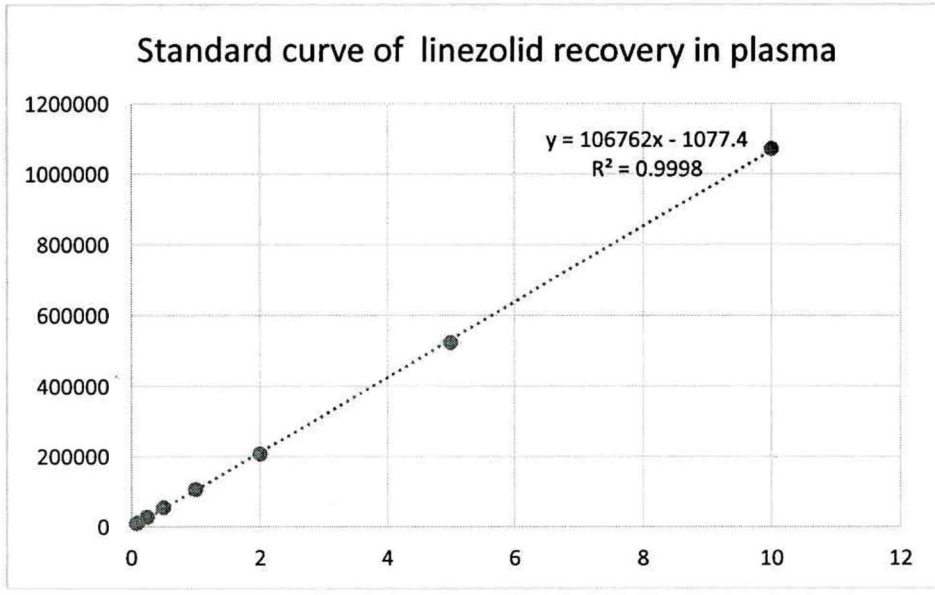


Figure lxxxxii: Standard curve of recovery of linezolid in plasma

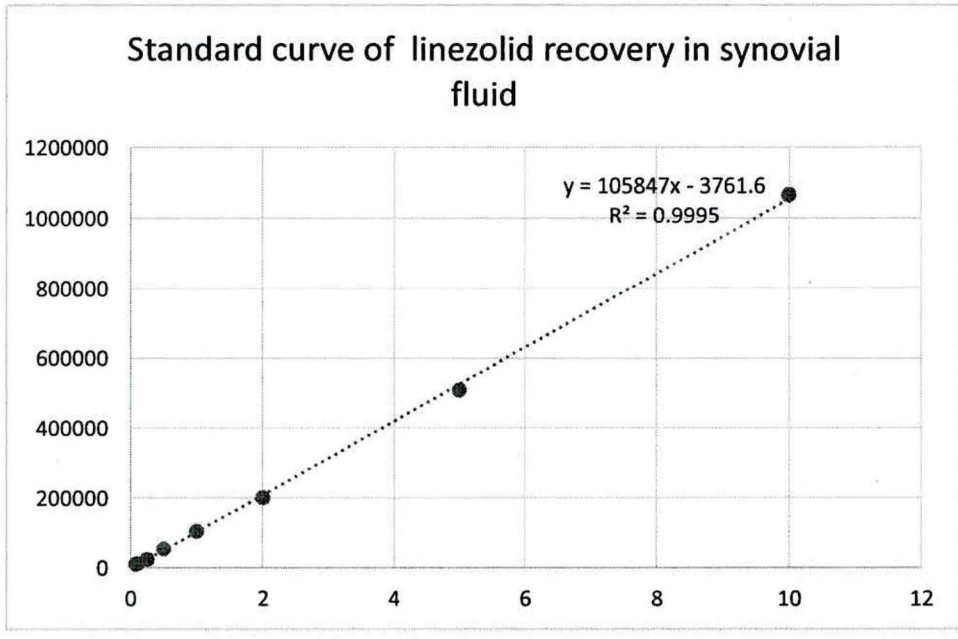


Figure lxxxxiii: Standard curve of recovery of linezolid in synovial fluid

Mean ±S.E. of plasma concentration of linezolid (µg ml^{-1}) following single dose oral administration of linezolid @75 mg kg^{-1} in healthy rabbits was depicted in table no. .Mean maximum plasma concentration (11.09 ± 0.15 µg ml^{-1}) of linezolid following single oral dosing @75 mg kg^{-1} was recorded at 2 hour followed by a declining concentration and minimum plasma concentration (1.15 ± 0.09 µg ml^{-1}) was detected at 6 hour post-dosing. Plasma concentration of linezolid (2.83 ± 0.09 µg ml^{-1}) was recorded at 0.25 hour, which gradually increased at 0.50 and 1 hour and achieved peak concentration at 2 hour.

