CLINICAL EVALUATION OF ANGIOTENSIN II INHIBITOR IN THE TREATMENT OF CONGESTIVE HEART FAILURE IN DOGS

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2004

CERTIFICATE

This is to certify that the thesis entitled **"CLINICAL EVALUATION OF ANGIOTENSIN II INHIBITOR IN THE TREATMENT OF CONGESTIVE HEART FAILURE IN DOGS"** submitted in part fulfilment to the requirements for the degree of **DOCTOR OF PHILOSOPHY in VETERINARY CLINICAL MEDICINE, ETHICS AND JURISPRUDENCE** to the Tamil Nadu Veterinary and Animal Sciences University, Chennai - 600 051 is a record of bonafide research work carried out by **K. JEYARAJA** under my supervision and guidance and that no part of the thesis has been submitted for the award of any other degree, diploma, fellowship or other similar titles or prizes and that the work has not been published in part or full in any scientific or popular journal or magazine.

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ABSTRACT

CLINICAL EVALUATION OF ANGIOTENSIN II INHIBITOR IN THE TREATMENT OF CONGESTIVE HEART FAILURE IN DOGS

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Congestive heart failure is one of the important cause of morbidity and mortality in dogs. It is often secondary to chronic degenerative mitral valve insufficiency (MVI) and dilated cardiomyopathy (DCM). Presently, judicious inhibition of renin - angiotensin - aldosterone - system remain as the ideal way of treating congestive heart failure. For this purpose, ACE inhibitors, diuretics and digitalis has become the standard therapy. Recently, Angiotensin II blockers were developed and tried in many human trials for hypertension and congestive heart failure. These agents block the angiotensin II at the receptor level thereby causing complete blockade of angiotensin II (eg. Chymase) apart from angiotensin I. With this backdrop, the present study was designed with the objective of assessing the efficacy of Losartan potassium, the first developed angiotensin II blocker in the treatment of CHF in dogs, and to compare its effect with existing standard treatment.

Dogs with DCM or MVI were randomly allotted to the treatment groups : group I - Enalapril + furosemide + with / without digoxin, group II - Losartan + furosemide + with / without digoxin and group III enalapril + losartan + furosemide + with / without digoxin. Therapy was carried out for eight weeks and clinical variables, radiography, echocardiography and serum biochemistry were evaluated.

In group I and III after 60 days of treatment more per cent of dogs had improved cough scores, respiratory effort, appetite, demeanor, mobility, attitude, activity, pulmonary edema and ascites scores compared to group II, with group III having a slight edge over group I. In heart rate, group I and group III had highly significant (P # 0.01) reduction in heart rate whereas no significant difference was observed in group II after 60 days of treatment.

A highly significant (P # 0.01) improvement in fractional shortening and ejection fraction was observed in group I and III whereas no significant changes was observed in group II in DCM dogs after 60 days of treatment. A highly significant (P # 0.01) reduction was observed in LA/Ao ratio in group I and group III whereas no significant difference was observed in group II in MVI dogs at 60 days of treatment. A significant (P # 0.05) difference was observed between group I and group III at 60 days of treatment.

In overall evaluation and class of heart failure group I and group III fared very well with group III slightly better than group I, but group II had very poor overall evaluation and improvement in class of heart failure. The level of azotemia was same in all the three treatment groups at 60 days of treatment. The survival rate was more in group III (80%) compared to group II (60%) whereas very poor survival rate (10%) was observed in group II.

With the above findings it was concluded that losartan monotherapy was not effective in the treatment of CHF in dogs when compared to the standard therapy and the combination of enalapril and losartan was better than the standard therapy.

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LIST OF ABBREVIATIONS

ACE	-	Angiotensin Converting Enzyme
Ao	-	Aorta dimension
ARBs	-	Angiotensin Receptor Blockers
ATPase	-	Adenosine - Tri - Phosphatase
BUN	-	Blood Urea Nitrogen
CHF	-	Congestive Heart Failure
COVE	-	Cooperative Veterinary Enalapril
DCM	-	Dilated Cardiomyopathy
ECG	-	Electrocardiograph
EDV	-	End Diastolic Volume
EF	-	Ejection Fraction
ELITE	-	Evaluation of Losartan In The Elderly
EPSS	-	End-Point Septal Separation
ESV	-	End Systolic Volume
FS	-	Fractional Shortening
IMPROVE	-	Invasive Multicenter PROspective Veterinary evaluation of Enalapril
LA	-	Left Atrium dimension
LA/Ao	-	Left Atrium/Aorta ratio
LIVE	-	Longterm Investigation of Enalapril
$LVID_d$	-	Left Ventricular Internal Dimension at end diastole
$LVID_s$	-	Left Ventricular Internal Dimension at end systole
MVI	-	Mitral Valvular Insufficiency
NYHA	-	Newyork Heart Association
RAAS	-	Renin-Angiotensin - Aldosterone system

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CHAPTER - I

INTRODUCTION

Congestive heart failure is a complex clinical syndrome wherein certain predictable hemodynamic changes develop as a consequence of impaired cardiac performance and the complex interplay of numerous neuroendocrine compensatory mechanisms (Packer *et al.,* 1993). Dilated cardiomyopathy (DCM) and mitral valvular insufficiency (MVI) are the two most common form of acquired heart disease in dogs which results in congestive heart failure (Ettinger, 1983 and Sisson, 1987).

Some of the more prominent neurohormonal perturbations identified in dogs and humans with heart failure include altered baroreceptor reactivity, decreased parasympathetic and increased sympathetic nervous system activities, activation of the renin-angiotensin-aldosterone system (RAAS) and increased production of antidiuretic hormone and atrial natriuretic peptide (Ware *et al.,* 1990 and Packer, 1993).

The trend in the therapy of heart failure due to systolic dysfunction had been changing from time to time. During 1948 to 1968, digitalis and diuretics were the only drugs used in heart failure. Later between 1968 to 1978, vasodilators such as alpha adrenergic blockers, nitrates, arteriodilators and calcium channel blockers were used. Then between 1978 to 1988 inotropic stimulation with beta adrenergic agonist, calcium sensitizing agents and phosphodiesterase inhibitors was the strategy.

After 1988, the concept of preserving the failing heart came into existence, where angiotensin converting enzyme (ACE) inhibitors and beta adrenergic blockers were used. Presently, judicious inhibition of renin-angiotensin - aldosterone system remain as the ideal way of treating congestive heart failure.

Activation of the RAAS increases the production of angiotensin II from angiotensin I. Circulating angiotensin II is a potent vasoconstrictor, and it also stimulates aldosterone secretion by the adrenal cortex resulting in increased sodium retention, volume expansion and ventricular remodeling. Angiotensin converting enzyme (ACE) is a dipeptidase that catalyzes the conversion of angiotensin I to angiotensin II. Inhibition of ACE decreases formation of circulating angiotensin II, inducing concomitant

decline in circulating aldosterone. By the above said mechanism ACE inhibitors block the production of angiotensin II from I. Recently other pathways by which angiotensin II is produced (eg. chymase) apart from angiotensin I were identified. Therefore, complete blockade of angiotensin II production was not achieved by ACE inhibitors.

To counteract the aforementioned lacunae, angiotensin II inhibitors or blockers were developed. These drugs block the actions of angiotensin II at the receptor level by blocking AT_1 receptors. Therefore, the action of angiotensin II, that was even produced from alternate pathways were nullified. Hence this remains as a novel way of blocking the action of angiotensin II.

Losartan potassium, was the first angiotensin II receptor blocker developed. This drug had undergone strenuous clinical trials in human beings in hypertension as well as in congestive heart failure. Although in humans it was approved for clinical use as an antihypertensive agent, its approval as an agent for congestive heart failure is still pending. In human beings, losartan was proved to be effective in improving the quality of life and survival in many short term clinical trials. In small animal medicine no published reports were available on the efficacy of this drug in congestive heart failure.

With this background, this pioneering study was designed with the following objectives:

1. To assess the efficacy of angiotensin II inhibitor (Losartan potassium) in the treatment of congestive heart failure in dogs.

2. To compare the efficacy of angiotensin II inhibitor with the existing standard therapeutic regimen for congestive heart failure in dogs.

CHAPTER - II

REVIEW OF LITERATURE

2.1 PATHOPHYSIOLOGY OF CONGESTIVE HEART FAILURE

Congestive heart failure is a complex clinical syndrome wherein certain predictable hemodynamic changes develop as a consequence of impaired cardiac performance and the complex interplay of numerous neuroendocrine compensatory mechanisms (Packer, 1993). Some of the more prominent neurohormonal perturbations identified in dogs and human with heart failure include altered baroreceptor reactivity, decreased parasympathetic and increased sympathetic nervous system activities, activation of renin-angiotensin-aldosterone system (RAAS) and increased production of antidiuretic hormone and atrial natriuretic peptide (Ware *et al.,* 1990, Packer, 1993).

2.1.1 Increased sympathetic activity

Thomas and Marks (1978) observed that the severity of left ventricular dysfunction and mortality were directly correlated with the extent of increased plasma norepinephrine concentration.

Cohn *et al.* (1984) concluded that it was not clear if there was a causal relationship between the increased circulating norepinephrine and the increased mortality or if the increased concentrations simply reflect the severity of congestive heart failure. Packer *et al.* (1987) reported that congestive heart failure was accompanied by an elevation in circulating catecholamines, particularly norepinephrine.

Ware *et al.* (1990) found that dogs had high plasma norepinephrine concentration in relation to the clinical severity of naturally acquired heart failure and added that dogs with dilated cardiomyopathy (DCM) had greater increase in plasma norepinephrine concentration than the dogs with primary mitral regurgitation which may indicate more severe decompensation and neurohumoral activation.

Re *et al.* (1999) and Borgarelli *et al.* (1999) observed that in symptomatic DCM dogs, plasma catecholamine concentration was significantly higher. Borgarelli *et al.* (1999) also observed increased concentrations of plasma catecholamines in asymptomatic DCM dogs.

Rose *et al.* (1985) and Hasking *et al.* (1986) attributed the increased plasma norepinephrine concentration to increased release from adrenergic nerve endings and the consequent `Spillover' into the plasma and it might also be attributed to reduced uptake by adrenergic nerve endings (Liang *et al.,* 1989).

Leimbach *et al.* (1986) correlated the adrenergic activity in human beings not only to symptomatology but also to physiological indices of severity of heart failure such as left and right atrial filling pressures.

Unger (2000) opined that the augmented levels of plasma catecholamines; in particular the release of norepinephrine by adrenergic cardiac nerves increased both myocardial contractility and heart rate and that response on the other hand lead to an increased cardiac workload and might accelerate the rates of myocardial cell death.

The increased adrenergic activity had several adverse effects on cardiac structure and function. Simpson and McGrath (1983) found that increased adrenergic activity had downregulation of myocardial $ß_1$-adrenoreceptor density and Floras (1993) observed a decreased ß-adrenergic responsiveness to endogenous or exogenous agonists.

Mann *et al.* (1992) observed that the increased levels of catecholamines had a trophic and toxic effects on cardiac myocytes.

Clark *et al.* (1993) reported exacerbation of arrhythmias and impairment of ventricular diastolic and systolic functions in response to increased adrenergic activity.

Bristow (1984) demonstrated that within 24-72 hours of increasing adrenergic drive, the $ß_1$-adrenoceptor of the heart underwent down regulation. Dzimiri (1999) opined that ß-adrenoceptors desensitization occurs as a result of two different alterations in their signalling, involving first reduced number of receptor (receptor downregulation) followed by an impairment in the function of the remaining receptors (receptors uncoupling). Re *et al.* (1999) concluded that the downregulation of ß-adrenoceptors in asymptomatic and symptomatic DCM dogs were not associated with alteration in receptor affinity.

Borgarelli *et al.* (1999) reported that dogs with symptomatic DCM and occult DCM had significantly lower density of total adrenoreceptors, $ß_1$- and $ß_2$-adrenoreceptor subtypes on their lymphocytes as compared to the control group. Dzimiri (1999) observed that the down regulation of ß-adrenoreceptors had been associated with an increased activity of α-adrenoreceptors in humans whereas in DCM affected dogs Re *et al.* (1999) observed that both in lymphocyte and myocardial cell membranes, all subtypes (total, $ß_1$-$ß_2$ and α_1-adrenoreceptor) appear to be downregulated.

2.1.2 Renin - angiotensin - aldosterone system (RAAS)

It is now widely accepted that CHF is associated with an increased RAAS activity in dogs and human (Kluger *et al.,* 1982; Teerlink, 1996). It is well known that systemic activation of the RAAS increases retention of sodium and water, leads to arteriolar vasoconstriction and elicits thirst (Lumbers, 1999).

Hirsch *et al.* (1991) and Dzau and Re (1994) observed that the major portion (90 to 99%) of angiotensin converting enzyme (ACE) in the body was found in the tissues.

Unger *et al.* (1987) and Foult *et al.* (1989) reported the local generation of angiotensin II through myocardial renin-angiotensin system (RAS). Dell' Italia *et al.* (1995) reported that the concentration of angiotensin II is considerably higher (approximately 1000 times) in myocardial tissue than in plasma of dogs with chronic mitral regurgitation and he also hypothesized chymase as other possible pathway for angiotensin II production. Malik *et al.* (1997) suggested local RAS as the most significant pathway of myocardial angiotensin II production.

Hirsch *et al.* (1991) opined that the increased activity of local (eg. myocardial) RAAS occurred early in the course of experimentally induced heart failure and these systems might play a major role in the progression of heart failure. At the local level, increased RAAS activity may lead to modification of myocytes and myocardial architecture (mainly through the action of angiotensin II and aldosterone) by inducing myocyte hypertrophy, myocyte necrosis (Tan *et al.,* 1991) and fibrosis, the latter mediated through increased collagen synthesis (cardiac remodelling). These changes lead eventually to systolic and diastolic dysfunction (Brilla & Rupp, 1994; Wilke *et al.,* 1996).

Malhotra *et al.* (1999) believed that the myocardial stretch activated the synthesis of Angiotensin II and other RAS components.

Koch *et al.* (1995) found that both plasma renin activity and plasma aldosterone concentration were increased in symptomatic DCM dogs and plasma renin activity was also increased in some DCM asymptomatic dogs too; while plasma aldosterone concentration was not.

2.2 Clinical characteristics of Dilated Cardiomyopathy (DCM)

2.2.1 Incidence

Calvert *et al.* (1982) reported DCM in Doberman Pinscher dogs, Gooding *et al.*, (1982) in English Cocker Spaniels and Harpster (1983) in Boxers. Sisson and Thomas (1995) in a survey of 1681 dogs, based on a search of Veterinary Medical data at Purdue University reported the most commonly affected breeds as Scottish Deerhound, Doberman Pinscher, Irish wolf hounds, Great Danes, Boxers, St.Bernard's, Afghan hound and New found land. Tidholm and Jonsson (1997) reported that no major breed specific differences concerning clinical, pathological or prognostic characteristics were found in a study of 189 dogs of 38 different breeds. Koch *et al.* (1996) reported dilated cardiomyopathy in German shepherd dogs.

A male predominance was reported in several studies (O'Grady and Horne, 1992; Sisson and Thomas 1995; Calvert *et al.*, 1997; Tidholm and Jonsson, 1997). Age at onset of clinical signs varies considerably although most dogs were initially presented at the age of five to seven years (Tidholm *et al.*, 2001).

