PATHOLOGICAL STUDIES ON THE AMELIORATIVE EFFECT OF *Curcuma longa* ON EXPERIMENTAL PASTEURELLOSIS IN RABBITS

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Thesis submitted in partial fulfilment of the requirement for the degree of

Master of Veterinary Science

Faculty of Veterinary and Animal Sciences
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DECLARATION

I hereby declare that the thesis entitled **"PATHOLOGICAL STUDIES ON THE AMELIORATIVE EFFECT OF *Curcuma longa* ON EXPERIMENTAL PASTEURELLOSIS IN RABBITS"** is a bonafide record of research work done by me during the course of research and that this thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

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CERTIFICATE

Certified that this thesis, entitled **"PATHOLOGICAL STUDIES ON THE AMELIORATIVE EFFECT OF *Curcuma longa* ON EXPERIMENTAL PASTEURELLOSIS IN RABBITS"** is a record of research work done independently by **Manjula V. James,** under my guidance and supervision and it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to her.

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Manjula V. James

Dedicated to My Beloved
Father and Mother

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Introduction

1. INTRODUCTION

In recent years, rabbit farming has become a burgeoning entrepreneurial area in our country due to a rise in global awareness on the virtues of meat, fur and skin besides their use as a laboratory animal for various investigations. Rabbit farming is found to be a viable alternative to other livestock activities with great scope as it provides a potential extra income to rural farmers.

However, diseases have always been posing problems in the management of rabbitries, and many a times been responsible for considerable economic losses on account of high mortality, lowered productivity and decreased performance. Pasteurellosis, an ubiquitously distributed bacterial disease of rabbits and one of the major causes of mortality, has been hindering the tradition to mass production units throughout the world. The disease is produced by *Pasteurella multocida* and is characterized by various clinical syndromes like respiratory distress, genital affections, abscesses and septicaemia, but infection by *P. multocida* can also appear without any clinical manifestation (Delong and Manning, 1994).

Under the routine production conditions, stress factors may alter susceptibility of rabbits to infectious diseases like pasteurellosis and thereby resulting in huge production loss. Stressors such as overcrowding, unhygienic environments, transportation and high ammonia concentrations in the air often stimulate latent *P. multocida* to cause disease (Digiacomo *et al.*, 1991). Indiscriminate use of antibiotics results in antibiotic resistant strains of microorganisms causing harmful effects. The antibiotic residues may cause deleterious effects in animals as well as human beings.

Indigenous drugs or plant derived compound formulations offer an alternative to antibiotics or chemical compounds and are considered to be nontoxic compared to their chemical counterparts for the treatment of various ailments.

One of the main strategies in using herbal products is to increase body's natural resistance to pathogens rather than directly neutralizing the agent itself and hence considered as safe alternatives to the consumers and environment.

India has a rich heritage of using plants for medicinal purposes. Turmeric (*Curcuma longa*), a perennial herb, is a medicinal plant extensively used in Ayurveda, Unani and Siddha medicine as home remedy for several common ailments (Eigner and Scholz, 1999). In recent years, traditional Indian medicine also uses turmeric powder for the treatment of biliary disorders, anorexia, coryza, diabetic wounds, hepatic disorders, rheumatism and sinusitis (Ammon *et al.*, 1992).

Curcumin (diferuloyl methane), the main yellow bioactive constituent of turmeric has been experimentally proved to have a wide range of therapeutic actions. Some pharmacological activities of turmeric and its extracts include anti-inflammatory, antioxidant, antibacterial, immunostimulant, hepatoprotective, antifungal, antiprotozoal, antimutagenic, antidiabetic, anticarcinogenic, antifibrotic, antiulcer, hypotensive and hypocholesteremic activities (Ishita *et al.*, 2004). Safety evaluation studies indicate that both turmeric and curcumin are well tolerated at a high dose without any toxic effects. Thus, both turmeric and curcumin have the potential to be successfully used in the treatment of various diseases. Keeping in view the significant importance of turmeric (*Curcuma longa*), this research study was conducted to support the possibility of using turmeric root powder as alternatives to antibiotic growth promoters.

Hence the present study was undertaken with the following objectives:

1. To study the symptoms and lesions of experimental pasteurellosis.

2. To evaluate the beneficial effects of turmeric in managing pasteurellosis.

Review of Literature

2. REVIEW OF LITERATURE

2.1 PASTEURELLOSIS

Diseases are impediment to the higher economic returns from rabbit farming since they lead to lowered productivity and increased mortality. According to Flatt (1974), pasteurellosis was the most common and important bacterial infectious disease complex in domestic rabbits.

2.1.1 Respiratory tract diseases associated with pasteurellosis

Flatt (1974) reported that rhinitis, conjunctivitis, otitis media, abscessation, chronic bronchopneumonia, acute fibrinous bronchopneumonia and septicaemia are the patterns of disease associated with *Pasteurella* infection in rabbits.

A postmortem survey of rabbits died within the first six months of life revealed that 19 % rabbits examined had respiratory disease. Bronchopneumonia was the most common condition diagnosed and *P. multocida* was the common agent isolated (Hinton, 1979).

Necropsy and histology of the rabbits that died of *P. multocida* pneumonia revealed hyperaemic and oedematous trachea and pneumonic changes in lungs. Engorgement and rupture of capillaries and extensive destruction of the mucosa and infiltration of inflammatory cells were observed microscopically in trachea. They also

noticed necrotic debris in the bronchioles with cuff of inflammatory cells around the bronchi. Erythrocytes, neutrophils and lymphocytic accumulations in the alveoli were also seen in the lungs (Nair *et al.* 1987).

Diseases of the upper respiratory tract including rhinitis and tracheitis are the major causes leading to morbidity and mortality due to pneumonia in rabbits (Sanyal and Gopikrishana, 1988).

Devi *et al.* (1990) reported the incidence of rabbit pasteurellosis to be 84.11% for a period of four years.

The response of rabbits to naturally acquired *P. multocida* infection varied from subclinical infection to death from systemic pasteurellosis and noticed atrophy of the maxilloturbinates of the nares with chronic rhinitis associated with *P. multocida* infection (Ronald *et al.*, 1991).

Dungsworth (1993) opined that larynx and trachea frequently get inflamed as part of inflammatory disease of either the upper or lower part of the respiratory tract.

Many species of bacteria like *Pasteurella, Actinobacillus, Haemophilus, Bordetella* and *Salmonella* were the main causes of clinically significant bronchopneumonia, most commonly after pulmonary defenses have been lowered by viral infection, severe stress or other predisposing factors (Jubb *et al.,* 1993).

Pasteurella multocida serotype A was isolated from cases of snuffles in rabbits (Koshimizu and Sato, 1994).

Bronchopneumonia was encountered in 47 % of the rabbit carcasses and *Pasteurella* organisms were isolated from lesions of acute bronchopneumonia (Sharma *et al.*, 1995).

Pawaiya *et al.* (1998) found congestion to consolidation with fibrinous adhesion in the lungs of rabbits died of pneumonia and histologically observed vascular and inflammatory reaction including macrophage reaction in the lungs. *Pasteurella* sp., *Klebsiella sp.*, *Pneumococcus* sp. and *E. coli* were isolated from the affected lungs.

An investigation conducted by Rekha (2003) on the pathology of respiratory system in rabbits revealed a high prevalence (80%) of respiratory disorders. Presence of bipolar bacteria indicative of *Pasteurella* sp. was isolated from the affected trachea and lungs.

Bhattacharya (2005) described necropsy lesions in natural duck cholera infection which included petechial haemorrhage on epicardium and enlarged liver with pinpoint necrotic foci on the surface.

2.1.2 Virulence of *P. multocida*

Greater virulence was observed for strains representing serotypes A: 1, A: 3 and A: 4 which were more commonly isolated from fowl cholera, as reported by Rimler (1987).

Breider *et al.* (1991) opined that several host and bacterial factors have been implicated as potential mediators of endothelial injury in pasteurellosis. Bacterial virulence factors included lipopolysaccharide and leukotoxin.

The variability in clinical signs as well as the course of the disease was influenced by different *P. multocida* virulence factors such as capsule, fimbriae, lipopolysaccharide (endotoxin), dermonecrotoxin and neuraminidase (Strauss *et al.*, 1996).

The presence of the capsule mainly increased the resistance of a *P. multocida* cell to phagocytosis and enhanced its virulence potency when compared with non-capsulated strains (Borrathybay *et al.*, 2003).

The mechanisms by which *P. multocida* invade the mucosa, evade innate immunity and cause systemic disease were elucidated. The capsule is clearly involved in bacterial avoidance of phagocytosis and resistance to complement, while complete lipopolysaccharide is critical for bacterial survival in the host (Marina *et al.*, 2006).

2.1.3 Pathogenicity studies

Mice could be used as the animal of choice for testing pathogenicity of *P. multocida* and an overwhelming increase in the number of organisms in visceral organs was the cause of death of mice when experimentally inoculated (Collins, 1976).

Yue-Shoung *et al.* (1991) observed that rabbits challenged intranasally with *P. multocida* inoculum containing 2.7 x 10^7organisms/ml, died 3 to 10 days post challenge and were depressed, anorectic and dyspneic before death.

Murugkar and Ghosh (1995) tested the pathogenicity of *P. multocida* serotypes A: 1 in different hosts such as pigeon and duck by intraperitoneal route and in mice and rabbit by subcutaneous route. The isolate killed all the hosts, though at varying time intervals and the rabbits died within 48 hrs when 0.1 ml of 6 hr broth culture was inoculated.

Capsulated type A strains of *P. multocida* caused 100% mortality in chicken when inoculated intramuscularly and intravenously both at lower and higher doses, whereas low doses of non-capsulated strain caused no mortality (Borrathybay *et al.*, 2003).

Kapoor *et al.* (2004) on assessing the pathogenicity of *P. multocida* A: 1 in mice found that the isolate killed the mice in 24 h following subcutaneous injection of an 18 hour broth culture.

Pasteurella multocida serotype A: 1 killed the mice inoculated with 0.3 x 10^8 organisms/ 0.1 ml intraperitoneally within eight hours and within 24 h when injected by subcutaneous route. The gross lesions observed were petechial haemorrhage in the epicardium and congestion of all the visceral organs, particularly lungs, liver and spleen (RajaGopal, 2007).

2.1.4 Isolation and characterization of *P. multocida.*

Adlam and Rutter (1989) reported that in tissues, exudates and recently isolated cultures, the bacteria showed characteristic bipolar staining with connecting strand with Methylene blue or Leishman stain.

Quinn *et al.* (1994) reported that *P. multocida* produced round, moderate sized grayish non haemolytic and characteristic dew drop like colonies on blood agar after 24 h of incubation at 37° C. Usually *P. multocida* type A selectively produced large mucoid colonies due to their large capsular hyaluronic acid.

Freshly isolated *P. multocida* produced smooth, grayish glistening transluscent colonies approximately one millimetre in diameter on blood agar after 24 h incubation at 37° C and it does not grow on Mac Conkey agar (OIE, 2004).

2.1.5 Haemato-biochemical alterations in experimental pasteurellosis

Weiss *et al.* (1991) in experimentally induced pneumonic pasteurellosis in calves noticed significant changes in haemoglobin and hematocrit values.

Mir *et al.* (2001) observed that during intranasal challenge with *P. multocida*, the blood parameters showed mild heterophilic leucocytosis and marked increase in the serum creatinine levels, ALP and ALT activities and slight increase in AST activity in young rabbits.

Haemato-biochemical alterations in rabbits experimentally infected by *P. multocida* serotype A: 3 through intra-nasal and intra-tracheal route showed a significant decrease in haemoglobin concentration and serum total protein and an increase in total leukocyte count, heterophils and monocytes (Rameshkumar *et al.*, 2006).

Haemato-biochemical changes in mice experimentally infected by *P. multocida* serotype A: 1 showed progressive leukocytosis with neutrophilia at late hours of post-infection and corresponding lymphocytopenia throughout the course of infection. Decrease in haemoglobin, an increased Packed Cell Volume, total protein and albumin: globulin ratio was noticed (Praveena *et al.*, 2007).

2.1.6 Gross and histopathological lesions in experimental pasteurellosis

Necropsy and histology of the rabbits that died of *P. multocida* intranasal inoculation, revealed severe pleuritis with the accumulation of a remarkable amount of fibrinopurulent exudate in the thoracic cavity, serous rhinitis and tracheitis and acute hepatitis with necrotic foci in the parenchyma (Glavits and Magyar, 1990).

After challenging rabbits intranasally with purified toxin obtained from type D strain of *P. multocida*, the role of toxin in the induction of pneumonia was determined. Pleuritis, pneumonia, acute hepatic necrosis was noticed (Chrisp and Foged, 1991).

Rhoades and Rimler (1991) observed that during the acute course of pasteurellosis in poultry, most of the post mortem lesions were associated with vascular disturbances which included epicardial and sub serosal haemorrhage mostly in lungs, abdominal fat and intestinal mucosa. Liver of the affected birds revealed multiple small focal areas of coagulative necrosis.

The acute stage of pneumonic pasteurellosis in cattle was characterized by severe endothelial damage to alveolar septal capillaries, which resulted in flooding of alveoli with plasma and erythrocytes (Wikse, 1995).

Al-Haddawi *et al.* (1999) observed loss of cilia, cellular swelling, goblet cell hyperplasia and endothelial cell damage in rabbits intranasally inoculated with *P. multocida* serotype A: 3 and opined that *P. multocida* infection was associated with inflammatory responses, which caused the tissue damage.

In rabbits experimentally infected with *P. multocida* serotype D: 1, clinical signs of septicaemia, mucopurulent nasal discharge and pneumonic lesions were observed. The ultrastructural changes detected were clumping of cilia of ciliated epithelium, cellular swelling, vacuolation and sloughing. The subepithelial capillaries showed congestion and endothelial injury. Heterophil, mast cells, vacuolated monocytes and macrophages infiltrated the lamina propria and between the degenerated epithelial cells (Al-Haddawi *et al.*, 2001).

Mir *et al.* (2001) described the gross and histopathological lesions in rabbits intranasally inoculated with *P. multocida* and recorded gross lesions like congestion, haemorrhage and emphysema in lungs and granular degenerative changes in liver and kidney. Microscopically, the trachea revealed engorgement of blood vessels, degeneration and sloughing of the mucosa. Mild haemorrhage, thickening of interalveolar septa, areas of emphysema, bronchitis and desquamation of bronchial epithelium were observed in the lungs.

Necropsy findings in rabbits experimentally infected by *P. multocida* serotype A: 3 through intra-nasal and intra-tracheal route showed that intranasally inoculated organism induced mild lesions and intratracheal route of infection produced moderate lesions of acute bronchopneumonia (Rameshkumar *et al.*, 2006).

Shilpa and Verma (2006) recorded gross lesions like congestion, haemorrhage and pneumonic changes in lungs of chickens experimentally inoculated with *P. multocida.* Heterophilic infiltration, focal areas of necrosis, fibrinous thickening of alveolar walls, degeneration of bronchi and desquamation of epithelial cells of bronchioles in lungs were noticed histopathologically.

2.2 BIOLOGICAL EFFECTS OF TURMERIC

Turmeric (*Curcuma longa*) a tropical herb of Zingiberaceae family, has a long tradition of use in cuisine as a spice, food preservative and colouring material. Curcumin, the active ingredient of turmeric, has shown a wide range of health-boosting properties. It is expected that curcumin may find application as a novel drug in the near future to control various diseases, including inflammatory disorders, carcinogenesis and oxidative stress- induced pathogenesis (Ishita *et al.*, 2004).

2.2.1 Influence on production performance

Soni *et al.* (1992) established the effect of turmeric in reversing the aflatoxin induced liver damage in ducklings and observed significant difference in body weight gain of birds supplemented with turmeric and curcumin.

Kurkure *et al.* (2001) observed that mean gain in body weight was numerically higher in the group fed with turmeric powder at the rate of 0.5 % upto twenty eighth day of age in an experimental study in White leghorn cockerels and reported the protective effect of turmeric on aflatoxin induced toxicity.

Mohan *et al.* (2001) demonstrated the efficacy of turmeric, curcumin and chitosan in reversing the aflatoxin induced liver damage in broiler chicks for a period of eight weeks. The body weight gain in groups fed with turmeric and curcumin were noted to be higher than the control group.

An investigation on the possible effect of *Curcuma longa* feed additive on the production performance of broiler chickens, indicated that higher body weight gain was observed in birds fed diet containing turmeric at level of 0.5% (Al-Sultan, 2003).

In an experiment conducted in broilers for a period of 42 days, indicated a significantly higher body weight gain in chicks supplemented with turmeric (1 g/kg feed) over those of control chicks (Kumar *et al.*, 2005).