Table 31: Mean ±S.E. plasma concentration of linezolid (µg ml^{-1}) following single dose oral administration of linezolid @75 mg kg^{-1}

Time(hour)	Concentration (µg/ml)
0.25	2.83 ± 0.09
0.50	4.55 ± 0.15
1	6.97 ± 0.09
2	11.09 ± 0.15
4	4.64 ± 0.11
6	1.15 ± 0.09
9	BDL
12	BDL
24	BDL
36	BDL
48	BDL
60	BDL
76	BDL
96	BDL

Table 31: *[BDL- Below Detectable Level]

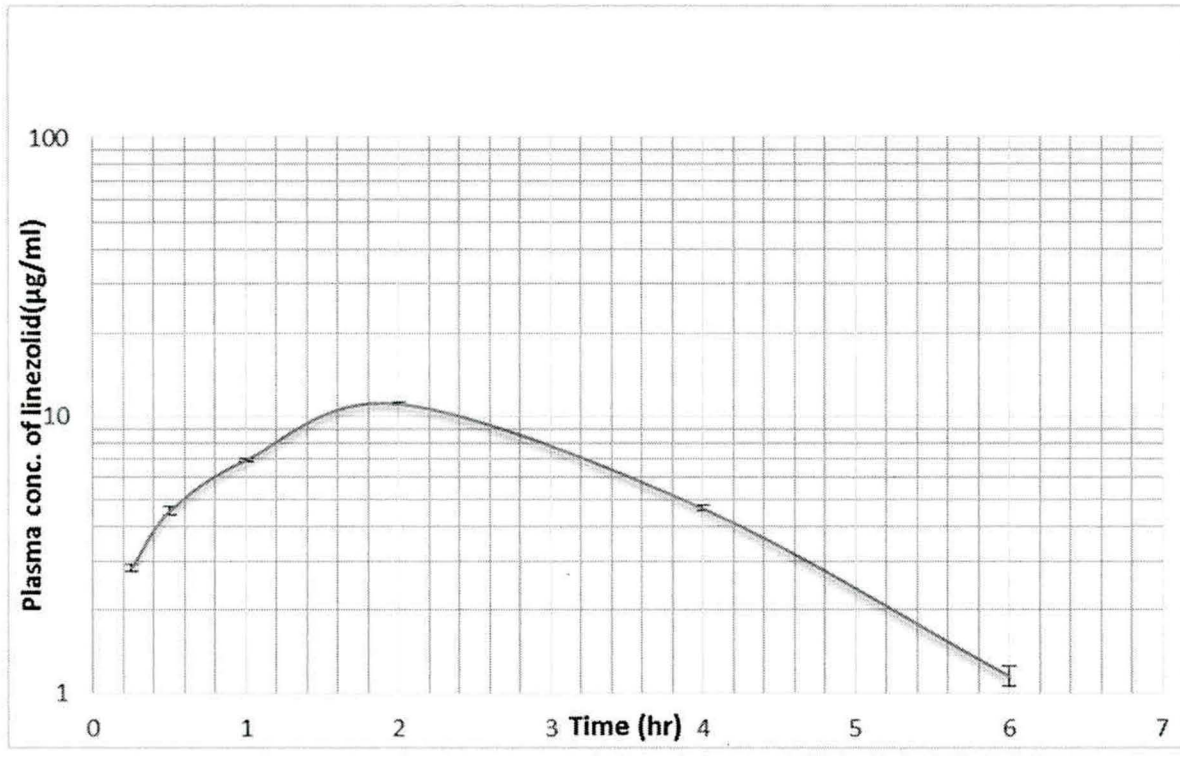


Figure lxxxxiv: Mean plasma concentration (µg ml^{-1}) in rabbits following single oral dose administration of linezolid @75 mg kg^{-1} body weight

Mean ± S.E. of synovial fluid concentration of linezolid (µg ml^{-1}) following single dose oral administration of linezolid @75 mg kg^{-1} in healthy rabbits was depicted in table..Mean maximum synovial fluid concentration (11.14 ± 0.74 µg ml^{-1}) of linezolid following single oral dosing @75 mg kg^{-1} was recorded at 2 hour followed by a declining concentration and minimum synovial fluid concentration (0.16 ± 0.004 µg ml^{-1}) was detected at 24 h. post-dosing. Concentration of linezolid concentration (µg ml^{-1}) in synovial fluid persisted up to 96 hour of collection period.

The MIC value of linezolid was found to be 1.5 µg ml^{-1} in the present study. So, linezolid maintained the MIC value up to 8 hour post dosing, which supported the fact that linezolid @75 mg/kg can be administered orally twice daily for treatment of susceptible bacterial infection.

Table 32: Mean ±S.E. synovial fluid concentration of linezolid (µg ml^{-1}) following single dose oral administration of linezolid @75 mg kg^{-1}

Time(hour)	Concentration (µg ml^{-1})
2	11.14 ± 0.74
4	5.35 ± 0.13
8	1.62 ± 0.06
24	0.16 ± 0.00
36	0.62 ± 0.01
48	0.20 ± 0.01
60	0.37 ± 0.01
76	0.48 ± 0.01
96	0.25 ± 0.01

Kinetic Parameters

The semi-logarithmic plot of mean plasma concentration of linezolid in healthy rabbits following single oral administration

Mean values of $t_{½}$ K_a and $t_{½}$ β were 0.71± 0.03 and 1.22 ± 0.06 h respectively. Mean values of AUC, $Vd_{area,}$ and Cl_B were found to be 34.64 ± 1.06 µg h ml^{-1}, 3.80 ± 0.09 L kg^{-1}, 34.08 ± 1.09 ml/min and mean MRT value was 2.710 ± 0.07 hr linezolid showed elimination half-life ($t_{½}$ β) of 1.22 ± 0.06 hr, while the absorption half-life ($t_{½}$ K_a) was found to be 0.71 ± 0.03 hr. Mean $t_{½}$ β value with mean Cl_B value 34.08 ± 1.09 ml/min indicated rapid clearance of linezolid in healthy rabbits. Mean Vd_{area} value 3.80 ± 0.09 L/kg indicated wide distribution of drug in the body of healthy rabbits.