2.2.2 Clinical findings

Presenting complaints in dogs with DCM included cough, depression, dyspnoea, weight loss, panting, syncope and polydipsia. Duration of clinical signs before presentation was commonly short, typically one to two weeks (Sisson and Thomas 1995; Tidholm and Jonsson, 1997).

Clinical presentation of DCM commonly included signs of left-sided or biventricular congestive heart failure i.e. dyspnoea caused by pulmonary

edema and/or pleural effusion and abdominal distension caused by ascites (Sisson and Thomas 1995; Tidholm and Jonsson, 1997).

A soft, regurgitant, systolic murmur was sometimes audible over the mitral valve region, along with a low-pitched proto diastolic (S3) gallop sound (Reddy 1985; Sisson *et al*., 1999).

2.2.3 Electrocardiography

Atrial fibrillation was the most commonly diagnosed electrocardiographic abnormality (Tilley and Liu, 1975; Fox, 1988; Sisson and Thomas 1995; Tidholm and Jonsson, 1997; Tidholm *et al*., 1997).

Ventricular premature depolarization and ventricular tachycardia were reported in a majority of Doberman Pinschers (Calvert *et al*., 1982; Calvert, 1986).

2.2.4 Radiography

Pulmonary edema was the most common finding on thoracic radiographs of dogs with symptomatic DCM, although signs of right-sided heart failure i.e. pleural effusion, pericardial effusion and ascites were reported to be common in giant breeds with DCM (Tidholm and Jonsson, 1997; Vollmar, 2000).

2.2.5 Echocardiography

Calvert *et al*. (1982) reported that M-mode abnormalities in DCM dogs include increased left atrial dimension, increased left ventricular systolic and diastolic internal dimensions, decreased left ventricular shortening fraction, increased EPSS and increased right ventricle internal dimension if the right heart is involved.

Lombard (1984) and Atkins and Snyder (1992) reported that echocardiographic evaluation of left ventricular systolic performance revealed increased end-systolic and end-diastolic dimensions, dilatation of the left atrium, and decreased fractional shortening.

Calvert *et al.* (1997) suggested that dogs with heart failure secondary to DCM always had severe disease and so had a fractional shortening less than 15 per cent.

Kittleson (1998) opined that shortening fraction in between 20 per cent and 25 per cent was considered evidence of mild disease, between 15 per cent and 20 per cent was moderate disease and less than 15 per cent was considered severe disease.

McEwan *et al.* (1999) in their proposed guidelines for the diagnosis of canine idiopathic dilated cardiomyopathy suggested the following ecohocardiographic abnormalities.

1. Left ventricular dilation especially in systole but also in diastole.

2. Depressed systolic function - M-mode fractional shortening of less than 20 per cent or 25 per cent and /or left ventricular ejection fraction less than 40 per cent.

3. Altered geometry of the left ventricle (increased sphericity)

4. Left or bi-atrial enlargement

5. Increased mitral valve M-mode E-point to septal separation (EPSS).

2.3 Clinical characteristics of Acquired Mitral Valve disease

2.3.1 Incidence

Myxomatous degeneration of the atrioventricular valve is by far the most common cardiovascular disease identified in small animals. In past studies, it had accounted for approximately 75 per cent of the cardiovascular disease seen in dogs (Detweiler and Patterson, 1965; Das and Tashjian, 1965).

Buchanan (1977) reported that the prevalence of mitral valve disease in dogs was age and breed related. Older dogs and small breeds had the highest incidence with males slightly more prone. In this report the small breeds include miniature and toy poodle, miniature Schnauzer, Cocker spaniel, Chihuahua, Fox terrier, Dachshund and Boston terrier and the incidence increased in dogs more than 12 years of age.

Kittleson (1998) opined that occasionally a large breed dog such as a Great Dane or a German Shepherd presented with mitral valve disease.

2.3.2 Clinical findings

Coughing was a common presenting complaint in dogs with a murmur of mitral regurgitation. The cough secondary to pulmonary edema in small dogs may be soft or harsh. In large dogs it was more commonly soft. Dogs with mitral regurgitation not only develop a cough secondary to pulmonary edema but also due to compression of the left main stem bronchus by the left atrium. Other clinical signs included tachypnoea, dyspnoea, exercise intolerance, weight loss and syncope (Abbott, 1998, Kittleson, 1998).

Physical examination findings included systolic cardiac murmur and S3 gallop especially in dogs with congestive heart failure on cardiac auscultation. Pulmonary auscultation might reveal respiratory sounds which might progress to crackles with the onset of alveolar edema. Hepatomegaly and ascites may be evident in dogs with right sided CHF. Precordial thrill was also palpated in the ventricular apex (Rush, 2002).

2.3.3 Radiography

In thoracic radiograph the most common finding was left atrial enlargement and pulmonary edema (Kittleson 1998, Rush, 2002).

2.3.4 Electrocardiography

Lombard and Spencer (1985) reported left atrial enlargement and left ventricular enlargement in electrocardiography. However, they concluded that left atrial enlargement in ECG may be detected in as few as 40 per cent of patients with moderate to severe enlargement and was usually normal in patients with mild left atrial enlargement. The detection of significant left ventricular enlargement was also poor, with less than 50 per cent of cases showing changes in electrocardiogram.

2.3.5 Echocardiography

Kittleson (1998) reported the following ecohocardiographic findings in dogs with severe mitral regurgitation.

1. Moderate to severely enlarged left atrium.
2. LA/Ao ratio greater than 2.0
3. Moderately enlarged left ventricle.
4. Fractional shortening of greater than 50 per cent in small dogs and a range of 25 per cent to 40 per cent in large dogs.

2.4 Strategies in the treatment of congestive heart failure

Jacobs (1989) suggested the following six basic components of treatment.

1. Decrease cardiac work
2. Identify and eliminate precipitating factors of CHF

3. Control cardiac arrhythmias, if necessary.

4. Decrease preload.

5. Increase contractility

6. Decrease afterload.

Strickland and Goodwin (1998) suggested the following goals in managing animals with chronic congestive heart failure.

1. Blunt the excessive activation of body's compensatory mechanism.

2. Promote tissue perfusion.

3. Control extracellular fluid volume.

4. Stabilize the heart rate and rhythm.

Kittleson (1998) opined that the primary aim of treating heart failure was to reduce the formation of edema and effusion and second goal was to increase cardiac output. He suggested the use of furosemide, angiotensin converting enzyme inhibitors, digoxin and with or with out salt restriction in the treatment of chronic, moderate to severe heart failure caused by mitral regurgitation or dilated cardiomyopathy. Use of digoxin in mitral regurgitation is limited to animals showing systolic dysfunction.

McEwan (2000) opined that the treatment of DCM was directed against the consequences of congestive heart failure rather than addressing or attempting to reverse the primary defect. Treatment involves:

1. Control of edema and effusions

2. Counteracting the detrimental neuroendocrine activation

3. Improving cardiac output

4. Controlling arrhythmias

Atkins (2001) suggested the following strategies in the medical management of factors contributing to signs of systolic heart failure in dogs:

1. Salt restriction diuresis
2. Venodilation
3. Blunting renin-angiotensin-aldosterone system and sympathetic nervous system.
4. Arterial vasodilation
6. Normalize heart rate and rhythm.

Keene (2002) stated that the hemodynamic state of heart failure patients can be modified with drugs that reduce the preload, reduce the afterload, slow the heart rate or alter the contractility.

2.5 Losartan Potassium

Losartan, the potassium salt of 2-n-butyl-4-chloro-1- [2' - (tetrazol-5-yl)-1, 1' - biphenyl-4ylmethyl] - 1H - imidazole - 5 - methanol was synthesized by E.I dupont de Nemours and Company (Wilmington, DE. USA) as a novel orally active non peptide angiotensin II receptor antagonist (Timmermans *et al.,* 1990; Wong *et al.,* 1990a).

2.5.1 Pharmacokinetics and pharmacodynamics

In vitro and *in vivo* studies of Chiu *et al.* (1990) showed that losartan was much more selective for the AT_1 site than the AT_2.

Wong *et al.,* (1990b) reported a significant decrease in blood pressure after oral or intravenous dose of losartan in conscious renal-ligated rats and same effect was observed by Wong *et al.* (1990c) in conscious spontaneously hypertensive rats.

Christ *et al.* (1990) reported EXP 3174, a 5-carboxylic acid as a major metabolite of losartan. Wong *et al.* (1990a) observed that direct

intravenous injection of EXP 3174 was far more potent than losartan itself as a inhibitor of angiotensin II pressor responses.

Christen *et al.* (1991) assessed the inhibitory effect of losartan on the pressor action of exogenous angiotensin I and II in healthy volunteers and they had suggested that losartan was a potent and long-acting antagonist of Angiotensin II in humans.

Munafo *et al.* (1992) observed that after oral administration of losartan, concentrations of EXP 3174 actually reached higher plasma concentration than the parent drug and it was eliminated more slowly and also have concluded that the principal long-term actions of the drug were most likely linked to the prolonged effects of the metabolite EXP 3174. He also concluded that losartan produces a dose-dependent, effective angiotensin II blockade that was largely determined by the active metabolite EXP 3174.

Timmermans *et al.* (1993) reported that Angiotensin receptor blockers (ARBs) potently and selectively inhibit, both *in vitro* and *in vivo*, most of the biological effects of angiotensin II, including angiotensin II induced : (1) contraction of vascular smooth muscle; (2) rapid pressor responses; (3) slow pressor responses; (4) thirst; (5) vasopressin release; (6) aldosterone secretion; (7) release of adrenal catecholamines; (9) increases in sympathetic tone; (10) changes in renal function; and (11) cellular hypertrophy and hyperplasia.

Timmermans *et al.* (1993) also opined that ARBs differ from ACE inhibitors in several important aspects : (1) ARBs reduce activation of AT_1 receptors more effectively than ACE inhibitors. (2) In contrast to ACE inhibitors, ARBs indirectly activate AT_2 receptors (3) ACE inhibitors may increase angiotensin (1-7) levels more than ARBs. (4) ACE inhibitors increase the levels of a number of ACE substrates, including bradykinin and AC-SDKP.

Csajka *et al.* (1997) reported the following pharmacokinetic-pharmacodynamic profile of losartan in humans: 1. approximately 14 per cent of an oral dose of losartan was converted to the 5-carboxylic acid metabolite, designated EXP 3174, which was more potent than losartan as an AT_1 receptor antagonist. 2. The metabolism of losartan to EXP 3174 and to inactive metabolites was mediated by CYP2C9 and 3A4. 3. Peak plasma levels of losartan and EXP 3174 were obtained approximately 1 to 3 hours

after oral administration and the plasma half lives were 2.5 and 6 to 9 hours, respectively. 4. The plasma clearance of losartan and EXP 3174 was affected by hepatic but not renal insufficiency.

Jackson (1999) recommended that losartan should be administered orally once or twice a day for a total daily dose of 25 to 100 mg.

2.5.2 Therapeutic trials

Kanda *et al.* (1995) suggested that long-term treatment with losartan might prevent thickening of left ventricular wall and cavity dimension in dilated cardiomyopathy in murine model and the efficacy of losartan appeared to be less than that of captopril.

Dickstein *et al.* (1995) concluded that losartan and enalapril were of comparable efficacy and tolerability in the short-term treatment of moderate or severe congestive heart failure in human volunteers and they also suggested that a trial designed to compare the efficacy, tolerability and effect on mortality of long-term angiotensin II receptor blockade with converting enzyme inhibition was both feasible and ethically responsible.

Regitz - Zagrosek *et al.* (1995) opined that AT_1 antagonists might counteract the effects of angiotensin II on the vasculature and therefore were effective vasodilators. He also added that subtype AT_2, which represents the dominant, receptors in both healthy and failing human myocardium was not blocked by AT_1 inhibition.

Pitt *et al.* (1996) suggested that the addition of an angiotensin II type 1 receptor blocking agent to an ACE inhibitor would theoretically block ACE as well as non-ACE dependant angiotensin II formation while maintaining the potential beneficial effect of ACE inhibitor - induced bradykinin formation.

Lang *et al.* (1997) concluded that losartan was generally well tolerated and comparable to enalapril in terms of exercise tolerance in short term (12 weeks) study of human patients with heart failure.

Hamroff *et al.* (1997) reported that combining AT_1 - receptor blocker losartan to maximally recommended or tolerated ACE inhibitor enalapril appeared to be safe and further leads to vasodilatation in symptomatic patients with congestive heart failure.

Pitt *et al.* (1997) in the Evaluation of Losartan In The Elderly (ELITE) study reported that in elderly patients with heart failure, losartan was as effective as captopril in improving symptoms and reduced mortality more than did captopril.

Hamroff *et al.* (1999) reported that losartan enhanced peak exercise capacity and alleviated symptoms in patients with CHF who were severely symptomatic despite treatment with maximally recommended or tolerated doses of ACE inhibitors.

Bastien *et al.* (1999) suggested that AT_1 receptor antagonists and ACE inhibitors were not necessarily equivalent or interchangeable in terms of their effects on cardiac hypertrophy and survival in selected progressive heart failure models.

Pitt *et al.,* (2000) reported the results of ELITE-II study which like ELITE-I, compared losartan and captopril in elderly patients, but had mortality as the primary end point. In contrast to the findings in ELITE-I, no significant difference in out come was noted between the treatment groups in ELITE-II.

Konstam *et al.* (2000) in the ELITE ventricular function sub study observed that both captopril and losartan prevented left ventricular dilation, representing adverse ventricular remodeling and also reverse remodeling in the captopril group. On the basis of this result he concluded that the relative effects on left ventricular remodelling did not provide a rationale for a survival benefit of losartan over captopril.

Petretta *et al.* (2000) reported that in patients with DCM, losartan treatment improved cardiac autonomic adaptation and increased urine output in response to volume over load.

Houghton *et al.* (1999) in a double blind ELITE trial sub study concluded that no substantial differences were observed between losartan and captopril on central or regional haemodynamics, neurohormones or exercise capacity in elderly patients with stable symptomatic heart failure.

Sharma *et al.* (2000) in a study to assess mortality in heart failure patients treated with losartan, reported that beneficial effect was provided by losartan upon survival. However, he added that a large confirmatory study is needed to assess the mortality benefit of losartan compared with an ACE inhibitor.

Cowley *et al.* (2000) observed a significant improvement in quality of life in elderly patients with symptomatic heart failure treated with losartan and captopril long-term. A trend favouring losartan in the composite measure of drug tolerability / quality of life was not significant but losartan was generally better tolerated than captopril and in that significantly fewer losartan patients discontinued therapy.

Houghton *et al.* (2000) in a double-blind, randomized, placebo-controlled trial concluded that in patients with mild to moderate heart failure, already maximally treated with an ACE inhibitor, additional treatment with losartan was well tolerated but no significant improvement in exercise capacity, quality of life, central and regional hemodynamics or neurohormones were observed.

Martineau and Goulet (2001) in their critical review on the studies comparing angiotensin II receptor antagonist with placebo or angiotensin - converting enzyme inhibitors in patients with congestive heart failure summarized the following facts:

1. Angiotensin II receptor antagonists inhibit the effects of angiotensin II at its sub-type 1 receptor independently of angiotensin II's synthesis pathway.