Emadi and Kermanshahi (2006) studied the effect of turmeric rhizome powder on performance and carcass characteristics of broiler chicken and opined that weight gain was not significantly affected by adding 0.25, 0.50 and 0.75 % turmeric in the diets.

Mekala *et al.* (2006) assessed the usefulness of curcumin and silymarin on aflatoxicosis in broiler chicken. Turmeric was fed at the rate of 10 mg/ bird/ day from fifteenth day to fourty second day. Only numerical increase in body weight gain was recorded at the end of fourth week but in the subsequent weeks significant difference was observed in this group.

2.2.2 Influence on haematological and biochemical parameters

Despande (1998) demonstrated the protective effect of turmeric extract in diet on carbon tetrachloride treated rats and observed that there was a reduction in AST, ALT, ALP, cholesterol and bilirubin levels compared to carbon tetrachloride alone treated group.

A study on the potential efficacy of Turmeric Antioxidant Protein (TAP) in protecting tissues from peroxidative damage in carbon tetrachloride treated rats showed decreased activity of ALT and AST in the liver of carbon tetrachloride treated rats and conferred protection of liver (Lalitha *et al.*, 1999).

Park *et al.* (2000) investigated the protective effects of curcumin on acute and subacute carbon tetrachloride induced liver damage in rats and observed that curcumin at 100 and 200 mg/ kg lowered the activity of AST, ALT and histopathological lesions compared to the control group.

A study on the effect of dietary turmeric treatment (0.5g/ kg feed) to counteract aflatoxin induced haematological and biochemical alterations in broiler chicks showed that the values of serum total protein, albumin and ALT levels were improved in aflatoxin plus turmeric fed group indicating the restorative effect of turmeric during aflatoxicosis (Kurkure *et al.*, 2001).

Mohan *et al.* (2001) conducted a feeding trial to determine the efficacy of turmeric, curcumin and chitosan in reversing the aflatoxin induced liver damage in broiler chicks. The value of haemoglobin was highest in the birds fed 50 mg turmeric/ bird/ day along with aflatoxin. TLC count was highest in the group fed turmeric extract at the rate of 10 mg/ bird/ day along with aflatoxin.

Pretreatment of rats with the ethanolic extract of *Curcuma longa* prior to paracetamol dosing statistically lowered the serum alanine aminotransferase (ALT), asparate aminotransferase (AST) and alkaline phosphatase (ALP) and the results

suggested that the extract has potent hepatoprotective effect against paracetamol-induced liver damage (Somchit *et al.*, 2002).

Al-Sultan (2003) found that higher levels of turmeric inclusion (0.5 and 1%) in diets increased both total erythrocyte count and total leukocyte count and concluded that the use of turmeric as feed additive at level of 0.5% enhanced overall performance of broiler chickens.

A study on the hepatoprotective effect of turmeric on D-galactosamine induced liver injury in rats proved that curcuminoids fraction suppressed the increase of liver enzymes like AST and ALT (Miyakoshi *et al.*, 2004).

Inclusion of turmeric rhizome powder (0.25, 0.50 and 0.75 %) into the broiler diets significantly increased haemoglobin level and decreased red blood cells and hence proved that administration of turmeric rhizome powder as herbal additive improved some of the components of the chicken's blood and possibly improved the health status of the chickens (Emadi *et al.*, 2007).

Simi (2007) investigated the effect of dietary turmeric on production performance in broilers and observed that the mean values of haemoglobin, TLC and total protein in the turmeric supplemented group were significantly higher than that of the control group. Decreased levels of liver enzymes like ALT and AST in the turmeric supplemented group were also noticed.

2.2.3 Antioxidant property

Antioxidant properties have been associated with turmeric powder by itself and its individual lipid soluble component curcumin and the aqueous soluble turmerin. Turmeric offered protection by inhibiting lipid peroxidation and scavenging free radicals (Shalini and Srinivas, 1987).

Amman and Wahl (1991) opined that curcumin is a potent scavenger of many reactive oxygen species and has the ability to protect lipids, haemoglobin and DNA against oxidative degradation and stated that curcumin protected DNA significantly and the activity was higher than that of the well known biological antioxidants like lipoate, alpha- tocopherol and beta- carotene.

A study was conducted to evaluate the effect of chitosan and turmeric to ameliorate the toxic effects of aflatoxin in chicken. The results revealed that there was 33.84 % reduction in aflatoxin concentration in chitosan treated feeds at 8% level and in turmeric treated ones, remarkable reduction (61.04 %) were noticed with 8 % turmeric in the feed (Mini *et al.*, 1993).

Sony and Kuttan (1993) proposed that curcumin partially reversed the lipid peroxidation and histopathological changes in lung damage caused by paraquat.

Dietary curcumin inhibited chemotherapy induced apoptosis through inhibition of reactive oxygen species which is needed for many chemotherapeutic drugs inducing apoptosis (Somasundaram *et al.*, 2002).

2.2.4 Anti-inflammatory property

Dinesh *et al.* (1972) proposed the anti-inflammatory and anti-arthritic activity of the volatile oil of *C. longa* against Freund's adjuvant induced arthritis in rats and talc induced teno-synovitis in pigeon.

Curcumin has been reported to be effective in the acute and chronic models of inflammation (Srimal *et al.*, 1973).

Amman and Wahl (1991) proved that curcumin possessed beneficial pharmacological activities against wide range of pathological conditions such as inflammation, rheumatism, thrombosis, cancer and mutation.

Curcumin, the colouring matter of *C. longa* has been reported to inhibit both lipooxygenase and cyclooxygenase pathway (Huang *et al.*, 1991).

Mujumdar *et al.* (2000) studied the anti-inflammatory activity of *Curcuma amada* rhizome extract (40mg/ kg and 80 mg/ kg) orally in albino rats and reported that the extract showed dose dependent anti-inflammatory activity in acute carrageenan induced rat paw oedema model and significant reduction in granular tissue formation in chronic model of cotton pellet implantation.

Oral administration of curcumin has important therapeutic implications in facilitating the early suppression of paraquat lung injury by blocking the rise in neutrophils and alkaline phosphatase and thus prevented the general toxicity and mortality induced by paraquat (Narayanan, 2000).

Sugimoto *et al.* (2002) reported that treatment of mice with curcumin at the rate of 0.5, 2 and 5% in diets prevented wasting and histopathological lesions of trinitrobenzene sulphonic acid (TNBS) induced colitis.

Babita and Balaram (2003) demonstrated that diferuloylmethane, a major active component of turmeric, exerts protective effect in high dose endotoxin shock by improving survival and reducing the severity of endotoxin shock symptoms following a challenge with lipopolysaccharide.

A study conducted on the anti-inflammatory activities of nine traditional medicinal plants against *Aspergillus* species and *Mucor* species revealed that *C. longa* was the most effective plant against *Mucor* species and exhibited significant activity against *Aspergillus* species (Perumal *et al.*, 2004).

A study on the anti-inflammatory effects of curcumin revealed that administration of curcumin significantly decreased reactive oxygen species production, nuclear factor Kappa B activation and tumor necrosis factor alpha secretion and increased glutathione content in myelomonocytic cells (Strasser *et al.*, 2005).

2.2.5 Antimicrobial property

According to Anu and Kapoor (1997), the anti-microbial activities of fresh juice and aqueous extracts of turmeric and ginger rhizomes against *Aspergillus niger* and *Penicillium digitatus in vitro*, arrested the growth of the fungi and showed a better anti-inflammatory activity.

C. longa rhizome extract was evaluated for antibacterial activity against pathogenic strains of bacteria by zone of inhibition assay and found that turmeric possessed significant antibacterial activity at very low concentration on pathogenic bacteria and clinical isolates were also found to be sensitive to this fraction. The study concluded that essential oil from turmeric is a potent antiseptic in prevention and treatment of bacterial infections (Rambir *et al.*, 2002).

Samarasinghe *et al.* (2003) clearly demonstrated that dried turmeric root powder when fed at 1g/ kg feed in broiler diets possessed antimicrobial effect *in vivo* against various harmful bacteria and fungal species and improved the growth and feed efficiency similar to antibiotics and hence can be used as a satisfactory substitute to antibiotics in broiler feeds.

Thakarae (2004) investigated the effect of dietary supplementation of different spices in broiler diets and pointed out that inclusion of turmeric extract at 0.29 % in the diet produced an effective antimicrobial effect.

2.2.6 Safety profile

Sub-acute toxicity experiments conducted using the total petroleum ether extract of powdered *C. longa* did not show any significant toxic side effects in rats when administered for four weeks at the dose rate of 1g and 2g/ kg. The oral LD_{50} was found to be 12.2g/ kg in rats (Arora *et al.*, 1971).

A study on curcumin in Sprague Dawley rats reported that when administered orally in a dose of 1g/ kg, curcumin was excreted in the faeces to about 75%, while negligible amounts of curcumin appeared in the urine. No apparent toxic effects were seen after doses of upto 5g/ kg (Wahlstrom *et al.*, 1978).

Sambaiah *et al.* (1982) reported that the whole spice turmeric when fed to rats at doses normally consumed or at much higher doses (2-125 times) than normal human intake (4g/adult/day) did not cause any adverse effects on growth, RBC, WBC, DLC and on the haemoglobin, total serum protein, albumin, globulin and serum aminotransferase and alkaline phosphatase.

2.2.7 Other protective effects

An investigation on the effect of 1% turmeric in diet of chicken infected with *Eimeria maxima* for three weeks of age showed that turmeric had a protective effect on weight gain in *E. maxima* infected chicken and reduced lesion scores and oocyst output (Allen *et al.*, 1998).

The immunomodulatory activity of curcumin administered orally in mice increased the macrophage phagocytic activity and indicated the immunostimulatory activity of curcumin (Antony *et al.*, 1999).

Kurkure *et al.* (2000) demonstrated the protective effect of turmeric at the rate of 0.5 g/ kg feed in induced aflatoxicosis in cockerels and proved the potential of turmeric to moderately reduce immune toxicity due to dietary aflatoxin B_1. Microscopically the histopathological changes in liver were found to be minimized in birds of turmeric treated group.

Lee *et al.* (2001) studied the effects of naturally occurring compounds from plants on biotransformation of aflatoxin B_1. It was shown that the formation of aflatoxin B_1 reductase product, aflatoxicol by chicken liver cytosols was strongly inhibited by curcumin.

Materials and Methods

3. MATERIALS AND METHODS

3.1. LOCATION

The experiment was designed and conducted at the Department of Veterinary Pathology, College of Veterinary and Animal Sciences, Mannuthy to evaluate the clinical signs and pathological lesions of experimental pasteurellosis in rabbits and to study the beneficial effects of *Curcuma longa* as dietary supplement in pasteurellosis. The experiment was carried out for a period of forty three days from September twenty-fourth to November fifth, 2007.

3.2 EXPERIMENTAL MATERIALS

3.2.1 Experimental animals

Thirty two healthy New Zealand white rabbits procured from the Small Animal Breeding Station, Mannuthy were used for the study.

3.2.2 Experimental feed

Commercial rabbit feed was procured from the Small Animal Breeding station, Mannuthy. Turmeric powder, prepared from sun dried rhizomes of *Curcuma longa* was purchased from Shaji's Viswanath Ayurveda Pharmacy, Ernakulam, Kerala, INDIA.

3.2.3 Bacteria

The *Pasteurella multocida* A: 1 strain (DP1) isolated from Niranam duck farm (Pathanamthitta district), serotyped at IVRI, Izatnagar and maintained in the Department of Veterinary Microbiology, COVAS, Mannuthy was used for the study. Purity of the isolate was confirmed as described by Barrow and Feltham (1993). Pathogenicity testing was carried out using an 18 h broth culture of *P. multocida* containing approximately 3×10^8 organisms/ml inoculated intraperitoneally at the rate of 0.1 millilitre in Swiss albino female mice, six to eight weeks of age (Antony, 2004).

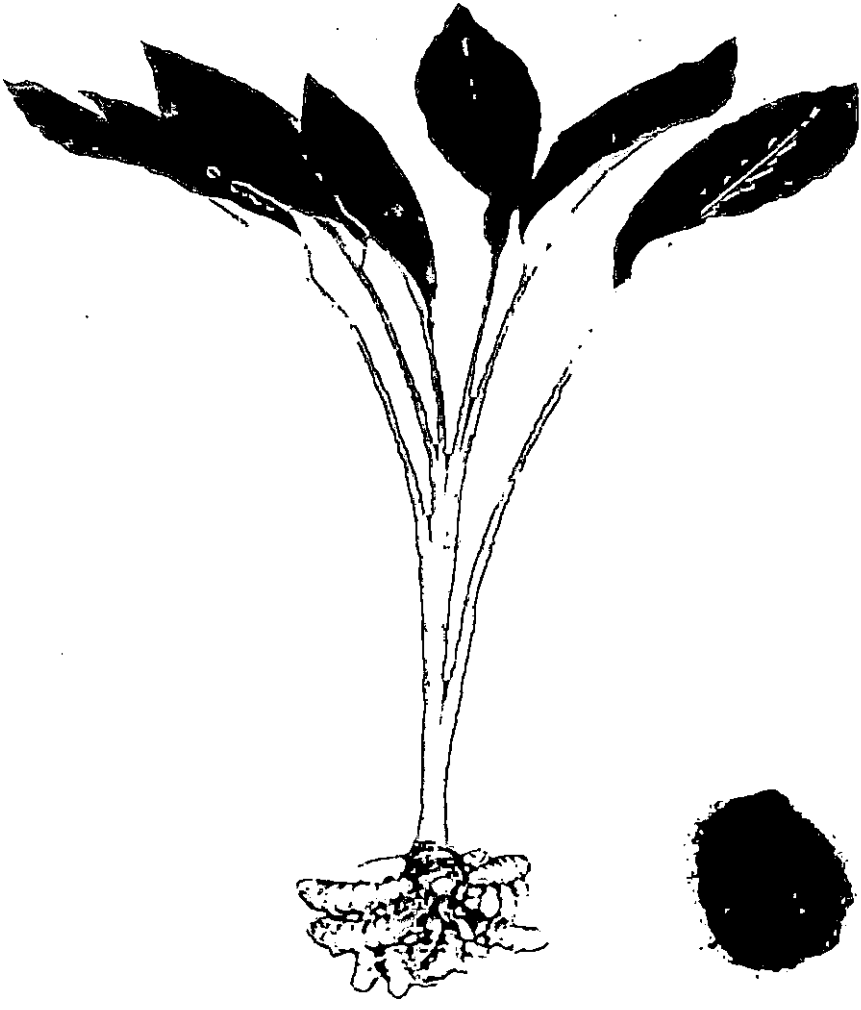

Fig.1 ***Curcuma longa***

3.3. EXPERIMENTAL DESIGN

Thirty two healthy rabbits aged one month were allotted randomly into four groups each having eight replicates as detailed below:

Group I	Control group with commercial feed alone.
Group II	Feed mixed with turmeric at the rate of 2 g/kg body weight was fed for the whole period of the experiment and on the thirty first day of the experiment animals were intranasally exposed to *P. multocida.*
Group III	Rabbits were intranasally exposed to *P. multocida* on the thirty first day of the experiment and turmeric was fed at the rate of 2 g/kg body weight after exposure until the end of the experiment.
Group 1V	Rabbits were fed on control diet alone for thirty days and then intranasally exposed to *P. multocida* on the thirty first day of the experiment.

3.3.1 Management

The experimental rabbits were reared in separate rabbit cages and maintained under standard conditions of feeding and management throughout the experimental period. Animals were allowed to acclimatize for one week before the experiment. Each group was fed with weighed quantity of experimental diets and had free access to green grass and wholesome water. During the first month of the experiment, body weight, haematological and biochemical parameters were recorded to study the influence of turmeric incorporation in feed with that of the control group.

3.3.2 Experimental infection of rabbits

Group I rabbits served as control. Rabbits in Groups II, III and IV were inoculated with 0.1 ml of 18 hr broth culture of *P. multocida* serotype A: 1 containing approximately 3×10^8 organisms/ml by intranasal route on the thirty first day of the experiment. The rabbits in each group were maintained in separate isolation units. The infected rabbits were regularly observed for clinical signs of the disease. Blood samples were collected for haematological and biochemical evaluations after eighteen hours of post inoculation from Groups II, III and IV and also from the control Group I.

3.4. PARAMETERS

3.4.1 Body Weight

The body weight of individual rabbit was recorded before the experiment (Day zero) and on the fourteenth and twenty eighth day of the experiment. From these data, the mean body weight was calculated.

3.4.2 Haematological Parameters

The blood samples were collected before the experiment (Day zero) and on the fourteenth day, twenty eighth day and thereafter at eighteenth hour of post inoculation from all the groups for haematological evaluation. One milliliter blood was collected from the median artery using 21 Gauge needle with sodium salt of Ethylene Diamine Tetra Acetic acid (EDTA) as an anticoagulant. The following parameters were studied.