Table 33: Kinetics parameters (values in mean ± S.E.) of linezolid following single oral administration @ 75mg kg^{-1} in rabbit

Kinetics parameters	values
C^0_P ($\mu g\ ml^{-1}$)	75.75 ± 3.76
A ($\mu g\ ml^{-1}$)	38.24 ± 1.32
B ($\mu g\ ml^{-1}$)	37.51 ± 1.67
K_a (h^{-1})	0.98 ± 0.04
$t_{½}$ K_a (h)	0.71 ± 0.03
β (h^{-1})	0.57 ± 0.02
$t_{½}$ β (h)	1.22 ± 0.06
AUC ($\mu g\ h\ ml^{-1}$)	34.64 ± 1.06
Vd_{area} ($L\ kg^{-1}$)	3.80 ± 0.09
Cl_B ($ml\ kg^{-1}\ min^{-1}$)	22.72 ± 0.79
MRT (h)	2.710 ± 0.07
Vd_c ($L\ kg^{-1}$)	0.99 ± 0.03
Vd_{ss} ($L\ kg^{-1}$)	1.05 ± 0.04
K_{12} (h^{-1})	0.05 ± 0.00
K_{21} (h^{-1})	0.77 ± 0.02
K_{el} (h^{-1})	0.73 ± 0.02
f_c	0.78 ± 0.02
T~P	0.25 ± 0.01
C_{max_calc}($\mu g\ ml^{-1}$)	7.257 ± 0.31
T_{max_calc} (h)	1.38 ± 0.03

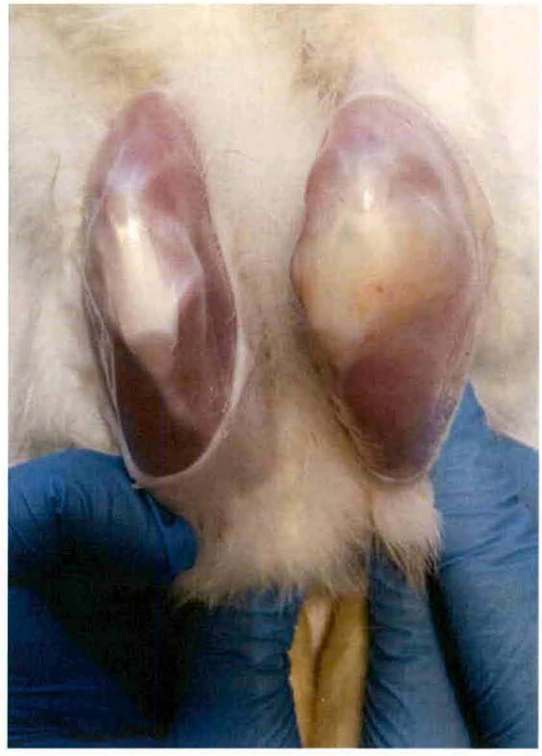


Figure lxxxxv: Left stifle joint of rabbit showing septic arthritis on day 16 following intra-articular inoculation of 10^4 CFU *S. aureus*

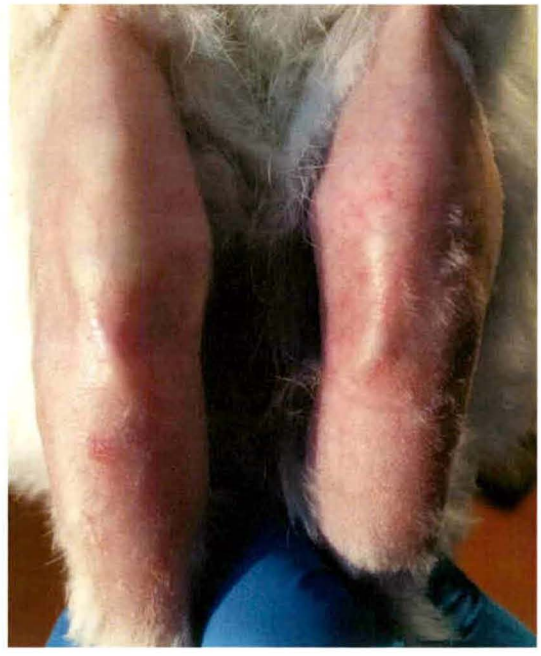
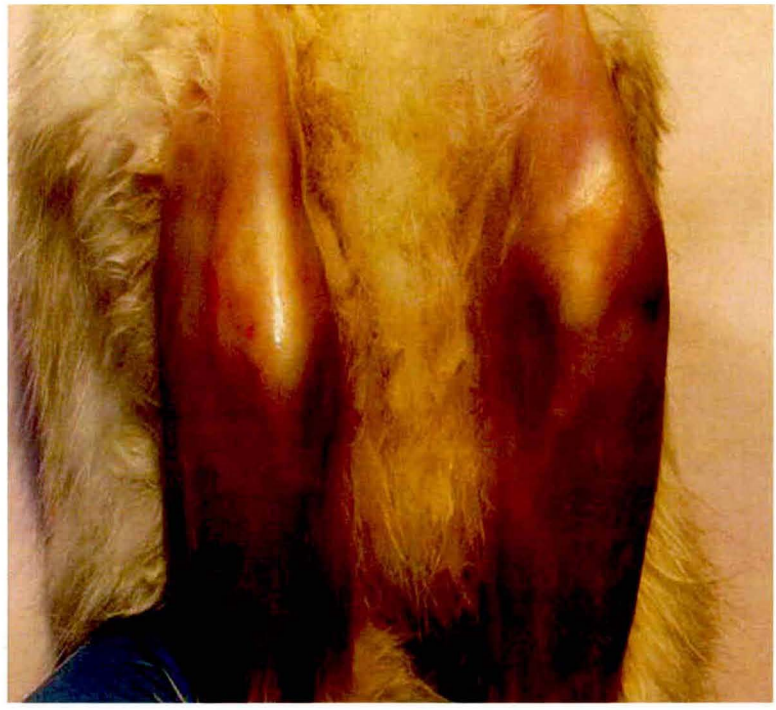


