EFFICACY OF *TERMINALIA ARJUNA* IN DILATED CARDIOMYOPATHY AND VALVULAR HEART DISEASE IN DOGS

T H E S I S

Submitted
in partial fulfillment of the requirements for the Degree of

M A S T E R OF V E T E R I N A R Y S C I E N C E

IN

VETERINARY PHARMACOLOGY AND TOXICOLOGY

BY

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Enrolment No : V/04/0233

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PGR-ANNEXURE-XIII

DECLARATION BY THE STUDENT

I hereby declare that the experimental Research work and interpretation of the thesis entitled, " **EFFICACY OF *TERMINALIA ARJUNA* IN DILATED CARDIOMYOPATHY AND VALVULAR HEART DISEASE IN DOGS** " or part thereof has not been submitted for any other degree or diploma of any University, nor the data have been derived from any thesis/publication of any University or scientific organization. The sources of materials used and all assistance received during the course of investigation have been duly acknowledged.

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Chairman, Advisory Committee
with date

PGR-ANNEXURE-XIV

DECLARATION OF ADVISORY COMMITTEE

Ku. PARANJAPE VAIDEHI VINAY has satisfactorily prosecuted her course of research for a period of not less than one semester and that the thesis entitled, **" EFFICACY OF *TERMINALIA ARJUNA* IN DILATED CARDIOMYOPATHY AND VALVULAR HEART DISEASE IN DOGS "** submitted by her is the result of research work is sufficient to warrant its presentation to the examination in the subject of Veterinary Pharmacology and Toxicology for the award of **MASTER OF VETERINARY SCIENCE** degree by the Maharashtra Animal and Fishery Sciences University, Nagpur.

We also certify that the thesis or part there of has not been previously submitted by her for a degree of any other University.

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PGR-ANNEXURE-XV

CERTIFICATE

This is to certify that the thesis entitled, **" EFFICACY OF *TERMINALIA ARJUNA* IN DILATED CARDIOMYOPATHY AND VALVULAR HEART DISEASE IN DOGS** ", submitted by **MISS PARANJAPE VAIDEHI VINAY** to the Maharashtra Animal and Fishery Sciences University in partial fulfillment of the requirement for the degree of **MASTER IN VETERINARY SCIENCE** has been approved by the Student's Advisory Committee after examination in collaboration with the External Examiner.

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Date:

Place: Mumbai-12 (Paranjape Vaidehi Vinay)

TABLE OF CONTENTS

LIST OF APPENDICES

LIST OF TABLES

LIST OF FIGURES

LIST OF FIGURES (Contd.)

LIST OF ABBREVIATIONS

2 D	Two Dimensional
ACE	Angiotensin Converting Enzyme
AETA	Aqueous extract *Terminalia arjuna*
AML	Anterior Mitral Leaflet
ANP	Atrial natriuretic peptide
AO	Aorta
AqE	Aqueous extract
ARVM	Adult rat ventricular myocyte
AV	Atrio-Ventricular
aVF	Augmented vector foot
aVL	Augmented vector left
aVR	Augmented vector right
bpm	beats per minute
B.P	Blood Pressure
BNP	B-Type natriuretic peptide
CAT	Catalase
CHF	Congestive Heart Failure
CL	Confidence Limits
CNS	Central Nervous System
CPK	Creatine phosphokinase
CRT	Capillary refill time
CT	Chordae Tendinae
D5	Dextrose 5%
DCM	Dilated Cardiomyopathy
ECG	Electrocardiogram
EF	Ejection Fraction
EPSS	End Point Septal Separation
FS	Fractional Shortening
GFR	Glomerular Filtration Rate
GI	Gastrointestinal
GSH	Glutathione

HCl	Hydrochloride
HDL	High density lipoprotein
HF	Heart Failure
HSP72	Heat shock protein 72
I.V	Intravenous
IVSd	Inter ventricular septum dimension at diastole
IVSs	Inter ventricular septum dimension at systole
kVp	Kilovolt peak
LA	Left atrium
LD_{50}	Lethal dose 50
LDL	Low density lipoprotein
LPS LAx	Left parasternal long axis
LPS SAx	Left parasternal short axis
LV	Left Ventricle
LV (d*P*/d*t*)	Integrated measure of LV pressure change
LVDd	Left ventricular dimension at diastole
LVDs	Left ventricular dimension at systole
LVEF	Left ventricular ejection fraction
LVIDd	Left ventricular internal dimension at diastole
LVIDs	Left ventricular internal dimension at systole
LVM	Left ventricular mass
LVOT	Left ventricular outflow tract
LVP	Left ventricular pressure
LVPWd	Left ventricular posterior wall thickness at diastole
LVPWs	Left ventricular posterior wall thickness at diastole
mAs	Milliampere second
MPO	Myelo peroxidase
MR	Mitral Regurge
MV	Mitral Valve
NSAID's	Non-Steroidal Anti-Inflammatory Drugs
NT-proBNP	N-terminal probrain natriuretic peptide
OPC's	Oligomeric proanthocyanidins
PDE	Phosphodiesterase

PO	Per Os
RA	Right atrium
PHT	Pulmonary hypertension
PO	Per Os
PT	Pulmonic Trunk
PV	Pulmonic Valve
PVS	Peripheral vagal stimulation
RA	Right atrium
RAAS	Renin-angiotensin-aldosterone system
RPS LAx	Right parasternal long axis
RPS SAx	Right parasternal short axis
RV	Right ventricle
S.C	Subcutaneous
S3	Third heart sound
SDH	Succinate dehydrogenase
SGOT	Serum glutamic oxaloacetic transaminase
SGPT	Serum glutamic pyruvic transaminase
SLE	Systemic lupus erythematosus
SOD	Superoxide dismutase
TA	*Terminalia arjuna*
TBARS	Thiobarbituric acid reactive substance
TNF-α	Tumour necrosis factor alpha
TRFV	Tricuspid regurgitant flow velocity
VHD	Valvular Heart Disease
VHS	Vertebral Heart Score
VPCs	Ventricular Premature Complexes
yGT	Glutamyl transpeptidase

1. INTRODUCTION

The past 25 years have witnessed remarkable advances in the discipline of veterinary cardiology and veterinary diagnostics have undergone a radical change, which has led to changes in therapeutic approach. Patients at risk can now be evaluated earlier and those identified as having a disease can be monitored carefully and treated appropriately. The ability to generate ever greater amounts of diagnostic information creates its own set of challenges. We must first understand the relevance of the information and, just as important, the limitations of the data that these technologies generate to effectively incorporate this information into successful patient management. Today's clinical environment offers an impressive array of technologic advances. High resolution, non-invasive imaging has become a gold standard for morphologic diagnosis. A broad range of therapeutic options are now available including a plethora of new cardiovascular drugs and related agents. The field of cardiology is one area in veterinary diagnostics that has seen tremendous advances. Examples include tissue Doppler echocardiography and a new set of blood-based tests that measure cardiac biomarkers, such as NTproBNP.

Cardiovascular disorders in dogs represent a substantial portion of diseases seen in the average veterinary practice all over the world but meagre information has been reported in India. Hence it became important to understand the principles of diagnosis and treatment of the numerous cardiovascular disorders affecting dogs. In India however, the recognition of dog cardiac diseases was delayed, and ignored on account of lack of awareness and knowledge by the owner and inadequate diagnostic facilities provided to a field veterinarian. Cardiac ailments were often termed as 'silent killers' , as they left owners wondering what caused the loss of their treasured pet and what could they have done to prevent it. During recent years, with the growth of dog population in urban India, alacrity exhibited by the owners to educate themselves about the illness of their pets and willingness to finance the medical therapy for their pets, aroused the need for variant diagnostic methods in the field of veterinary cardiology. Pet owners began visiting a veterinarian regularly to ensure

early diagnosis and immediate treatment for cardiac diseases to prolong life of their pets.

The most common cause of heart failure in dogs is valvular heart disease (VHD), also known as chronic degenerative AV valve disease, endocardiosis, mucoid or myxomatous valvular degeneration, and chronic valvular fibrosis. The mitral valve is usually affected most, but occasionally the tricuspid valves are also involved. Isolated tricuspid valve degenerative disease is uncommon. Aortic or pulmonic valve thickening is seen in some older animals, but insufficiency is usually only mild. Dilated cardiomyopathy (DCM) is the second most commonly encountered cardiac disease in dogs leading to congestive heart failure. It is an idiopathic disease of the myocardium that is characterized by impaired myocardial contractility with dilation of the left ventricle or both ventricles. Cardiac chamber dilation follows progressive deterioration of systolic pump function, as cardiac output declines and compensatory volume expansion mechanisms are activated. Typically, all chambers dilate, although left atrium and left ventricle enlargement may predominate. Eventually, as the myopathy (heart muscle disease) progresses, the heart itself enlarges and the heart valves start to leak. Basic diagnostics to investigate a VHD or a DCM include detailed physical examination (cardiac auscultation primarily), thoracic radiology, Doppler blood pressure measurement, electrocardiography and 2D and M mode echocardiography.

Congestive heart failure is the inability of the heart to provide adequate circulation to meet the body's needs. It is the end result of a weakened cardiac muscle. A diseased heart can compensate for many months or years without signs of failure. When failure does occur, it may appear suddenly and unexpectedly, sometimes immediately post strenuous exercise, when the heart is unable to keep up with the body's demands. In toy and small-breed dogs, VHD with mitral regurgitation is the most common cause of congestive heart failure. In large-breed dogs it is DCM. Sometimes both these cardiac ailments occur simultaneously. The early signs of congestive heart failure are lethargy, decrease in activity level, and intermittent coughing. The coughing occurs during periods of exertion or excitation. Dogs may be restless-pacing instead of quickly settling down to sleep. As heart failure progresses the dog develops other signs, such as

lack of appetite, rapid breathing, ascites and a marked loss of weight. Because the heart no longer pumps effectively, blood backs up in the lungs, liver, limbs and other organs. Pulmonary oedema indicates failure of the left ventricle. With failure of the right ventricle, symptoms like ascites, dependent oedema and pleural effusion occur. Once CHF advances, it is difficult to revert it. Hence early diagnosis and therapeutic management aids in identifying the cardiac disease and delays its progression to an advanced stage.