3.4.2.1 Haemoglobin concentration

The haemoglobin concentration was measured by acid haematin method (Benjamin, 1985).

3.4.2.2 Total Leukocyte Count

The total leukocytes were counted by standard dilution technique using Thomas Fluid as a diluent. Counting was done using the haemocytometer placed under the low power of the microscope (Benjamin, 1985).

3.4.2.3 Differential Leukocyte Count

Blood smears were prepared from freshly drawn blood by slide method. The smears were stained with Wright's stain and the leukocytes were counted under the oil immersion objective of the microscope (Benjamin, 1985).

3.4.3 Serum Biochemistry

Blood samples were collected on the twenty eighth day of the experiment and thereafter at the eighteenth hour of post inoculation from all the groups for biochemical evaluation. Three milliliter blood was collected from median artery using 21 Gauge needle for the separation of serum. For serum collection, clotted blood was kept in the refrigerator for half an hour and then centrifuged at 3000 rpm for 10 minutes. Serum was separated and the following parameters were estimated.

3.4.3.1 Serum total protein, albumin and albumin : globulin ratio

Serum total protein and albumin were estimated by Biuret method (Henry *et al.*, 1957) and Doumas method (Doumas *et* al., 1971) respectively using commercial kits supplied by Agappe Diagnostics Pvt. Ltd. Agappe Hills, Ernakulam, Kerala, INDIA. Serum globulin content was calculated as the difference between serum total protein and albumin contents. Albumin: Globulin ratio was calculated by dividing albumin values with globulin level.

3:4.3.2 Serum creatinine

Serum creatinine was estimated by Modified Jaffe's method using commercial kits purchased from Agappe Diagnostics and the final reading was taken spectrophotometrically.

3.4.3.3 Serum enzymes

Alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) were assayed spectrophotometrically using Agappe Diagnostic Kits.

3.5 MICROBIOLOGICAL STUDIES

For bacterial re-isolation, heart blood, trachea, liver, spleen and lungs from dead rabbits were removed and confirmation by cultural and morphological characters in blood agar, followed by incubation at 37°C under five per cent carbon dioxide tension and staining by using Gram's staining were done as described by Barrow and Feltham (1993). Impression smears from heart blood and lungs of dead animals, were prepared, fixed and stained with Leishman's stain to demonstrate bipolar organisms.

3.6 GROSS AND HISTOPATHOLOGICAL EXAMINATION

A detailed and systematic autopsy of both the dead and sacrificed animals of all the groups was conducted and gross lesions were recorded. Tissue samples of appropriate sizes from trachea, lungs, heart, liver and kidney were collected and fixed in 10% formalin for histopathological investigations. They were then processed and paraffin embedded as described by Sheehan and Hrapchak (1980). After processing conventionally, the paraffin embedded blocks were sectioned at four micron thickness and stained with routine Haematoxylin and Eosin stain as per the technique followed by Bancroft and Cook (1995). The stained sections were examined in detail under light microscope and the lesions were classified.

3.7 STATISTICAL ANALYSIS

The data obtained from the present study was analyzed statistically as per the method outlined by Snedecor and Cochran (1994).

4. RESULTS

The experiment was conducted to study the symptoms and lesions of experimental pasteurellosis in rabbits and to evaluate the beneficial effects of turmeric in managing pasteurellosis. The results are analysed and presented in tables and figures in this chapter.

Thirty two healthy New Zealand white rabbits aged four weeks were divided randomly into four groups each having eight replicates. Group I rabbits served as control group with commercial feed alone. Group II rabbits were given feed mixed with turmeric at the rate of 2 g/kg body weight for the entire study period and on the thirty first day animals were intranasally exposed to *Pasteurella multocida.* Group III rabbits were intranasally exposed to *P. multocida* on the thirty first day and turmeric was fed at the rate of 2 g/kg body weight simultaneously until the end of the experiment. Group 1V rabbits were fed on control diet alone for thirty days and then intranasally exposed to *P. multocida* on the thirty first day of the experiment.

4.1 EFFECT OF TURMERIC ON BODY WEIGHT

The mean body weight of rabbits in control and turmeric incorporated groups showed a gradual increase during the experimental period. The mean body weight in the control and turmeric incorporated group on day zero (at the beginning of the experiment) was 616.25±11.08 and 612.50±17.68 g, respectively and the corresponding mean body weight on 14^{th} day were 796.67±11.12 and 816.25±17.08 g, respectively. No significant difference was noticed between the two groups in the body weight on 14^{th} day. The values obtained on 14^{th} day in the experimental groups

were statistically comparable. Thereafter an increase in the body weight was seen in the turmeric incorporated group (1022.50±15.09 g) compared to the control group (970.42±10.49 g) on 28th day of the experiment. Statistical studies revealed a significant ($P<0.05$) increase in body weight of turmeric incorporated group from that of the control group on 28th day. The values of mean body weight are presented in Table 1. and graphically in Fig. 2.

4.2 BIOLOGICAL EFFECTS OF TURMERIC (*Curcuma longa*) RHIZOME POWDER BEFORE AND AFTER EXPERIMENTAL INFECTION

4.2.2 Haematological parameters

4.2.2.1 Haemoglobin concentration

On day zero, the mean haemoglobin concentration for control and turmeric incorporated group was 12.74±0.15 and 12.75±0.15 g/dl, respectively and thereafter on 14th day was 12.98±0.15 and 13.43±0.17 g/dl, respectively. The mean haemoglobin concentration for control and turmeric incorporated group on 28th day was 13.39±0.14 and 14.05±0.22 g/dl, respectively. Statistical studies revealed a significant ($P<0.05$) increase in haemoglobin concentration in turmeric incorporated group from that of the control group on day 14th and 28th of the experiment. The values of mean haemoglobin concentration recorded are presented in Table 2 and graphically in Fig. 3.

The haemoglobin concentration after challenge with *P. multocida* in Groups I, II, III and IV were 13.46±0.23, 13.79±0.22, 13.19±0.22 and 13.05± 0.28 g/dl, respectively. No significant difference was noticed between any of the groups. The values of the mean haemoglobin concentration after challenge with *P. multocida* are presented in Table 4.

4.2.2.2 Total Leukocyte Count (TLC)

The mean total leukocyte count observed in control and turmeric incorporated group on day zero was 6837.50±117.42 and 6887.50±232.58 numbers/cumm, respectively and thereafter on 14th day were 7017.08±116.74 and 7105.00±221.49 numbers/cumm, respectively. The mean TLC on 28th day was 7039.75±107.72 and 7657.50±203.61 numbers/cumm, respectively. No significant difference was noticed between the two groups in the total leukocyte count on 14th day and 28th day. The values of mean total leukocyte count are presented in Table 2.

The mean TLC after challenge with *P. multocida* in Groups I, II, III and IV were respectively 7040.12±209.48, 7841.25±162.48, 7065.25±157.00 and 7315.88±205.09 numbers/cumm. Statistical studies revealed a significant ($P<0.05$) increase in total leukocyte count in the Group II followed by Group IV from that of the control Group I. The values in Group III were statistically comparable with the control. The mean total leukocyte count recorded after challenge with *P. multocida* is presented in Table 4.

4.2.2.3 Differential Leukocyte Count (DLC)

The mean neutrophil and lymphocyte counts on day zero were 32.83±0.89 and 63.17±0.99% in control group and 34.63±1.84 and 60.88±1.82% in turmeric incorporated group, respectively. The corresponding counts on 14th day were 33.25±0.92 and 62.88 ±1.04% in control group and 36.50±1.87 and 59.00±1.96% in turmeric incorporated group. On 28th day the corresponding counts were 32.79±0.89 and 62.54±0.99% in control compared to 34.38±1.96 and 61.13±2.07% in turmeric incorporated group. The values are presented in table 3. The mean monocyte and eosinophil count on day zero was 2.25± 0.11 and1.75±0.12% in control group and 2.50±1.89 and 2.00±0.27% in turmeric incorporated group. On14th day the values

were 2.21± 0.10 and 1.67±0.13 in control and 2.50± 0.19 and 2.00±0.27% in turmeric incorporated group, respectively and thereafter on 28th day was 2.79± 0.18 and 1.88 ±0.15 in control and 2.62±0.26 and 1.88±0.23% in turmeric incorporated group, respectively. Statistical analysis revealed that the DLC on day zero, 14th and 28th of the experiment did not differ significantly between the two groups.

The mean neutrophil counts after challenge with *P. multocida* in Groups I, II, III and IV were 35.25±2.02, 37.25±1.91, 44.25±1.59 and 46.50±2.70%, respectively and the corresponding mean lymphocyte counts were 60.00±1.81, 58.13±1.83, 50.00±1.48 and 48.50±2.54%. Statistical studies revealed a significant ($P<0.05$) increase in neutrophil and lymphocyte counts in the Groups III and IV from that of the Group II and control values. The values in Group II were statistically comparable with that of control. The mean monocyte counts after challenge in Groups I, II, III and IV were 2.75±0.25, 3.25±0.45, 4.13±0.29 and 3.50±0.42%, respectively and the corresponding mean eosinophil counts were 2.00±0.27, 1.38±0.18 , 1.63±0.26 and 1.50±0.27%. Statistical studies revealed no significant difference in monocyte and eosinophil counts among the Groups I, II, III and IV. The results are presented in Table 4.

4.2.3 Serum biochemistry

4.2.3.1 Total Serum Protein

The mean total serum protein values on 28th day of the experiment were 5.29±0.04 and 6.74±0.27 g/dl in control and turmeric fed group, respectively. The turmeric fed group showed a significantly ($P<0.05$) higher total serum protein on 28th day of the experiment compared to the control group. The mean serum biochemistry values on twenty eighth day are presented in tables 5. and graphically in Fig. 4.

The serum average total protein values after challenge with *P. multocida* in Groups I, II, III and IV were 5.41±0.09, 6.16±0.22, 4.93±0.14 and 4.80± 0.08 g/dl, respectively. Statistical studies revealed a significant difference ($P<0.05$) between the Groups II, III and IV from that of the healthy control Group I in total serum protein values. The mean serum biochemistry values after challenge with *P. multocida* are presented in tables 6.

4.2.3.2 Albumin

The mean albumin value on 28th day of the experiment was 3.17±0.05 in control group and 3.58±0.13 g/dl in turmeric treated group. The turmeric treated group showed significantly ($P<0.05$) higher albumin values on 28th day of the experiment compared to the control group. The values obtained are presented in tables 5. and graphically in Fig. 4.

The serum average albumin values after challenge with *P. multocida* in Groups I, II, III and IV were 3.25±0.06, 3.13±0.12, 2.54± 0.13 and 2.43±0.14 g/dl, respectively. Statistical studies revealed a significant difference ($P<0.05$) between the Group III and IV from that of the healthy control Group I in albumin values. The values obtained in Group II were statistically comparable with that of the control. The results are presented in table 6.

4.2.3.3 Albumin : Globulin ratio

The mean albumin : globulin ratio on 28th day of the experiment was 1.05±0.05 in control group and 1.22±0.08 in turmeric incorporated group. The turmeric fed group showed a significantly ($P<0.05$) higher albumin : globulin ratio on 28th day of the experiment compared to the control group. The result is presented in table 5. and graphically in Fig. 5.

The serum average albumin : globulin ratio after challenge with *P. multocida* in Groups I, II, III and IV were 1.52±0.06, 1.00±0.04, 1.08±0.07 and 1.05±0.09, respectively. Statistical studies revealed a significant difference ($P<0.05$) between the Groups II, III and IV from that of the healthy control Group I. The results are presented in table 6.

4.2.3.4 Serum creatinine

The mean creatinine values on 28th day of the experiment were 0.95±0.03 in control group and 1.00±0.00 mg/dl in turmeric incorporated group. Serum creatinine levels of both the groups were within the normal range throughout the experiment. No significant difference was noticed between the two groups. The result is presented in tables 5.

The serum average creatinine values after challenge in Groups I, II, III and IV were 1.01±0.03, 1.38±0.09, 1.68±0.11 and 1.84±0.12 mg/dl, respectively. Statistical studies revealed a significant difference ($P<0.05$) between the Groups II, III and IV from that of the healthy control Group I in creatinine values. The values are presented in table 6.

4.2.3.5 Serum enzymes

The average ALT value on 28th day of the experiment was 36.83±1.51 in control and 34.25±2.94 U/L in turmeric incorporated group. The corresponding average AST values were 76.00±2.47 in control and 72.79±2.21 U/L in turmeric incorporated group. The average ALP values were 60.71±2.71 in control and 50.62 ±5.52 U/L in turmeric incorporated group, respectively. Though no statistical difference was noticed between the two groups, the turmeric incorporated group showed slightly lower ALT, AST and ALP activities on 28th day of the experiment compared to the control group. The mean serum enzyme profile (U/L) on twenty eighth day of the experiment is presented in table 7. and graphically in Fig. 6.

The serum average ALT levels after challenged with *P. multocida* in Groups I, II, III and IV were 39.00±2.55, 61.00±5.22, 61.38±3.42 and 65.38±4.46 U/L, respectively and corresponding AST levels were 73.00±4.65, 90.50±1.46, 94.25±5.96 and 98.75±3.59 U/L, respectively. The average ALP levels were 63.75±5.42, 85.00±3.53, 101.88±7.29 and 109.50±6.94 U/L, respectively. Statistical studies revealed a significant difference ($P<0.05$) between the Groups II, III and IV from that of the healthy control Group I in all these enzyme levels. The results are presented in Table 8 and graphically in Fig. 7.

Table1. Mean body weight (g) of experimental rabbits

Group	Day 0	Day 14	Day 28
Control group	$616.25^{a} \pm 11.08$	$796.67^{a} \pm 11.12$	$970.42^{a} \pm 10.49$
Turmeric incorporated group	$612.50^{a} \pm 17.68$	$816.25^{a} \pm 17.08$	$1022.50^{b} \pm 15.09$

Means bearing the same superscript within a column do not differ significantly ($P<0.05$)

Table 2. Mean haemogram values of experimental rabbits

Parameter	Control group			Turmeric incorporated group		
	Day 0	Day 14	Day 28	Day 0	Day 14	Day 28
Hb (g/dl)	$12.74^{a} \pm 0.15$	$12.98^{a} \pm 0.15$	$13.39^{a} \pm 0.14$	$12.75^{a} \pm 0.15$	$13.43^{b} \pm 0.17$	$14.05^{b} \pm 0.22$
TLC (numbers/cumm)	$6837.50^{a} \pm 117.42$	$7017.08^{a} \pm 116.74$	$7039.75^{a} \pm 107.72$	$6887.50^{a} \pm 232.58$	$7105.00^{a} \pm 221.49$	$7657.50^{a} \pm 203.61$

Means bearing the same superscript within a row do not differ significantly ($P<0.05$)

Table 3. Mean differential leukocyte count of experimental rabbits

Group	Differential leukocyte count (%)			
	Neutrophil	**Lymphocyte**	**Monocyte**	**Eosinophil**
Day 0 Control group	32.83[a] ± 0.89	63.17[a] ± 0.99	2.25[a] ± 0.11	1.75[a] ± 0.12
Turmeric incorporated group	34.63[a] ±1.84	60.88[a] ± 1.82	2.50[a] ± 1.89	2.00[a] ± 0.27
Day 14 Control group	33.25[a] ± 0.92	62.88[a] ± 1.04	2.21[a] ± 0.10	1.67[a] ±0.13
Turmeric incorporated group	36.50[a] ± 1.87	59.00[a] ± 1.96	2.50[a] ± 0.19	2.00[a] ± 0.27
Day 28 Control group	32.79[a] ± 0.89	62.54[a] ± 0.99	2.79[a] ± 0.18	1.88[a] ± 0.15
Turmeric incorporated group	34.38[a] ± 1.96	61.13[a] ± 2.07	2.62[a] ± 0.26	1.88[a] ± 0.23

Means bearing the same superscript within a column do not differ significantly ($P<0.05$)

Table 4. Mean haemogram values after challenge with *Pasteurella multocida*

Group	Hb (g/dl)	TLC (numbers/ cumm)	Differential leukocyte count (%)			
			Neutrophil	Lymphocyte	Monocyte	Eosinophil
I	13 .46^{a} ± 0.23	7040.12^{a} ± 209.48	35.25^{a} ± 2.02	60.00^{b} ± 1.81	2.75^{a} ± 0.25	2.00^{a} ± 0.27
II	13.79^{a} ± 0.22	7841.25^{b} ± 162.48	37.25^{a} ± 1.91	58.13^{b} ± 1.83	3.25^{a} ± 0.45	1.38^{a} ± 0.18
III	13.19^{a} ± 0.22	7065.25^{a} ± 157.00	44.25^{b} ± 1.59	50.00^{a} ± 1.48	4.13^{a} ± 0.29	1.63^{a} ± 0.26
IV	13.05^{a} ± 0.28	7315.88^{ab} ± 205.09	46.50^{b} ± 2.70	48.50^{a} ± 2.54	3.50^{a} ± 0.42	1.50^{a} ± 0.27