2. Angiotensin II receptor antagonists present a hemodynamic profile similar to that of ACE inhibitors, without reflex neurohormonal activation.

3. They were at least as effective as ACE inhibitor in improving symptoms, exercise capacity and NYHA functional class.

4. Although the ELITE-I (Evaluation of Losartan In The Elderly) trial suggested that losartan improved survival compared with captopril, ELITE-II, an adequately powered study, showed no difference in mortality rates between the two.

5. Combination of angiotensin II receptor antagonists and ACE inhibitors provided additional benefit on blood pressure lowering and prevention of ventricular remodeling.

6. Angiotensin II receptor antagonists were tolerated with an incidence of adverse effects similar to or lower than that of ACE inhibitors and their lack of effect on bradykinin degradation might explain their lower incidence of cough.

With the above facts, they concluded that in patients with CHF, ACE inhibitors must remain as the treatment of choice and the angiotensin II receptor antagonists might be considered as an acceptable alternative for patients who were intolerant to ACE inhibitors.

Dickstein (2001) in his review on the role of losartan in the management of patients with heart failure concluded that there was strong evidence for the broad applicability of angiotensin II antagonist in heart failure and for the use of angiotensin II-antagonist in the treatment of a broader population of patients, not only those who were unable to tolerate treatment with ACE inhibitors.

Pitt (2002) opined that the possible reasons for lack of angiotensin receptor blockers superiority over ACE inhibitors were insufficient dosing, differences in effects mediated through angiotensin II type 2 receptors, interaction with beta-blockers, and bradykinin mediated effects specific to ACE inhibitors.

Xiu *et al.* (2002) reported that in myocardium, enalapril failed to significantly inhibit aldosterone production whereas losartan significantly inhibited aldosterone production compared to untreated chronic heart failure rats.

Cocco *et al.* (2002) reported that after six weeks of treatment, the combination of enalapril plus losartan was more effective than enalapril alone in improving myocardial function both at rest and after stress.

Shimizu *et al.* (2002) reported that long term treatment with enalapril and valsartan combination improved cardiac function and survival compared to placebo (or) enalapril monotherapy in cardiomyopathic hamsters.

Pascual Figal *et al.* (2002) reported that after maximum ACE

inhibitor doses, the addition of losartan was safe and associated with an improvement in ventricular function and NYHA functional class but with no change in neurohormonal status.

Chen *et al.* (2003) opined that AT_1 receptor antagonism preserved glomerular filtration rate and renal blood flow and enhanced sodium excretion during acute diuretic therapy in addition to inhibiting aldosterone secretion and added that these findings support the use of AT_1 receptor blockade for human CHF requiring acute diuretics to improve renal hemodynamics and tubular function and to suppress aldosterone.

Koji *et al.* (2003) reported that endogenous bradykinin partially contributed to the synergistic improvement of cardiovascular function in congestive heart failure with additional treatment of angiotensin II receptor antagonist to angiotensin converting enzyme inhibitor.

2.5.3 Adverse effects

Burnier and Brunner (1996) reported that in normal human subjects ACE inhibitors and angiotensin II receptors antagonist had comparable renal properties.

Hamroff *et al.*(1997) in a study on combining losartan with enalapril, reported that serum potassium, serum sodium and parameters of renal functions remained unchanged.

Jackson (1999) reported that in patients whose arterial blood pressure or renal function was highly dependent on the RAS, ARBs caused hypotension, oliguria, azotemia and acute renal failure.

Blake and Devereux (2000) reported that the addition or substitution of losartan potassium in patients with ACE induced azotemia and hyperkalemia resulted in statistically insignificant reduction in blood urea nitrogen and creatinine and a significant reduction in potassium and they concluded that there appeared to be fundamental differences between the effects of losartan potassium and ACE inhibitors on potassium excretion in congestive heart failure patients with mild to moderate renal insufficiency.

2.6 ENALAPRIL

2.6.1 Actions

Enalapril maleate is an orally active angiotensin converting enzyme inhibitor. It is a prodrug which is hydrolysed after absorption to form active angiotensin converting enzyme inhibitor enalaprilat. Enalapril administered as the maleate salt, was designed as a prodrug to improve the systemic availability of the active ACE inhibitor enalaprilat (Todd and Heel, 1986). Enalaprilat prevents the conversion of angiotensin I to angiotensin II by binding competitively to the angiotensin I binding sites on angiotensin converting enzyme. The affinity for enalaprilat was approximately 200,000 times that of angiotensin converting enzyme (AHFS Drug information, 1995).

2.6.2 Pharmacokinetics

Bioavailability was approximately 60 per cent. Enalapril was metabolized to enalaprilat. Peak serum concentration of this active form occurs 3 to 4 hours after an oral dose. The half life of accumulation was approximately 11 hours and duration of effect is 12 to 14 hours. Steady-state serum concentration was achieved on the fourth day of administration. Excretion of enalapril and enalaprilat was primarily renal (40 per cent) although 36 per cent is excreted in the faeces (Tocco *et al.,* 1982).

Sweet and Ulm (1988) studied the pharmacodynamics of enalapril in experimental dogs and reported that a dose of 0.3 mg/kg administered per os resulted in approximately 75 per cent inhibition of the pressor response to angiotensin I and this effect lasted for at least 6 hours and is completely dissipated by 24 hours after administration. A dose of 1 mg/kg produced only slightly better inhibition (approximately 80 per cent) for at least 7 hours.

Benitz *et al.* (1991) performed dose ranging of enalapril in dogs with surgically induced mitral regurgitation and heart failure and reported that a dose of 0.5 m/kg enalapril per os acutely produced a greater decrease is pulmonary capillary pressure than a dose of 0.25 g/kg. A dose of 0.75 m/kg produced no better response.

Kittleson (1998) recommended a dose 0.5 mg/kg twice a day to dogs in heart failure approximately 12 hours apart.

2.6.3 Therapeutic trials

Sisson (1992) reported that enalapril maleate plus conventional therapy was determined to be superior to conventional therapy alone.

Allworth *et al.* (1995) found that in pacing - induced heart failure in dogs, enalapril treatment had a qualitative evidence of clinical and radiographic improvement and quantitative improvement in echocardiography.

The IMPROVE study group (1995) evaluated the efficacy of enalapril maleate in dogs with naturally acquired class III or class IV heart failure in a multicenter study and reported that enalapril treated dogs had significantly greater decrease in class of heart failure, pulmonary edema score, mobility score relative to baseline and had significantly better over all evaluation scores when compared with placebo treated dogs and this study showed the beneficial clinical effects of adding enalapril to conventional therapy of dogs with heart failure.

The COVE study group (1995) evaluated the clinical efficacy and safety of enalapril in dogs with moderate or severe heart failure due to acquired mitral valvular disease or dilated cardiomyopathy and reported that clinical variables measured improved significantly in the enalapril group compared with placebo and in placebo group 68.6 per cent of the dogs completed the study compared with 84.9 per cent in the enalapril group.

Keene and Bonagura (1995) opined that the ACE inhibitors should be prescribed together with appropriate doses of digoxin and furosemide in most dogs with acquired heart failure caused by dilated cardiomyopathy or mitral regurgitation.

Watson and Church (1995) reported that the most preferred vasodilator by veterinarians in USA and Australia was enalapril.

Hamlin *et al.* (1996) evaluated the effects of enalapril on exercise capacity and longevity in dogs with left sided heart failure produced by iatrogenic mitral regurgitation and reported that the dogs received enalapril had significantly increased exercise tolerance when compared with controls

and at 357 days 22 per cent of dogs receiving placebo were alive compared with 67 per cent of dogs receiving enalapril.

Jacob (1996) opined that ACE inhibitor in people and dogs with CHF relieved the signs of congestion and increased the exercise capacity and survival.

Ettinger *et al.* (1998) in the Long Term Investigation of Veterinary Enalapril (LIVE) study evaluated the effects of enalapril maleate on survival of dogs with naturally acquired heart failure associated with chronic degenerative mitral valvular disease or dilated cardiomyopathy. They reported that enalapril in combination with standard treatment appears to be beneficial over an extended period compared with standard treatment alone.

Kvart *et al.* (2002) evaluated the long-term effect of early angiotensin converting enzyme inhibition as monotherapy to postpone or prevent congestive heart failure in asymptomatic dogs with mitral regurgitation attributable to myxomatous valvular disease in a prospective, randomized double-blinded, placebo-controlled multicenter trial. They concluded that long-term treatment with enalapril in asymptomatic dogs with myxomatous valvular disease did not delay the onset of heart failure regardless of whether or not cardiomegaly was present at initiation of the study.

2.6.4 Adverse effects

Keene and Rush (1995) in a study out of 429 dogs treated with naturally occurring class II, class III or class IV heart failure reported that the incidence of clinical azotemia occurred in 9.4 per cent of those treated with furosemide and enalapril, as compared with 8.3 percent of those treated with furosemide and placebo.

Kittleson (1998) opined that chronic enalapril toxicity appears to be confined to the kidneys.

Atkins *et al.* (2002) reported that administration of enalapril for up to two years did not had any demonstrable adverse effect on renal function in dogs with severe compensated mitral regurgitation.

2.7 DIGOXIN

2.7.1 Actions

Mason *et al.* (1972) reported the following mechanism: at therapeutic concentration digoxin "poisons" approximately 30 percent of the Na^+ K^+ - ATPase pumps in the myocardium. Thus, the cell loses some of its ability to extrude sodium from the intracellular space during diastole, resulting in an increase in intracellular sodium concentration. The cell counters by exchanging intracellular sodium for extracellular calcium via the Na^+ / Ca^{++} cation exchanger. The net result was an increase in the number of calcium ions within the cell. In a normal cell, these excess calcium ions are bound by sarcoplasmic reticulum during diastole. They were subsequently released onto the contractile proteins during systole, causing increased contractility.

The digitalis glycosides increases contractility in normal myocardium and may also do so in failing myocardium. However, their ability to increase contractility in normal myocardium was only about one third that of sympathomimetics (eg. dopamine, dobutamine) and bipyridine compound (e.g. amrinone, milirinone) (Mahler *et al.,* 1974). The positive inotropic effect of digitalis was thought to be caused by the effect of digitalis on the Na^+ K^+ - ATPase pumps located on myocardial cell membranes (Hougen and Smith, 1978).

The digitalis glycosides were used as antiarrhythmic agents, mostly for controlling supraventricular tachyarrhythmias. These agents increased parasympathetic nerve activity to the sinus node, atria and atrioventricular (AV) node when the digitalis serum concentration was within therapeutic range (Moe and Farah, 1970). Ferrari *et al.* (1981) reported that the digitalis glycosides had the ability to increase baroreceptor function in normal cats and dogs. Ferguson *et al.* (1989) reported that digitalis glycosides decreased plasma catecholamine concentration which might be related to increased baroreceptor activity. Lloyd *et al.* (1992) examined the renal effects of a digitalis glycoside and found that it had diuretic properties.

2.7.2 Pharmacokinetics

Digoxin is well absorbed after oral administration. Approximately 60 per cent of the tablet was absorbed and an average of 27 per cent of digoxin was bound to albumin (Baggot and Davis, 1973). Weildler *et al.* (1987) reported that in dogs serum half-life of digoxin was 23 to 39 hours.

Rick *et al.* (1978) opined that serum concentration of digoxin between 1.0 and 2.5 ng/mL was generally considered to be within the therapeutic range. Pedersoli (1978) reported that in dogs digoxin at the dose rate of 0.022 mg/kg every 24 hours maintained the serum concentration within therapeutic range by the second day.

Gierke *et al.* (1978) reported that the renal failure reduced renal clearance, total body clearance and volume of distribution and resulted in increased serum digoxin concentration.

Button *et al.* (1980) opined that digoxin does not distribute well into ascitic fluid and therefore the dose of digoxin must be reduced in patients with ascites if total body weight was used to calculate the dose.

2.7.3 Therapeutic trials

Kittleson (1986) reported that dogs with DCM responded to digoxin by increased contractility i.e. fractional shortening and lived significantly longer than those that did not respond.

The PROVED (Uretsky *et al.*, 1993) and RADIANCE (Packer *et al.*, 1993) trials indicated that the efficacy of digoxin might be dose dependent, as better results seem to be associated with lower plasma concentrations than previously recommended.

The digitalis investigation group (1997) in a large placebo-controlled trial involving human heart failure patients concluded that cardiac glycosides did not reduce overall mortality rates.

2.7.4 Adverse effects

Teske *et al.* (1976) opined that the problems from digitalis intoxication fall into three general classes: those referable to the central nervous system, those to the gastrointestinal system and those to the myocardium.

Borison *et al.* (1984) reported anorexia and vomiting as the common manifestations of digitalis intoxication and were probably due to the direct effect of the digitalis molecule on the chemoreceptor trigger zone located in the area postrema in the medulla.

Clinically myocardial toxicity can take the form of almost every known rhythm disturbance. In the dog ventricular tachyarrhythmias and bradyarrhythmias were most common (Kittleson, 1998).

Lidocaine is the drug of choice for treating ventricular tachyarrhythmias caused by digitalis intoxication (Smith and Willerson, 1971).

2.8 Furosemide

In Veterinary medicine, diuretics especially the loop diuretics are the most important and efficacious class of drugs used for treating heart failure. Furosemide is the most commonly used diuretic in small animal veterinary medicine (Kittleson, 1998).

2.8.1 Actions

Puschett (1981) reported that the loop diuretics were capable of increasing the maximal fractional excretion of sodium to 15 per cent - 25 per cent of the filtered load, making them the most powerful natriuretic agents available.

White *et al.* (1981) reported that furosemide at a dose of 1mg/ kg to normal anaesthetized dogs increased sodium excretion from a baseline average of 144 μEq/min to 2419 μEq/min, approximately a 17-fold increase.

Loop diuretics act by inhibiting the $Na^+/K^+/2Cl$ transporter on the luminal surface of the thick ascending loop of Henle, (Puschett, 1994).

In addition to its diuretic effects, furosemide acts as a venodilator decreasing venous pressures before diuresis takes place (Kittleson, 1998).

2.8.2 Pharmacokinetics

Furosemide was highly protein bound (86 per cent to 91 per cent) and small amount of furosemide (1 per cent to 14 per cent) was metabolised to a glucuronide derivative in dogs, but this metabolism does not take place in the liver (Cohen *et al*., 1976 and Verbeeck *et al*., 1981).

Furosemide was rapidly but incompletely absorbed after oral administration, with a bioavailability of 40 per cent to 50 per cent. After oral administration, onset of action occurs within 60 minutes, peak effects occur within 1 to 2 hours and duration of effect was approximately 6 hours (Yakatam *et al.,* 1979).

Kittleson (1998) recommended the oral dose for treating chronic heart failure ranging from 1mg/kg every other day for very mild heart failure to 4 mg/kg every 8 hours for severe heart failure.

2.8.3 Adverse effects

Furosemide should be judiciously used when combined with digoxin, ACE inhibitors or both. Concurrent use of aminoglycoside antibiotic and furosemide was contraindicated because of enhanced potential for aminoglycoside induced renal toxicity. Common electrolyte disturbances in furosemide therapy was hypokalemia and hyponatremia (Keene and Rush, 1995).

CHAPTER - III

MATERIALS AND METHODS

This study was conducted in the Centre of Advanced Studies in Veterinary Clinical Medicine, Ethics and Jurisprudence at Madras Veterinary College for a period of four semesters during the year 2003 and 2004.