Once diagnosed, the standard treatment then involves a use of positive inotropes, diuretics, and ACE inhibitors. The widely used positive inotrope overseas in veterinary cardiology is pimobendan. Pimobendan sensitizes and increases the binding efficiency of cardiac myofibril to the calcium ions that are already present without increasing the consumption of oxygen and energy. It also causes peripheral vasodilation by inhibiting the function of phosphodiesterase III. Hence it is classified as an inodilator. It results in decreased pressure, translating into smaller cardiac preload and afterload (decreases the failing heart's workload) and modulates cytokines to improve survivability. Based on pimobendan's pharmacodynamic profile, it appears to be ideal to improve quality of life and extend survivability in dogs with VHD and DCM in dogs. In spite of these qualities, it's not widely used in India by the veterinarians. The reason being it is difficult to procure this drug in time as it has to be shipped from the UK, USA and Canada only on the order of a licensed veterinarian and also it being expensive for pet owners in India. Hence, in India, it is still replaced by digoxin in spite of it giving better results than digoxin. This study was hence planned with an objective for providing an easily available, affordable and effective remedy that could substitute the modern contemporary treatment i.e. pimobendan along with enalapril and furosemide in treatment of VHD or DCM leading to CHF in dogs.

Ayurvedic therapies enjoy a respectable position today, especially in the developing countries, where modern health services are limited. Safe, effective and inexpensive indigenous remedies are gaining popularity among the people of both urban and rural areas especially in India. One such ayurvedic wonder drug is *Terminalia arjuna.* Apart from having medicinal effects on the digestive, respiratory, integumentry, urinary, reproductive and musculo-skeletal systems, it possesses a prime effect on the cardiovascular system. Researchers

have proven it to possess positive inotropic, hypotensive, anti-oxidant, hypolipidaemic and a variety of other cardiotonic properties. Gatne *et al.* (2009) in their unpublished work demonstrated the cardiac stimulant property of arjuna on isolated rat heart. It is widely used in human medicine to treat angina pectoris, congestive cardiac failure, ischemic heart disease, cardiomyopathy and hypercholesterolemia. It would be thus interesting to validate the available data by putting it to use in clinical cardiac patients in the field veterinary cardiology. The use of arjuna in veterinary cardiology has not been validated in spite of it possessing these versatile properties and its use in human cardiology. *Terminalia arjuna* was therefore selected for the study as a positive inotropic and hypotensive herb, to determine its efficacy in VHD and/or DCM progressing into CHF in clinical dog patients, so as to evaluate it clinically as an alternative to pimobendan.

With due consideration of the above background, the present project was proposed with following objective:

1. To compare, evaluate and study if the extract of Terminalia arjuna along with enalapril and furosemide can substitute pimobendan along with enalapril and furosemide as an alternative therapy in treatment of valvular heart disease and/or dilated cardiomyopathy resulting in congestive heart failure in dog patients.

2. REVIEW OF LITERATURE

Ancient Indian physicians used the powdered tree bark of *Terminalia arjuna* for alleviating “hritshool” (angina) and other cardiovascular conditions. Experimental studies have revealed its bark exerting significant inotropic and hypotensive effect, increasing coronary artery flow and protecting myocardium against ischemic damage thus making *Terminalia arjuna* unique amongst currently used medicinal plants in cardiology.

The literature pertaining to the present research work has been reviewed under following categories.

1. Pharmacognosy of *Terminalia arjuna*
2. Phytochemistry of *Terminalia arjuna*
3. Mechanism of action of *Terminalia arjuna*
4. Animal studies explaining cardiac properties of *Terminalia arjuna*
5. Other medicinal properties of *Terminalia arjuna*
6. Research using *Terminalia arjuna* in dogs
7. Incidence of valvular heart disease (VHD) and/or dilated cardiomyopathy (DCM) leading to congestive heart failure (CHF) in dogs
8. Diagnosis of VHD and/or DCM in dogs
9. Treatment of VHD and/or DCM in dogs
10. Pimobendan in dogs

2.1 Pharmacognosy of *Terminalia arjuna*

Terminalia arjuna is a deciduous and evergreen tree, standing 20–30m above ground level (Fig.1). It belongs to Combretaceae family (Chopra and Ghosh, 1929; Caius et al., 1930; Nadkarni and Nadkarni, 1954). It is found in abundance throughout Indo-sub-Himalayan tracts of Uttar Pradesh, South Bihar, Madhya Pradesh, parts of Maharashtra, Delhi and Deccan region near ponds, rivers, ravines and dry water beds. It is also found in forests of Sri Lanka, Burma and Mauritius (Chopra *et al*., 1958) where it was introduced by early Indian migrants (Pettit *et al*., 1996; Gurib- Fakim *et al*., 1997). Remarkably the tree is pest and disease free.

The bark of *Terminalia arjuna* is smooth, pinkish-grey from outside and flakes off in large, curved and rather flat pieces (Shah and Bhavsar, 1956) (Fig.2).The histology of *Terminalia arjuna* bark reveals the presence of single layered epidermis with hair like projections and few scattered lenticels. Underlying the epidermis is a thin layer of cortex. Periderm and secondary phloem are present in the old bark (Prasad, 1941). Leaves are simple, borne sub-opposite coriaceous, often crenulating, oblong or elliptic (Fig. 3). Their upper face is pale or dark green and the lower face is pale brown. Leaf measures 10–15 cm long and 4–7 cm broad. A network of 10–15 pairs of nerves is arranged in reticulate fashion. Petioles are 6–10mm long with one or usually two prominent glands at the top, immediately below the leaf. This is a unique pharmacognostic feature of *Terminalia arjuna*. The tree bears white sessile flowers arranged in short axillary spikes or in terminal pannicule. The flowers are bisexual. Each flower consists of 10 stamens and an ovary which is disk-clothed with yellowish or reddish hairs. Linear, lanceolate-like bracteoles are present. Calyx is glabrous. Its fruit is a drupe, 2.5–5 cm long, ovoid or oblong, fibrous-woody, smooth-skinned with five hard angles or wings (Fig.2). The lines of the wings are oblique and curved upwards.

2.2 Phytochemistry of *Terminalia arjuna*

As the bark was considered to be the most important constituent from medicinal point of view, most of the early studies were limited to bark stem of the plant. It was initially reported that the bark had 34% ash content consisting entirely of pure calcium carbonate. The aqueous extract revealed 23% calcium salts and 16% tannins, whereas the alcoholic extract contained very little colouring matter and tannins. Later chemical analysis of the bark showed evidence of sugar, tannins (12%), colouring matter, a glycoside, and carbonates of calcium, sodium and traces of chloride of alkali metals (Ghoshal, 1909). Subsequently presence of an alkaloid as well as a glycoside was confirmed. The glycoside was capable of increasing the force of contraction of the frog heart (Ghosh, 1926). Attempt to isolate the glycoside resulted into finding of an organic acid with a high melting point, a phytosterol, an organic ester easily hydrolysed by mineral acids, 12% tannins consisting largely of pyrocatechol tannins, large quantities of calcium, small amounts of aluminium and magnesium salts,

colouring matter and sugar (Chopra and Ghosh, 1929). Major chemical constituents of various parts of *Terminalia arjuna* have been studied by various researchers and have been summarised by Dwivedi and Udupa (1989) as follows:

2.2. a Stem bark - B Sitosterol, Triterpenoids like arjunin, arjunic acid, arjunolic acid, arjungenin, terminic acid. Glycosides like arjunetin, arjunoside I, arjunoside II, arjunaphthanoloside, terminoside A. Flavonoids like arjunolone, arjunone, bicalein, luteolin, gallic acid, ethyl gallate, quercetin, kempferol, pelorgonidin, oligomeric proanthocyanidins. Tanins like pyrocatechols, punicallin, punicalagin, terchebulin, terflavin C,castalagin, casuariin, casuarinin. Minerals/trace elements like calcium, aluminium, magnesium, silica, zinc, and copper.

2.2. b Roots - B Sitosterol, Triterpenoids like arjunic acid, arjunolic acid, oleanolic acid, terminic acid. Glycosides like arjunoside I, arjunoside II, arjunoside III and arjunoside IV.

2.2. c Leaves and fruits - Glycosides and Flavonoids like luteolin.

2.3 Mechanism of action of *Terminalia arjuna*

Terminalia arjuna has been used in Ayurveda since 2500 B.C. It has remarkable cardioprotective and heart muscle strengthening properties**.** Improvement of cardiac muscle function and subsequent improved pumping activity of the heart seems to be the primary benefit of *Terminalia arjuna* (Colabawalla, 1951). It is thought that saponin glycosides might be responsible for the inotropic effects of *Terminalia arjuna*, while flavonoids and oligomeric proanthocyanidins (OPC's) provide free radical anti-oxidant activity and vascular strengthing. Bark of arjuna tree has been found to be rich in Co-enzyme Q-10 which is highly prescribed in now a days to prevent heart problems. High amounts of Co-enzyme Q-10 prevent incidence of heart attacks. It also has a tonic effect in cases of cirrhosis of the liver. It induces a drug-dependent decrease in blood pressure and heart rate (Singh *et al*., 1982). It has been reported to possess protective cardiovascular and hypolipidemic properties (Shaila, 1998). No toxicity has been documented.