Figure lxxxxvi: Left stifle joint of rabbit after completion of treatment on day 16 with oral linezolid @75 mg kg^{-1} for 10 days twice daily with single intra-articular injection of betamethasone @0.5 mg kg^{-1} in septic arthritis



Figurelxxxxvii: Left stifle joint of rabbit after completion of treatment on day 16 with oral linezolid @75 mg kg^{-1} for 10 days twice daily in septic arthritis

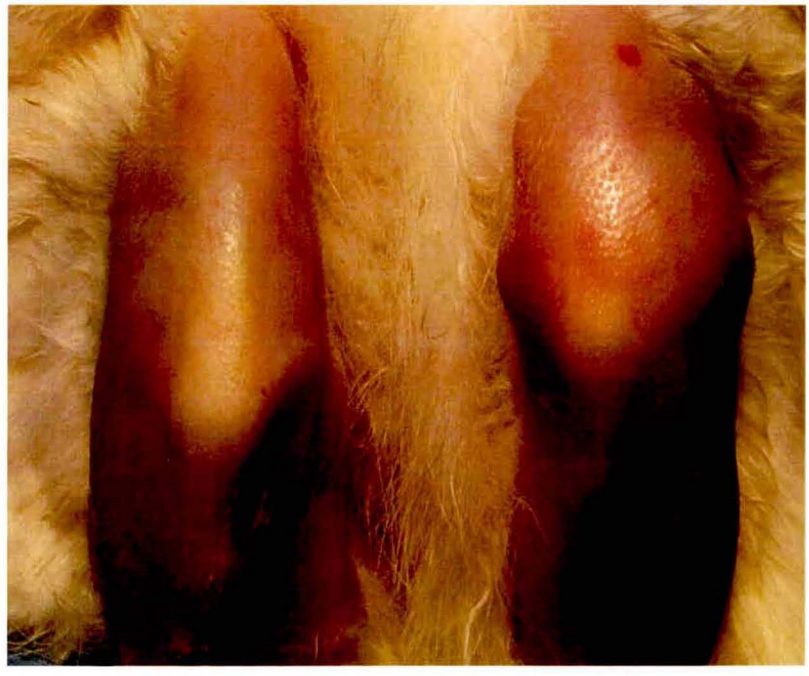
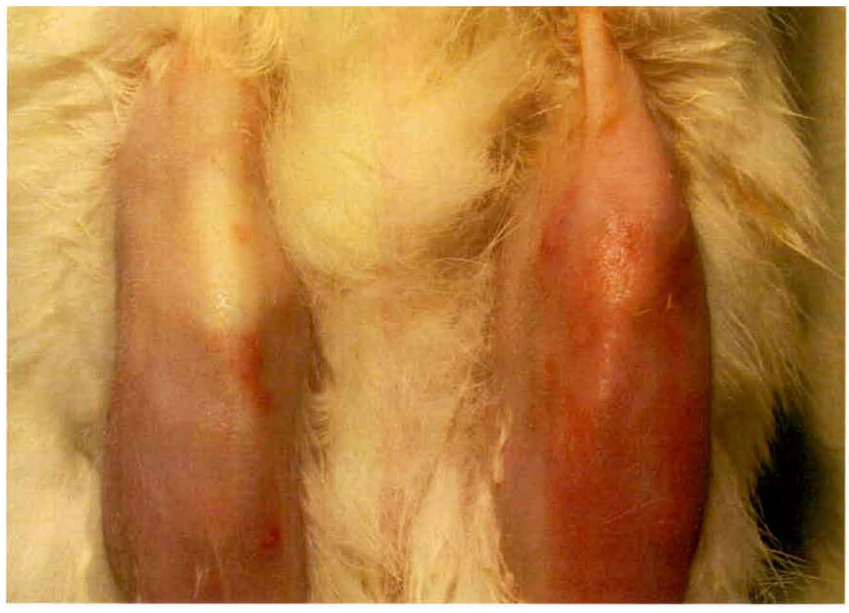


Figure lxxxxviii: Left stifle joint of rabbit after completion of treatment on day 16 with ethanolic extract of *Tamarindus indica* L. @500 mg/kg for 14 days in septic arthritis



Figurelxxxxix: Left stifle joint of rabbit after completion of treatment on day 16 with ethanolic extract of *Tamarindus indica* L. @1000 mg/kg for 14 days in septic arthritis