3.1 MATERIALS

Dogs attending the out-patient unit of Madras Veterinary College Hospital with history and clinical signs suggestive of congestive heart failure were subjected to radiography, electrocardiography, echocardiography, haematological and bio-chemical studies. The cases diagnosed as congestive heart failure due to Dilated Cardiomyopathy (DCM) or Mitral Valvular Insufficiency (MVI) were included in the treatment trials.

Selection criteria for inclusion in the treatment trials

i. Cases diagnosed as having class III heart failure of New York Heart Association (NYHA).

ii. Normal haematological parameters, BUN levels not exceeding 50 mg/dl and creatinine levels not more than 2.5 mg/dl.

iii. No life threatening arrhythmias should be present. Atrial fibrillation and occasional ventricular premature contractions were the only arrhythmias allowed.

A total of 36 dogs including 18 dogs of Dilated Cardiomyopathy and 18 dogs of Mitral Valvular Insufficiency of different breeds, age and sexes were randomly allotted to the three treatment groups.

3.2 EXPERIMENTAL DESIGN

36 dogs with congestive heart failure were randomly allotted to the treatment groups taking into consideration the type of disease, body weight and the level of systolic function based on fractional shortening.

Group I - Enalapril+ Furosemide + with or without Digoxin
DCM - 6 dogs + MVI - 6 dogs = 12 dogs.

Group II - Losartan + Furosemide + with or without Digoxin
DCM - 6 dogs + MVI - 6 dogs = 12 dogs

Group III - Enalapril + Losartan + Furosemide + with or without Digoxin.

DCM - 6 dogs + MVI - 6 dogs = 12 dogs.

3.3 THERAPY

3.3.1 Group I

Dogs in this group were treated with enalapril maleate at the dose rate of 0.5 mg/kg b.wt every 12 hours orally, furosemide at the dose rate of 2mg/kg b.wt every 12 hours orally and digoxin 0.006 mg/kg b.wt every 12 hours not exceeding 0.25 mg per dog every 12 hours orally (Keene, 2002). The use of digoxin was limited to the dogs having systolic failure.

3.3.2 Group II

The dose of losartan was extrapolated from the human dose of 50 mg total body dose every 12 hours as per Van Miert (1986). This extrapolation was based on the relationship between adult man and dogs' body surface area as shown in the table below.

Species	Body wt (Kg)	Surface area (m^2)	Km Factor	Dose equivalent (Kg^{-1})	Actual dose (mg/kg)
Adult man	60	1.6	37.5	1	0.8
Dog	8	0.4	20	1.88	1.5
	16	0.65	24.5	1.5	1.2
	32	1.02	31.37	1.2	1.0

Dogs in this group were treated with losartan at the dose rate of 1.2mg/kg every 12 hours orally, furosemide at the dose rate 2mg/kg b.wt every 12 hours orally and digoxin at the dose rate of 0.006 mg/kg every 12 hours not exceeding 0.25 mg/dog every 12 hours orally. The use of digoxin was limited to the dogs having systolic failure.

3.3.3 Group III

Dogs in this group were treated with losartan at the dose rate of 1.2mg/kg every 24 hours orally, enalapril maleate at the dose rate of 0.5 mg/kg every 12 hours orally, furosemide at the dose rate of 1.2mg/kg b.wt every 12 hours orally and digoxin at the dose rate of 0.006 mg/kg every 12 hours not exceeding 0.25 mg/dog every 12 hours orally. The use of digoxin was limited to the dogs having systolic failure.

3.4 EVALUATION OF TREATMENT

The treatment was carried out for lifetime and the dogs were evaluated pre-treatment on zero day, 30 days post-treatment and 60-days post-treatment. The following parameters were studied.

3.4.1 History and physical examination variables

Evaluation was done based on the scoring system adapted from IMPROVE study group 1995 and modified with addition of ascites as a clinical variable.

a. Cough score

0 - None

1 - Occasional

2 - Frequent

3 - Persistent

b. Respiratory effort

0 - Normal

1 - Mildly increased effort

2 - Labored

3 - Respiratory distress

c. Appetite

1 - Increased

2 - Normal

3 - Decreased

4 - Markedly decreased

d. Demeanor

0 - Alert, responsive

1 - Mildly depressed

2 - Moderately depressed

3 - Minimally responsive

4 - Unresponsive.

e. Mobility

1 - Very good : ambulates well, runs, capable of some strenuous activity

2 - Good : ambulates well, will run a short distance or pulls on lead rope but unable to do strenuous activity

3 - moderate : will walk, but for a limited distance before needing to rest

4 - Poor: can only walk a few yards before needing to rest

5 - Very poor: to get up and move is a major effort, only able to move a few steps before resting

f. Attitude

1 - Increased: has stronger desire and interest than in the past to go out for walks or play with owner, appears more alert and responsive to surrounding environment

2 - Remained the same: has approximately the same degree of interest and desire to go out for walks and play with the owner as in the past, is alert to the surrounding environment as before

3 - Decreased: has some interest but plays less often and has decreased interest to go for a run or walk

g. Activity

1 - High: moves around with ease, capable of climbing stairs or running short distance, alert and responsive to external stimuli.

2 - Moderate: Tends to be inactive but moves around a few times a day, has difficulty with stairs and avoids long walks.

3 - Low: Generally inactive, tending to remain in one place most of the day and unable to climb stairs or walk more than short distance.

4 - Minimal: remains inactive all day, only gets up to eat, drink or urinate.

5 - Incapacitated: will get up or move if only encouraged by the owner.

h. Ascites

0 - Normal

1 - Mild

2 - Moderate

3 - Severe

3.4.2 Radiography

The dogs were subjected to radiography of the thorax in the lateral view to assess the pulmonary edema (Plates 1 & 2) and scored as below

0 - Normal

1 - Mild interstitial

2 - Moderate interstitial

3 - Alveolar pattern

4 - Severe consolidation

3.4.3 Electrocardiography

Dogs were subjected to six lead electrocardiography to assess the heart rate and rhythm.

3.4.4 Echocardiography

3.4.4.1 Instrumentation

Echocardiography was performed using `Scanner 200 Vet'. A mechanical sector transducer of 3.5 mHz frequency was used for obtaining the image.

3.4.4.2 Patient preparation and positioning

Transthoracic echocardiograms were obtained with unsedated dog on right lateral recumbency. Access to the right side of the thorax was facilitated by use of a table with a special cut-out to allow the transducer to be directed upward toward the site of maximal cardiac pulsation (Allworth *et al.,* 1995).

Transducer location is right `parasternal window' between 3rd and 6th intercostal space within sternum and costochondral junctions (Thomas *et al.,* 1994). The right parasternal window was closely clipped and acoustic coupling gel was liberally applied on the transducer as well as over the transducer locations on the animal body during the procedure.

3.4.4.3 Procedure of Echocardiography

B-Mode

Right parasternal window was accessed to obtain a four chamber view. This was done with the beam plane oriented nearly perpendicular to the long axis of the body and parallel to the long axis of the heart. In this

four chamber view (Plate 4) mitral valve structures were completely examined for abnormalities of the valve which includes irregular, smooth, thickening and knobby enlargements at the end of the leaflets which may have echogenicity similar to or less than the normal valve. In addition to these abnormalities, mitral valve prolapse and mitral valve flail can also be identified in this view (Kienle and Thomas, 1995).

M-Mode

The standard M-mode echocardiographic images were obtained from the right parasternal view in correlation with the 2-D images.

The ultrasound beam was directed from the right parasternal position through the heart at the level of the middle of the left ventricle, to obtain real time motions of the interventricular septum and left ventricular posterior wall in diastole and systole. When the beam was angled dorsally, mitral valve leaflets appear with in the left ventricle. From the mitral valve level cranial and slight dorsal angulation of the transducer brings the aortic root and left atrium in view (Feigenbaum, 1986).

Measurement of the left ventricular internal dimension (Plate 3) at the end diastole ($LVID_d$) and end systole ($LVID_s$), was intraluminal from the trailing edge of the septal wall image to the leading edge of the left ventricular free wall (Allworth *et al.,* 1995).

E-point septal separation was measured from the point of maximal cranial motion of the anterior mitral valve leaflet (E-point) to the inter-ventricular septum during the rapid filling phase of diastole (Calvert and Brown, 1986). All measurements were made in millimeters (Plate 3).

The level of aortic valve (Plate 4) was used for measurements of aortic root (Ao), and left atrial dimensions (LA). The aortic root was measured at end-diastole and the left atrium was measured at its maximal upward excursion near the end of systole (Bonagura *et al.,* 1985).

The following indices were also calculated using the above measurements (Kienle and Thomas 1995).

i. Fractional shortening (FS%)

$$FS = \frac{LVID_d - LVID_s}{LVID_d} \times 100$$

ii. Left atrium and aortic root ratio

LA/Ao

iii. End diastolic volume

$EDV = (LVID_d)^3$

iv. End systolic volume

$ESV = (LVID_s)^3$

v. Ejection fraction (%)

$$EF = \frac{EDV - ESV}{EDV} \times 100$$

3.4.5 Serum Bio-chemistry

Ten millimeters of blood was collected taking all precautions for avoiding haemolysis as suggested by Alleman (1990) into a centrifuge tube without anticoagulant for separation of serum and the following estimations were undertaken on day 30 and day 60.

3.4.5.1 Blood urea nitrogen

Blood urea nitrogen was quantitated by Berthelot method with Ranbaxy Urea/BUN kit.

3.4.5.2 Serum Creatinine

Serum creatinine was estimated by Jaffe's kinetic colorimetric method with Stangens creatinine kit.

3.4.5.3 Serum Potassium

Serum potassium was estimated by colorimetric method with Accurex potassium kit.

3.4.5.4 Azotemia and Hyperkalemia

Dogs with BUN value greater than 50 mg/dl and /or creatinine 2.5 mg/dl were declared azotaemic (Longhofer *et al.,* 1993). Dogs with potassium level more than 6.0 mEq/L were declared as having hyperkalemia.

3.4.6 Class of Heart failure (IMPROVE study group, 1995)

Evaluation of class of heart failure was done on day 30 and day 60.

I - Exercise capacity limited only during strenuous exercise, athletic activity.

II - Fatigue, shortness of breath become evident when ordinary exercise is exceeded.

III - Comfortable at rest but exercise capacity is minimal.

IV - No capacity for exercise, disabling signs are present even at rest.

3.4.7 Overall Evaluation (IMPROVE study group, 1995)

Overall evaluation was done on day 60.

1 - greatly improved with treatment

2 - improved with treatment

3 - remained the same

4 - is worse with treatment

3.4.8 Survival Rate

The survival rate at five months post treatment was calculated as below:

$$\frac{\text{Number of dogs alive}}{\text{Number of dogs evaluated}} \times 100$$

3.5 STATISTICAL ANALYSIS

The data collected were statistically analysed as per Snedecor and Cochran (1994). The data from history and physical examination findings, radiographic findings, heart rate, serum biochemistry, class of heart failure, overall evaluation and survival rate from DCM dogs and MVI dogs were clubbed together for analysis whereas the data from echocardiographic findings were analysed separately for DCM and MVI dogs.

CHAPTER - IV

RESULTS

4.1 INCIDENCE

This study comprised of a total of 36 dogs, out of which 18 dogs were affected with DCM and 18 dogs were affected with MVI. The breed of dogs affected with DCM included eight Doberman, four German Shepherd, four Labrador retriever, one Boxer and one non-descript (Fig.1) and in case of MVI the breeds were seven Spitz, five non-descript, three German Shepherd, two Labrador retriever and one Boxer (Fig.2). Males were more prone for both diseases with involvement of 13 males and five females in each disease (Fig.3). The average age of dogs affected with DCM was 7.2 years with a range of 2.5 to 15 years and with MVI was 10.4 years with a range of 8 to 17 years (Fig.4).

4.2 HISTORY AND PHYSICAL EXAMINATION FINDINGS

The percentage of dogs with improved scores compared with baseline for history and physical examination parameters on day 30 and day 60 for all three treatment groups are given in the table 1 and 2 respectively.

The percentage of dogs that showed improved cough scores compared with base line on day 30 were 83, 75 and 83 and on day 60 were 92, 66 and 92 for the treatment groups I, II and III respectively.

The percentage of dogs that showed improved respiratory effort compared with baseline on day 30 were 75, 66, 83 and on day 60 were 83, 58, 92 for the treatment groups I, II and III respectively.

The percentage of dogs that showed improved appetite compared with baseline on day 30 were 75, 58, 92 and on day 60 were 83, 50, 92 for the treatment groups I, II and III respectively.

The percentage of dogs that showed improved demeanor compared with baseline on day 30 were 75, 58, 83 and on day 60 were 83, 50, 92 for the treatment groups I, II and III respectively.

The percentage of dogs that showed improved mobility compared with baseline on day 30 were 66, 33, 75 and on day 60 were 75, 33, 83 for the treatment groups I, II and III respectively.

The percentage of dogs that showed improved attitude compared with baseline on day 30 were 66, 41, 66 and on day 60 were 66, 41, 66 for the treatment groups I, II and III respectively.

The percentage of dogs that showed improved activity compared with baseline on day 30 were 75, 58, 83 and on day 60 were 75, 50, 83 for the treatment groups I, II and III respectively.

The percentage of dogs that showed improved ascites scores compared with baseline on day 30 were 92, 75, 92 and on day 60 were 92, 66, 92 for the treatment groups I, II and III respectively.

4.3 RADIOGRAPHIC EVIDENCE OF PULMONARY EDEMA

The percentage of dogs that showed improved pulmonary edema scores compared with baseline on day 30 were 66, 33, 83 and on day 60 were 75, 33, 83 for the treatment groups I, II and III respectively (Table 1 and 2).

4.4 HEART RATE

The mean ∀ SE values of heart rate for all three treatment groups are given in the Table 3.

The mean ∀ SE values of heart rate for treatment group I were 163.33 ∀ 4.32, 144.17 ∀ 3.36, 139.17 ∀ 4.68, for treatment group II were 155.83 ∀ 4.21, 151.25 ∀ 4.09, 158.75 ∀ 6.72 and treatment group III were 150.83 ∀ 5.14, 135.00 ∀ 3.99; 126.67 ∀ 6.32 on zero day, 30 days and 60 days respectively (Fig.5).

In group I, a highly significant (P # 0.01) difference was observed between 0 day and 30 days and between zero day and 60 days whereas no significant difference was observed between 30 days and 60 days. In group II no significant difference was observed within and between treatment groups.

In group III, a highly significant (P # 0.01) difference was observed between zero day and 60 days whereas no significant difference was observed between zero day and 30 days and between 30 days and 60 days. On zero day and 30 days no significant difference was observed between treatment groups. On 60 days, a highly significant (P#0.01) difference was observed between group I and II and between group II and group III whereas no significant difference was observed between group I and group III.

4.5 CLASS OF HEART FAILURE

The percentage of dogs that improved in class of Heart failure compared with baseline on day 30 were 58, 33, 66 and on day 60 were 66, 25, 75 for the treatment groups I, II and III respectively (Table 1 and 2).

4.6 ECHOCARDIOGRAPHIC FINDINGS IN DILATED CARDIOMYOPATHY

4.6.1 Left ventricular dimensions and EPSS

The mean ∀ SE values of left ventricular dimensions in millimeters and EPSS in millimeters for all 3 treatment groups are given in the Table 4.