2.4 Animal studies explaining cardiac properties of *Terminalia arjuna*

The therapeutic use of *Terminalia arjuna* for cardiac ailments was based on empirical observations recorded in various treatises of ancient medicine. However, scientific investigations into the physiological and pharmacological actions and critical evaluation of its cardioprotective effects were done only in 20th century.

2.4.1 Cardiac stimulant properties

Early physiological studies carried on the isolated frog and rabbit heart revealed that the bark of *Terminalia arjuna* had cardiotonic and stimulant actions (Ghoshal, 1909). Later on it was detected that the bark powder, in addition to cardiotonic property, also possessed diuretic properties (Caius *et al*., 1930). Subsequent experimental studies in isolated frog heart revealed that the aqueous extract of the bark had chronotropic and inotropic activities (Chopra *et al*., 1958). This was later on confirmed by another study wherein water soluble portion of total alcoholic extract of *Terminalia arjuna* caused increase in force of contraction of frog heart (Gupta, 1974). The same group again reported that intravenous administration of alcoholic extract of *Terminalia arjuna* enhanced auricular and ventricular contraction in rabbits (Gupta *et al*., 1976).

Radhakrishnan *et al.* (1993) reported that an aqueous, ethanolic, chloroform and petroleum ether extracts of *Terminalia arjuna* increased contractile force of rat isolated atria. The aqueous extract produced a substantial positive inotropic effect (EC_{50} = 0.25 mg/ml) but no change in the rate. The ethanol, chloroform and petroleum ether extracts all decreased the rate of contraction. A slight increase in force was seen with ethanol and chloroform extracts. It was thus concluded that *Terminalia arjuna* had a significant positive inotropic property on rat atria *in vitro* which could contribute to its efficacy in treating cardiovascular disorders.

Karamsetty *et al.* (1995) studied effects of an aqueous extract of *Terminalia arjuna* to characterize its positive inotropic effect in an isolated paced rat left atria and effects on a vascular smooth muscle preparation, the rat thoracic

aorta. The crude and semipurified aqueous extracts produced a positive inotropic effect of rat atria and the maximum contraction was comparable to that produced by isoprenaline. The positive inotropic effect of the extract was completely blocked by a β-adrenoceptor blocker, propranolol, and an uptake-1 blocker, cocaine. In precontracted aorta, the aqueous extract produced contraction followed by relaxation. Propranolol did not block the relaxant effect of the aqueous extract. It was hence concluded that the positive inotropic effect of the aqueous extract were mediated via an action on β_1-adrenoceptors and were likely to be due to the release of noradrenaline from the sympathetic nerve endings increasing the heart rate to some extent. The vasorelaxant effect of the extract, however, was not mediated via an action on β_2 adrenoceptor. Oberoi and Liu (2007) stated that aqueous extract of *Terminalia arjuna* bark (AETA) exerted a positive ionotropic effect on adult rat ventricular myocytes and contained a potential novel class of cardiac tonic molecules.

After Dwivedi and Jauhari (1997) reported that extract of *Terminalia arjuna* improved left ventricular ejection fraction and possessed left ventricular mass lowering effect in humans suffering from angina, to validate similar data in dogs, Rao *et al.* (2009) proved that extract of *Terminalia arjuna* improved left ventricular ejection fraction and fractional shortening considerably in dogs suffering from congestive heart failure.

2.4.2 Effect on coronary flow

Injection of the aqueous extract of the bark in isolated rabbit heart preparation (Langendorff's) was noted to produce increase in coronary flow. The concentration which caused maximum increase in coronary flow was 1024 ug/ml (Bhatia et al., 1998).

2.4.3 Hypotensive effects

Experimental studies to evaluate its effect on blood pressure started quite late whereas its clinical use as an anti-hypertensive/diuretic started much earlier (Colabawalla, 1951). Singh *et al*. (1982) reported dose-dependent sustained hypotension and bradycardia following intracerebro-ventricular and

intravertebral injection of aqueous and alcoholic extract of its bark in chloralose anaesthetized dogs, in doses as small as 1/10th and 1/20th, respectively to that of intravenous dose. Since the bradycardia and hypotension could be blocked by prior bilateral vagotomy and as the intravertebral administration could produce these effects in a lower dose, it was proposed that the active constituent in the extract acts centrally. Further, the hypotensive effect of the alcoholic extract in dogs was abolished by pre-treatment with atropine. These observations got further support by another study conducted in dogs wherein intravenous administration of aqueous extract of *Terminalia arjuna* resulted in dose-dependent fall in blood pressure (Srivastava *et al*., 1992). In another experiment the aqueous extract in different doses was administered intravenously by a femoral cannula to the anaesthetized dogs. It was observed that 40 mg/kg dose produced a sustained fall of blood pressure for about 90 min. Its response to various neurohumoral agents like acetylcholine, adrenaline and niacin suggested that the drug acts by modifying the autonomic responses in the body (Bhatia *et al*., 2000). The effect of 70% alcoholic extract of *Terminalia arjuna* on blood pressure of anaesthetized dog was also investigated. The drug was administered in doses 5–10 mg/kg. It was observed that the drug produced dose-dependent hypotension which was blocked by propranolol (Nammi *et al*., 2003). A study on the rat thoracic aorta using aqueous extract revealed contraction of isolated rat thoracic aorta followed by relaxation. The initial contraction was blocked by propranolol whereas the vasorelaxant effect was unaffected. The aqueous extract as well as the fraction of the extract containing tannin-related compounds (F2) produced hypotensive effects (Takahashi *et al*., 1997).

2.4.4 Effect on aortic prostaglandins

The effect of *Terminalia arjuna* bark on prostaglandins was studied in rabbits subjected to isoproterenol induced myocardial ischemia. It was observed that aortic prostaglandin E_2 like activity was enhanced in those rabbits that were administered *Terminalia arjuna* compared to those that were on placebo. The finding of raised PGE_2 like activity was significant because PGE_2 is known to produce coronary vasodilation. This explained the pharmacological basis of the increased coronary flow following *Terminalia arjuna* infusion (Bhatia *et al*., 1998).

and contributed to the beneficial effect of *Terminalia arjuna* in coronary artery diseased patients (Dwivedi, 1998).

2.4.5 Cardio protective and anti-oxidant properties

The cardioprotective effects of *Terminalia arjuna* have been studied in isoproterenol-induced myocardial ischemia model in rats, rabbits and mice by several authors. It has also been compared with two other medicinal plants namely *Inula racemosa* and *Saussurea lappa*. Bark powder of *Terminalia arjuna*, root powder of *Inula racemosa* and *Saussurea lappa* 500 mg twice daily in emulsion form were administered as a gavage to three different group of rabbits. Another group was put on placebo powder along with standard feed. After 90 days of drug pre-treatment the rabbits were challenged with isoproterenol infusion through intravenous route. The onset of myocardial ischemia and its severity, both were reduced in rabbits pre-treated with *Terminalia arjuna* compared to placebo control rabbits (Dwivedi *et al*., 1988). The rabbits on *Inula racemosa* and *Saussurea lappa* did not show any significant cardioprotection. In another study, effect of a compound formulation (abana) containing *Terminalia arjuna* 30 mg per tablet, was studied in isoproterenol-induced myocardial necrosis in rats. Increase in serum CPK, SGOT, SGPT and yGT following myocardial necrosis were significantly reversed by abana (Tandon *et al*., 1995). Effect of arjunolic acid derived from *Terminalia arjuna* (15 mg/kg body weight) on antiplatelet activity, electrocardiographic changes, serum marker enzymes, antioxidant status, lipid peroxide and myeloperoxidase (MPO) was assessed and compared with the acetyl salicylic acid (ASA) in rats subjected to isoproterenol challenge. Arjunolic acid was proven the possible reason to protect the heart against the damage caused by myocardial necrosis (Sumitra *et al*., 2001).

Role of *Terminalia arjuna* as an antioxidant agent on ischemic perfused rat heart was studied by Gauthaman and his colleagues. The pulverised bark powder in 2% carboxy methyl cellulose was given in doses of 500 and 700 mg/kg orally 6 days per week by gavage up to 12 weeks. At the end of the experiment rats were subjected to ischemic injury by isoproterenol infusion for 2 days. The rats were then sacrificed and heart was isolated for estimation of thiobarbitutates, SOD, GSH and catalase. Hearts harvested from 500 mg/kg *Terminalia arjuna*

orally for 12 weeks treatment group were significantly protected from the oxidative stress caused by isoproterenol-induced ischemic reperfusion. It was thus concluded that crude bark of *Terminalia arjuna* augmented endogenous antioxidant compounds of rat heart and prevented it from oxidative stress (Gauthaman *et al*., 2001).

Karthikeyan *et al*. (2003) studied cardioprotective effect of the alcoholic extract of *Terminalia arjuna* bark in an in vivo model of myocardial ischemic reperfusion injury. The study was designed to investigate the effects of chronic administration of the alcoholic extract of *Terminalia arjuna* (TAAE) bark on isoproterenol induced myocardial injury. The study demonstrated that the 6.75 mg/kg TAAE augmented endogenous antioxidant compounds of the rat heart and also prevented the myocardium from isoproterenol induced myocardial ischemic reperfusion injury. A study conducted on similar lines in rabbits by Gauthaman *et al*. (2001) showed that *Terminalia arjuna* (TA) protected the heart against ischemic-reperfusion injury and role of antioxidant enzymes and heat shock protein**.** Oral administration of TA for 12 weeks in rabbits caused augmentation of myocardial antioxidants; superoxide dismutase (SOD), catalase (CAT) and glutathione (GSH) along with induction of heat shock protein72 (HSP72). It was thus concluded that in vivo ischemic-reperfusion injury induced oxidative stress while tissue injury of heart and haemodynamic effects were prevented in the TA treated rabbit hearts. The study provided scientific basis for the putative therapeutic effect of TA in ischemic heart disease.