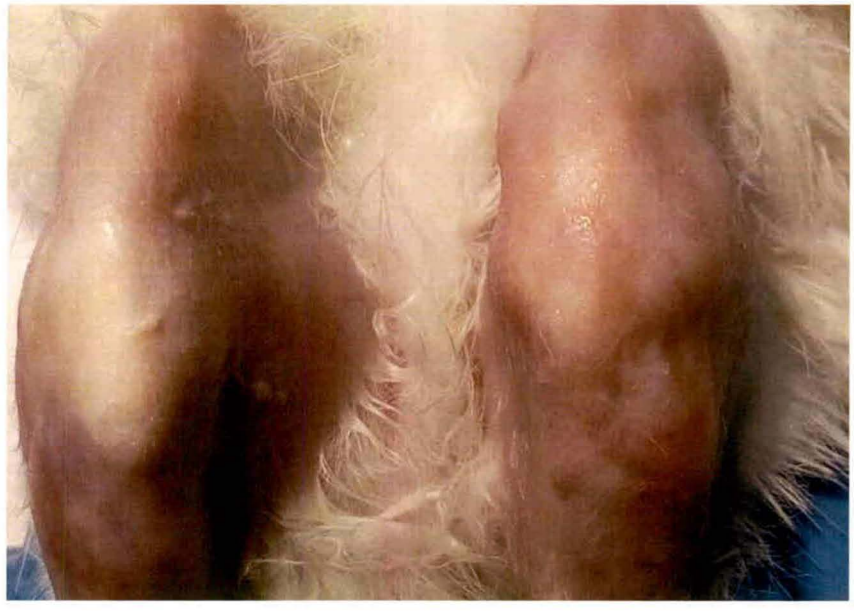


Figure C: Left stifle joint of rabbit after completion of treatment on day 16 with aqueous extract of *Tamarindus indica* L. @500 mg kg^{-1} for 14 days in septic arthritis

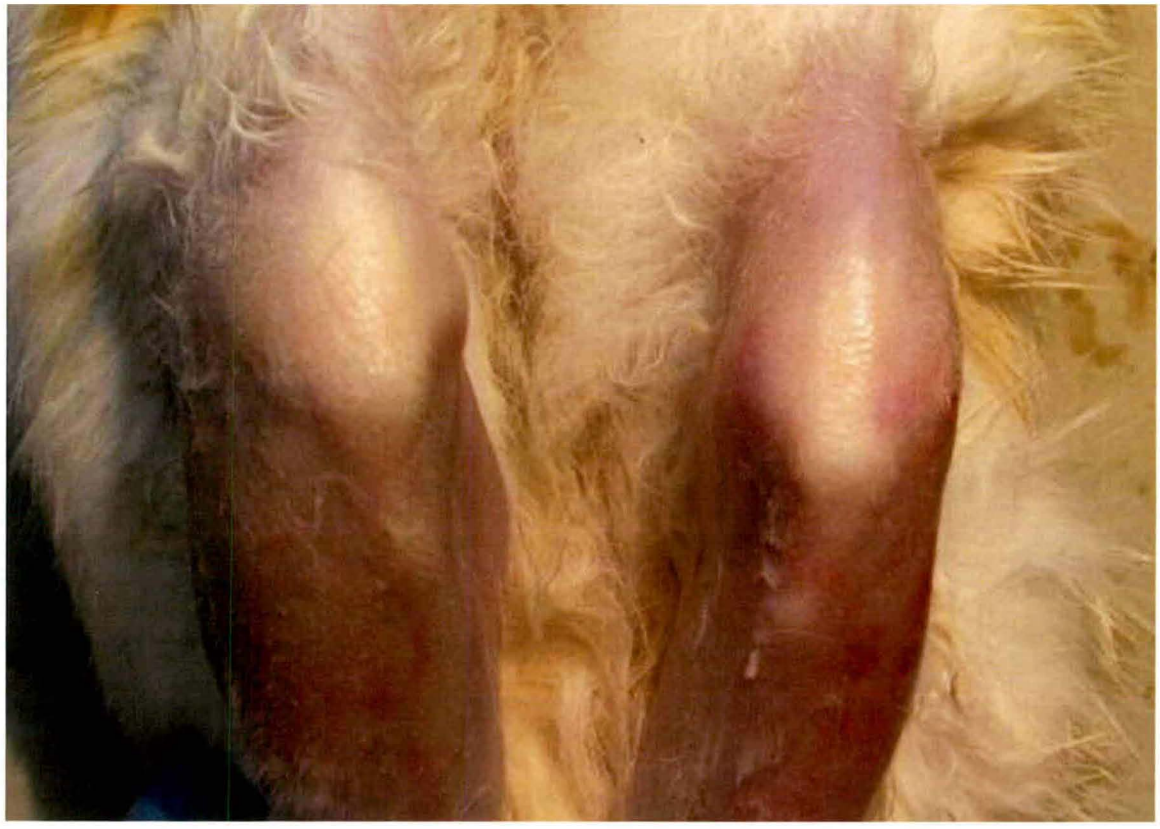
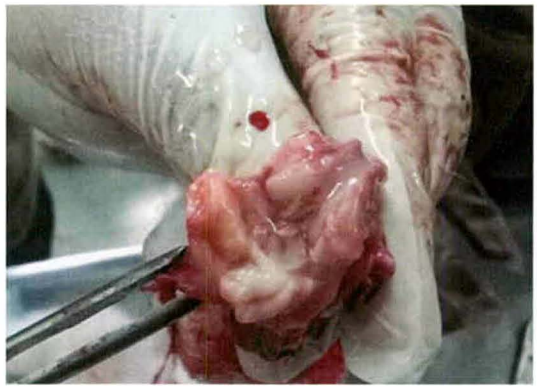
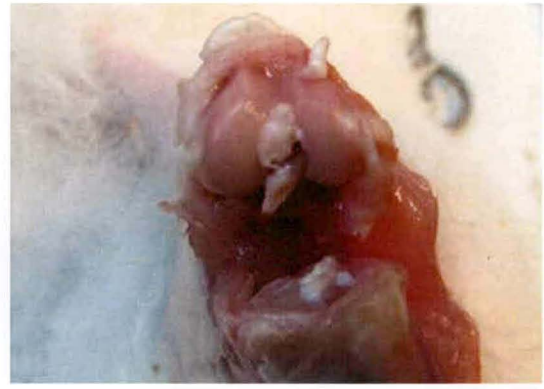