The mean ∀ SE values of $LVID_d$ for treatment group I were 62.00 ∀ 2.96, 59.17 ∀ 2.27, 56.50 ∀ 2.14, for treatment group II were 61.33 ∀ 4.01, 60.00 ∀ 3.33, 61.83 ∀ 3.51 and for treatment group III were 61.83 ∀ 2.47, 57.67 ∀ 2.11. 54.33 ∀ 1.82 on zero day, 30 days and 60 days respectively. There was no significant difference within and between the three treatment groups.

The mean ∀ SE values of $LVID_s$ for treatment group I were 54.83 ∀ 3.12, 49.50 ∀ 2.28, 45.17 ∀ 2.12, for treatment group II were 54.67 ∀ 4.23, 50.67 ∀ 3.77, 53.83 ∀ 3.90 and for treatment group III were 54.33 ∀ 2.56, 47.33 ∀ 1.89, 42.00 ∀ 1.98 on zero day, 30 days and 60 days respectively.

In group I and group III there was no significant difference between zero day and 30 days and between 30 days and 60 days but there was a significant (P # 0.05) difference between zero day and 60 days whereas in group II there was no significant difference between zero day, 30 days and 60 days.

There was no significant difference between treatment groups on zero day and 30 days whereas a significant (P # 0.05) difference was observed on 60 days between group I and group II and between group II and group III. There as no significant difference between group I and group III on day 60.

The mean ∀ SE values of EPSS for treatment group I were 20.67∀1.36, 18.33 ∀ 1.17, 16.83 ∀ 1.01, for treatment group II were 19.83∀1.82, 19.50 ∀ 1.48, 20.17 ∀ 1.51 and for treatment group III were 20.33 ∀ 1.12, 18.17 ∀ 1.08, 15.17 ∀ 1.30 on zero day, 30 days and 60 days respectively. There was no significant difference within and between the three treatment groups.

4.6.2 Left atrium (LA), Aorta (Ao), and Left atrium/Aorta ratio (LA/Ao)

The mean ∀ SE values of LA, Ao in millimeters and LA/Ao for all the three treatment groups are given in Table 4.

The mean ∀ SE values of LA for treatment group I were 34.67 ∀ 1.54, 32.50 ∀ 1.34, 31.17 ∀ 1.35, for treatment group II were 33.50 ∀ 1.91, 32.67 ∀ 1.69, 33.33 ∀ 1.63 and for treatment group III were 34.00 ∀ 1.37, 31.83∀1.14, 29.83 ∀ 1.28 on zero day, 30 days and 60 days respectively. There was no significant difference within and between the three treatment groups.

The mean ∀ SE values of Ao for treatment group I were 19.00 ∀ 0.68, 20.50 ∀ 0.72, 21.83 ∀ 0.91, for treatment group II were 20.67 ∀ 0.42, 20.50 ∀ 0.67, 20.33 ∀ 0.71 and for treatment group III were 19.33 ∀ 0.67, 20.33∀0.80, 22.00 ∀ 1.03 on zero day, 30 days and 60 days respectively. There was no significant difference within and between the three treatment groups.

The mean ∀ SE values for LA/Ao ratio for treatment group I were 1.82 ∀ 0.09, 1.59 ∀ 0.08, 1.45 ∀ 0.11, for treatment group II were 1.63 ∀ 0.11, 1.61 ∀ 0.12, 1.66 ∀ 0.12 and for treatment group III were 1.81 ∀ 0.09, 1.64 ∀ 0.10, 1.55 ∀ 0.11 on zero day, 30 days and 60 days respectively. There was no significant difference within and between the three treatment groups.

4.6.3 Fractional shortening (FS) and Ejection fraction (EF)

The mean ∀ SE values of FS and EF in percentage for all three treatment groups are given in Table 5.

The mean ∀ SE values of FS for treatment group I were 12.00 ∀ 0.82, 16.33 ∀ 0.71, 20.00 ∀ 1.00, for treatment group II were 12.17 ∀ 1.35, 15.83 ∀ 1.62, 13.17 ∀ 1.92 and for treatment group III were 12.00 ∀ 0.77, 18.00 ∀ 0.52, 22.83 ∀ 1.14 on zero day, 30 days and 60 days respectively (Fig.6).

In group I a highly significant (P # 0.01) difference was observed

between zero day and 30 days and between zero day and 60 days and no significant difference was observed between 30 days and 60 days. In group II, no significant difference was observed between zero day, 30 days and 60 days. In group III, a highly significant (P # 0.01) difference was observed between zero day and 30 days, zero day and 60 days and between 30 days and 60 days.

On zero day and 30 days no significant difference was observed between treatment groups whereas, on 60 days a highly significant (P#0.01) difference was observed between group I and group II, group II and group III and no significant difference was observed between group I and group III.

The mean ∀ SE values of EF for treatment group I were 31.17 ∀ 2.14, 39.83 ∀ 1.17, 49.17 ∀ 1.78, for treatment group II were 32.17 ∀ 3.07, 40.00 ∀ 3.48, 34.33 ∀ 4.20 and for treatment group III were 32.33 ∀ 1.61, 43.50 ∀ 1.23, 50.50 ∀ 2.59 on zero day, 30 days and 60 days respectively (Fig.7).

In group I, a highly significant (P # 0.01) difference was observed between zero day and 60 days but there was no significant difference between zero day and 30 days and between 30 days and 60 days. In group II, no significant difference was observed between zero day, 30 days and 60 days. In group III, a highly significant (P # 0.01) difference was observed between zero day and 30 days and between zero day and 60 days. There was no significant difference between 30 days and 60 days.

On zero day and 30 days no significant difference was observed between treatment groups. On 60 days, a highly significant (P # 0.01) difference was observed between group I and group II and between group II and group III but no significant difference was observed between group I and group III.

4.7 ECHOCARDIOGRAPHIC FINDINGS IN MITRAL VALVE INSUFFICIENCY

4.7.1 Left ventricular dimensions

The mean ∀ SE values of left ventricular dimensions in millimeters for all three treatment groups are given in the Table 6.

The mean ∀ SE values of $LVID_d$ for treatment group I were 52.67 ∀ 2.33, 49.67 ∀ 2.44, 48.83 ∀ 2.40, for treatment group II were 53.00 ∀ 2.52, 52.17 ∀ 2.40, 53.50 ∀ 2.39 and for treatment group III were 52.50 ∀ 2.62, 49.50 ∀ 2.72, 46.50 ∀ 2.40 on zero day, 30 days and 60 days respectively. There was no significant difference within and between the three treatment groups.

The mean ∀ SE values of $LVID_s$ for treatment group I were 33.50 ∀ 3.63, 31.33 ∀ 2.78, 30.00 ∀ 1.89, for treatment group II were 34.00 ∀ 3.77, 35.00 ∀ 2.94, 36.00 ∀ 2.54 and for treatment group III were 32.50 ∀ 3.53, 29.50 ∀ 2.62, 28.17 ∀ 1.82 on zero day, 30 days and 60 days respectively. There was no significant difference within and between the three treatment groups.

4.7.2 Left atrium (LA), Aorta (Ao) and Left atrium/Aorta ratio (LA/Ao)

The mean ∀ SE values of LA in millimeters, Ao in millimeters and LA/Ao ratio for all three treatment groups are given in the Table 6.

The mean ∀ SE values of LA for treatment group I were 35.17 ∀ 2.48, 32.67 ∀ 2.33, 28.67 ∀ 1.26, for treatment group II were 35.50 ∀ 2.23, 35.67 ∀ 2.65, 36.67 ∀ 2.53 and for treatment group III were 35.33 ∀ 2.30, 31.50 ∀ 2.23, 28.33 ∀ 2.46 on zero day, 30 days and 60 days respectively. There was no significant difference within and between the three treatment groups.

The mean ∀ SE values of Ao for treatment group I were 18.00 ∀ 1.18, 20.50 ∀ 1.34, 22.33 ∀ 1.26, for treatment group II were 18.17 ∀ 1.40, 18.00 ∀ 1.13, 18.33 ∀ 1.20 and for treatment group III were 18.50 ∀ 1.61, 21.33 ∀ 1.48, 23.83 ∀ 1.62 on zero day, 30 days and 60 days respectively.

In group I, a significant (P # 0.05) difference was observed between zero day and 60 days and no significant difference was observed between zero day and 30 days and between 30 days and 60 days. In group II there was no significant difference between zero day, 30 days and 60 days. In group III a significant (P # 0.05) difference was observed between zero day and 60 days whereas there was no significant difference between zero day and 30 days and between 30 days and 60 days.

On zero day and 30 days there was no significant difference between the three treatment groups. On 60 days there was a significant (P#0.05) difference between group I and group II and between group II and group III whereas no significant difference was observed between group I and group III.

The mean ∀ SE values of LA/Ao ratio for treatment group I were 1.96 ∀ 0.08, 1.59 ∀ 0.05, 1.36 ∀ 0.05, for treatment group II were 1.96 ∀ 0.04, 1.97 ∀ 0.03, 1.99 ∀ 0.03 and for treatment group III were 1.93 ∀ 0.04, 1.48 ∀ 0.04, 1.20 ∀ 0.03 on zero day, 30 days and 60 days respectively (Fig.8).

In group I, a highly significant (P # 0.01) difference was observed between zero day and 30 days, zero day and 60 days and between 30 days and 60 days. In group II there was no significant difference between 0 day, 30 days and 60 days. In group III, a highly significant (P # 0.01) difference was observed between zero day and 30 days, zero day and 60 days and between 30 days and 60 days.

On zero day, there was no significant difference between treatment groups. On 30 days a highly significant (P # 0.01) difference was observed between group I and group II and between group II and group III whereas no significant difference was observed between group I and group III.

On 60 days, a highly significant (P # 0.01) difference was observed between group I and group II and between group II and group III whereas a significant (P # 0.05) difference was observed between group I and group III.

4.7.3 Fractional shortening (FS)

The mean ∀ SE values of FS in percentage for all three treatment

groups are given in Table 6.

The mean ∀ SE values of FS for treatment group I were 37.00 ∀ 4.13, 37.67 ∀ 2.53, 38.67 ∀ 1.52, for treatment group II were 37.67 ∀ 4.63, 34.83 ∀ 2.79, 35.00 ∀ 2.54 and for treatment group III were 38.50 ∀ 4.57, 40.50 ∀ 3.58, 39.50 ∀ 1.59 on zero day, 30 days and 60 days respectively. There was no significant difference within and between the three treatment groups.

4.8 AZOTEMIA AND HYPERKALEMIA

In all the three treatment groups at the baseline on day zero no animals were azotemic and hyperkalemic. On day 30, two animals in group I and one animal in group II and III were azotemic. On day 60, two animals in all three treatment groups were azotemic.

No animals showed hyperkalemia in all three treatment groups both on 30 days and 60 days post treatment.

4.9 OVERALL EVALUATION

The overall evaluation of dogs in percentage on 60 days for all three treatment groups are given in Table 7.

In group I, 33 per cent were greatly improved, 50 per cent were improved, 17 per cent remained the same and zero per cent worsened. In group II, zero per cent were greatly improved, 33 per cent were improved, 25 per cent remained the same and 42 per cent worsened. In group III, 42 per cent were greatly improved, 50 per cent were improved, 8 per cent remained the same and zero per cent worsened.

4.10 SURVIVAL RATE

The survival rate of dogs in percentage for all the three treatment groups are given in Table 7.

In group I 60 per cent of the dogs, in group II 10 per cent of the dogs and in group III 80 per cent of the dogs survived five months post-treatment.

CHAPTER - V

DISCUSSION

5.1 INCIDENCE

In DCM, Doberman Pinscher (8) was the most commonly affected breed followed by German Shepherd (4) and Labrador retriever (4). This finding is in agreement with Sisson and Thomas (1995) who reported in a survey of 1681 dogs based on a search of Veterinary medical data at Purdue University that one of the most commonly affected breed was Doberman Pinscher. In COVE study group (1995) trial also Doberman was the most commonly affected breed with DCM followed by Great Dane, German Shepherd and Labrador retriever.

In MVI, Spitz (7) was the most commonly affected followed by non-descript (5). This is in agreement with Buchanan (1977) who reported highest incidence in small breeds. In the present study MVI was also reported in German Shepherd and Labrador retriever and this concur with Kittleson (1998) who opined that occasionally large breed of dogs such as German Shepherd may be presented with mitral valve disease.

A male predominance of around 72 per cent was present in both the diseases. This concur with the findings of O'Grady and Horne (1992), Sisson and Thomas 1995, Calvert *et al.* (1997) and Tidholm and Jonsson (1997) who all reported male predominance in DCM and Buchanan (1977) who reported male predominance in mitral valve disease.

The average age of dogs affected with DCM in this study was 7.2 years. This finding concur with Tidholm *et al.* (2001) who reported that the average age was seven years.

In MVI, the average age of dogs affected was 10.4 years. This is in partial agreement with Buchanan (1977) who reported increased incidence in dogs more than 12 years of age.

5.2 HISTORY AND PHYSICAL EXAMINATION FINDINGS

In group I and group III, an appreciable improvement in the history and physical examination parameters such as cough score, respiratory effort, appetite, deameanor, mobility, attitude, activity and ascites score was observed on day 30 compared with day zero. A marginal improvement in the history and physical examination parameters was observed on day 60 compared to day 30. The above findings show that the progression of disease was arrested and stabilized in these treatment groups.

In group II, although an less appreciable improvement compared to group I in the history and physical examination parameters was observed on day 30 when compared to day zero, a marginal reduction was observed on day 60 compared to day 30. These findings suggest that the disease was progressing in group II and the treatment was not effective in stabilizing the heart failure.

When compared to baseline on day 60, group II had 66 per cent, 58 per cent, 50 per cent, 50 per cent, 33 per cent, 41 per cent, 50 per cent and 66 per cent of improved cough score, respiratory effort, appetite, demeanor, mobility, attitude, activity and ascites score respectively compared to group I scores of 92 per cent, 83 per cent, 83 per cent, 83 per cent, 75 per cent, 66 per cent, 75 per cent and 92 per cent. This shows that less percentage of dogs improved in group II when compared to group I.

The findings in group I are in agreement with IMPROVE study group (1995) and COVE study group (1995). They evaluated the efficacy of enalapril maleate in dogs with naturally acquired class III or class IV heart failure in a multicenter study and reported that enalapril treated dogs had significantly greater decrease in clinical variables measured when compared to placebo treated dogs and this study showed the beneficial clinical effects of adding enalapril to conventional therapy of dogs with heart failure.

The findings in group II are in contrast to human studies by Dickstein *et al.* (1995), Lang *et al.* (1997) and Pitt *et al.* (1997). Dickstein *et al.* (1995) reported that losartan and enalapril were of comparable efficacy in short term treatment of moderate to severe congestive heart failure. Lang *et al.* (1997) reported that losartan was generally well tolerated and comparable to enalapril in term of exercise tolerance in short term (12 weeks) study of human patients with heart failure. Pitt *et al.* (1997) in the evaluation of losartan in the elderly (ELITE-I) reported that losartan was as effective as captopril in improving symptoms of heart failure.