Means bearing the same superscript within a column do not differ significantly ($P<0.05$)

Table 5. Mean serum biochemistry on twenty eighth day

Group	Albumin (g/dl)	Total protein (g/dl)	Albumin : Globulin ratio	Creatinine (mg/dl)
Control group	$3.17^{a} \pm 0.05$	$5.29^{a} \pm 0.04$	$1.05^{a} \pm 0.05$	$0.95^{a} \pm 0.03$
Turmeric incorporated group	$3.58^{b} \pm 0.13$	$6.74^{b} \pm 0.27$	$1.22^{b} \pm 0.08$	$1.00^{a} \pm 0.00$

Mean values bearing the same superscript within a column do not differ significantly ($P<0.05$)

Table 6. Mean serum biochemistry after challenge with *Pasteurella multocida*

Group	Albumin (g/dl)	Total protein (g/dl)	Albumin : Globulin ratio	Creatinine (mg/dl)
I	$3.25^{b} \pm 0.06$	$5.41^{b} \pm 0.09$	$1.52^{b} \pm 0.06$	$1.01^{a} \pm 0.03$
II	$3.13^{b} \pm 0.12$	$6.16^{c} \pm 0.22$	$1.00^{a} \pm 0.04$	$1.38^{b} \pm 0.09$
III	$2.54^{a} \pm 0.13$	$4.93^{a} \pm 0.14$	$1.08^{a} \pm 0.07$	$1.68^{c} \pm 0.11$
IV	$2.43^{a} \pm 0.14$	$4.80^{a} \pm .08$	$1.05^{a} \pm 0.09$	$1.84^{c} \pm 0.12$

Mean values bearing the same superscript within a column do not differ significantly ($P<0.05$)

Table 7. Mean serum enzyme profile (U/L) on twenty eighth day

Group	ALT	AST	ALP
Control group	$36.83^{a} \pm 1.51$	$76.00^{a} \pm 2.47$	$60.71^{a} \pm 2.71$
Turmeric incorporated group	$34.25^{a} \pm 2.94$	$72.79^{a} \pm 2.21$	$50.62^{a} \pm 5.52$

Mean values bearing the same superscript within a column do not differ significantly ($P<0.05$)

Table 8. Mean serum enzyme profile (U/L) after challenge with *Pasteurella multocida*

Group	ALT	AST	ALP
I	$39.00^{a} \pm 2.55$	$73.00^{a} \pm 4.65$	$63.75^{a} \pm 5.42$
II	$61.00^{b} \pm 5.22$	$90.50^{b} \pm 1.46$	$85.00^{ab} \pm 3.53$
III	$61.38^{b} \pm 3.42$	$94.25^{b} \pm 5.96$	$101.88^{b} \pm 7.29$
IV	$65.38^{b} \pm 4.46$	$98.75^{b} \pm 3.59$	$109.50^{b} \pm 6.94$

Mean values bearing the same superscript within a column do not differ significantly ($P<0.05$)

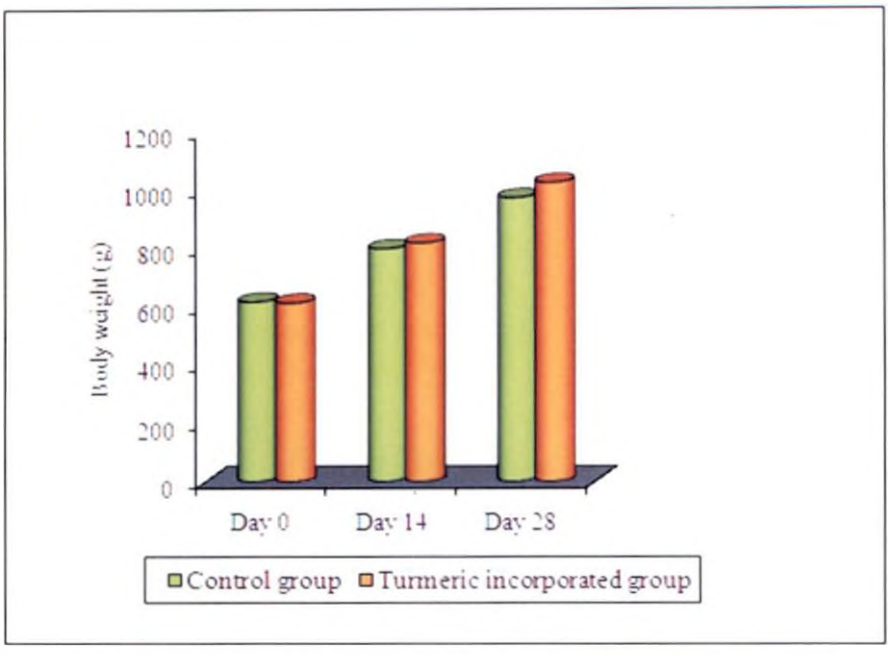

Fig.2 Mean body weight of experimental rabbits

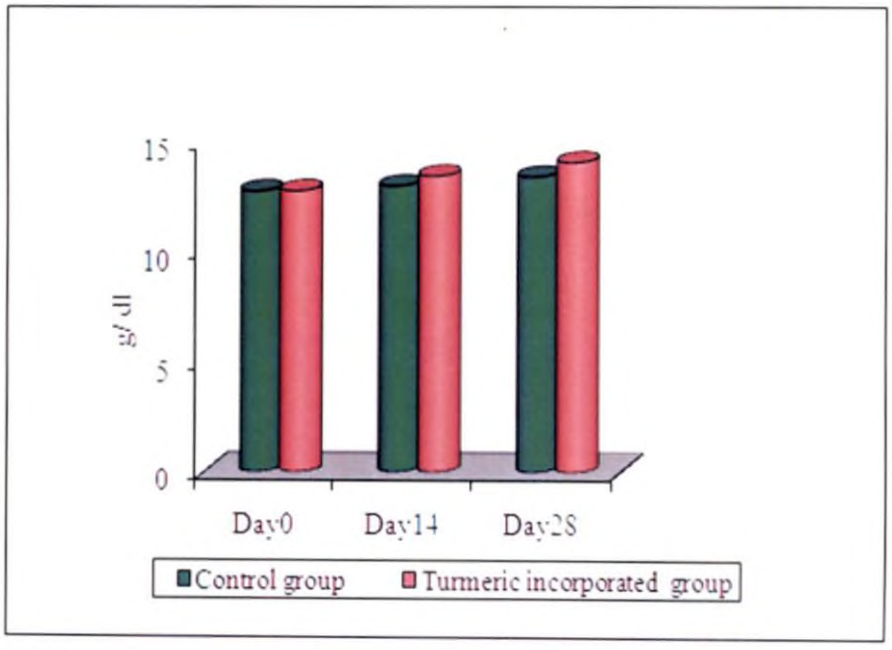

Fig.3 Mean haemoglobin concentration of experimental rabbits

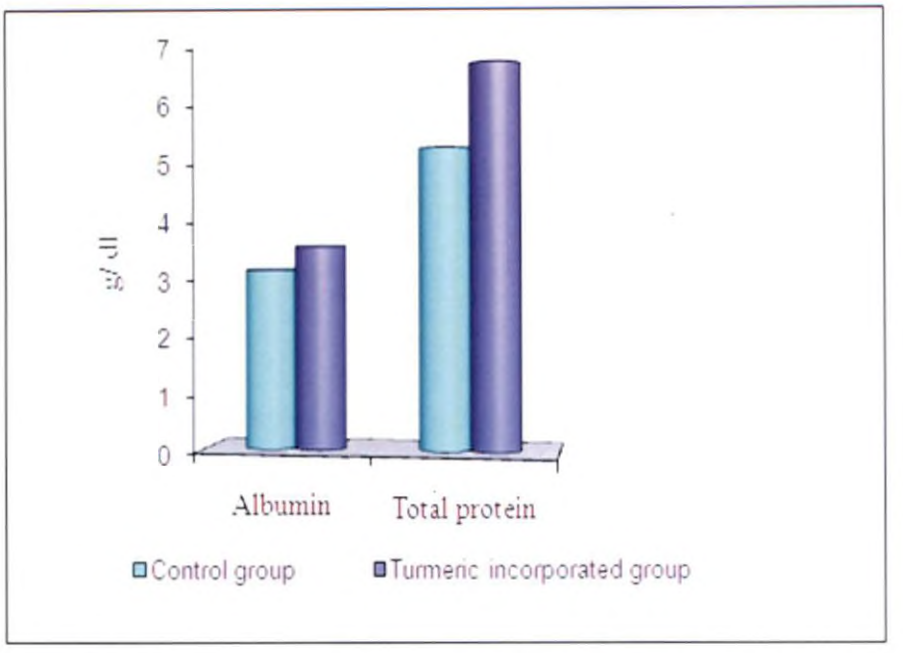

Fig.4 Mean serum protein profile on twenty eighth day

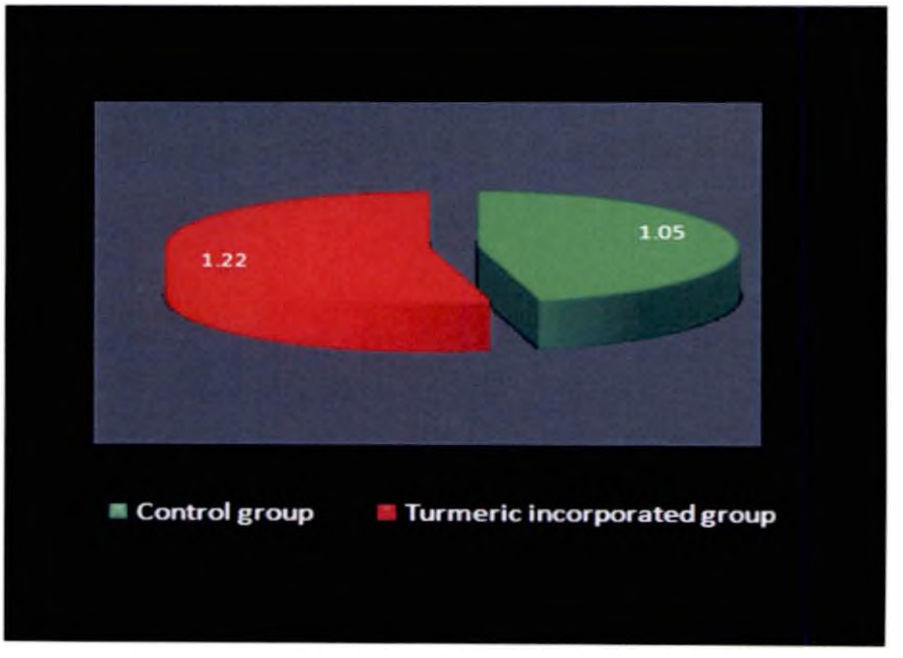

Fig.5 Albumin : Globulin ratio on twenty eighth day

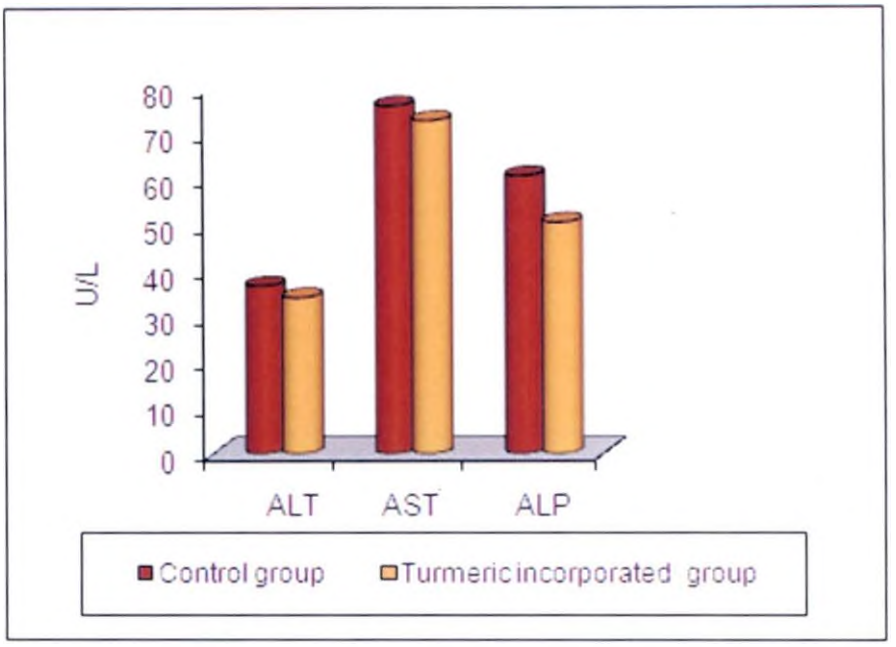

Fig.6 Serum enzyme profile on twenty eighth day

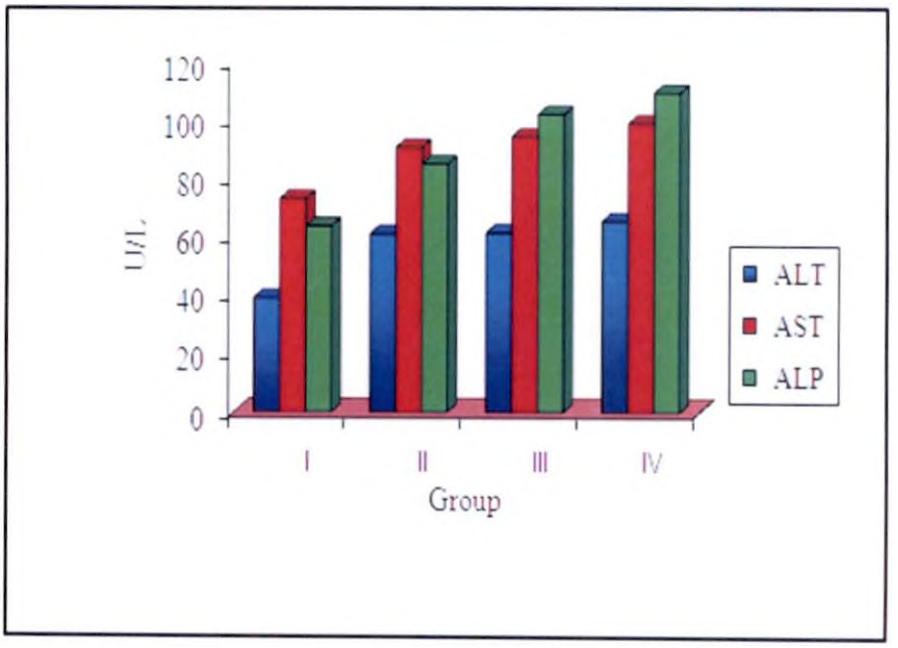

Fig.7 Serum enzyme profile after challenge with *Pasteurella multocida*

4.3 PATHOGENICITY STUDIES

The isolate was able to kill weaned mice, on inoculation with 0.1 ml of 18 h broth culture of *P. multocida* serotype A: 1 containing approximately 3×10^8 organisms/ ml intraperitoneally. The same isolate was able to kill a rabbit, when administered with the same dose. The mice died within eight hours when injected intraperitoneally and rabbit within 24 h when administered intranasally. The gross lesions observed in the internal organs of the dead mice were petechiae on the pericardium and congestion of lung, liver and spleen. Fluid accumulation was also noticed in the peritoneal cavity of mice when inoculated intraperitoneally. The gross lesions observed in the rabbit were copious amount of blood mixed frothy exudate in the tracheal lumen, severe congestion and consolidation of lungs, epicardial and endocardial haemorrhage and varying degrees of congestion, degeneration and necrotic areas in the liver and kidney. Blood smear and impression smears from spleen and liver revealed large number of bipolar stained organisms. Colonies suggestive of *P. multocida* were isolated from the heart blood, lung, trachea, liver and spleen on bovine blood agar, following incubation at 37°C under five per cent carbon dioxide tension.