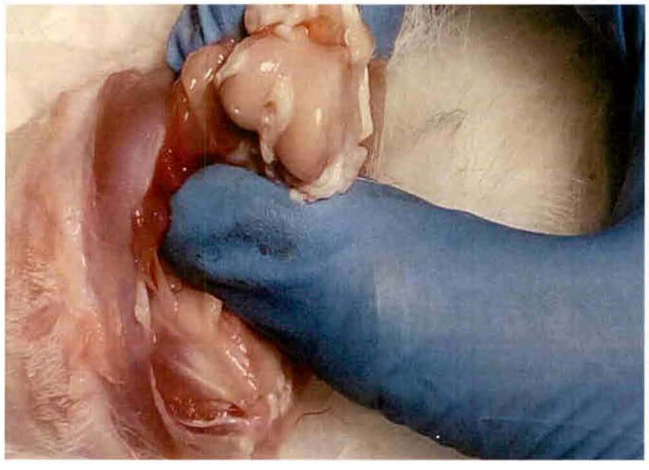
Figure Ci: Left stifle joint of rabbit after completion of treatment on day 16 with aqueous extract of *Tamarindus indica* L. @1000 mg kg^{-1} for 14 days in septic arthritis



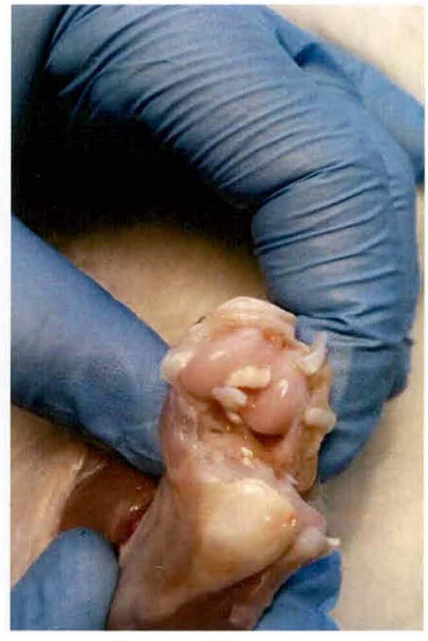
[a]



[b]



[c]



[d]

Figure cii: [a] : Left stifle joint surfaces of rabbit showing erosion following induction of septic arthritis in rabbit; [b] : Left joint surfaces of rabbit showing very less erosion while treated with linezolid (@75 mg kg^{-1}, P.O., bd) and intra-articular betamethasone following induction of septic arthritis; [c] : Left stifle joint surfaces of rabbit showing less erosion when treated with ethanolic extract of *T. indica* L. @1000 mg kg^{-1} b.w. for 14 days; [d] : Left stifle joint surfaces of rabbit showing less erosion when treated with aqueous extract of *T. indica* L. @1000 mg kg^{-1} b.w. for 14 days

SUMMARY AND CONCLUSION

Summary and conclusion

Septic arthritis is inflammation of a joint caused by a bacterial infection. Arthritis is a degenerative joint disease affecting socio-economic life of human as well as of animals. The condition is most commonly caused by staphylococcal or streptococcal bacteria. There are another species of bacteria too cause septic arthritis. From various study it was found that in more than 50% cases the causative organism is *S. aureus*.

The current preferred therapeutic drug treatments (antibiotics and corticosteroids), showed couple of adverse effects in prolonged use. Employing medicinal plants in the treatment of various human and veterinary diseases is an ancient idea. Therefore, an herbal therapy could be an alternative of conventional antibiotics and corticosteroids to combat septic arthritis.

Tamarindus indica L. leaves have been reported to have good antimicrobial as well as anti-inflammatory, antioxidant and anti-nociceptive properties. Therefore, aqueous and ethanolic extracts of *Tamarindus indica* L. leaves were prepared by standardized protocol with subsequent safety evaluation and fixation of dosage regimen to be used as an alternative therapy in septic arthritis. It was observed that the two extracts of *Tamarindus indica* L. leaves were practically nontoxic and can be used at the recommended dosage regimen safely in septic arthritis. Oral linezolid and intra-articular betamethasone as well as only oral linezolid were used as conventional therapy to compare the efficacy between alternative herbal and conventional synthetic drug therapy.

In the present study, the pus sample was collected from an affected joint of a kid suffering with septic arthritis. *S. aureus* was isolated from the sample. The strain was found to be resistant against methicillin and intermediately sensitive against vancomycin in AST. The study was divided mainly into 3 parts, viz., [1] Induction and confirmation of septic arthritis in rabbit. [2] Efficacy study of ethanolic and aqueous extract of *Tamarindus indica* L. leaf and Compare it with only oral linezolid and oral linezolid with single intra-articular betamethasone combine therapy. [3] Pharmacokinetics study of oral linezolid from rabbit plasma with monitoring its concentration in synovial fluid also.

Induction of septic arthritis had been done by intra-articular inoculation of 10^4 c.f.u. *S. aureus* in the left stifle joint of rabbits. The septic arthritis was confirmed by Studying of clinical signs, biochemical (LDH, Glucose,Total protein), haematological (Hb, TC, DC, ESR, PCV, MCV, MCHC, MCH) and microbiological (Bacterial culture and colony count from synovial fluid, ELISA for Rabbit's specific CRP and PCT) parameters from collected serum and synovial fluid at 0,2,7,16 days. Digital radiographs were taken to measure the joint space and observe the joint condition. Monitoring of body weight, body temperature, measurement of joint radius (externally) at regular intervals were also performed. Euthanasia of all animals for histopathological scoring of menisci with the help of different staining techniques had been performed after 16 days of study.