On day 60, group III had a slightly better scores of respiratory effort, appetite, demeanor, mobility and activity when compared to group I. This shows that group III was slightly better than group I in improving the signs of heart failure. These findings are in agreement with Hamroff *et al.* (1999) and Pascual Figal *et al.* (2002).

Hamroff *et al.* (1999) reported that losartan enhanced peak exercise capacity and alleviated symptom in patients with CHF who were severely symptomatic despite treatment with maximally recommended or tolerated doses of ACE inhibitors.

Pascual Figal *et al.* (2002) reported that after maximum ACE inhibitor doses, the addition of losartan was safe and associated with an improvement in ventricular function and NYHA functional class.

5.3 RADIOGRAPHIC EVIDENCE OF PULMONARY EDEMA

When compared to baseline, on day 60, 75 per cent of the dogs in group I, 33 per cent of the dogs in group II and 83 per cent of the dogs in group III had improved scores of pulmonary edema i.e. it had reduction in the level of pulmonary edema in radiography. These findings showed that treatment group I and III had a substantial improvement of signs of heart failure when compared to group II. The finding in group I concur with the COVE study group (1995) which reported a significant reduction in the radiographic evidence of pulmonary edema when treated with enalapril plus conventional therapy. The finding in group II is in contrast to Cowley *et al.* (2000) who observed a significant improvement in quality of life in elderly patients with losartan treatment.

Treatment group III was slightly better than group I i.e. standard treatment, which is in agreement with Hamroff *et al.* (1999). The substantial reduction in radiographic evidence of pulmonary edema in group I and group III showed that the drugs in these groups were very effective than the drugs in group II where very less percentage of dogs improved.

5.4 HEART RATE

A highly significant (P # 0.01) reduction in heart rate was observed in group I from 163.33 to 150.83 and in group III from 150.83 to 126.67 between 0 day and 60 days whereas in group II there was no significant difference in heart rate between 0 day and 60 days. A highly significant (P # 0.01) difference was also observed between group I and II and between

group II and group III whereas no significant difference in heart rate was observed between group I and group III on day 60. These findings showed that in group II the disease was progressing and heart failure was not stabilized whereas in other two treatment groups the treatment was effective in transiently arresting the progression.

5.5 CLASS OF HEART FAILURE

The percentage of dogs that improved in class of heart failure compared with baseline on day 30 were 58, 33, 66 and on day 60 were 66, 25, 75 for the treatment group I, II and III respectively.

These findings showed that the treatment in group I and group III were effective in controlling signs of heart failure than group II. The finding in group I concur with IMPROVE study group (1995) which observed a significant improvement in class of heart failure in enalapril plus conventional therapy, whereas finding in group II is in contrast to Dickstein *et al.* (1995) who observed a comparable efficacy between losartan and enalapril in improving the class of heart failure. The finding in group III is in partial agreement with Pascual Figal *et al.* (2002) who observed an improvement with NYHA functional class when losartan was added to patients treated with maximum doses of ACE inhibitor.

5.6 ECHOCARDIOGRAPHIC FINDINGS IN DILATED CARDIOMYOPATHY

5.6.1 Left ventricular dimensions and EPSS

A non-significant reduction in left ventricular internal dimensions in diastole was observed in group I and group III and a significant (P#0.05) reduction in left ventricular internal dimensions in systole was observed in group I and group III on day 60 when compared to day zero. This is in agreement with Errikson *et al.* (1990) who reported a non significant reduction in left ventricle dimensions in human patients after treatment with enalapril. This can be explained as improved ventricular emptying as a result of enalapril and losartan induced arteriodilatation and decreased systemic vascular resistance causing a decrease in ventricular dimension (Todd and Karen, 1989). Thus, the aforementioned non significant and significant reduction in chamber size may indicate a positive therapeutic effect in these groups.

The finding in group I is in agreement with Allworth *et al.* (1995) who observed similar findings in the treatment of dogs with pacing-induced heart failure.

In group II, there was no significant difference in ventricular dimension in diastole and systole. This indicate a progressive nature of the disease without responding to the treatment. This is in contrast to Konstam *et al.* (2000) who in a ELITE II sub study observed that losartan and captopril prevented left ventricular dilation representing adverse ventricular remodeling and also reverse remodeling in the elderly patients with heart failure.

A non-significant reduction in EPSS was observed in treatment groups I and III at 60 days of treatment compared to zero day. Decrease in EPSS indicates a positive response to the treatment. This may be explained as increase in mitral value excursion due to increased diastolic inflow resulting from increased systolic outflow and decrease in left ventricle dimensions bringing the septum closer to the valve resulting in decreased EPSS (Calvert and Brown, 1986).

5.6.2 Left atrium, Aorta and Left Atrium / Aorta ratio

A non-significant reduction in LA dimension was observed on 60 days post treatment in group I and group III and a non-significant increase in Ao dimension was also observed in group I and group III 60 days post treatment. As a reflection of these changes in group I and group III, a non-significant reduction of LA/Ao ratio was observed 60 days post treatment. These findings in group I and group III suggest that the treatment was showing positive response. Left atrial dimension has a strong correlation with left ventricular end diastolic pressure or the degree of preloading of the left ventricle (Haendchen *et al.*, 1982). Therefore, the slight reduction of LA dimension may indicate a positive response reflecting unloading of the ventricle, placing the heart in a more favourable part of the Frank Starling length - tension curve (Mirsky, 1974). This concur with the findings of Errikson *et al.* (1990) who observed a non-significant decrease in left atrial dimension in response to enalapril treatment of congestive heart failure in human beings.

In group II, there was even no non-significant changes in the LA, Ao and LA/Ao ratio 60 days post treatment. This shows that the treatment in this group was not effective in improving the systolic function and thereby

not showing any positive changes in the above said parameters.

5.6.3 Fractional Shortening and Ejection Fraction

In group I and group III, a highly significant (P # 0.01) increase in fractional shortening was observed from zero day to 30 days and 30 days to 60 days and in group II no significant difference was observed between zero day, 30 days and 60 days.

With regard to ejection fraction, a highly significant (P # 0.01) increase from zero day to 60 days was observed in group I and group III whereas no significant difference was observed within treatment in group II.

A highly significant (P # 0.01) difference in FS and EF was observed on 60 days post treatment between group I and group II and between group II and group III whereas no significant difference was observed between group I and group III.

These findings suggest that treatment in group I and group III was effective in improving the ventricular function in dogs with DCM. The improvement in ventricular function was reflected as highly significant increase in fractional shortening and ejection fraction substantially towards normal ranges.

The findings in group I are in agreement with Allworth *et al.* (1995) who observed a improved systolic function in dogs treated with enalapril. The above effect in group I might be attributed to the combination of enalapril induced decreased aortic impedance and digoxin induced increased myocardial contractility.

In group II, the findings are in contrast to Konstam *et al.* (2000) ELITE ventricular function sub study who observed that both captopril and losartan prevented left ventricular dilatation and improved left ventricular function with comparable efficacy.

In group III the findings are in contrast to Cocco *et al.* (2002), Shimizu *et al.* (2002) and Pascual Figal *et al.* (2002). Cocco *et al.* (2002) reported that after six weeks of treatment the combination of enalapril plus losartan was more effective than enalapril alone in improving myocardial

function both at rest and after stress. Shimizu *et al.* (2002) reported that long term treatment with enalapril and valsartan combination improved cardiac function and survival compared to placebo (or) enalapril monotherapy in cardiomyopathic hamsters.

Pascual Figal *et al.* (2002) reported that after maximum ACE inhibitor doses, the addition of losartan was safe and associated with an improvement in ventricular function but with no change in neurohormonal status.

5.7 ECHOCARDIOGRAPHIC FINDINGS IN MITRAL VALVULAR INSUFFICIENCY

5.7.1 Left ventricular dimensions

A non-significant reduction in left ventricular dimensions in diastole and systole was observed in group I and group III at 60 days of treatment when compared to zero day. This finding shows that in group I and group III the dogs were showing positive response to the treatment probably due to reduced peripheral resistance and thereby reduced preload. In group II even a non-significant reduction in ventricular dimension was not observed indicating that the treatment was not effective.

The findings in group I are in agreement with IMPROVE study group (1995) which studied the treatment of enalapril in combination with digoxin and furosemide in mitral valvular insufficiency and reported a non-significant improvement in ventricular dimensions and also with Allworth *et al.* (1995) who reported a similar finding in pacing induced heart failure.

In group II, there was no non-significant difference in ventricular dimension in diastole and systole. This indicates the progressive nature of the disease without responding to the treatment. This is in contrast to Konstam *et al.* (2000) who reported ventricular reverse remodeling with losartan treatment.

5.7.2 Left atrium (LA), Aorta (Ao) and Left Atrium/Aorta ratio (LA/Ao)

A non-significant reduction in LA dimension was observed in group I and group III on day 60 compared to day zero whereas in group II a

non-significant increase was observed on day 60 compared to day zero.

A significant (P # 0.05) increase in Ao was observed between day 60 and day zero in group I and group III whereas no significant difference was observed in group II between zero day and 60 days. A significant (P # 0.05) difference was observed on day 60 between group I and group II, and between group II and group III whereas no significant difference was observed between group I and group III.

In LA/Ao ratio, a highly significant (P # 0.01) difference was observed between zero day and 60 days in group I and group III respectively. In group II, no significant difference was observed between zero day and 60 days of treatment. On 60 days post treatment a highly significant difference was observed between group I and II and between group II and group III whereas a significant difference was observed between group I and group III.

In group I, the enalapril could have caused reduced vascular resistance which in turn could have reduced the systolic pressure in left ventricle. This reduced systolic pressure in ventricle might lead to reduced left atrial pressure which in turn, could have prevented dilatation of left atrium. When there is reduced dilatation of LA naturally there will be increased dimension of Ao leading to reduction in LA/Ao ratio. In group II, the effect of losartan alone was not sufficient to cause any appreciable reduction in LA/Ao ratio at 60 days of treatment. In group III, addition of losartan to enalapril could have augmented the aforementioned effect of enalapril leading to increased reduction in LA/Ao ratio than group I and there by making it a superior combination in the treatment of mitral valvular insufficiency.

5.7.3 Fractional shortening

There was no significant difference in fractional shortening within as well as between treatment groups.

In the treatment groups, small dogs as well as large dogs were distributed in equal numbers. The mechanism of systolic dysfunction in mitral valve insufficiency of small dogs was slightly different from larger ones. In large dogs some degree of systolic dysfunction will be present in severe mitral regurgitation with the fractional shortening range of around 25 per cent to 40 per cent whereas in small dogs there will not be any systolic dysfunction, infact increased fractional shortening of more than 50

per cent will be present due to sympathetic stimulation (Kittleson, 1998). Therefore, reduced fractional shortening in larger dogs will be compensated for increase in smaller ones in the treatment groups before treatment. After treatment in larger dogs because of the treatment there will be increased FS whereas in smaller dogs there will be reduction in FS towards the normal level. This might be the reason for fractional shortening not being significant in any of the treatment groups before and as well as after treatment.

5.8 AZOTEMIA AND HYPERKALEMIA

On day 60 the level of azotemia was same in all the three treatment groups.

This may be attributed to the effects of enalapril, losartan and furosemide either individually or together. In chronic congestive heart failure, glomerular filtration rate may be preserved despite reduced renal blood flow. This may be due to effects of angiotensin II effecting relatively greater vasoconstriction on the efferent than afferent renal arteriole. Enalapril by blocking the conversion of angiotensin I to angiotensin II or losartan by blocking the effect of angiotensin II at receptor level, may thereby reduce this compensatory mechanism and predispose renal insufficiency. Furosemide can cause azotemia by volume depletion.

The findings in group I are in agreement with Keene and Rush (1995) who in a study of 429 dogs treated for congestive heart failure reported that the incidence of clinical azotemia occurred in 9.4 per cent of those treated with furosemide and enalapril, as compared with 8.3 percent of those treated with furosemide and placebo. In contrast to the present findings in group I Atkins *et al.* (2002) reported that administration of enalapril for upto two years did not had any demonstrable adverse effect on renal function in dogs with severe compensated mitral regurgitation.

In group II, the dogs had the same level of azotemia when compared to standard therapy in group I. This finding is in agreement with Burnier and Brunner (1996) who reported that in normal human subjects ACE inhibitors and Angiotensin II receptor antagonist had comparable renal properties.

The finding in group III is in contrast with Hamroff *et al.* (1997) who reported that in a study of combining losartan with enalapril the parameters of renal function remained unchanged. In another study by

Blake and Devereux (2000) it was reported that addition or substitution of losartan potassium in patients with ACE induced azotemia resulted in statistically insignificant reduction in blood urea nitrogen and creatinine, which concur with the present study.

In the present study hyperkalemia was not observed in any of the treatment groups. In group I and III the effect the enalapril in retaining potassium was counteracted by the effect of furosemide which depletes potassium. Therefore, the effect of enalapril was nullified. The present study is in agreement with IMPROVE study (1995) and COVE study (1995) which observed no significant difference in the level of potassium after treatment with enalapril and conventional therapy for two weeks and four weeks respectively.

The findings in group II and III was in agreement with Hamroff *et al.* (1997) and Blake and Devereux (2000).

Hamroff *et al.* (1997) in a study on combining losartan with enalapril reported that serum potassium remained unchanged.

Blake and Devereux (2000) reported that the addition or substitution of losartan potassium in patients with ACE induced hyperkalemia resulted in a significant reduction in potassium and they concluded that there might be fundamental differences between the effects of losartan and ACE inhibitors on potassium excretion in congestive heart failure patients with mild to moderate insufficiency.

5.9 OVERALL EVALUATION

In group I, 33 per cent of dogs were greatly improved, 50 per cent of the dogs were improved and 17 per cent remained the same. This finding is in partial agreement with IMPROVE study group in which 26 per cent of dogs were classified as greatly improved and 44 per cent were classified as improved.

In group II, zero percent of the dogs greatly improved, 33 per cent improved, 25 per cent remained the same and 42 per cent worsened. These findings indicate that the treatment with losartan was not effective in delaying the progression.

The reason for failure of losartan in this study may be attributed to the pharmacokinetic differences which may exist between human beings and dogs.

In human beings, the losartan gets converted to an active metabolite EXP 3174 which has 24 hours of sustained action and 15 times more potency than losartan which has only less than 8 hours of action. Therefore, in human beings the effect of losartan is mainly because of its metabolite, and therefore in human trials its efficacy was comparable to ACE inhibitors. In dogs, probably this active metabolite may not be produced or negligibly produced and effect may be only through losartan.

In group III, 42 per cent of the dogs greatly improved, 50 per cent improved and 8 per cent remained the same. This findings showed that group III was slightly better than group I. The reason for this augmentation may be due to complete blockade of angiotensin II i.e. angiotensin II which is formed through other alternative pathways may also be blocked by adding losartan to enalapril. In addition to this ACE inhibitor's action on bradykinin was also preserved.

5.10 SURVIVAL RATE

The survival rate 5 months post treatment in group I was 60 per cent and group II was 10 per cent and group III was 80 per cent.

The survival rate of 60 per cent in group I with enalapril and conventional therapy treatment is comparable to the previous studies by Ettinger *et al.* (1998) whereas survival rate in group II is in contrast to human studies by Pitt *et al.* (1997) who reported comparable efficacy with ACE inhibitors in improving the survival rate.