Another study conducted by Singh et al. (2008) suggested that the butanolic fraction of *Terminalia arjuna* bark had protective effects against doxorubicin -induced cardiotoxicity and a cardioprotective potential. Parveen *et.al* (2010) determined mechanistic clues in the cardioprotective effect of *Terminalia arjuna* bark extract in isoproterenol-induced chronic heart failure in rats. The study demonstrated prophylactic and therapeutic potential of *Terminalia arjuna* bark extract in isoproterenol-induced chronic heart failure.

2.4.6 Effect on lipids

A case controlled study in rabbits fed on high cholesterol diet and administered Terminalia arjuna bark powder 250 mg/kg twice daily was carried out to determine its hypolipidaemic effect. It was found that the rabbits receiving *Terminalia arjuna* had a marked reduction in total cholesterol ($P < 0.02$) than control rabbits (Tiwari *et al*., 1990). Khanna *et al*. (1996) stated that *Terminalia arjuna,* regulated lipid metabolism in hyperlipidemic rats. The lipid lowering action was reported to be mediated through inhibition of hepatic cholesterol biosynthesis, increased faecal bile acid excretion and enhanced plasma lecithin:cholesterol acyltransferase activity and stimulation of receptor mediated catabolism of low density lipoproteins. In another experimental study this time using its ethanolic extract in doses of 100 and 500 mg/kg a significant reduction in total and LDL cholesterol were noted in hypercholesterolemic rabbits. At 500 mg/kg doses it also reduced total:HDL and LDL:HDL ratio. It was also found that fat deposition in heart, liver and kidney was significantly low in those who received the drug. Significantly the extract did not adversely affect biochemical tests of liver and renal functions and haematological parameters (Ram *et al*., 1997). However, it did not show any change in HDL levels. Besides hypolipidemia, it also induced partial inhibition of aortic atherosclerosis, thus showing anti-atherogenic properties (Shaila *et al*., 1998).

2.5 Other medicinal properties of *Terminalia arjuna*

Arjuna is very helpful in treating various health related problems. Below are actions of arjuna other than its cardiotonic properties on the body's different organ systems:

2.5.1 Local action

In view of its supposed beneficial effect on ischemic heart disease, effect of *Terminalia arjuna* on haemostyptic parameters were also investigated in rabbits (Chaturvedi, 1967). Nadkarni (1976) stated that arjuna could be widely used in stopping external haemorrhages as it has the power to coagulate blood and constrict the blood vessels locally. It can be applied on wounds to get instant

results. Arjuna is also one of the most powerful herbal supplements that is known for its healing powers. Good prognosis has been seen in cases of wounds and injuries.

2.5.2 Digestive system

Kirtikar and Basu (1935) stated that *Terminalia arjuna* is kshaya (astringent) in nature. Bakhru (2008) further confirmed that due to its astringent properties it is very helpful in treatment of diarrhoea and dysentery. It regulates the peristaltic movements in the body and prevents dehydration due to electrolyte imbalance. It may also be protective against gastric ulcers, such as those caused by NSAID's. One study demonstrated that the aqueous extract of the bark of *Terminalia arjuna* could protect the liver and kidney tissues probably by increasing anti-oxidative defence activities. It is also a good remedy in improving liver conditions, especially cirrhosis.

2.5.3 Respiratory system

Kumar *et al*. (1987) explained that *Terminalia arjuna* was found to have some expectorant and decongestant action and thus helped in expulsion of excess of mucous from respiratory passage. It is also helpful in maintaining normal tone of respiratory system (Colabawalla, 1951).

2.5.4 Nervous system

Nadkarni (1976) stated *Terminalia arjuna* as a good nervine tonic. It provides strength to the neurons and also strengthens the reflexes.

2.5.5 Reproductive system

Terminalia arjuna being astringent in nature helps in thickening of the spermic fluid that is very essential for the proper fertilization of the ovum. It is also helpful in increasing the sperm count and increasing the overall stamina of the body (Bakhru, 2008).

2.5.6 Endocrine system

Terminalia arjuna regulates the hormones of the body and maintains proper stimulation to the endocrine glands (Nadkarni, 1976).

2.6.7 Excretory system

Terminalia arjuna is proved to have a diuretic effect. It helps in toning up of urinary tract (Colabawalla, 1951).

2.5.8 Integumentary system

*Terminalia arjuna i*s extremely beneficial in treating all kinds of skin related problems. Due to its cooling effect, it is highly recommended in skin ailments. Ailments like eczema, itching, rashes scars and serious skin conditions like psoriasis can also be treated with the regular use of arjuna (Bakhru, 2008). *Terminalia arjuna* is also found to be having antibacterial property (Samy *et al.,* 1998).

2.5.9 Anti-mutagenic agent

Of late several publications regarding potential anti-mutagenic properties of *Terminalia arjuna* have also been reported (Pettit *et al*., 1996; Woodman and Chan, 2004).

2.6 Research using *Terminalia arjuna* in dogs

Gupta *et al.* (1976) carried out an in vivo study in dogs. They injected an alcoholic extract of the bark of *Terminalia arjuna*, intravenously in normal dogs and proved that it caused enhanced auricular and ventricular contractions.

Singh *et al.* (1982) stated that an aqueous and alcoholic bark extract of *Terminalia arjuna*, intravenous, intra cerebral and intravertebral in normal dogs, in vivo caused dose-dependent decrease in blood pressure.

Bhatia *et al.* (2000) experimentally evaluated the effect of *Terminalia arjuna* on blood pressure of anesthetized dogs. The study was designed to investigate the effect of different doses (10-40 mg/kg, IV.) of the aqueous extract (AqE) of the bark of *T.arjuna* on the blood pressure (B.P.) of anaesthetised dogs (n=6). Further, the effect of AqE on a standard bracket comprising recording of responses to adrenaline (Adr: 2 µg/kg), acetylcholine (Ach: 2 µg/kg), nor-adrenaline (N-Adr: 2 µg/kg), isoprenaline (Iso: 2 µg/kg), nicotine (5 ug/kg), peripheral vagal stimulation (PVS) and carotid occlusion was also studied. 10 mg/kg dose of AqE did not have any effect on B.P. while the 40 mg/kg dose produced a sustained fall in B.P. which lasted for approximately 90 min. On the basis of the results it was proved that the AqE of *T. arjuna* brought about beneficial B.P. lowering effect by modifying the autonomic responses in the body.

Nammi *et al.* (2003) studied possible mechanisms of hypotension produced by 70% alcoholic extract of *Terminalia arjuna* in anaesthetized dogs. Six dogs were anaesthetized with intraperitoneal injection of thiopental sodium and the blood pressure of each dog (n = 6) was measured from the left common carotid artery connected to a mercury manometer on kymograph. The femoral vein was cannulated for administration of drug solutions. The extract of *T. arjuna* (dissolved in propylene glycol) in the dose range of 5 to 15 mg/kg was administered intravenously in a pilot study and the dose (6 mg/kg) which produced appreciable hypotension was selected for further studies. Intravenous administration of *T. arjuna* produced dose-dependent hypotension in anaesthetized dogs. The hypotension produced by 6 mg/kg dose of the extract was blocked by propranolol but not by atropine or mepyramine maleate. This indicated that muscarinic or histaminergic mechanisms were not likely to be involved in the hypotension produced by the extract. The blockade by propranolol of the hypotension produced by *T. arjuna* indicated that the extract probably contained active compound(s) possessing adrenergic β_2-receptor agonist action that acted directly on the heart muscle. The results indicated the likely involvement of peripheral mechanism for hypotension produced by the 70% alcoholic extract of *Terminalia arjuna* and supported the claims of its traditional usage in cardiovascular disorders.

Rao *et al.* (2009) conducted a study to determine the cardiotonic property of *Terminalia arjuna* in dogs with congestive heart failure. 30 dogs with dilated cardiomyopathy were divided into groups I, II and III and treated with digoxin (0.22 mg/m^2), enalapril (0.5 mg/kg) and furosemide (2 mg/kg); bark extract of *T. arjuna* (250 mg/dog); and bark extract of *T. arjuna* with enalapril (0.25 mg/kg) and furosemide (2 mg/kg) twice daily for 60 days. Results revealed that clinical signs were minimized, exercise capacity significantly improved and risk of clinical deterioration of heart failure decreased in all treatment groups. Clinical improvement was observed between 15 and 30 days of treatment. It is concluded that the combination of *T. arjuna*, enalapril and furosemide is effective in the therapeutic management of congestive heart failure in dogs.

2.7 Incidence of valvular heart disease (VHD) and/or dilated cardiomyopathy (DCM) leading to congestive heart failure (CHF) in dogs

Congestive heart failure (CHF) is caused by an abnormality in the structure or function of the heart and occurs when high diastolic pressures in the heart “back up” into the veins and capillaries causing fluid to leak out of these vessels (oedema). Heart failure is the end-result of many different cardiac and pericardial diseases. Most important causes to CHF include either decreased myocardial contractility which is commonly seen with dilated cardiomyopathy, valvular regurgitation as seen with degenerative valvular heart disease or increased myocardial stiffness impairing the heart’s ability to fill with blood as seen with feline hypertrophic cardiomyopathy.