The rabbits inoculated with 10^4 c.f.u. *S. aureus* in the left stifle joint showed marked swelling and redness of the particular joint, lameness and restricted movement, restlessness, elevated body temperature and severe anorexia. Presence of pus was observed in the inoculated joint. Subsequent culturing of the collected pus from the particular joint showed the same organism with which the infection was given which confirmed induction of septic arthritis.

A total of 42 rabbits were divided into 7 different groups each containing 6 animals. Following induction of septic arthritis in all the rabbits, treatment was started after 48 hours with oral Linezolid @75 $mgkg^{-1}$ for 10 days twice daily (Gr – II), oral Linezolid @75 for 10 days $mgkg^{-1}$ twice daily with a single intra-articular injection of Betamethasone @0.5 $mgkg^{-1}$ (Gr – III), Ethanolic Extract of *Tamarindus indica* L. leaves @ 500 $mgkg^{-1}$ for 14 days(Gr – IV), Ethanolic Extract of *Tamarindus indica* L. leaves @ 1000 $mgkg^{-1}$ for 14 days(Gr – V), Aqueous Extract of *Tamarindus indica* L. leaves @ 500 $mgkg^{-1}$ for 14 days (Gr – VI) and Aqueous Extract of *Tamarindus indica* L. leaves @ 1000 $mgkg^{-1}$ for 14 days (Gr – VII) while Gr- I rabbits were kept untreated.

Bacterial colony count was performed on day 2, 7 and 16. On day 2 the bacterial colonies were ranged from $1.08 \pm 0.47 \times 10^5$ to $1.28 \pm 0.51 \times 10^5$ in different groups. After starting therapies in different groups (Gr – II to Gr – VII) bacterial colonies were found to be decreased in number on day 7 and subsequently decreased to nil (Gr – II and Gr – III) or very less (Gr – V and Gr – VII)

amount on day 16. So, it could be reported that leaves extracts (ethanolic and aqueous) of *Tamarindus indica* L. was found to have good antimicrobial property.

The mean serum glucose level did not alter significantly in any group except in rabbits of Gr-III, where it was increased significantly from day 7 to day 16. While significant alteration was found in synovial glucose level in all the groups. The simultaneous difference in glucose level between serum and synovial fluid was found to be ranged from 11.38 ± 0.69 to 14.14 ± 1.07 in healthy rabbits. The difference was gradually increased up to day 7 in all the groups but it was subsequently reduced (<25 mg dl^{-1}) significantly in rabbits of Gr - II, Gr – III, Gr – V, Gr – VI and Gr – VII and non-significantly (>25 mg dl^{-1}) in Gr - I and Gr – IV. So, the leaves extracts (ethanolic and aqueous) of *T. indica* L. is having enough potency in restoration of glucose level in synovial fluid.

The serum total protein level did not alter significantly in any group. But significant alteration was found in synovial total protein level in all the groups. In all the treatment groups, a trend of declining of synovial fluid total protein level after 4 days post-inoculation of 10^4 c.f.u. *S. aureus* in the left stifle joints of rabbits were noticed. But in untreated group synovial total protein level was found to be increased on different days. Total protein level in synovial fluid was observed to be above 4.5 g dl^{-1} on day 4 which indicated significant inflammation in the left stifle joints of rabbits of all the experimental groups. However, the total protein level in synovial fluid of rabbits of Gr-II, Gr-III and Gr- VII (Aqueous Extract of *Tamarindus indica* L. leaves treatment @ 1000 $mgkg^{-1}$ for 14 days) were significantly reduced to < 2.5 g dl^{-1} on day 16. In Gr – V, the level was also found to be very close to 2.5. So, from the findings it could be suggested that the leaves extracts (ethanolic and aqueous) of *T. indica* L. is having enough potentiality to subside higher total protein level in synovial fluid.

The synovial fluid LDH level was ranged from 49.40 ± 11.79 to 61.00 ± 7.63 in healthy rabbits. The LDH level of synovial fluid was increased gradually in all the groups up to day 7 and subsequently decreased on day 16. The LDH level was reduced more markedly in different treatment groups while it was found less markedly in rabbits of Gr-I.

Mean ESR level was gradually increased up to day 7 following intra-articular inoculation of 10^4 c.f.u. *S. aureus* in the left stifle joint of rabbits and significantly declined on day 16 in all the

groups. The intensity of reduction of all the treatment groups was observed to be more compared to untreated arthritic group. So, it could be opined that the leaves extracts (ethanolic and aqueous) of *T. indica* L. is also effective to reduce increased ESR level.

Body temperature was significantly increased up to day 5 following intra-articular inoculation of 10^4 c.f.u. *S. aureus* in the left stifle joint of rabbits of all the groups except Gr- VII. Thereafter the body temperature of the animals was found to be decreased in all the groups on day 16. A significant rise in temperature was found between day 8 to day 12 in animals of Gr – IV, VI and VII while the body temperature of animals only of Gr – II was found to be significantly decreased between day 8 and day 12.