4.4 CLINICAL OBSERVATION AND MORTALITY PATTERN

Experimentally infected rabbits (Groups II, III and IV) showed varying degrees of clinical signs and responded differently to the lethal challenge in the mortality pattern observed in these groups. Groups III and IV rabbits exhibited similar clinical symptoms like sneezing, anorexia, dyspnea, loss of condition, mucopurulent nasal discharges and abdominal breathing (Fig. 8). The rabbits appeared dull, depressed, off-feed, stood aloof and showed increased respiratory rate, reduced spontaneous activities and signs of coma. Only two rabbits of Group II

showed similar clinical signs while others of this group showed less severe clinical signs without any abnormal manifestations when compared to the other challenged groups. The mortality pattern varied between the challenged groups. The clinical symptoms exhibited were more severe in rabbits of Groups III and IV which died within twenty six hours of post inoculation whereas Group II rabbits which withstood the infection for another six hours and died within thirty two hours of post inoculation.

4.5 BACTERIOLOGICAL EXAMINATION

Bipolar organisms were detected from blood smears and lung impression smears by Leishman's staining. Colonies suggestive of *P. multocida* was re-isolated from heart blood, trachea, liver, spleen and lungs from dead rabbits on blood agar following incubation at 37°C under five per cent carbondioxide tension. The isolate produced typical colonies on blood agar which were smooth, convex, translucent and butyraceous and one to three millimeters in diameter, after 18 to 24 h of incubation. Gram's staining revealed Gram negative coccobacillary organisms arranged singly or in pairs.

4.6 PATHOANATOMICAL STUDIES

The gross and histopathological lesions in various experimental Groups I, II, III and IV are described in detail.

4.6.1 Gross pathology

Group I

The control rabbits of Group I revealed no apparent gross lesions on postmortem examination.

Group II

The animals treated with turmeric for one month and challenged with *P. multocida* revealed slight hyperemia of the tracheal mucosa. Mild to moderate congestion, focal areas of collapse and emphysema were the lesions observed in the lungs. No apparent gross lesions could be detected on the heart. In the liver, there were focal grayish white areas indicating degeneration. Areas of mild degenerative changes in kidney could also be noticed.

Group III

Grossly, the animals in this group had conjunctivitis, nasal discharges and revealed petechial haemorrhage subcutaneously on the neck region. Moderate to severe pulmonary congestion and congestion of tracheal mucosa was seen. Blood mixed frothy exudate was present in the tracheal lumen. The lungs were oedematous and appeared wet, heavy and from the cut surfaces fluid oozed out. The lungs were severely congested and had focal consolidation indicating pneumonic changes along with focal areas of collapse and compensatory emphysematous areas. Moderate enlargement and congestion of liver and kidney could be observed. The livers were swollen with necrotic foci and petechial haemorrhage. Epicardial and endocardial haemorrhage were also seen.

Group IV

In rabbits challenged with *Pasteurella* alone group, the animals revealed conjunctivitis and intense mucopurulent nasal discharges grossly. Petechial haemorrhage in the subcutaneous area on the neck region was also present (Fig. 9).

Copious amount of blood mixed frothy exudate was present in the tracheal lumen. Severe pulmonary congestion, oedema, areas of consolidation and extensive haemorrhages were seen in the lungs. In some of the severely oedematous lungs, on tracing the airways the bronchi and the bronchioles were also found to be filled with blood tinged froth. Areas of oedema appeared wet, heavy and from the cut surfaces fluid oozed out. The lungs showed focal areas of consolidation, collapse and emphysematous areas. Liver in most cases showed white pin point necrotic spots, swollen and congestion. Kidneys exhibited varying degrees of congestion, haemorrhage and necrosis. Petechial haemorrhages on the epicardium and endocardium was evident.

4.6.2 Histopathology

Histopathological lesions of varying intensities were seen in the trachea, lungs, heart, liver, and kidney of Groups II, III and IV rabbits. The lesions observed in the different groups were.

Group I

In healthy control Group I, the organs like trachea, lungs, heart, liver and kidneys revealed no apparent lesions at microscopic level.

Group II

Trachea

In the turmeric treated and challenged with *Pasteurella* group of rabbits there were only mild changes in the trachea. The mucosa appeared intact throughout and there were mild congestion and infiltration with focal inflammatory cells. Tracheal epithelium showing nodular hyperplasia was also seen. Mild degenerative changes and presence of oedema in the tracheal mucosa was also observed.

Lungs

Animals treated with turmeric and challenged with *Pasteurella* exhibited focal areas of inflammatory changes in the lungs characterized by infiltration with neutrophils and mononuclear cells with predominant macrophages in the alveoli and caused moderate thickening of the interalveolar septum. Focal areas of collapse and compensatory emphysema were observed. An increase in the peribronchial accumulation of lymphoid cells were also seen.

Heart

The turmeric treated and challenged animals did not show much changes except for mild degenerative lesions in the myocardium.

Liver

The histological changes in the liver of turmeric treated and challenged animals were sinusoidal dilatation, focal centrilobular degeneration and moderate fatty change of surrounding hepatocytes. Mild to moderate granular and vacuolar degenerative changes in hepatocytes could be observed in the liver parenchyma.

Kidney

In the kidney of Group II rabbits, mild degeneration of tubular lining cells and in the glomeruli mild degenerative changes and infiltration with few inflammatory cell infiltrations were seen.

Group III

Trachea

Animals challenged with *Pasteurella* and simultaneously treated with turmeric showed extravasation of blood in the tracheal mucosa, submucosal area and in the lumen of the trachea. Capillaries on the tracheal wall were markedly dilated. Infiltration with polymorphonuclear leukocytes, macrophages and plasma cells were seen in the tracheal mucosa and submucosa along with focal desquamation of the tracheal epithelium.

Lungs

Pulmonary lesions in the Group III animals were characterized by vascular changes like congestion and presence of free erythrocytes in the lumen of the alveoli as well as in the interstitial space. Most of the alveoli were ruptured and at certain places the alveoli and interstitial space were filled with oedema fluid. Also areas of collapse, emphysema and denuded bronchial epithelium were seen. Rupture of the wall of the alveoli and fusion with the neighbouring alveoli were also seen. Peribronchial accumulation of lymphocytes were found to be more intense. Thrombosis of pulmonary artery followed by extravasation of erythrocytes and exudation of plasma could also be observed.

Heart

Severe congestion and haemorrhage in between the myocardial fibres and endocardium could be observed. Degenerative lesions in the myocardial fibres and focal areas of fragmentation and lysis of muscle bundles could also be observed in the heart.

Liver

Vascular changes were predominant in the liver of Group III animals. The lesions seen were varying degrees of congestion and multifocal haemorrhage. Diffuse areas of degeneration of hepatocytes characterized by cloudy swelling to focal necrosis and central venous congestion were observed in certain areas. Focal areas of infiltration with inflammatory cells like neutrophilic and mononuclear cell infiltration and lymph stasis were pronounced.

Kidney

Degeneration of tubular epithelium, congestion, haemorrhage and oedema of glomeruli, clumping of glomerular tufts, atrophy, congestion and presence of inflammatory cells were the main histopathological lesions seen in the kidneys of Group III animals.

Group IV

Trachea

The histological lesions in the trachea of animals challenged with *Pasteurella* alone, the lesions were very severe. Extravasated erythrocytes were scattered in the tracheal mucosa, submucosal area and in the lumen of the trachea. Focal degeneration and desquamation of the tracheal epithelium could also be seen. The lumen of the capillaries on the tracheal wall revealed marked engorgement. Inflammatory changes characterized by oedema, infiltration with polymorphonuclear leukocytes and macrophages in the tracheal mucosa and submucosa were evident.

Lungs

In the animals challenged with *Pasteurella* alone, there were very severe vascular changes predominated to inflammatory and necrotic changes in the lungs. Pulmonary artery, arterioles, pulmonary vein and alveolar capillaries were engorged with blood. Hypertrophy of the smooth muscle in the wall of medium and small sized pulmonary arteries and rhexis of pulmonary artery with subsequent leakage of erythrocytes could be observed in certain areas. Extensive haemorrhage was seen in the alveoli, interstitial tissues and in a few bronchioles. Focal areas of collapse of the alveoli were noticed along with emphysema. In the collapsed areas the alveolar walls were found to be in close apposition and the alveolar lumen was greatly reduced in size. Desquamated bronchial epithelium and necrotic debri could be observed. Peribronchial collections of lymphoid cells were found to be more intense than normal. Focal lobular type of inflammation characterized by infiltration with neutrophils, lymphocytes and macrophages and subsequent moderate thickening of the alveolar septum could also be observed in the lung parenchyma.

Heart

Endocardial haemorrhage and focal areas of congestion and haemorrhage in myocardium were evident. Moderate to severe degeneration of myocardial fibres could also be seen. Areas of fragmentation of muscle fibres and myolysis were also observed in the heart of rabbits challenged with *Pasteurella* alone.

Liver

Severe vascular changes like congestion and diffuse haemorrhage were predominant in the liver. Varying degrees of degeneration and necrosis of hepatocytes characterized by cloudy swelling, focal necrosis of midzonal areas and central venous congestion were observed. Diffuse necrotic changes of hepatic cells in certain areas could be seen. Dilated lymphatics and focal infiltration of inflammatory cells like neutrophils and mononuclear cells could be observed.

Kidney

Vascular lesions predominated with disorganized renal architecture in most areas. Vascular lesions like congestion and multiple haemorrhage both in cortical and medullary areas and in the interstitium could be observed. Varying degrees of degeneration and scattered coagulative necrotic areas were also seen in the tubular epithelium. Focal infiltration of inflammatory cells was also evident. Hypocellularity of the glomeruli, oedema of the glomerular wall and atrophy of few glomerular tufts were also seen. In some proximal convoluted tubules, there was desquamation of tubular epithelial cells. Varying degrees of necrotic changes in renal tubular epithelium and diffusely in the cortical glomerular tissues were also noticed.

4.6.3 Comparison of gross and histopathological lesions in different challenged groups

The gross and histopathological lesions observed in the rabbits of Groups III and IV was almost similar. The intensity of these lesions in Group II was very much less compared to the Groups III and IV rabbits. Grossly, in the Group II rabbits, there were only mild changes in the trachea compared to the other two challenged Groups III and IV. There was mild congestion of the tracheal mucosa in Group II (Fig. 10). Whereas, copious amount of blood mixed frothy exudate was present in the tracheal lumen in Group IV (Fig. 11). Mild congestion was observed in the lungs of Group II rabbits (Fig. 12). Whereas, severe pulmonary congestion (Fig. 13), areas of consolidation and extensive haemorrhage were seen in Group IV. No apparent gross lesions could be detected on the heart of Group II rabbits. Whereas, petechial haemorrhage was evident on the epicardium and endocardium in Group IV (Fig. 14). On the liver of Group II rabbits, there were focal grayish white areas indicating degenerative areas (Fig. 15). Whereas, Group IV liver was swollen and haemorrhagic (Fig. 16). Areas of mild degenerative changes on kidney were seen in Group II rabbits. Whereas, Group IV exhibited varying degrees of renal congestion (Fig. 17).

Histopathologically, in the Group II rabbits, there were only mild changes in the trachea compared to the Groups III and IV. In Group II, the tracheal mucosa appeared intact throughout and there was mild congestion of capillaries (Fig. 18). Tracheal epithelium showing nodular hyperplasia was also observed in Group II (Fig. 19). Group III trachea revealed dilated capillaries and loss of epithelium (Fig. 20). Presence of inflammatory cells in the trachea was also noticed in Group III (Fig. 21). In Group IV, severely desquamated tracheal epithelium along with engorged blood vessels and extravasation of erythrocytes could be observed (Fig. 22&23).

In the lungs, the vascular changes and other degenerative lesions were not prominent in Group II compared to that of Groups III and IV. In the lungs of Group II animals, focal areas of collapse and compensatory emphysema were observed (Fig. 24). Inflammatory changes characterized by infiltration with inflammatory cells were also evident in Group II (Fig. 25). Pulmonary thrombus was observed in Group III lungs (Fig. 26). Also areas of collapse, emphysema and denuded bronchial epithelium were seen in Group III (Fig. 27). Whereas, desquamated bronchial epithelium and necrotic debri was evident in Group IV (Fig. 28). Pulmonary congestion and haemorrhage (Fig. 29) and rhexis of pulmonary artery and escape of erythrocytes were also observed (Fig. 30). Erythrocytes and desquamated bronchial epithelium were prominent in the lungs of Group IV (Fig. 31).

In the heart, mild degenerative changes were seen in Group II (Fig. 32) Whereas, endocardial haemorrhage and congestion and haemorrhage in myocardium were seen in Group III and IV rabbits (Fig. 33&34). A mild degenerative area in liver with dilated sinusoids was evident in Group II (Fig. 35&36). Presence of erythrocytes and inflammatory cells and scattered areas of degeneration and necrosis were noticed in Group III liver (Fig. 37&38). Whereas, severe diffuse areas of haemorrhage and infiltration of inflammatory cells in the liver were prominent in Group IV (Fig. 39&40). Group II kidney revealed mild degenerative changes in tubular epithelium (Fig. 41). Clumping of glomerular tufts, atrophy, congestion and presence of inflammatory cells were noticed in Group III kidney (Fig. 42&43).Whereas, Group IV kidney revealed severe congestion and haemorrhage (Fig. 44) and areas of glomerular and tubular necrosis (Fig. 45)

Fig.8 Rabbit showing clinical signs when challenged with *Pasteurella multocida*

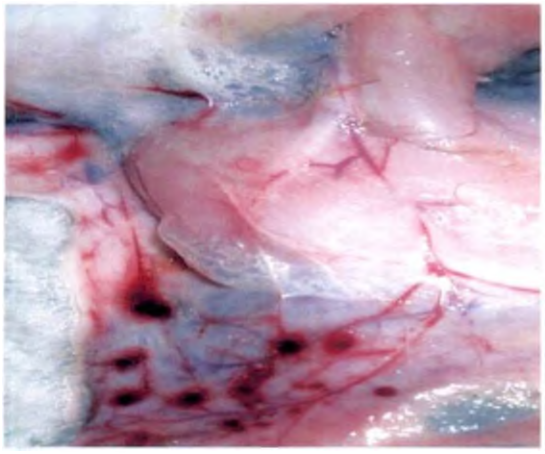

Fig.9 Subcutaneous haemorrhage on the neck region

Fig.10 Mild congestion of tracheal mucosa- Group II

Fig.11 Blood mixed frothy exudate in the tracheal lumen- Group 1V

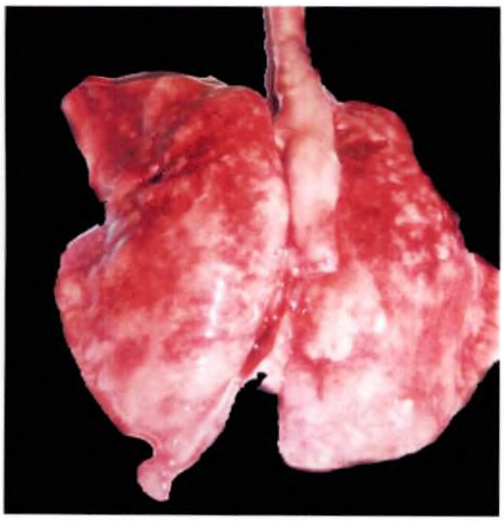

Fig.12 Mildly congested lungs- Group II

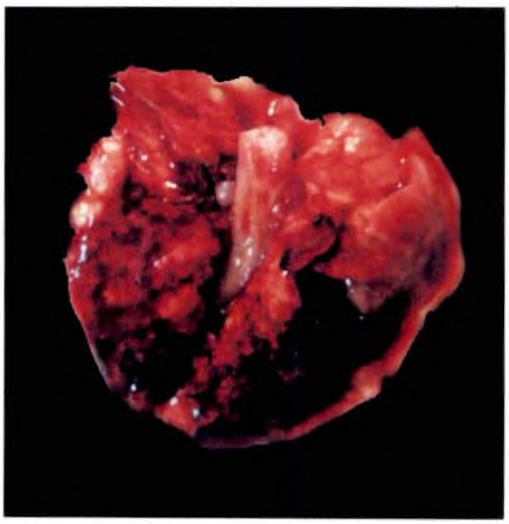

Fig.13 Pulmonary congestion - Group IV

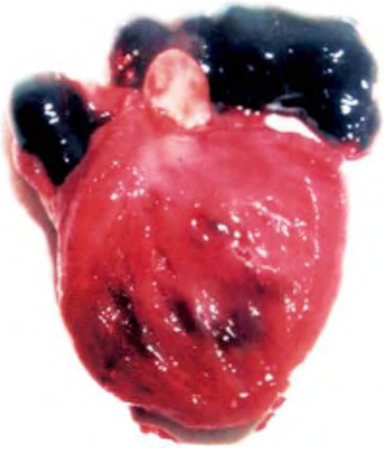

Fig.14 Endocardial haemorrhage- Group IV

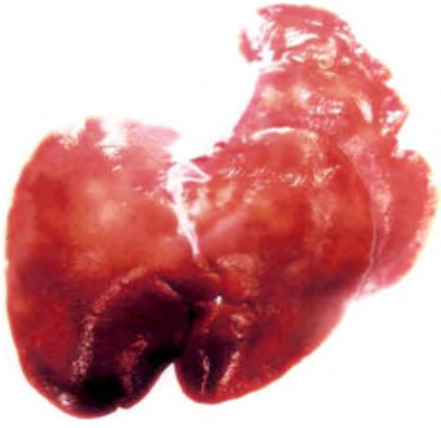

Fig.15 Mild degenerative changes on liver- Group II

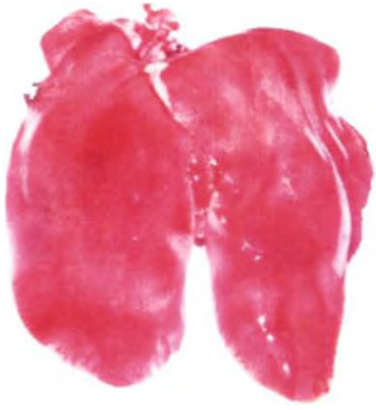

Fig.16 Swollen and haemorrhagic liver- Group 1V

Fig.17 Renal congestion- Group 1V

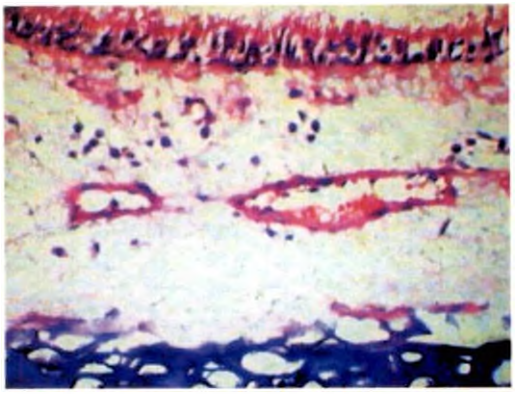

Fig.18 Intact tracheal epithelium and mild congestion of capillaries, Group II- H&Ex400