Chronic valvular heart disease (VHD) (Fig.4), also known as endocardiosis, mitral valve disease, acquired valve disease, myxomatous valvular degeneration is a chronic, degenerative process that affects the valves in the heart, primarily the atrio-ventricular valves (commonly mitral) causing valvular insufficiency. These valves prevent the back flow of blood from the ventricles into the atria during heart contractions. The result of this degenerative process is that the affected valves become thickened and irregular or thin and dysfunctional and no longer close properly. Blood is then allowed to flow back (regurgitation) into the atria, causing the heart to work harder by pumping additional blood volumes. This valvular regurgitation is a chronic problem, which unfortunately, continues to

progress. The prevalence and severity of the disease increases with age. At post mortem, more than half the population of old dogs have markedly distorted mitral valves. This is a most common cardiac disease and is seen in middle-aged and older dogs. Smaller breed dogs are more commonly affected than large breed dogs (Olsen *et al*., 2009).

VHD (mostly mitral, rarely tricuspid) is the most common cardiac disease in the dog; it is an acquired disease, and the prevalence is greatest in the geriatric population (Olsen *et al*., 2009). VHD may affect any breed of dog, but clinical consequences of VHD are observed most often in small- to medium sized breeds of dogs. Cavalier King Charles Spaniels and Dachshunds are over-represented and sometimes clinically evident at a young age. Miniature Poodles, Pomeranians, Yorkshire Terriers, Chihuahuas, and other small dogs are commonly affected. It accounts for around 75% of all cases of CHF in dogs. Clinical evidence of VHD is detected in approximately 30% of dogs aged 13 years and older. The incidence is reported as being between 11% (clinical determination) and 42% (necropsy determination) depending on the method of examination (Abbott, 2008). VHD is a progressive disease, and subtle changes in valve structure precede the development of clinically evident valvular dysfunction. The incidence of VHD is increased further, to above 60%, in aged dogs. The mitral valve alone is affected in 60% of dogs, with the mitral and tricuspid valves both affected in 30% of cases and the tricuspid valve alone in 10% of dogs (Abbott, 2008). The aortic and pulmonic valves may be affected but clinically important disease is uncommon. Signs of mitral valve disease and left-sided cardiac dysfunction predominate in most cases. Postmortem evidence of advanced degenerative valvular disease was found in 58% of dogs older than 9 years; when mild degenerative changes are included, the postmortem prevalence exceeds 90% in dogs older than 13 years. VHD also occurs in large breed dogs (i.e., German Shepherd, Doberman pinscher), but DCM is much more common in these breeds than VHD. The incidence of VHD is increased in male dogs relative to females (1.5 to 1.0). This is a slowly progressive disease in which lesions may begin in the first half of life (e.g., 2 to 3 years), but clinical disease is unlikely before middle age. In the early stages, the only clinical sign may be a cardiac murmur detected on routine examination. Cardiac decompensation and CHF

typically occurs in later life (e.g., 6 to 10 years of age or older) (Atkins *et al*., 2009).

Dilated cardiomyopathy (DCM) (Fig.5) is the second most commonly encountered cardiac disease in veterinary medicine and is an idiopathic disease of the myocardium characterized by progressive myocardial failure (pathologic decrease in contractility) and its inability to pump blood into the arteries. As a consequence of this reduced contractility, more blood remains into the heart chambers after each heart beat, causing a so-called "volume overload", which results in dilation of the heart, hence called dilated cardiomyopathy. The progressive myocardial failure results in enlargement of the cardiac chambers (sometimes one side worse than the other) in association with the compensatory mechanisms (renin-angiotensin-aldosterone system, sympathetic nervous system, and eccentric ventricular hypertrophy) that become activated in response to sustained cardiac dysfunction. This disease scenario is likely to be multiple diseases that are lumped into a group of idiopathic diseases that result in progressive myocardial failure, CHF, and occasionally, sudden death (Meurs, 2009).

It is the second most common cause of CHF other than VHD (Oyama, 2008). In general, DCM is seen in middle-age and older (median age is between 4–8 years) large and giant breed dogs (Meurs, 2009). Males seem to be affected more commonly than females. Breeds that are predisposed for this condition areDoberman Pinschers, Irish Wolfhounds, Great Danes, Newfoundlands, German Shepherds, Old English Sheepdogs, Boxer Dogs, St. Bernards, and Afgan Hounds, among others. Medium size breeds may also be affected including the Portuguese Water dog, the American and English Cocker Spaniel (associated with nutritional deficiency) and Dalmatians (Oyama, 2008). Geographic variations in the prevalence of this disease may suggest genetic and/or environmental factors that may significantly increase the risk of developing this condition. Dietary causes include deficiency of taurine and L- carnitine. Taurine helps to regulate heartbeat, helps calcium absorption during times of reduced oxygen and protects the heart from calcium overload. L-carnitine, on other hand helps by bringing fatty acids into muscle cells which are then converted into the required energy for the heart. In certain breeds, the prevalence

of DCM is remarkably high. Approximately 25% of Irish Wolfhounds, 50% of male Doberman Pinschers, and 33% of female Doberman Pinschers develop DCM. The typical age at diagnosis is between 6 and 8 years; however, it is not uncommon to diagnose DCM in dogs as young as 3 years and as old as 12 years (Oyama, 2008). Screening is recommended for dogs with prevalence of DCM to identify dogs with cardiac abnormalities (Meurs, 2009).

2.8 Diagnosis of VHD and/or DCM leading to CHF in dogs

Diagnosis of DCM and VHD terminating into CHF is done on basis of a good medical history of the patient along with clinical presentation, detailed physical examination and highly diagnostic techniques like thoracic radiology, electrocardiography, Doppler B.P measurement and 2-D and M mode echocardiography which is the interaction between ultrahigh- frequency sound waves and the heart allows the depiction of cardiac morphology, information on the movement of myocardium and valves, and blood flow within the heart. As the disease progresses, it often aids in selecting appropriate therapy (Fuentes, 2008).

Detection of VHD generally begins with auscultation of thorax on physical examination in an asymptomatic dog. Identification of a heart murmur of particular timing, quality and location is sufficient to accurately diagnose VHD in most dogs, particularly in breeds considered at high risk for the disease (Abbott, 2008). An acquired, left apical, systolic murmur in an older, small-breed dog is almost always due to VHD (usually affecting the mitral valve). An exaggerated apical impulse is often evident on precordial palpation of patients with moderate or severe mitral regurge (MR) with murmur intensity grade V/VI or greater. A high-frequency, mid-systolic click is sometimes heard in older, small-breed dogs (Olsen *et al*., 2009).These clicks may be associated with prolapse of the mitral valve. In contrast, a S_3 gallop usually reflects severe MR and generally is heard in patients with loud murmurs. Very severe MR can be associated with diminished femoral arterial pulse strength. Crackles may be heard in patients with pulmonary oedema (Haggstrom *et al*., 2004). In most cases, thoracic radiography is the most important element of the diagnostic approach to VHD. If clinically consequential MR develops, then there is enlargement of the cardiac silhouette.

Cardiomegaly can be easily determined in thorax radiographs and can be objectively measured with vertebral heart score (VHS) (Buchanan *et al*., 1995). In general, the left atrium can be assessed with the greatest certainty. This is fortunate because, in the overwhelming majority of cases, left atrial enlargement precedes the development of CHF. A diagnosis of left-sided CHF secondary to VHD rarely can be supported in the absence of radiographic left atrial enlargement. The radiographic finding of pulmonary venous distention reflects increases in pulmonary venous pressure. Pulmonary venous distention suggests pulmonary congestion and may precede the development of pulmonary edema. Electrocardiography is useful primarily for the diagnosis of arrhythmias but also can provide indirect insensitive evidence of chamber enlargement. Arrhythmias can complicate the presentation of VHD. Most often, arrhythmias take the form of supraventricular tachyarrhythmias that reflect atrial stretch. Atrial premature complexes and paroxysms of atrial tachycardia are relatively common. Atrial fibrillation develops occasionally and generally indicates advanced disease with marked atrial dilation. Ventricular arrhythmias (ventricular premature complexes) may develop in association with left ventricular dilation and myocardial fibrosis (Abbott, 2008). Echocardiographic examination of patients with VHD demonstrates variable degrees of left atrial and left ventricular dilation and increased EPSS in advanced stages. Hypertrophy is usually adequate to preserve a near-normal relationship between the diastolic luminal dimension and wall thickness. The mitral leaflets may be noticeably thicker than normal, and prolapse of the leaflets into the left atrium in systole is commonly observed. The echogenicity of affected leaflets is generally uniform and nodular thickening is diffuse (Moise and Fox, 1999). Often, the tricuspid leaflets are affected, although seldom as markedly as the mitral valve. Ejection phase indices of systolic performance such as ejection fraction and fractional shortening are often elevated because these variables are highly load-dependent. When MR is present, impedance to ventricular emptying is reduced because the ventricle is able to eject blood into the low-pressure reservoir of the left atrium. Additionally, end-diastolic ventricular stretch associated with MR increases the force of contraction and contributes to the finding of hyperdynamic ventricular performance. A normal or subnormal fractional shortening in the setting of moderate or severe MR suggests systolic myocardial dysfunction. Doppler echocardiography is used to evaluate velocity, direction, and character of blood

flow. Assessment of the severity of MR can be evaluated quantitatively or more often, semi-quantitatively by Doppler echocardiograph and can be rated as mild, moderate or severe (Abbott,2008).