Mean value of joint radius of healthy rabbits was ranged from 1.358 ± 0.006 to 1.403 ±0.006. Swelling of the inoculated stifle joint caused increase of the circumference of the particular joint, leading to increase of joint radius. Gradual increased joint radius was recorded up to day 8 following intra-articular inoculation of 10^4 c.f.u. *S. aureus* in the left stifle joint of rabbits followed by decrease in joint radius in the same arthritic joint. However, interestingly the joint radius of the inoculated stifle joint was observed to be reduced in all the treatment groups (Gr – II, Gr – III, Gr – V, Gr – VI and Gr – VII) except Gr – IV from day 5. So, it could be suggested that the leaves extracts (ethanolic and aqueous) of *Tamarindus indica* L. is also efficacious to reduce swelling, which may be due to its anti-inflammatory property.

The affected menisci were removed after 16 days post inoculation of 10^4 c.f.u. *S. aureus* in the left stifle joint of rabbits. A marked erosion in both of the meniscus were found in septic arthritis group. But the menisci were not found to be badly eroded compare to untreated group after treatment with antibiotic and steroid (group - III), ethanolic extract of Tamarindus indica L. leaves @1000 $mgkg^{-1}$ (group - V) and aqueous extract of *Tamarindus indica* L. leaves @1000 $mgkg^{-1}$ (group - VII). The groups treated with only antibiotic (group - II), ethanolic extract of *Tamarindus indica* L. leaves @500 $mgkg^{-1}$ (group - IV) and aqueous extract of *Tamarindus indica* L. leaves @500 $mgkg^{-1}$ (group - VI) also showed a less erosion compare to the untreated group but not up to group – III, group – V and group – VII. So, it could be inferred that the leaves extracts (ethanolic and aqueous) of *T. indica* L. in higher dose rate (@1000 $mgkg^{-1}$ b.w.) is having a remarkable chondroprotective potential, whereas the leaves extracts (ethanolic and

aqueous) of *T. indica* L. in lower dose rate (@500 $mgkg^{-1}$ b.w.) is also having chondroprotective potential, but a bit lesser in comparison with higher dose rates.

Intra-articular inoculation of 10^4 c.f.u. *S. aureus* in the left stifle joint of rabbits produced serum procalcitonin level more than 0.4 $ngml^{-1}$ on day 2,7,16, which confirmed induction of septic arthritis in these animals. But treatment with Linezolid, Linezolid with Betamethasone decreased the values less than 0.4 $ngml^{-1}$ on day 16. The ethanolic and aqueous extracts (@1000$mgkg^{-1}$ for 14 days) of *T. indica* L. leaves also reduced serum PCT levels to less than 0.4 $ngml^{-1}$ on day 16, which indicated probable recovery of septic arthritis in rabbits. However, the lower dosages of ethanolic and aqueous extracts (@500$mgkg^{-1}$ for 14 days) of *T. indica* L. leaves could reduce serum PCT level to less than 0.6 and less than 0.5 respectively on day 16. The findings suggested that both the extracts of T. indica L. leaves at higher dosage i.e. @1000 $mgkg^{-1}$ for 14 days were found to be more effective compare to lower dosage i.e. @500$mgkg^{-1}$.

The C-Reactive protein in serum was below detectable level in all the groups on day 0. The level was markedly increased (>50 $ngml^{-1}$) in all the groups following intra-articular inoculation of 10^4 c.f.u. *S. aureus* in the left stifle joint of rabbits on day 2. However the level of CRP in serum was found to be maintained more than 20 $ngml^{-1}$ on day 16 in septic arthritis induced rabbits (group-I). On the other hand, rabbits of different treatment groups (Gr- I,II,III,IV,V,VI and VII) showed decreased CRP level on day 7 and 16. The serum CRP level was below 5 $ngml^{-1}$ in all the treated groups indicating possible recovery of inflammation of joint. So, it could be concluded that the leaves extracts (ethanolic and aqueous) of *T. indica* L. is also efficacious to optimize C-Reactive protein level in serum.

Induction of septic arthritis produced histomorphological changes like loss of matrix, loss of cellularity, cloning of chondrocytes, adhesion of pannus and loss of proteoglycans in the meniscal cartilage in left stifle joint of rabbits. However, oral linezolid with single intra-articular betamethasone dosage regimen, only oral linezolid dosage regimen, aqueous extract of *Tamarindus indica* L. leaves at higher dosage regimen and ethanolic extract of *Tamarindus indica* L. leaves at higher dosage regimen caused significant improvement of septic arthritis. Lower dosage regimen of aqueous and ethanolic extract of *Tamarindus indica* L. leaves also showed promising result.

Narrowing of joint space and erosion of joint cartilage in the left stifle joint were recorded in septic arthritis induced rabbits. Noticeable erosion of menisci was also recorded in the same animals. While oral linezolid with single intra-articular betamethasone dosage regimen, aqueous extract of *Tamarindus indica* L. leaves at higher dosage regimen, only oral linezolid dosage regimen, aqueous and ethanolic extract of *Tamarindus indica* L. leaves at higher dosage regimen were found to prevent narrowing of joint space and erosion of the meniscal cartilage.

So, from the present study, it can be recommended that the induction of septic arthritis by *s. aureus* in rabbits can be utilized as a good model for research purpose for further study. The efficacy study of both the alternative herbal therapy (aqueous and ethanolic extract of *T. indica* L. leaves) and conventional antibiotic (linezolid) and or antibiotic (linezolid) and corticosteroid (betamethasone) therapy showed more or less comparable efficacy in treatment of septic arthritis. However, monitoring of different parameters and markers suggested that both the extract produced better effect at a higher dosage regimen (@ 1000 $mgkg^{-1}$ body weight for 14 days) compare to lower dosage regimen (@ 500 $mgkg^{-1}$ body weight for 14 days). Further research on these two extracts of *T. indica* L. leaves may help to discover more safer and effective therapy for septic arthritis.

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