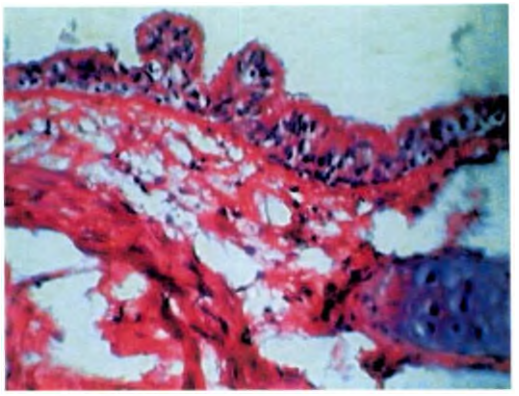

Fig.19 Tracheal epithelium showing nodular hyperplasia, Group II- H&Ex400

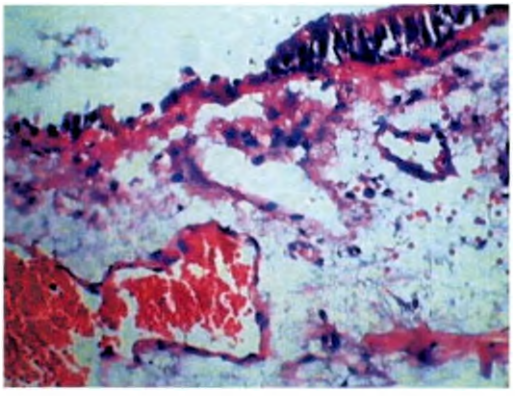

Fig. 20 Dilated capillaries and loss of tracheal epithelium, Group III- H&Ex400

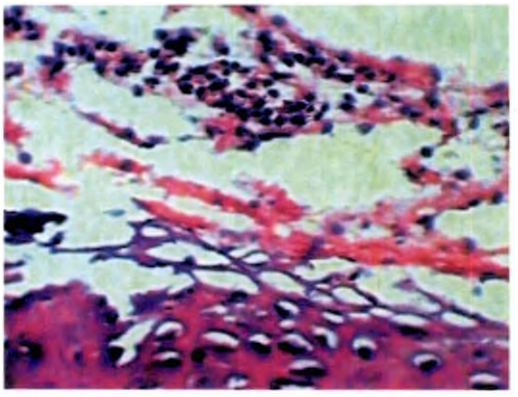

Fig..21 Presence of inflammatory cells in trachea, Group III- H&Ex400

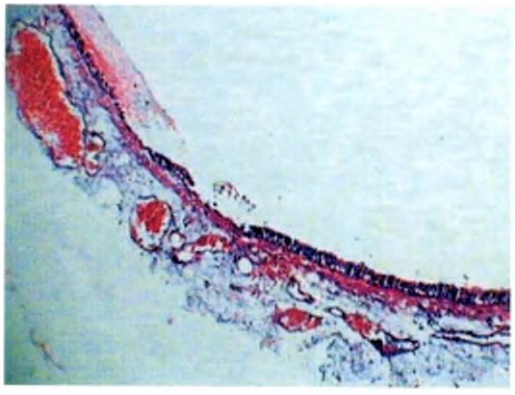

Fig.22 Desquamated tracheal epithelium and engorged blood vessels, Group IV- H&Ex100

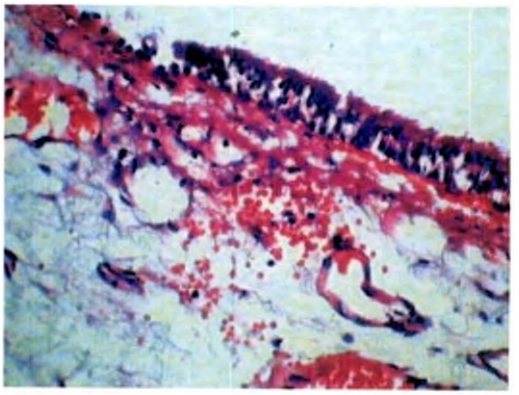

Fig.23 Loss of continuity in tracheal epithelium and extravasation of erythrocytes, Group IV- H&Ex400

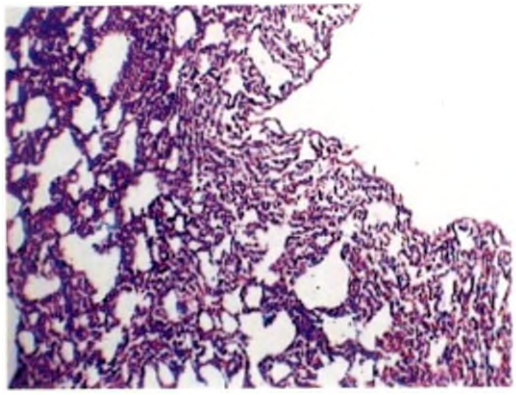

Fig.24 Pulmonary collapse and emphysema, Group II- H&Ex100

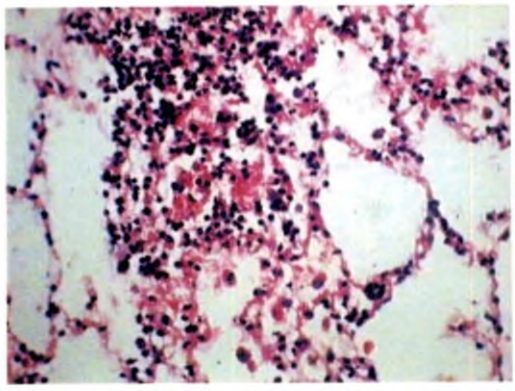

Fig.25 Inflammatory cells in the lungs , Group II- H&Ex400

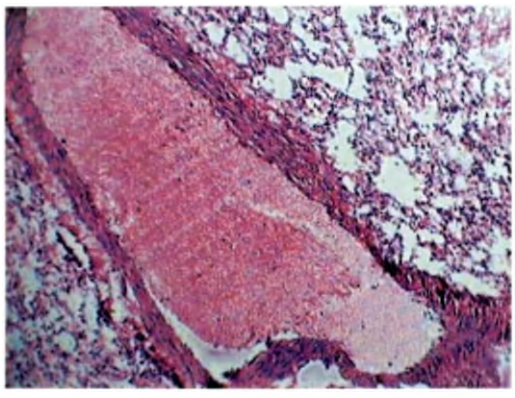

Fig.26 Pulmonary thrombi, Group III- H&Ex400

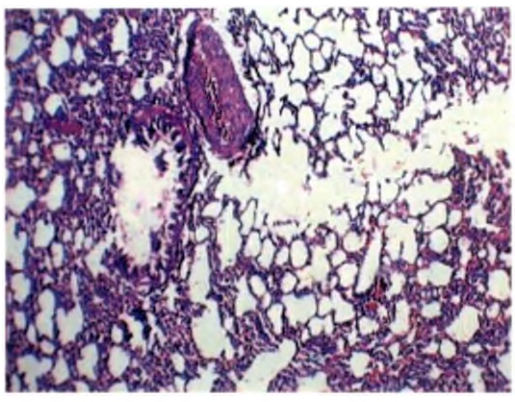

Fig.27 Pulmonary collapse , emphysema and denuded bronchial epithelium, Group III- H&Ex100

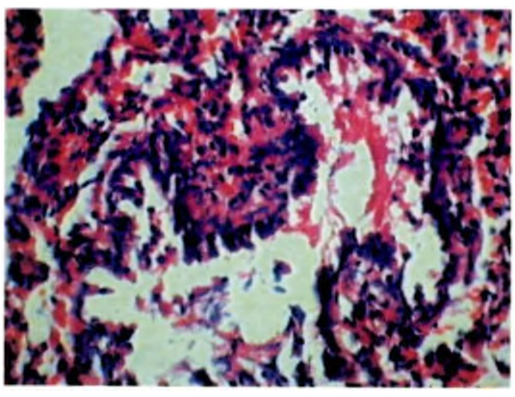

Fig.28 Desquamated bronchial epithelium and necrotic debri, Group IV- H&Ex400

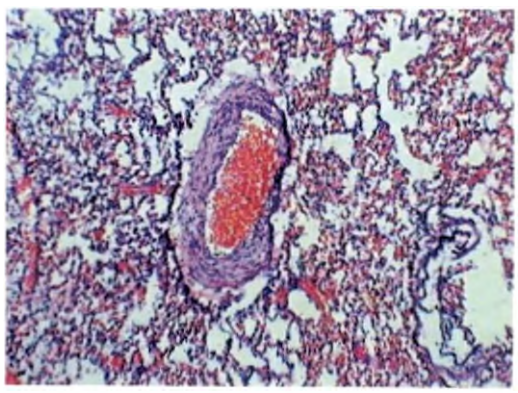

Fig.29 Pulmonary congestion and haemorrhage, Group IV- H&E 100

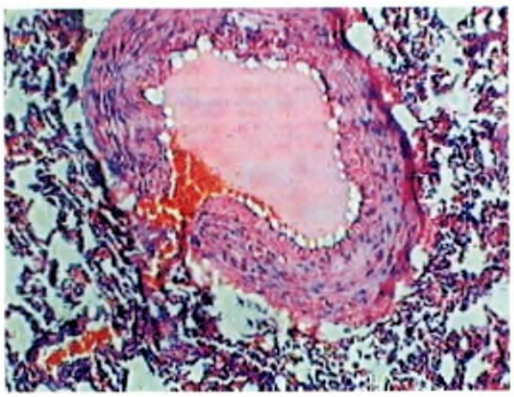

Fig.30 Rhexis of pulmonary artery and escape of erythrocytes, Group IV- H&Ex400

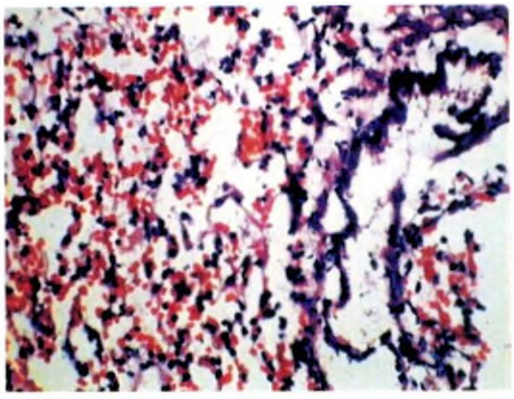

Fig.31 Erythrocytes in lung and desquamated bronchial epithelium, Group IV- H&Ex400

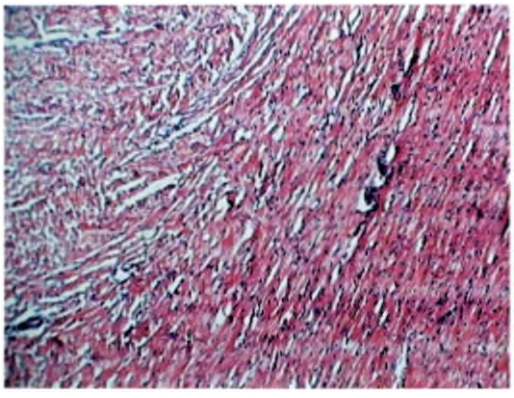

Fig. 32 Mild degenerative changes in heart, Group II- H&Ex100

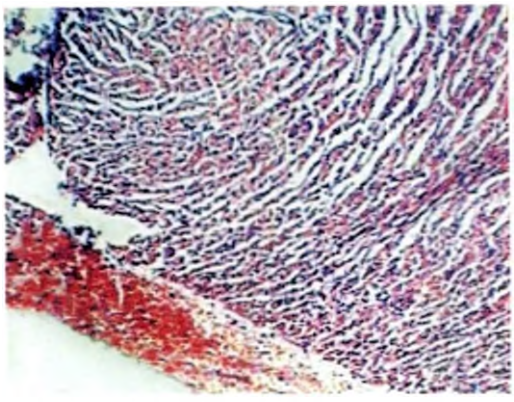

Fig.33 Endocardial haemorrhage, Group III- H&Ex100

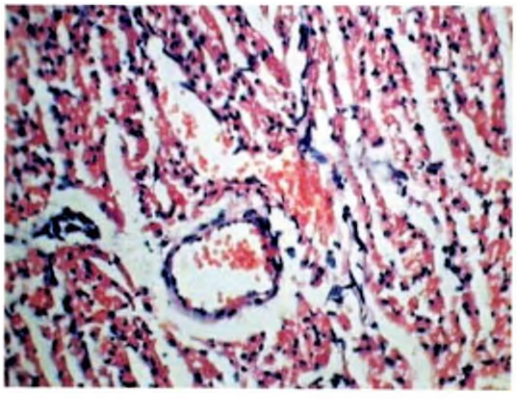

Fig.34 Congestion and haemorrhage in myocardium, Group IV- H&Ex400

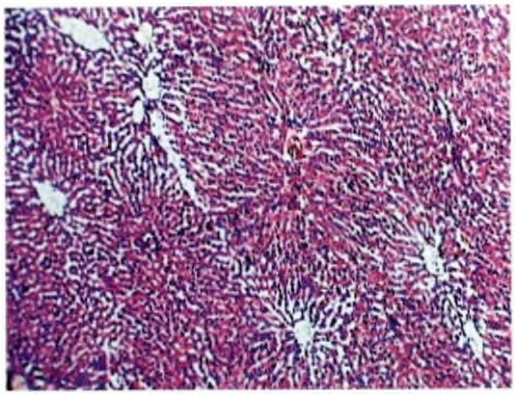

Fig.35 Mild degenerative areas in liver, Group II-H&Ex100

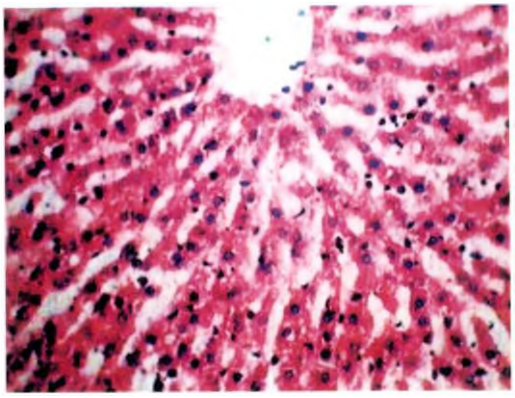

Fig.36 Dilated sinusoids in liver , Group II -H&Ex400

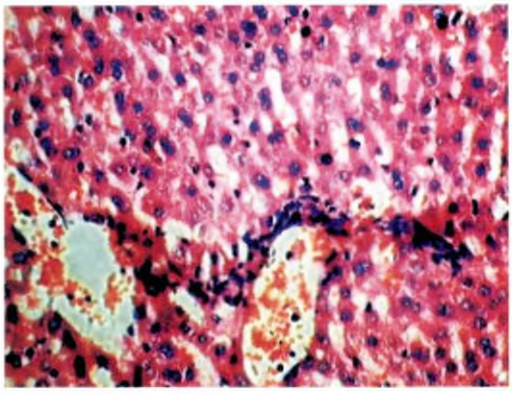

Fig.37 Presence of erythrocytes and inflammatory cells in liver, Group III- H&Ex400