DCM is an adult onset disease with a clinical presentation that may be as subtle as a gradual development of exercise intolerance and weight loss. However, more commonly the early signs of the disease are overlooked and the disease is not diagnosed until CHF develops and the patient presents with coughing, respiratory distress and occasionally, ascites (Harpster, 1983). The clinical evaluation of DCM starts with the signalment. It is extremely rare to diagnose DCM in small breed dogs. A history of syncope, exercise intolerance, coughing, or tachypnoea/dyspnoea may be noted. The clinical progression of DCM is best described as occurring in two distinct phases. Asymptomatic occult phase is a phase where no clinical signs are evident; however, myocardial or electrical abnormalities are present and may include increased left ventricular and atrial dimensions, decreased myocardial contractility, and ventricular premature beats (O'Grady and Horne, 1998).The duration of the occult phase is highly variable and thought to last for months to years. During this phase, progressive heart enlargement and worsening arrhythmias occur. Occult phase ends with the appearance of the first clinical signs of disease. While overt clinical phase is a phase where clinical signs of congestive heart failure develop and symptoms like syncope, exercise and activity intolerance are noted. Arrhythmias in the form of ventricular premature beats, ventricular tachycardia, and atrial fibrillation are common. Death is due either to advanced congestive heart failure that is refractory to medical therapy or sudden death (Oyama, 2008). History and physical examination findings in the asymptomatic occult phase include soft systolic heart murmur, irregular heart rhythm with pulse deficits and occasionally diastolic gallop rhythm, decreased intensity of heart sounds, weak femoral pulse quality jugular vein distension or pulses. While in the overt clinical phase symptoms like exercise intolerance, syncope, lethargy, anorexia, difficulty breathing, coughing, abdominal distension may be noted along with moderate intensity systolic heart murmur, irregular heart rhythm with pulse deficits, pulse alterans, increased respiratory rate and effort, increased bronchovesicular sounds, decreased intensity of heart sound and weakness are observed. Occasional findings include jugular vein distension or pulses, hepatomegaly,

ascites, pale mucous membranes, hypothermia, pulmonary crackles and depressed mentation (Oyama, 2008). Blood pressure measurement may reveal hypertension. The electrocardiogram (ECG) may be normal or may demonstrate a variety of rhythm disturbances and chamber enlargement/conduction abnormality patterns. Atrial fibrillation, characterized by a rapid, irregularly irregular narrow complex tachyarrhythmia with no P-waves, is common in dogs with DCM (Meurs, 2009). Ventricular premature complexes (VPCs) are common in dogs with DCM and may be associated with an increased risk of sudden death (depending on the severity and complexity of the arrhythmia) (O'Grady and Horne, 1998). If left atrial enlargement is present, the P-wave may be wide (P-mitrale). Increased R-wave amplitude indicates left ventricular enlargement. A right axis shift may indicate a conduction abnormality or cardiomyopathy primarily affecting the right side of the heart (Meurs, 2009). Thoracic radiographs are useful for detecting and assessing the presence of congestive heart failure. DCM can be associated with left sided CHF (pulmonary oedema), biventricular failure (combination of pleural effusion and pulmonary oedema), or right sided CHF (ascites). Radiographic CHF is usually associated with cardiomegaly and venous congestion. Echocardiographic evaluation of dogs with DCM reveals varying degrees of myocardial dysfunction as evidenced by a increased left ventricular dimensions, decreased fractional shortening, increased EPSS, increased end-systolic volume index, and reduced ejection fraction. Hemodynamically significant chronic myocardial failure results in chamber enlargement (eccentric hypertrophy) i.e. dilated left and right atria and ventricles are seen therefore allowing for larger volumes without significant increases in diastolic pressures (Moise and Fox, 1999). AV valvular insufficiency is common in cases with moderate to severe chamber enlargement. The jet of MR is typically centrally located in this scenario (compared to the typical eccentric jet seen with degenerative valve disease) (Oyama, 2008).

Apart from a blood biochemistry profile and urine analysis aiding in diagnosis of cardiac failure, there are now cardiac neurohormones and biomarkers that are measured to evaluate heart function and can be used for early detection of heart disease in dogs. Cardiac troponin I and T are specific markers of myocyte injury, ischemia, and necrosis. Atrial natriuretic peptide (ANP) and B-type natriuretic peptide (BNP) are produced by myocardial tissue in

response to increased pressure and wall stress and are markers for cardiac dysfunction and heart failure. Other special diagnostic techniques for evaluating cardiac diseases include holter monitoring and implant cardiac event recorders, continuous in-hospital electrocardiographic monitoring, provocative electrocardiographic techniques like vagal maneuver that may demonstrate sinus arrest or AV block, suggestive of parasympathetic hypersensitivity or primary nodal disease. Non selective angiography is occasionally helpful to identify congenital and/or acquired abnormalities of intracardiac or intravascular blood flow. Cardiac catheterization is defined as a combined angiographic and hemodynamic study undertaken for therapeutic or diagnostic purposes that includes measurement of intra cardiac pressures, blood oximetry, and selective angiocardiography. Newer cardiac imaging techniques include computed tomography, magnetic resonance imaging and nuclear cardiology (Sleeper, 2008).

2.9 Treatment of VHD and/or DCM in dogs

Heart disease is an acquired or congenital abnormality of the cardiovascular system that can be structural, infective, degenerative, inflammatory and often genetic. Many heart diseases have a prolonged preclinical stage characterized by the presence of underlying cardiac disease and the absence of any clinical signs attributable to heart disease. During this stage the cardiovascular system adapts and compensates for the underlying abnormality in an effort to maintain a state that is free from clinical signs of cardiovascular disease. However, more often these adaptive mechanisms contribute to the eventual development of clinical signs and can thus be considered maladaptive. When heart disease is severe it overwhelms the ability of the heart and body to compensate and clinical signs of heart failure appear.

Standard treatment involves the use of diuretics, positive inotropes, and ACE inhibitors. Treatment depends on the breed, stage of disease, and presence of congestive heart failure or arrhythmias. Diuretic therapy alleviates signs of congestion and oedema. As disease worsens, use of multiple diuretics helps achieve increased diuresis. Diuretic monotherapy increases activity of the renin angiotensin-aldosterone system (RAAS) and concomitant angiotensin

converting enzyme (ACE) inhibitors should be used. Positive inotropic-vasodilator therapy is used to improve contractility. ACE inhibitors blunt activity of the RAAS, reduce salt and water retention, and elicit arterial vasodilation. Venous vasodilators are used to reduce preload and arterial vasodilators to reduce afterload. Other drugs like the Beta blocking drugs are added to further decrease heart rate and slow progression of heart enlargement and systolic dysfunction. Anti-arrhythmic drugs can also be prescribed to control life-threatening ventricular arrhythmias and control the ventricular rate during atrial fibrillation. It is wise to try supplementation with L-carnitine and taurine in diets as nutritional deficiencies of these is a contributing cause of DCM. (Oyama, 2008)

2.9.1 Enalapril

L.D. Byers and R. Wolfenden followed by M.Ondetti and his co-workers helped develop the first ACE inhibitor, captopril, but it had adverse effects such as a metallic taste (which it turned out was due to the sulfhydryl group). Merck research group led by Dr. Arthur A. Patchett developed enalapril as a competing product that lacked the SH thiol group (Ravina and Kubinyi, 2011).

(Plumb, 2008)

2.9.1.a. Chemistry

ACE inhibitors, enalapril maleate and enalaprilat are structurally related to captopril. Enalapril is a prodrug and is converted in vivo by the liver to enalaprilat. Enalapril maleate occurs as a white to off white crystalline powder. Enalaprilat occurs as a white to off white crystalline powder that is slightly soluble in water.

2.9.1.b. Storage/stability/compatibility

The commercially available tablets should be stored at temperatures less than 30 ºC and in tight containers. When stored properly, the tablets have an expiration date of 30 months after manufacture. Enalaprilat injection should be stored at temperatures less than 30 ºC. After dilution with normal saline or D_5 in lactated Ringers, it is stable for up to 24 hours at room temperature. Enalaprilat

has been documented to be incompatible with amphotericin B or phenytoin sodium. Much other medication has been noted to be compatible with enalaprilat at various concentrations. Compatibility is dependent upon factors such as pH, concentration, temperature and diluents used.

2.9.1.c. Pharmacology

Enalapril is converted in the liver to the active compound enalaprilat. Enalaprilat prevents the formation of angiotensin II (a potent vasoconstrictor) by competing with angiotensin I for the ACE. ACE has a much higher affinity for enalaprilat than for angiotensin I. Because angiotensin II concentrations are decreased, aldosterone secretion is reduced and plasma renin activity is increased. The cardiovascular effects of enalaprilat in patients with CHF include decreased total peripheral resistance, pulmonary vascular resistance, mean arterial and right atrial pressures, and pulmonary capillary wedge pressure, no change or decrease in heart rate, and increased cardiac index and output, stroke volume, and exercise tolerance. Renal blood flow can be increased with little change in hepatic blood flow.

2.9.1.d. Pharmacokinetics

Enalapril/enalaprilat has different pharmacokinetic properties than captopril in dogs. It has a slower onset of action (4-6 hours), but a longer duration of action (12-14 hours). In humans, enalapril is well absorbed after oral administration, but enalaprilat is not. Both enalapril and enalaprilat are distributed poorly into the CNS and are distributed into milk in trace amounts. Enalaprilat crosses the placenta. In humans, the half life of enalapril is about 2 hours; enalaprilat about 11 hours. Half lives are increased in patients with renal failure or severe CHF.

2.9.1.e. Dosage and administration

As a vasodilator in heart failure in dogs, 0.25- 0.5 mg/kg, PO, q12 hrs.

2.9.1.f. Uses/indications

The principal uses of enalapril/enalaprilat in veterinary medicine at present are as a vasodilator in the treatment of heart failure and in the treatment of hypertension. It may also be of benefit in treating the effects associated with valvular heart disease, and left to right shunts. It is being explored as adjunctive treatment in chronic renal failure and in protein losing nephropathies.