In group III the survival rate was 80 per cent which was better than group I. This is in agreement with Shimizu *et al.* (2002) who reported that long term treatment with enalapril and valsartan combination improved cardiac function and survival compared to placebo (or) enalapril monotherapy in cardiomyopathic hamsters.

CHAPTER - VI

SUMMARY AND CONCLUSIONS

Congestive heart failure is one of the important cause of morbidity and mortality in dogs. It is most often secondary to chronic degenerative mitral valve disease and dilated cardiomyopathy.

Activation of the sympathetic nervous system and the renin angiotensin - aldosterone system early in the course of heart failure is a compensatory mechanism that is initially beneficial, but with chronic activation it becomes maladaptive and is associated with increased morbidity and decreased survival time in patients with CHF. Modulating these maladaptive compensating mechanism with agents such as ACE inhibitor, diuretics and digitalis has become a standard therapy in managing CHF in small animals.

Recently, angiotensin II blockers were developed and tried in many human trials for hypertension and congestive heart failure. These agents block the angiotensin II at the receptor level there by causing complete blockade of angiotensin II that are even produced by alternate pathways (e.g chymase) apart from angiotensin I. In dogs, no published reports are available on the efficacy of angiotensin II receptor blockers. With this back drop, the present study was designed with the objective of assessing the efficacy of losartan potassium, the first developed angiotensin II blocker in the treatment of CHF in dogs and to compare its effect with the existing standard treatment.

Dogs attending the out-patient unit of Madras Veterinary College Hospital with history and clinical signs suggestive of CHF were subjected to ultrasound, X-ray, ECG, haematology and biochemical studies. The cases confirmed as DCM or MVI were used as materials for the study. The cases of DCM or MVI were randomly allotted to the experimental design based on body weight, type of disease and level of systolic function.

EXPERIMENTAL DESIGN

Group I	-	Enalapril+ Furosemide + with or without Digoxin DCM - 6 dogs + MVI - 6 dogs = 12 dogs.

Group II - Losartan + Furosemide + with or without Digoxin
DCM - 6 dogs + MVI - 6 dogs = 12 dogs

Group III - Enalapril + Losartan + Furosemide + with or without Digoxin.

DCM - 6 dogs + MVI - 6 dogs = 12 dogs.

The dogs in the treatment groups were evaluated on zero day, 30 days and 60 days post treatment.

The parameters studied were

1. History and physical examination variables such as cough score, respiratory effort, appetite, mobility, activity, demeanor, attitude, ascites score.

2. Radiography - pulmonary edema

3. ECG for heart rate

4. Echocardiography
DCM - $LVID_d$, $LVID_s$, FS, EPSS, LA, Ao, LA/Ao, EF
MVI - $LVID_d$, $LVID_s$, LA, Ao, LA/Ao, FS

5. Serum biochemistry - BUN, Creatinine and Potassium

6. Class of heart failure

7. Overall evaluation

8. Survival rate at five months post treatment

This study comprised of a total of 36 dogs inclusive of 18 dogs with DCM and 18 dogs with MVI. Males (26/36) were more prone for both the diseases. In DCM, common breeds affected were Doberman (8/18) followed by German Shepherd (4/18) and Labrador (4/18). In MVI common breeds affected were Spitz (7/18) followed by non-descript (5/18) and German

Shepherd (3/18). The average age of dogs affected in DCM was 7.2 years and MVI was 10.4 years.

On 60 days of treatment, in group I and III more percentage of dogs had improved cough scores, respiratory effort, appetite, demeanor, mobility, attitude, ascites score and activity whereas in group II less percentage of dogs had improved. Group III had more percentage of dogs with improved respiratory effort, appetite, demeanor, mobility and activity when compared to group I. Reduction in pulmonary edema was observed in 75 per cent of dogs in group I, 33 per cent in group II and 83 per cent in group III. A highly significant (P#0.01) reduction in the heart rate was observed in group I and group III whereas no significant difference was observed in group II after 60 days of treatment. These findings showed that the treatment was very effective in group I and III with group III having slight edge over group I, but less effective in group II where only losartan was used.

In DCM, group I and group III dogs had non-significant reduction in left ventricular dimensions, left atrial dimensions, EPSS and LA/Ao ratio on 60 days of treatment whereas in group II no such changes were appreciated. Fractional shortening and ejection fraction significantly (P # 0.01) improved in group I and group III i.e. FS from 12 per cent to 20 per cent and 12 per cent to 22 per cent in group I and group III respectively and EF from 31.17 per cent to 49.17 per cent and 32.33 per cent to 50.50 per cent in group I and group III respectively, whereas in group II no significant difference was observed. Significant (P # 0.01) change was observed between group I and group II and between group II and group III at 60 days of treatment.

In MVI, at 60 days of treatment group I and group III dogs had non-significant reduction in LV dimensions and LA dimensions, significant (P # 0.05) increase in Ao dimensions and significant (P # 0.01) reduction in LA/Ao ratio whereas in group II no such changes were appreciated. In LA/Ao ratio, a significant (P # 0.01) difference was observed between group I and II and between group II and group III and a significant (P # 0.05) difference was observed between group I and group III at 60 days of treatment.

These findings clearly showed that the treatment was very effective in group I and III whereas less effective in group II. In MVI, group III fared slightly better than group I.

The level of azotemia was almost same in all three treatment groups

with two animals developing azotemia after 60 days of treatment whereas hyperkalemia was not observed at any stage of treatment.

On 60 days of treatment, 66 per cent of dogs in group I, 25 per cent of dogs in group II and 75 per cent of dogs in group III had improved class of heart failure compared to baseline.

In overall evaluation, in group I, 33 per cent of dogs were classified as greatly improved, 50 per cent as improved and 17 per cent remained the same, in group II zero per cent of dogs were classified as greatly improved, 33 per cent as improved, 25 per cent remained the same and 42 per cent worsened and in group III, 42 per cent of dogs were classified as greatly improved, 50 per cent as improved and 8 per cent remained the same.

The survival rate five months post treatment was 60 per cent in group I, 10 per cent in group II and 80 per cent in group III. In class of heart failure, overall evaluation and survival rate group II fared very poorly compared with standard therapy in group I whereas group III was slightly better than the standard therapy.

CONCLUSIONS

1. In DCM, Doberman Pinscher was the most affected breed and in MVI, Spitz was the most affected breed. Males were more prone for both the diseases. The average age of dogs affected in DCM was 7.2 years and in MVI was 10.4 years.

2. Losartan, furosemide and with or without digoxin therapy was inferior to the standard treatment with enalapril, furosemide and with or without digoxin.

3. Combination of enalapril, losartan, furosemide and with or without digoxin was better than the standard treatment.

4. Level of azotemia was same in all the three treatment groups.

5. Survival rate at five months post treatment was more with losartan and enalapril combination (80%) compared to standard treatment (60%). Poor survival rate (10%) was observed in losartan monotherapy.

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APPENDIX

PROFORMA FOR CARDIOVASCULAR RESEARCH

Centre of Advanced Studies in Veterinary Clinical Medicine
Madras Veterinary College, Chennai - 600 007.

Case No. : Date :

Owner's Name and Address :

Breed : Colour : Age : Sex : B.Wt.

History

General Clinical Examination

Temperature :
Mucous membrane :
Heart rate :
Pulse :
CRT :
Hydration :
Lung auscultation :
Heart auscultation :
Abdominal palpation :
Abdominal percussion :
Other Systems :

Special Examination

Radiography

Echocardiography

Electrocardiography

Heart rate :
Rhythm :
P-wave-amp :
dur :
QRS-
Complex dur :
R-amp :
P-R interval :
Q-T interval :
S-T segment :

Result :

Haematology

Hb :
PCV :
RBC :
WBC :
N :
B :
E :
M :

Serum Biochemistry

BUN :
Creatinine :
Potassium :
SGPT :
Total protein :
Albumin :

Diagnosis
Treatment group
Evaluation Post Treatment

Clinical Variables

	0 day	**30 days**	**60 days**
Cough score			
Respiratory effort			
Appetite			
Demeanor			
Mobility			
Attitude			
Activity			
Ascites			

Radiography

Pulmonary edema			

Electrocardiography

Rhythm			
Rate			

Echocardiography

$LVID_d$ (mm)			
$LVID_s$ (mm)			
FS (%)			
EPSS (mm)			
LA (mm)			
Ao (mm)			
LA / Ao			
EF (%)			

Serum Biochemistry

BUN			
Creatinine			
Potassium			

Class of Heart Failure

Overall Evaluation

Survival Rate

Table - 1

Percentage of dogs with improved scores compared with baseline on day 30 for clinical variables in treatment groups

S.No	Variable	Group I (n=12)	Group II (n=12)	Group III (n=12)
1.	Cough scores	83%	75%	83%
2.	Respiratory effort	75%	66%	83%
3.	Appetite	75%	58%	92%
4.	Demeanor	75%	58%	83%
5.	Mobility	66%	33%	75%
6.	Attitude	66%	41%	66%
7.	Activity	75%	58%	83%
8.	Ascites score	92%	75%	92%
9.	Radiographic evidence of Pulmonary edema	66%	33%	83%
10.	Class of heart failure	58%	33%	66%

Table - 2

Percentage of dogs with improved scores compared with baseline on day 60 for clinical variables in treatment groups

S.No	Variable	Group I (n=12)	Group II (n=12)	Group III (n=12)
1.	Cough scores	92%	66%	92%
2.	Respiratory effort	83%	58%	92%
3.	Appetite	83%	50%	92%
4.	Demeanor	83%	50%	92%
5.	Mobility	75%	33%	83%
6.	Attitude	66%	41%	66%
7.	Activity	75%	50%	83%
8.	Ascites score	92%	66%	92%
9.	Radiographic evidence of Pulmonary edema	75%	33%	83%
10.	Class of heart failure	66%	25%	75%

Table -3

Mean ∀ SE values of Heart rate in treatment groups

Parameters	Groups	O day	30 days	60 days	F ratio
Heart rate (Beats/ min)	I	163.33 ∀ 4.32^{e}	144.17 ∀ 3.36^{abcd}	139.17 ∀ 4.68^{abc}	6.01**
	II	155.83 ∀ 4.21^{cde}	151.25 ∀ 4.09^{bcde}	158.75 ∀ 6.72^{de}	
	III	150.83 ∀ 5.14^{bcde}	135.00 ∀ 3.99^{ab}	126.67 ∀ 6.32^{a}	

** - Highly Significant (P # 0.01).

Mean values bearing the same superscripts in the rows and columns do not vary significantly for individual parameters.

Table - 4

Mean ∀ SE values of Ventricular and Atrial dimensions of DCM dogs in treatment groups

Parameters	Groups	O day	30 days	60 days	F ratio
$LVID_d$ (mm)	I	62.00 ∀ 2.96	59.17 ∀ 2.27	56.50∀ 2.14	0.94
	II	61.33 ∀ 4.01	60.00 ∀ 3.33	61.83 ∀ 3.51	
	III	61.83 ∀ 2.47	57.67 ∀ 2.11	54.33 ∀ 1.82	
$LVID_s$ (mm)	I	54.83 ∀ 3.12^{a}	49.50 ∀ 2.28^{abc}	45.17 ∀ 2.12^{bc}	2.41*
	II	54.67 ∀ 4.23^{a}	50.67 ∀ 3.77^{ac}	53.83 ∀ 3.90^{a}	
	III	54.33 ∀ 2.56^{a}	47.33 ∀ 1.89^{abc}	42.00 ∀ 1.98^{c}	
LA (mm)	I	34.67 ∀ 1.54	32.50 ∀ 1.34	31.17 ∀ 1.35	1.01
	II	33.50 ∀ 1.91	32.67 ∀ 1.69	33.33 ∀ 1.63	
	III	34.00 ∀ 1.37	31.83 ∀ 1.14	29.83 ∀ 1.28	
Ao (mm)	I	19.00 ∀ 0.68	20.50 ∀ 0.72	21.83 ∀ 0.91	1.7
	II	20.67 ∀ 0.42	20.50 ∀ 0.67	20.33 ∀ 0.71	
	III	19.33 ∀ 0.67	20.33 ∀ 0.80	22.00 ∀ 1.03	
LA/Ao ratio	I	1.82 ∀ 0.09	1.59 ∀ 0.08	1.45 ∀ 0.11	1.23
	II	1.63 ∀ 0.11	1.61 ∀ 0.12	1.66 ∀ 0.12	
	III	1.81 ∀ 0.09	1.64 ∀ 0.10	1.55 ∀ 0.11	
EPSS (mm)	I	20.67 ∀ 1.36	18.33 ∀ 1.17	16.83 ∀ 1.01	1.88
	II	19.83 ∀ 1.82	19.50 ∀ 1.48	20.17 ∀ 1.51	
	III	20.33 ∀ 1.12	18.17 ∀ 1.08	15.17 ∀ 1.30	

* - Significant (P # 0.05).

Mean values bearing the same superscripts in the rows and columns do not vary significantly for individual parameters.

Table - 5

Mean ∀ SE values of Fractional shortening and Ejection fraction of DCM dogs in treatment groups

Parameters	Groups	O day	30 days	60 days	F ratio
FS (%)	I	12.00 ∀ 0.82^{a}	16.33 ∀ 0.71^{bc}	20.00 ∀ 1.00^{cd}	10.92**
	II	12.17 ∀ 1.35^{a}	15.83 ∀ 1.62^{abc}	13.17 ∀ 1.92^{ab}	
	II	12.00 ∀ 0.77^{a}	18.00 ∀ 0.52^{c}	22.83 ∀ 1.14^{d}	
EF (%)	I	31.17 ∀ 2.14^{a}	39.83 ∀ 1.17^{abc}	49.17 ∀ 1.78^{cd}	8.21**
	II	32.17 ∀ 3.07^{a}	40.00 ∀ 3.48^{abc}	34.33 ∀ 4.20^{ab}	
	III	32.33 ∀ 1.61^{a}	43.50 ∀ 1.23^{bcd}	50.50 ∀ 2.59^{d}	

** - Highly Significant (P # 0.01).

Mean values bearing the same superscripts in the rows and columns do not vary significantly for individual parameters.

Table - 6

Mean ∀ SE values of Ventricular and Atrial dimensions of MVI dogs in treatment groups

Parameters	Groups	O day	30 days	60 days	F ratio
$LVID_d$ (mm)	I	52.67 ∀ 2.33	49.67 ∀ 2.44	48.83 ∀ 2.40	0.96
	II	53.00 ∀ 2.52	52.17 ∀ 2.40	53.50 ∀ 2.39	
	III	52.50 ∀ 2.62	49.50 ∀ 2.72	46.50 ∀ 2.40	
$LVID_s$ (mm)	I	33.50 ∀ 3.63	31.33 ∀ 2.78	30.00 ∀ 1.89	0.83
	II	34.00 ∀ 3.77	35.00 ∀ 2.94	36.00 ∀ 2.54	
	III	32.50 ∀ 3.53	29.50 ∀ 2.62	28.17 ∀ 1.82	
LA (mm)	I	35.17 ∀ 2.48	32.67 ∀ 2.33	28.67 ∀ 1.26	1.75
	II	35.50 ∀ 2.23	35.67 ∀ 2.65	36.67 ∀ 2.53	
	III	35.33 ∀ 2.30	31.50 ∀ 2.23	28.33 ∀ 2.46	
Ao (mm)	I	$18.00 \forall 1.18^{a}$	$20.50 \forall 1.34^{ab}$	$22.33 \forall 1.26^{b}$	2.57^{*}
	II	$18.17\ 1.40^{a}$	$18.00 \forall 1.13^{a}$	$18.33 \forall 1.20^{a}$	
	III	$18.50 \forall 1.61^{a}$	$21.33 \forall 1.48^{ab}$	$23.83 \forall 1.62^{b}$	
LA/Ao ratio	I	$1.96 \forall 0.08^{d}$	$1.59 \forall 0.05^{c}$	$1.36 \forall 0.05^{ab}{}_{*}$	44.48^{**}
	II	$1.96 \forall 0.04^{d}$	$1.97 \forall 0.03^{d}$	$1.99 \forall 0.03^{d}$	
	III	$1.93 \forall 0.04^{d}$	$1.48 \forall 0.04^{bc}$	$1.20 \forall 0.03^{a}{}_{*}$	
FS (%)	I	37.00 ∀ 4.13	37.67 ∀ 2.53	38.67 ∀ 1.52	0.30
	II	37.67 ∀ 4.63	34.83 ∀ 2.79	35.00 ∀ 2.54	
	III	38.50 ∀ 4.57	40.50 ∀ 3.58	39.50 ∀ 1.59	

* - Significant (P # 0.05)
** - Highly Significant (P # 0.01).