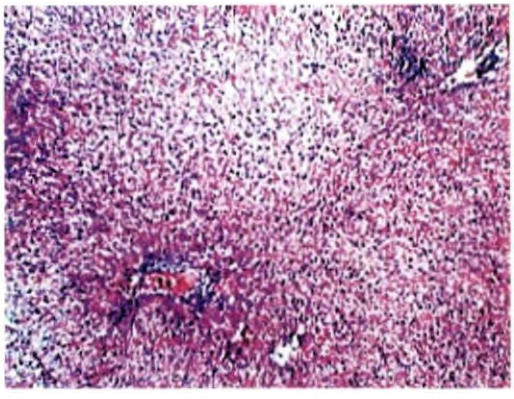

Fig.38 Scattered areas of degeneration and necrosis in liver, Group III- H&Ex100

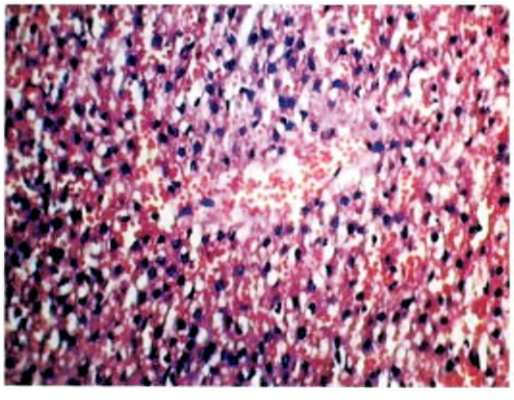

Fig.39 Diffuse areas of haemorrhage in liver, Group IV- H&Ex400

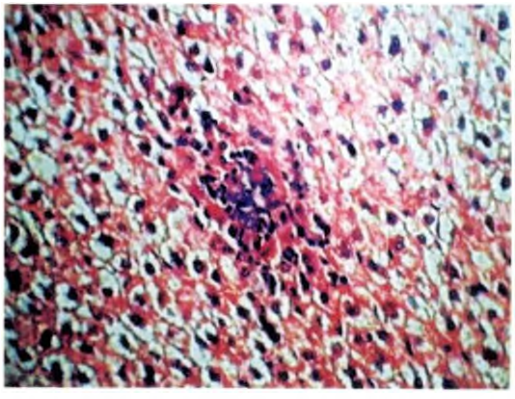

Fig.40 Infiltration of inflammatory cells in liver, Group IV- H&Ex400

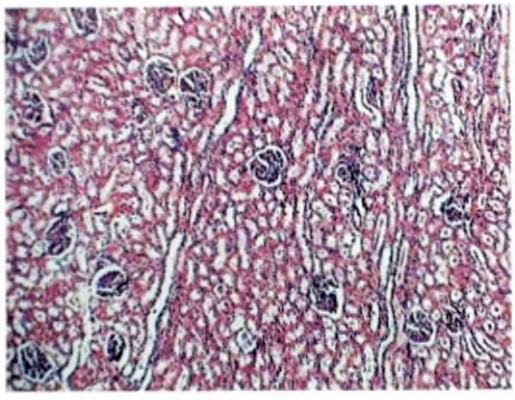

Fig.41 Mild degenerative changes in renal tubular epithelium, Group II-H&Ex100

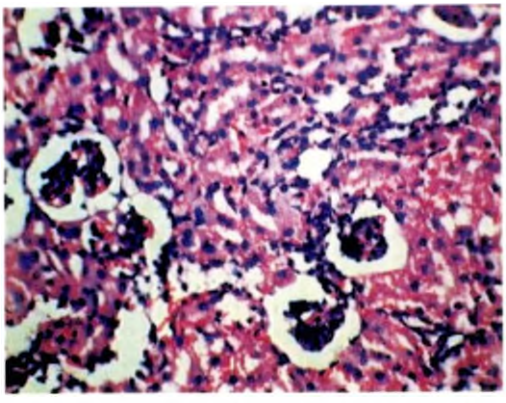

Fig.42 Clumping of glomerular tufts in kidney, Group III-H&Ex400

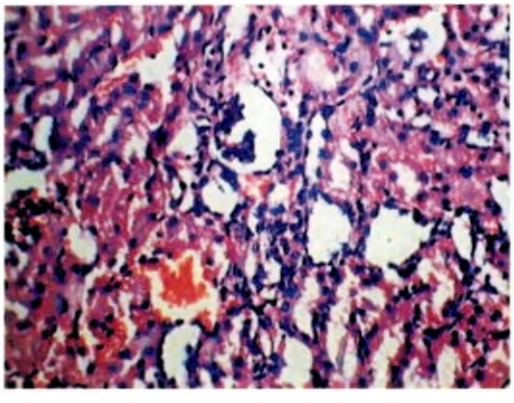

Fig.43 Glomerular atrophy, congestion and inflammatory cells in kidney, Group III-H&Ex400

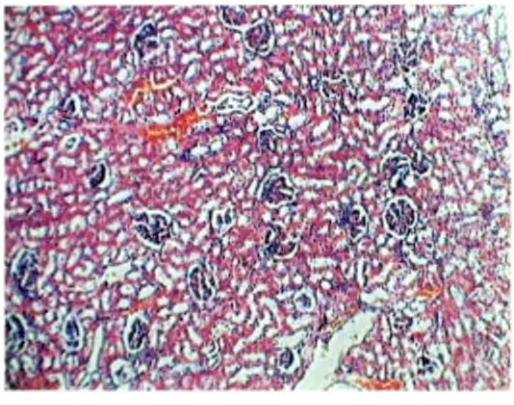

Fig.44 Congestion and haemorrhage in kidney, Group IV-H&Ex100

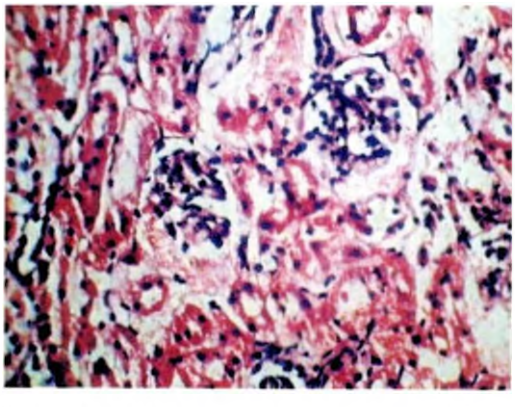

Fig.45 Glomerular and tubular necrosis in kidney, Group IV-H&Ex400

Discussion

5. DISCUSSION

Rabbit farming is a progressive enterprise in our country. Health care forms an integral part for the success of rabbit industry besides breeding, nutrition and management. Rabbit pasteurellosis is a septicaemic disease caused by strains of *Pasteurella multocida*. Because of its heavy toll, pasteurellosis is a major concern to the rabbit industry, resulting in huge economic loss. The disease is often latent; however, under various conditions of stress, severe clinical expressions of disease occur (Yue-Shoung *et al.*, 1988).

The rhizome of *Curcuma longa* (turmeric) is one of the most common ingredients in the indigenous system of medicine. Turmeric has been used as a traditional remedy for the treatment of inflammation and it could be successfully employed for therapeutic management of inflammation (Ishita *et al.*, 2004).

Hence, a systematic investigation in the present study was undertaken to elucidate the symptoms and lesions of experimental pasteurellosis and to study the beneficial effects of dietary supplementation of *Curcuma longa* (turmeric) in managing pasteurellosis. The results obtained in the present study are discussed in this chapter. The parameters like body weight, haemogram and serum profile of the animals treated with turmeric were compared with the control during the first month of the experiment. Haematological, biochemical and pathological alterations thereafter lethal challenge were also studied.

5.1 EFFECT OF TURMERIC ON BODY WEIGHT

The body weight of rabbits in the turmeric incorporated group did not show significant variation compared to control group on 14th day of the experiment but numerical increase was recorded in turmeric incorporated group. Similar findings have been reported by Kurkure *et al.* (2001) and Mekala *et al.* (2006) in broilers dosed with turmeric.

The rabbits in the turmeric treated group showed a significant increase in body weight on the 28th day of the experiment. These findings closely agreed with earlier reports by Mohan *et al.* (2001), Samarasinghe *et al.* (2003) and Kumar *et al.* (2005). Whereas, Emadi and Kermanshahi (2006) observed no alteration in body weight in broilers incorporated with turmeric at different doses. Al-Sultan (2003) recorded a higher body weight gain in birds fed with diet containing turmeric. He opined that the higher body weight gain observed was due to the antioxidant activity of curcumin that stimulated protein synthesis by body's enzymatic system which increased feed intake and improved feed efficiency.

The present study revealed that the increased body weight of rabbits fed turmeric rhizome powder could be due to increased secretion of digestive juices, resulting in increased nutrient utilization. It may be inferred from the present study that supplementation of turmeric increased body weight and is effective in improving production performance of rabbits.

5.2 HAEMATOLOGICAL PARAMETERS

From this study, it was observed that the supplementation of turmeric rhizome powder improved the haematological parameters. The experimental infection affected adversely the haematological parameters viz., total leukocyte count and differential leukocyte count while the haemoglobin concentration did not reveal significant difference among the Groups II, III and IV when compared with the control Group I.

The concentration of haemoglobin observed in turmeric fed group was significantly higher on the 14th and 28th day of the experiment which indicated the stimulating effect of turmeric rhizome powder on haemoglobin synthesis and the better efficacy of blood for oxygen transfer in the circulatory system. This finding is in accordance with the findings of Emadi *et al.* (2007). On the contrary, Sambaiah *et al.* (1982) reported that the whole spice turmeric or curcumin when fed to rats did not make any difference to the haematological picture. The haemoglobin concentration of rabbits post challenge in Groups I to IV showed no significant variations among the challenged groups from that of the control group. Mir *et al.* (2001), Rameshkumar *et al.* (2006) and Praveena *et al.* (2007) have also reported similar findings. The values in haemoglobin concentration were higher in Group II numerically than that of Groups III and IV rabbits. The comparatively better higher haemoglobin level in Group II following the lethal challenge indicates the protective effect of turmeric. The salient features of these findings are in close agreement with Mohan *et al.* (2001).

No significant difference was noticed between the two groups in the total leukocyte count on 14th and 28th day of the experiment. Though the values obtained were statistically comparable, the turmeric fed group showed numerical increase in TLC on 14th and 28th day which denotes the immunomodulatory activity of turmeric.

This finding was in accordance with those of Kurkure *et al.* (2001), Mohan *et al.* (2001) and Al-Sultan (2003). They opined that the increase in TLC was due to the immunomodulatory activity of curcumin, the active principle of turmeric. Simi (2007) observed that the mean value of haemoglobin and TLC in turmeric supplemented group were significantly higher than that of the control group. The mean differential leukocyte count (DLC) values observed in both these groups were within the normal range and did not reveal significant difference.

After experimental pasteurellosis, the blood parameters showed mild heterophilic leukocytosis. The mean total leukocyte count (TLC) observed in Groups III and IV was satistically comparable with the values of the control group while Group II showed significant increase in TLC compared to the other groups. Differential leukocyte count in the challenged Groups III and IV revealed a significant increase in neutrophil count and slight increase in monocyte count. A significant decrease in lymphocyte count and a slight reduction in eosinophil count were also observed in the challenged groups when compared to the control. In Group II, the mean differential leukocyte count observed was within the normal range and did not reveal significant difference from that of the control. These observations indicate that Group II rabbits showed a better immune response when compared to the other challenged rabbits of Groups III and IV. Similar haematological picture was observed by Praveena *et al.* (2007). Rabbits experimentally infected with *P. multocida*, showed a significant increase in total leukocyte count, heterophil and monocyte counts (Rameshkumar *et al.*, 2006). The mild heterophilic leukocytosis might be viewed as the primary response to bacterial infection and presence of the organisms in the respiratory tract (Mir *et al.*, 2001).

5.3 SERUM BIOCHEMISTRY

From the results of the present study, it could be observed that there was a significant increase in the mean value of total protein, albumin and albumin : globulin ratio in turmeric incorporated group compared to that of control group on 28th day of the experiment. This is in consonance with the observations made by Simi (2007). Findings following lethal challenge showed significant changes in serum protein profile among the challenged groups from that of the control. Similar observations have been made by Praveena *et al.* (2007). Contrary to this, Mir *et al.* (2001) observed that serum levels of total protein, albumin, globulin and albumin : globulin ratio remained unaffected upto 4 weeks post inoculation with *P. multocida.* The hypoalbuminemia in the present study may be attributed to liver damage caused from the lethal action of the organism. Liver is the only site of albumin synthesis and hypoalbuminemia is an important feature of liver disease (Kaneko *et al.*, 1997). The decrease in protein values were mild in Group II when compared to the other challenged groups where marked decrease was noticed. The findings of this study closely agreed with the findings of Kurkure *et al.* (2001). The present observation indicates the protective effect of turmeric on liver. Biochemical changes in the liver also correlated with this observation.

The mean value of creatinine observed in turmeric incorporated group when compared to the control group on 28th day of the experiment was within the normal range. No significant difference was noticed between the two groups. Significantly higher level of creatinine was observed in the challenged groups when compared to the control. The marked increase in serum creatinine levels could be due to kidney damage as revealed by histopathological changes. The findings of this study closely agreed with the findings of Mir *et al.* (2001) where marked increase in creatinine

level in *P. multocida* infected rabbits was observed. The inclusion of turmeric powder for the entire study period in Group II resulted in a comparatively lower creatinine level compared to the Groups III and IV. This could be attributed to the protective effect of turmeric on kidney.

The mean value of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) estimated on 28th day of the experiment revealed no significant difference between the turmeric fed group from that of the control. Numerically the values recorded in the turmeric incorporated group were lower from that of the control. The present study indicated that the supplementation of turmeric powder did not significantly affect the enzyme activities in rabbits. These findings are in consonance with Simi (2007).

In the present study, a marked elevation of ALT, AST and ALP levels were found in the serum of challenged animals when compared to the control. The increased serum ALT, AST and ALP levels observed after lethal challenge is suggestive of liver damage caused by the virulent organism. The elevated enzyme levels can be attributed to the pathological changes produced by the virulent organism on the hepatobiliary system which resulted in increased cellular permeability and release of these enzymes into the serum. The results of the present study are in consonance with Mir *et al.* (2001). The serum levels of these enzymes were comparatively lower in the turmeric fed for the entire study period (Group II) than the other challenged groups. This might be due to the protective effect of turmeric against the pathological changes induced by the virulent organism. Similar observations were made by Despande (1998), Park *et al.* (2000), Kurkure *et al.* (2001), Somchit *et al.* (2002) and Miyakoshi *et al.* (2004).

In the present study, the marked increase in serum creatinine, ALP, AST and ALT activities in Groups III and IV, could be due to kidney and liver damage as revealed by histopathological changes in these organs. The results obtained are indicative of an injury to the liver and kidneys incited by the virulent organism. All these observations suggest that biochemical changes produced in pasteurellosis are attributed to the pathological effects on the various organs studied. The degree of damage was comparatively reduced in the Group II rabbits. This suggests the beneficial effects of turmeric to modulate the hepato-renal system.

5.4 PATHOGENECITY STUDIES

In the present study, the pathogenicity of the isolate *P. multocida* serotype A: 1 was confirmed in mice and rabbit. The mice inoculated with 0.1 ml of 18 h broth culture of *P. multocida* serotype A: 1 containing approximately 3×10^8 organisms/ ml intraperitoneally died within eight hours and rabbit within 24 h when administered with the same dose by intranasal route. The gross lesions observed in the mice were petechial haemorrhage on the epicardium and general congestion of all the visceral organs. Mice could be used as the animal of choice for testing pathogenicity of *P. multocida* and an overwhelming increase in the number of organisms in visceral organs was the cause of death of mice when experimentally inoculated (Collins, 1976). The lesions observed in the rabbit were haemorrhagic tracheitis, severe congestion and consolidation of lungs, epicardial and endocardial haemorrhage and varying degrees of congestion and degeneration on the liver and kidney, which were indicative of a severe septicaemic condition. Murugkar and Ghosh (1995) tested the pathogenicity of *P. multocida* serotypes A: 1 in rabbit and reported that the rabbit died within 48 hrs when 0.1 ml of 6 hr broth culture was inoculated. These findings agreed with the observations made in the present study.