2.9.1.g. Contraindications/precautions/reproductive safety

Enalaprilat is contraindicated in patients who have demonstrated hypersensitivity to the ACE inhibitors. It should be used with caution and close supervision, in patients with renal insufficiency and doses may need to be reduced. Enalaprilat should also be used with caution in patients with hyponatremia or sodium depletion, coronary or cerebrovascular insufficiency, preexisting hematologic abnormalities or a collagen vascular disease (e.g., SLE). Patients with severe CHF should be monitored very closely upon initiation of therapy. Enalapril crosses the placenta. High doses in rodents have caused decreased fetal weights and increases in fetal and maternal death rates; teratogenic effects have not been reported.

2.9.1.h. Adverse Effects/Warnings

Principally GI distress (anorexia, vomiting, diarrhea). Potentially, hypotension, renal dysfunction and hyperkalemia could occur. Because it lacks a sulfhydryl group (unlike captopril), there is less likelihood that immune-mediated reactions will occur, but rashes, neutropenia and agranulocytosis have been reported in humans.

2.9.1.i. Overdosage/acute toxicity

In dogs, a dose of 200 mg/kg was lethal, but 100 mg/kg was not. In overdose situations, the primary concern is hypotension; supportive treatment with volume expansion with normal saline is recommended to correct blood pressure. Because of the drug, long duration of action, prolonged monitoring and

treatment may be required. Recent overdoses should be managed using gut emptying protocols when warranted.

2.9.1.j. Drug interactions

Concomitant diuretics or other vasodilators may cause hypotension if used with enalapril/enalaprilat; titrate dosages carefully. Some clinicians recommend reducing furosemide doses (by 25 - 50%) when adding enalapril to therapy in CHF. Hyperkalemia may develop if given with potassium or potassium sparing diuretics (e.g.,spironolactone).

2.9.2 Furosemide

Furosemide, the N-furfuryl derivative of 5- sulphamoylanthranilic acid, was synthesized by Dr. Heinrich Ruschig, Chairman of Farbwerke Hoechst Chemical division at the beginning of 1960's as a carbonic anhydrase inhibitor and studied in dogs by Dr. Roman Muschawek and his associates (Ravina and Kubinyi, 2011).

(Plumb, 2008)

2.9.2.a. Chemistry

A loop diuretic related structurally to the sulfonamides, furosemide occurs as an odourless, practically tasteless, and white to slightly yellow, fine, crystalline powder. Furosemide has a melting point between 203 ºC- 205 ºC with decomposition, and a pKa of 3.9. It is practically insoluble in water, sparingly soluble in alcohol and freely soluble in alkaline hydroxides. The injectable product has its pH adjusted to 8 - 9.3 with sodium hydroxide. Furosemide may also be known as frusemide.

2.9.2.b Storage/stability/compatibility

Furosemide tablets should be stored in light-resistant, well closed containers. The oral solution should be stored at room temperature and protected from light and freezing. Furosemide injection should be stored at room

temperature. A precipitate may form if the injection is refrigerated, but will resolubolize when warmed without alteration in potency. The human injection (10 mg/ml) should not be used if it is a yellow-color. The veterinary injection (50 mg/ml) normally has a slight yellow color. Furosemide is unstable at an acid pH, but is very stable under alkaline conditions. Furosemide injection (10 mg/ml) is reportedly compatible with all commonly used intravenous solutions and the following drugs: amikacin sulfate, cimetidine, kanamycin sulfate, tobramycin sulfate and verapamil. It is reportedly incompatible with the following agents: ascorbic acid solutions, dobutamine, epinephrine, gentamicin sulfate, netilmicin sulfate and tetracylines. It should generally not be mixed with antihistamines, local anesthetics, alkaloids, hypnotics, or opiates.

2.9.2.c. Pharmacology

Furosemide reduces the absorption of electrolytes in the ascending section of the loop of Henle, decreases the reabsorption of both sodium and chloride and increases the excretion of potassium in the distal renal tubule, and directly effects electrolyte transport in the proximal tubule. It has no effect on carbonic anhydrase nor does it antagonize aldosterone. Furosemide increases renal excretion of water, sodium, potassium, chloride, calcium, magnesium, hydrogen, ammonium, and bicarbonate. It causes some renal venodilation and transiently increases glomerular filtration rates (GFR). Renal blood flow is increased and decreased peripheral resistance may occur. Furosemide can cause hyperglycemia, but to a lesser extent than the thiazides.

2.9.2.d. Pharmacokinetics

The pharmacokinetics of furosemide has been studied in a limited fashion in domestic animals. In dogs, the oral bioavailability is approximately 77% and the elimination half-life approximately 1 - 1.5 hours. In humans, furosemide is 60-75% absorbed following oral administration. The diuretic effect takes place within 5 minutes after IV administration and within one hour after oral dosing. Peak effects occur approximately 30 minutes after IV dosing, and 1-2 hours after oral dosing. The drug is approximately 95% bound to plasma proteins in both

azotemic and normal patients. The serum half-life is about 2 hours, but is prolonged in patients with renal failure, uremia, CHF, and in neonates.

2.9.2.e. Dosage and administration

As a general diuretic when coupled with ACE inhibitor and an inotrope in dogs, 1.1mg/ kg ,PO ,q12 hrs in cases of mild heart failure and 4.4 mg/kg PO q12 hrs in cases of severe heart failure.

2.9.2.f. Uses/indications

Furosemide is used for its diuretic activity in all species. It is used in small animals for the treatment of CHF, pulmonary edema, hypercalcuric nephropathy, uremia, as adjunctive therapy in hyperkalemia and, occasionally, as an antihypertensive agent. In cattle, it is approved for use for the treatment of post-parturient udder edema. It has been used to help prevent or reduce epistaxis (in exercise-induced pulmonary hemorrhage) in race horses.

2.9.2.g. Contraindications/precautions

Furosemide is contraindicated in patients with anuria or who are hypersensitive to the drug. The manufacturer states that the drug should be discontinued in patients with progressive renal disease if increasing azotemia and oliguria occur during therapy. Furosemide should be used with caution in patients with pre-existing electrolyte or water balance abnormalities, impaired hepatic function (may precipitate hepatic coma) and diabetes mellitus. Patients with conditions that may lead to electrolyte or water balance abnormalities (e.g., vomiting, diarrhea, etc.) should be monitored carefully. Patients hypersensitive to sulfonamides may also be hypersensitive to furosemide (not documented in veterinary species).

2.9.2.h. Adverse effects/warnings

Furosemide may induce fluid and electrolyte abnormalities. Patients should be monitored for hydration status and electrolyte imbalances (especially

potassium, calcium and sodium). Other potential adverse effects include ototoxicity (especially in cats with high dose IV therapy), gastrointestinal disturbances, hematologic effects (anemia, leukopenia), weakness and restlessness.

2.9.2.i. Overdosage/acute toxicity

The LD_{50} in dogs after oral administration is > 1000 mg/kg and after IV injection > 300 mg/kg. Chronic overdosing at 10 mg/kg for six months in dogs led to development of calcification and scarring of the renal parenchyma. Acute overdosage may cause electrolyte and water balance problems, CNS effects (lethargy to coma and seizures) and cardiovascular collapse. Treatment consists of emptying the gut after recent oral ingestion, using standard protocols. Avoid giving concomitant cathartics as they may exacerbate the fluid and electrolyte imbalances that can occur. Aggressively monitor and treat electrolyte and water balance abnormalities supportively. Additionally, monitor respiratory, CNS, and cardiovascular status. Treat supportively and symptomatically if necessary.

2.9.2.j. Drug interactions

Pharmacologic effects of theophylline may be enhanced when given with furosemide. Ototoxicity and nephrotoxicity associated with the aminoglycoside antibiotics may be increased when furosemide is also used. If used concomitantly with corticosteroids, corticotropin or amphotericin B, furosemide may increase the chance of hypokalemia development. Furosemide-induced hypokalemia may increase chances of digitalis toxicity. Patients on aspirin therapy may need dosage adjustment as furosemide competes for renal excretory sites. Furosemide may inhibit the muscle relaxation qualities of tubocurarine. Enhanced effects may occur if furosemide is used concomitantly with other diuretics. The uricosuric effects of probenecid or sulfinpyrazone may be inhibited by furosemide. furosemide may alter the requirements of insulin or other anti-diabetic agents in diabetic patients.

2.9.3 Pimobendan

(Plumb, 2008)

2.9.3.a. Chemistry

The chemical name of pimobendan is 4,5- dihydro-6-[2-(4-methoxyphenyl)-1H-benzimidazole- 5-yl]-5-methyl-3(2H)-pyridazinone. It appears as a white, off white or slightly yellowish hygroscopic powder. It is practically insoluble in water but slightly soluble in acetone and methyl alcohol. The molecular weight is 334.3718 and the molecular formula is C19H18N4O2. Density is 1.36g /cm3 and the percent composition is - C 68.25%, H 5.43%, N 16.76%, and O 9.57%. The derivative type is hydrochloride and the melting point is 311°(dec). The molecule contains one asymmetrical centre. However, the active constituent is a racemic mixture and optically inactive.

2.9.3.b. Storage

Vetmedin (pimobendan) is supplied as oblong half-scored chewable tablets containing 1.25 or 5 mg pimobendan per tablet or capsules containing 1.25, 2.5 or 5 mg pimobendan. It is stored at controlled room temperature below 25°C in a dry place.