Mean values bearing the same superscripts in the rows and columns do not vary significantly for individual parameters.

Means carrying * in the subscript differ significantly at 5% level (P # 0.05).

Table - 7

Percentage of overall evaluation and survival rate in treatment groups

S.No	Variable	Group I (n=12)	Group II (n=12)	Group III (n=12)
1.	Greatly improved	33%	0%	42%
2.	Improved	50%	33%	50%
3.	Remained the same	17%	25%	8%
4.	Worse	0%	42%	0%
S.No	**Variable**	**Group I (n=10)**	**Group II (n=10)**	**Group III (n=10)**
1.	Survival rate (5 months post treatment)	**60%**	**10%**	**80%**

FIGURE - 1
BREED DISTRIBUTION IN DCM DOGS

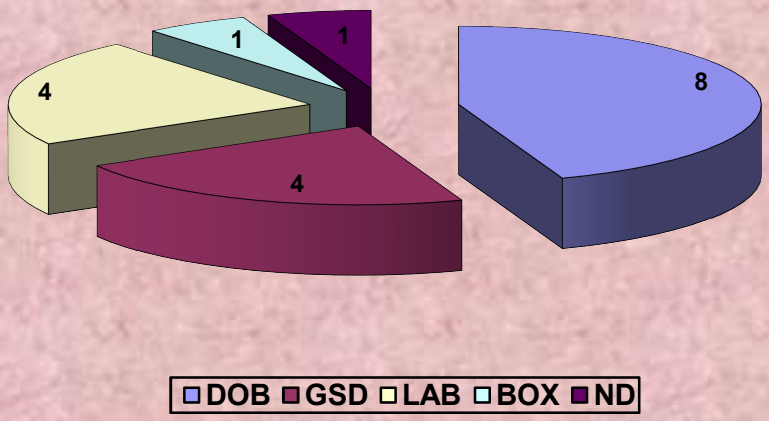

FIGURE - 2
BREED DISTRIBUTION IN MVI DOGS

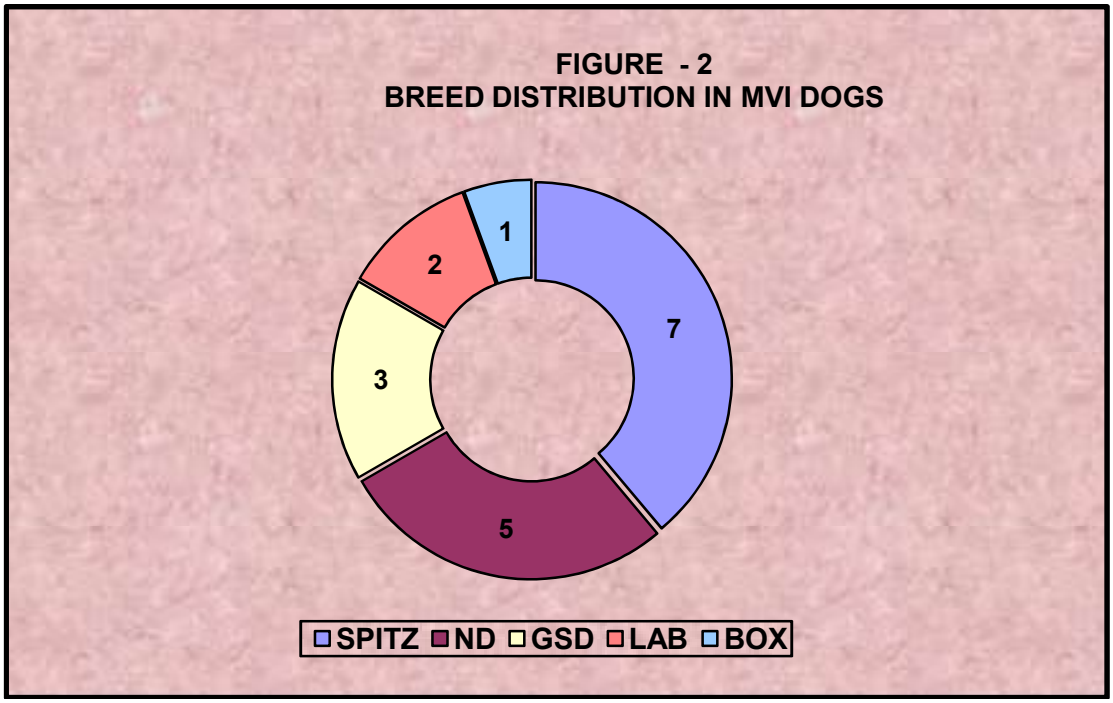

FIGURE - 3
SEX DISTRIBUTION IN CHF DOGS

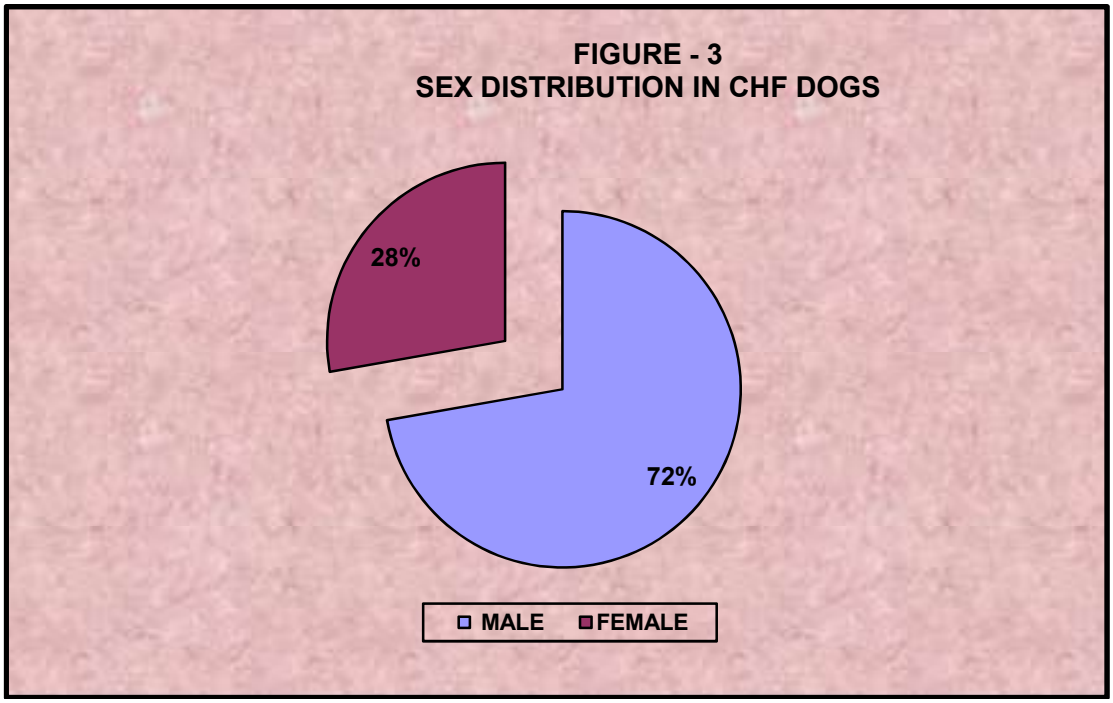

FIGURE - 4
AVERAGE AGE OF DOGS AFFECTED IN DCM AND MVI

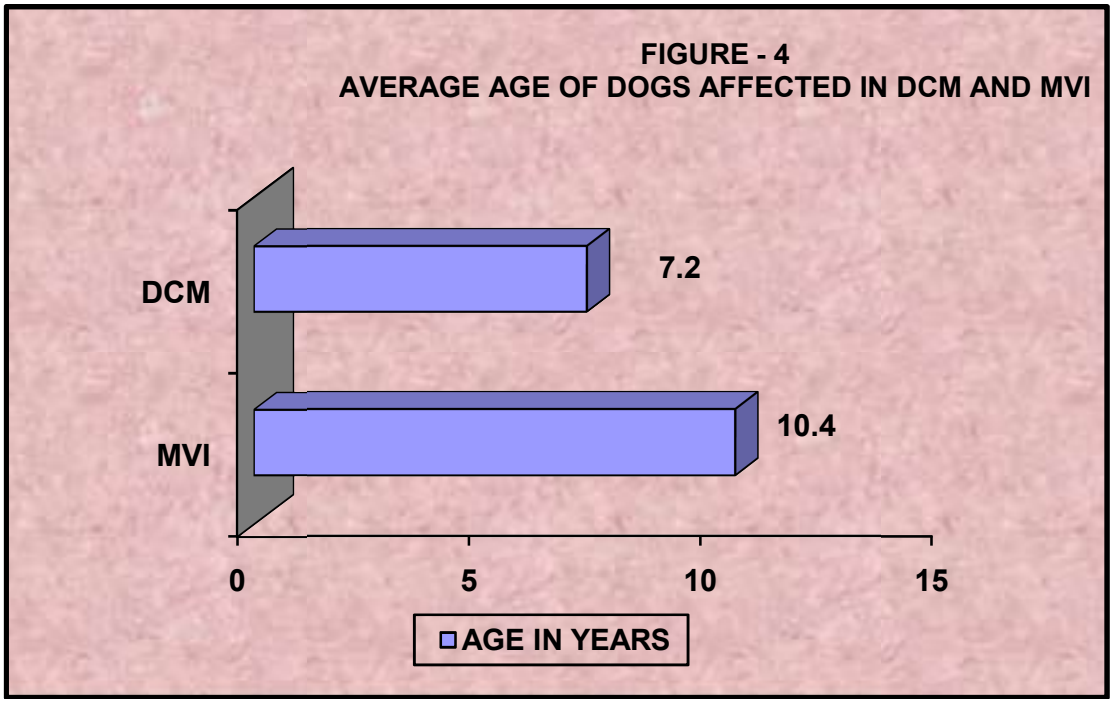

FIGURE - 6
COMPARISION OF FRACTIONAL SHORTENING BETWEEN TREATMENT GROUPS IN DCM DOGS

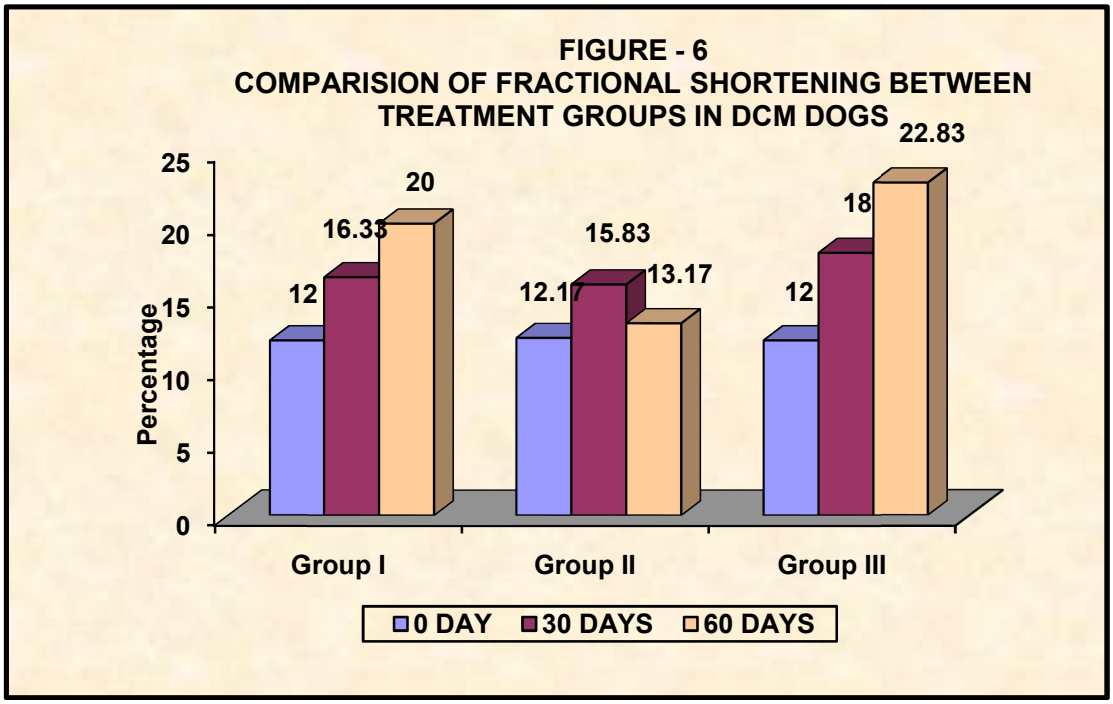

FIGURE - 7
COMPARISION OF EJECTION FRACTION BETWEEN TREATMENT GROUPS IN DCM DOGS

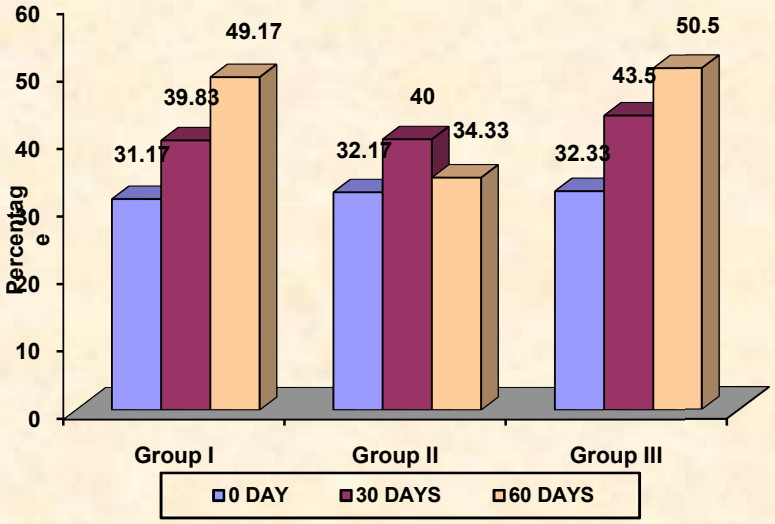

FIGURE - 8
COMPARISION OF LA/Ao RATIO BETWEEN TREATMENT GROUPS IN MVI DOGS