5.5 BACTERIOLOGICAL EXAMINATION

The isolate from the Groups II, III and IV, produced typical colonies of *P. multocida* on blood agar after incubation at 37°C for 24 h and Gram's staining revealed Gram negative cocco-bacillary organisms arranged singly or in pairs. These findings are in agreement with the observations of Quinn *et al.* (1994).

5.6 PATHOANATOMICAL STUDIES

5.6.1 Clinical observation and mortality pattern

Experimentally infected rabbits (Groups II, III and IV) showed varying degrees of clinical signs. The clinical signs observed in the Groups III and IV were almost similar. The clinical signs exhibited were sneezing, anorexia, dyspnea, mucopurulent rhinitis, conjunctivitis, abdominal breathing, off-feed, loss of condition and signs of coma. These findings are in agreement with the observations of Mir *et al.* (2001) and Rameshkumar *et al.* (2006) on experimental pneumonia in rabbits. Similar findings were also observed by Ronald *et al.*, (1991) in naturally acquired *P. multocida* infection in rabbits. Only two rabbits of Group II showed similar clinical signs while rest of the animals exhibited less severe clinical signs without any abnormal manifestations when compared to the other challenged groups. Many active principles in turmeric might have stimulated protective immune responses in Group II rabbits which reduced the intensity of clinical signs exhibited. Similar observations have been made by Antony *et al.* (1999).

The rabbits of Groups III and IV showed 100% mortality within 26 hours whereas Group II rabbits withstood the infection for another six hours and died within thirty two hours of post inoculation. There was no mortality in the healthy control rabbits which indicates that the mortality was due to the lethal challenge. Similar observations have been made by Borrathybay *et al.* (2003) and Kapoor *et al.* (2004).

5.6.2 Gross pathology

The animals of Groups III and IV showed conjunctivitis, nasal discharges and subcutaneous petechial haemorrhage on the neck region on necropsy. The gross lesions were well pronounced in Groups III and IV rabbits when compared to that of Group II rabbits.

The main lesions were observed in the trachea and lungs in the present study. The mucosa of the entire length of the trachea was diffusely hyperaemic and oedematous in Groups III and IV. This observation in the trachea was in line with the findings of Nair *et al.* (1987) and Mir *et al.* (2001). Severe congestion and haemorrhage in tracheal mucosa and lungs indicated the vascular damage caused by the organism. In the lungs, varying degrees of pneumonic changes was also seen. The lungs of Groups III and IV rabbits showed severe hyperaemia of the apical, cardiac and anterior portion of diaphragmatic lobes. This is in agreement with the results of the work conducted by Nair *et al.* (1987) and Rameshkumar *et al.* (2006). The lungs were severely congested and pneumonic and revealed emphysematous areas as well. Similar observations were reported by Mir *et al.* (2001).

Lesions in other organs were epicardial and endocardial haemorrhage, enlarged and congested liver with necrotic spots. The gross changes observed in the heart were supported by findings of Rhoades and Rimler (1991).

The kidneys revealed congestion and degenerative lesions. The gross lesions observed in the present study in the liver and kidneys were similar to the findings of Nair *et al.* (1987) and Mir *et al.* (2001).

5.6.3 Histopathology

Histopathological examination of the trachea revealed mild congestion of capillaries with intact mucosa in the Group II rabbits whereas, engorged capillaries on the tracheal wall of Groups III and IV animals were seen. This finding agreed with the reports of Nair *et al.* (1987) and Mir *et al.* (2001). They noticed similar lesions in the tracheal mucosa of the rabbits died of pneumonia caused by *P. multocida*. In the present study lesions like infiltration of inflammatory cells, capillary congestion and haemorhage were recorded among the challenged groups. There were degeneration and necrosis of tracheal epithelium with disruption of the normal pseudostratified pattern in the trachea. Nair *et al.* (1987) reported diffuse hyperaemia and foci of blood clots in the trachea and exudate in the larger bronchi in rabbits infected with *P. multocida*.

Histopathological examination of the lungs revealed that in Group II animals, there were focal areas of inflammatory changes in the lungs along with collapse and compensatory emphysema. Group III and Group IV lungs revealed thrombotic vessels, oedema, places of alveolar collapse and bronchiectasis in addition to varying degrees of pulmonary congestion and haemorrhage. Pulmonary thrombosis observed in the challenged Groups III and IV might be due to the severe vascular endothelial damage induced by the virulent organism. Areas of collapse, emphysema and necrosis were frequently seen along with hypertrophy of the smooth muscle in the wall of medium and small sized pulmonary arteries. Rameshkumar *et al.* (2006)

noticed serous exudates along with erythrocytes in alveolar lumen and infiltration of heterophils, proliferation of histiocytes leading to thickening of interalveolar septae and alveolar walls in rabbits. Mir *et al.* (2001) reported extensive haemorrhage, thickening of interalveolar septae and areas of emphysema in affected lungs on experimental pasteurellosis in rabbits. Loss of alveolar architecture and acute bronchitis with sloughing of lining epithelium into bronchial lumen was observed by Nair *et al.* (1987). In the present study, inflammatory reaction in the bronchi was characterized by presence of exudates in the lumen of the bronchi with varying number of neutrophils, mononuclear cells and necrotic cell debri. Similar findings were reported by Dungsworth (1993) and Shilpa and Verma (2006).

Moderate to severe degeneration of myocardial fibres, focal areas of congestion, haemorrhage and areas of fragmentation of muscle fibres and myolysis were observed in the Groups III and IV. The lesions like endocardial and myocardial haemorrhage observed in the present study could be attributed to the virulent organism used for the lethal challenge which might have led to septicaemia. Similar findings have been reported by Rhoades and Rimler (1991).

Severe circulatory changes like congestion and haemorrhage were predominant in the liver of Groups III and IV. Varying degrees of degeneration, necrosis, infiltration of inflammatory cells and tendency for individualization could be observed. These findings are in agreement with the hepatic lesions observed in *P. multocida* induced changes in rabbits by Nair *et al.* (1987) and Mir *et al.* (2001). The necrotic change in liver suggests the septicaemic effect caused by the virulent organism which might have directly disrupted the functional anatomy and physiology of the hepatocytes. Also the *Pasteurella* organisms isolated from the liver in challenged rabbits indicated a hematogenous spread of the disease. Similar findings have been reported by Heddleston and Watko (1963). The histological changes in the

liver of turmeric treated and challenged animals were focal centrilobular degeneration, sinusoidal dilatation and moderate fatty change of surrounding hepatocytes. No central vein congestion and necrotic changes could be seen as observed in Groups III and IV. Turmeric and curcumin were found to reverse biliary hyperplasia, fatty changes and necrosis induced by aflatoxin production (Soni *et al.*, 1992). The hepatoprotective effects of turmeric were evident by reduced serum enzyme levels as well as the less severe histopathological changes in liver. The biochemical changes also correlated with this observation. Kurkure *et al.* (2000) observed that the histopathological changes in liver were found to be minimized in birds of turmeric group on induced aflatoxicosis in cockerels.

Varying degrees of degenerative changes were observed in the kidney of Group II rabbits. The intensity of renal lesions in Group II was less compared to the Groups III and IV rabbits. The findings indicated the nephroprotective effect of turmeric. Severe congestion, haemorrhage, leukocytic infiltration, tubular necrosis and glomerular lesions like oedema, hypocellularity and atrophy were seen in the Groups III and IV. These findings are in agreement with the renal lesions observed by Nair *et al.* (1987) and Mir *et al.* (2001).

The present study revealed gross and histopathological lesions of varying intensities. Circulatory, degenerative and inflammatory changes in the lungs, trachea, liver, heart and kidney were noticed in the Groups III and IV rabbits. In the light of the above findings, the acute lesions observed in the present study were suggestive of septicaemia. The intensity of these lesions in Group II rabbits were very much less compared to the Groups III and IV which could be attributable to the active principles in turmeric.

The findings of the present study confirm the role of turmeric rhizome powder in improving health status of the rabbits by increased body weight and positive responses in the haematological and biochemical parameters. The intensity of lesions were much less in turmeric fed group which correlated with the less intense clinical signs which might be due to anti-inflammatory, antioxidant and immunostimulatory properties of turmeric. From the alterations recorded in the haematology, serum biochemistry, clinical signs, mortality pattern and severity of lesions in histopathology revealed that heavy infection with *P. multocida* was lethal to rabbits and it is evident that turmeric has the potential to restore or protect the systems of the body from the pathological effects caused by *P. multocida*. The systematic investigation conducted brought to the light that apart from the beneficial effects on body weight, haematology and biochemical profile, turmeric also revealed partial protective role in managing pasteurellosis. The present study is only a preliminary investigation and further studies with different doses of turmeric powder would be helpful to clarify the beneficial effects of turmeric on immune system and its therapeutic importance in rabbits.

Summary

6. SUMMARY

An experiment was designed to evaluate the symptoms and lesions of experimental pasteurellosis and to study the beneficial effects of dietary supplementation of *Curcuma longa* (turmeric) in managing pasteurellosis in rabbits. Thirty two healthy New Zealand white rabbits aged four weeks were divided randomly into four groups each having eight replicates. Group I rabbits served as control group with commercial feed alone. Group II rabbits were given feed mixed with turmeric at the rate of 2 g/kg body weight for the entire study period and on the thirty first day animals were intranasally exposed to *Pasteurella multocida.* Group III rabbits were intranasally exposed to *P. multocida* on the thirty first day and turmeric was fed at the rate of 2 g/kg body weight after exposure until the end of the experiment. Group 1V rabbits were fed on control diet alone for thirty days and then intranasally exposed to *P. multocida* on the thirty first day of the experiment.

During the first month of the experiment, body weight and haematological parameters were recorded on day zero, 14^{th} and 28^{th} and biochemical parameters on 28^{th} day of the experiment were estimated. The rabbits in Groups II, III, and 1V were challenged with 0.1 ml of 18 h broth culture of *P. multocida* serotype A: 1 containing approximately 3×10^8 organisms/ml by intranasal route. Blood samples were collected for haematological and biochemical evaluations after eighteen hours of post inoculation from all the groups. The parameters observed included body weight, clinical signs, haemogram and biochemical parameters, gross pathology and histopathology. The haemoglobin concentration, total leukocyte count and differential leukocyte count were evaluated on day zero, 14^{th}, 28^{th} and thereafter lethal challenge from all the groups. Serum levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), creatinine, total protein and albumin in serum were estimated on 28^{th} day of the experiment and thereafter

following the lethal challenge with *P. multocida*. Gross and histopathology of trachea, lungs, heart, liver and kidney were systematically conducted at the end of the experiment.

Based on the results obtained in the present study, the following conclusions were made. The mean body weight of rabbits on day 14 and 28 in turmeric fed group were 816.25 and 1022.50 g, respectively and that of control were 796.67 and 970.42 g, respectively. The values were statistically comparable on day 14 and significantly ($P<0.05$) higher in turmeric incorporated group on day 28 than that of the control group. The mean haemoglobin concentration on day 14 and 28 in turmeric fed group were 13.43 and 14.05 g/dl, respectively and that of control were 12.98 and 13.39 g/dl, respectively. The mean value of haemoglobin in the turmeric supplemented group was significantly ($P<0.05$) higher than that of the control group on day 14 and 28 of the experiment. The mean value of TLC and DLC in the turmeric supplemented group showed no significant difference from that of the control. The serum protein profile in turmeric fed group was significantly ($P<0.05$) higher than control group on 28^{th} day of the experiment. Serum creatinine levels of both the groups were within the normal range and no significant difference was noticed. The mean value of serum enzymes like ALT, AST and ALP showed no significant difference between the two groups.

Following the lethal challenge, the haemoglobin concentration revealed no significant difference among the Groups II, III and IV from that of the control Group I. Total leukocyte count revealed a significant ($P<0.05$) increase in Group II followed by Group IV from that of the control Group I. The values in Groups III were statistically comparable with the control. The differential leukocyte count revealed a significant ($P<0.05$) increase in neutrophil and lymphocyte counts in Groups III and IV from that of the Group II and Group I values. The values in Group II were statistically comparable with control. The monocyte and eosinophil counts revealed no significant difference among the Groups I, II, III and IV. The biochemical

parameters revealed significant difference ($P<0.05$) in total protein, A: G ratio and creatinine levels between the Groups II, III and IV from that of the control Group I. There was a significant difference ($P<0.05$) between the Groups III and IV from that of the healthy control group I in albumin values. The values obtained in Group II were statistically comparable with that of the control. The serum average ALT levels after challenge in Groups I, II, III and IV were 39.00, 61.00, 61.38 and 65.38 U/L, respectively and corresponding AST levels were 73.00, 90.50, 94.25 and 98.75 U/L, respectively. The average ALP levels respectively were 63.75, 85.00, 101.88 and 109.50 U/L. A significant difference ($P<0.05$) between the challenged groups from that of the control were noticed in all these enzyme levels.

Clinical symptoms observed in the challenged groups included sneezing, anorexia, dyspnea, loss of condition, mucopurulent rhinitis and abdominal breathing. The clinical symptoms exhibited were more severe in rabbits of Groups III and IV which died within twenty six hours of post inoculation than Group II rabbits which withstood the infection for another six hours and died within thirty two hours of post inoculation. Gross lesions of varying intensities of vascular changes such as severe congestion of tracheal mucosa, pulmonary congestion and haemorrhage, congestion, degeneration and necrotic areas on liver and kidney, petechial haemorrhage on heart and subcutaneous area of the neck were observed. Histopathological lesions varied from severe vascular, degenerative, necrotic and inflammatory changes in the organs studied. The intensity of these lesions in Group II rabbits were very much reduced when compared to the Groups III and IV rabbits.

Based on the results obtained in the present study, the beneficial effects during the first month of the study indicate improved health status in turmeric supplemented rabbits. From the results following the lethal challenge, it can be concluded that all the rabbits challenged died of septicaemia caused by the virulent organism and the intensity of damage caused was very much less by the supplementation of turmeric in rabbit diets.

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* originals not seen

PATHOLOGICAL STUDIES ON THE AMELIORATIVE EFFECT OF *Curcuma longa* ON EXPERIMENTAL PASTEURELLOSIS IN RABBITS

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ABSTRACT

An experiment was conducted to evaluate the symptoms and lesions of experimental pasteurellosis and to study the beneficial effects of *Curcuma longa* (turmeric) in managing pasteurellosis. Thirty two New Zealand white rabbits were divided into four groups each having eight replicates. Group I served as control. Group II were given feed mixed with turmeric at the rate of 2 g/kg body weight for the whole period of experiment and on the thirty first day of the experiment, animals were intranasally exposed to *P. multocida.* Group III rabbits were intranasally exposed to *P. multocida* on the thirty first day of the experiment and turmeric was fed at the rate of 2 g/ kg body weight after exposure until the end of the experiment. Group IV rabbits were fed on control diet alone for thirty days and then intranasally exposed to *P. multocida* on the thirty first day of the experiment. Body weight and haematological parameters were recorded on day zero, 14^{th} and 28^{th} day of the experiment and biochemical parameters on 28^{th} day were estimated. The rabbits were challenged with 0.1 ml of 18 h broth culture of *P. multocida* serotype A: 1 containing approximately 3×10^8 organisms/ml by intranasal route. Blood was collected for haematological and biochemical evaluations after eighteen hours of post inoculation from all the groups.

Supplementation of turmeric significantly ($P<0.05$) increased the body weight on 28^{th} day of the experiment. Turmeric supplementation significantly ($P<0.05$) increased the haemoglobin concentration on day 28. The total leukocyte count and differential leukocyte count did not reveal significant difference between the dietary groups. The serum total protein, albumin and albumin : globulin ratio were significantly ($P<0.05$) higher in turmeric fed group on the 28^{th} day. The serum creatinine values were within the normal range and no significant difference was noticed. The levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) were comparatively lower in turmeric fed group but no significant difference were noticed on the 28^{th} day.

Results of the present study after challenge with *P. multocida* indicated that the haemoglobin concentration did not reveal significant difference except for numerical decrease in values noted in challenged groups compared to the control. The total leukocyte count and differential leukocyte count significantly ($P<0.05$) increased in challenged groups compared to the control. Also the levels of serum total protein, albumin: globulin ratio and creatinine were significantly ($P<0.05$) higher in challenged groups. The levels of ALT, AST and ALP were also significantly ($P<0.05$) higher in the challenged groups. Pathological studies on the trachea, lungs, heart, liver and kidney revealed acute lesions suggestive of septicaemia. The intensity of damage in Group II rabbits was found to be much less in these organs which were evident in the haemato-biochemical values and gross and histopathological lesions.

Overall evaluation of the results of the study indicated that supplementation of turmeric was advantageous. Also the study brought to the light that apart from the beneficial effects on body weight, haematology and biochemical profile, turmeric revealed partial protective role on pasteurellosis. The results of the study prove promising and need further investigation using different levels of turmeric in diets.