2.9.3.c. **Pharmacology** (Gordon and Kittleson , 2008)

Pimobendan is a benzimidazole pyridazinone derivative and is classified as an inodilator (i.e. positive inotrope and balanced systemic arterial and venous dilator) (Fujimoto, 1994) a non-sympathomimetic, non-glycoside inotropic drug with vasodilatative properties. In failing hearts, it exerts its positive inotropic effects primarily through sensitization of the cardiac contractile apparatus to intracellular calcium. As a phosphodiesterase (PDE) III inhibitor it can potentially increase intracellular calcium concentration and increase myocardial oxygen consumption. Pimobendan's calcium sensitization of the contractile apparatus is achieved by enhancement of the interaction between calcium and the troponin C complex, resulting in a positive inotropic effect that does not increase myocardial oxygen consumption. Overall, pimobendan

enhances systolic function by improving the efficiency of contraction, limiting the potential arrhythmogenic side effects of other positive inotropes whose sole mechanism of action is to increase myocardial intracellular calcium. Calcium sensitizers such as pimobendan may thus represent the only class of inotropic agents that 'safely' augment contractility. Phosphodiesterase (PDE) III and V are found in vascular smooth muscle. Inhibitors of PDE III such as pimobendan result in balanced vasodilation (combination of venous and arterial dilation) leading to a reduction of both cardiac preload and afterload, a cornerstone of therapy in heart failure. In addition, pimobendan may have some PDE V inhibition effects. PDE V concentrations are relatively high in the vascular smooth muscle of pulmonary arteries, so PDE V inhibition might help ameliorate elevations in pulmonary artery pressure (pulmonary hypertension) that tend to parallel longstanding elevations in left atrial pressure, a clinically important complication of chronic VHD. Cytokine modulation-The significance of alterations in proinflammatory cytokine concentrations such as tumor necrosis factor-β, interleukins 1β and 6 on the progression of heart failure has been documented in many forms of heart disease. Maladaptive alterations in these cytokine concentrations are associated with increased morbidity and mortality and pimobendan has demonstrated beneficial modulation of several such cytokines in various models of heart failure. Pimobendan reportedly may have some platelet inhibitory effect in the dog. The clinical significance of this property is not yet clear. Positive lusitropic effects occur via PDE III inhibition in cardiomyocytes. Pimobendan increases intracellular cAMP, which facilitates phosphorylation of receptors on the sarcoplasmic recticulum and enhances the diastolic re-uptake of calcium and the speed of relaxation.

2.9.3.d. Pharmacokinetics

Pimobendan is oxidatively demethylated to a pharmacologically active metabolite which is then conjugated with sulphate or glucuronic acid and excreted mainly via faeces. The mean extent of protein binding of pimobendan and the active metabolite in dog plasma is >90%. Following a single oral administration of 0.25 mg/kg, the maximal mean (± 1 SD) plasma concentrations (C_{max}) of pimobendan and the active metabolite were 3.09 (0.76) ng/ml and 3.66 (1.21) ng/ml, respectively. Individual dog C_{max} values for pimobendan and the

active metabolite were observed 1 to 4 hours post-dose (mean: 2 and 3 hours, respectively). The total body clearance of pimobendan was approximately 90 mL/min/kg, and the terminal elimination half-lives of pimobendan and the active metabolite were approximately 0.5 hours and 2 hours, respectively. Plasma levels of pimobendan and active metabolite were below quantifiable levels by 4 and 8 hours after oral administration, respectively. The steady-state volume of distribution of pimobendan is 2.6 L/kg, indicating that the drug is readily distributed into tissues. Food decreased the bioavailability of an aqueous solution of pimobendan, but the effect of food on the absorption of pimobendan from Vetmedin tablets is unknown. In normal dogs instrumented with left ventricular (LV) pressure transducers, pimobendan increased LV dP/dt_{max} (a measure of contractility of the heart) in a dose-dependent manner between 0.1 and 0.5 mg/kg orally. The effect was still present 8 hours after dosing. There was a delay between peak blood levels of pimobendan and active metabolite and the maximum physiologic response (peak LV dP/dt_{max}). Blood levels of pimobendan and active metabolite began to drop before maximum contractility was seen. Repeated oral administration of pimobendan did not result in evidence of tachyphylaxis (decreased positive inotropic effect) or drug accumulation (increased positive inotropic effect). Laboratory studies indicate that the positive inotropic effect of pimobendan may be attenuated by the concurrent use of a ß-adrenergic blocker or a calcium channel blocker.

2.9.3.e. Dosage and administration

Pimobendan should be administered orally at a daily dose of 0.2-0.6 mg/ kg, q12 hrs in dogs, one hour before food (Fuentes, 2004).

2.9.3.f. Contraindications

Pimobendan should not be given in cases of hypertrophic cardiomyopathy, aortic stenosis, or any other clinical condition where an augmentation of cardiac output is inappropriate for functional or anatomical reasons.

2.9.3.g. Warnings

Only for use in dogs with clinical evidence of heart failure. At 3 and 5 times the recommended dosage, administered over a 6-month period of time, pimobendan caused an exaggerated hemodynamic response in the normal dog heart, which was associated with cardiac pathology.

2.9.3.h. Precautions

The safety of pimobendan has not been established in dogs with asymptomatic heart disease or in heart failure caused by aetiologies other than atrioventricular valvular insufficiency or DCM. The safe use of pimobendan has not been evaluated in dogs younger than 6 months of age, dogs with congenital heart defects, dogs with diabetes mellitus or other serious metabolic diseases, dogs used for breeding, or pregnant or lactating bitches.

2.9.3.i. Adverse reactions

Clinical findings/adverse reactions were recorded in a 56-day field study of dogs with congestive heart failure (CHF) due to VHD (256 dogs) or DCM (99 dogs). Dogs were treated with either pimobendan (175 dogs) or the active control enalapril maleate (180 dogs). Dogs in both treatment groups received additional background cardiac therapy. The pimobendan group had the following prevalence (percent of dogs with at least one occurrence) of common adverse reactions/new clinical findings (not present in a dog prior to beginning study treatments): poor appetite (38%), lethargy (33%), diarrhoea (30%), dyspnoea (29%), azotemia (14%), weakness and ataxia (13%), pleural effusion (10%), syncope (9%), cough (7%), sudden death (6%), ascites (6%), and heart murmur (3%). Prevalence was similar in the active control group. The prevalence of renal failure was higher in the active control group (4%) compared to the pimobendan group (1%). Adverse reactions/new clinical findings were seen in both treatment groups and were potentially related to CHF, the therapy of CHF, or both. The following adverse reactions/new clinical findings are listed according to body system and are not in order of prevalence: CHF death, sudden death, chordae tendineae rupture, left atrial tear, arrhythmias overall, tachycardia, syncope, weak

pulses, irregular pulses, increased pulmonary oedema, dyspnoea, increased respiratory rate, coughing, gagging, pleural effusion, ascites, hepatic congestion, decreased appetite, vomiting, diarrhoea, melena, weight loss, lethargy, depression, weakness, collapse, shaking, trembling, ataxia, seizures, restlessness, agitation, pruritus, increased water consumption, increased urination, urinary accidents, azotemia, dehydration, abnormal serum electrolyte, protein, and glucose values, mild increases in serum hepatic enzyme levels, and mildly decreased platelet counts.

2.9.3.j. Palatability

In a laboratory study, the palatability of pimobendan was evaluated in 20 adult female Beagle dogs offered doses twice daily for 14 days. Ninety percent (18 of 20 dogs) voluntarily consumed more than 70% of the 28 tablets offered. Including two dogs that consumed only 4 and 7% of the tablets offered, the average voluntary consumption was 84.2%.

2.9.3.k. Animal safety

In a laboratory study, pimobendan chewable tablets were administered to 6 healthy Beagles per treatment group at 0 (control), 1, 3, and 5 times the recommended dosage for 6 months. The cardiac pathology/histopathology noted in the 3X and 5X dose groups is typical of positive inotropic and vasodilator drug toxicity in normal dog hearts, and is associated with exaggerated hemodynamic responses to these drugs. None of the dogs developed signs of heart failure and there was no mortality.

2.9.3.l. Drug interactions

Pimobendan can be administered safely with diuretics, ACE inhibitors, and digoxin (Fuentes, 2004). The modest vasodilator action of pimobendan is additive to that produced by ace inhibitors. Additive vasodilator action should be expected with nitrates (isosorbide dinitrate or nitroglycerin), amlodipine or carvedilol .It can be used in a combination with ace inhibitor (enalapril or benazepril), spironolactone, and furosemide treatment in dogs with congestive

heart failure. In fact, improved heart function resulting from pimobendan treatment may permit a small reduction of furosemide dosage. Concurrent use of pimobendan with a beta-blocker or calcium channel blocker may attenuate the positive inotropic action of pimobendan.

Theoretically, pimobendan may increase the rate of intestinal digoxin absorption. The co-administration of pimobendan with digoxin has neither increased paired serum digoxin concentrations nor resulted in concentrations within the upper 40th percentile of our reference range (Aiba *et al*., 2005). In addition, the combination of pimobendan with amiodarone or mexiletine or both has, in clinical experience, been well-tolerated in Boxers and Doberman pinschers with advanced dilated cardiomyopathy and severe ventricular arrhythmias.

2.9.3.m. Role in dilated cardiomyopathy and valvular heart disease in dogs (Kleeman *et al*., 1998)

Pimobendan's strongest indication is to treat advanced dilated cardiomyopathy. Patients with advanced dilated cardiomyopathy have poor left ventricular systolic function and reduced ejection fraction. They are subject to increased afterload as a result of dilation of the left ventricle with inadequate wall hypertrophy and arteriolar constriction (caused by activation of the renin-angiotensin-aldosterone system and increased plasma norepinephrine concentrations). This increased afterload negatively affects stroke volume and ejection fraction. Through phosphodiesterase III and V inhibition, pimobendan promotes both arteriolar and venous dilation (Fugimoto and Matsuda, 1990), reducing afterload and preload, respectively. However, pimobendan's myocardial phosphodiesterase inhibition is probably attenuated in the face of chronic, advanced dilated cardiomyopathy due to beta-receptor downregulation (Hagemeijer, 1993).Patients with valvular heart disease have good contractility as assessed by echocardiography, even when the left heart is severely dilated. Thus, the inotropic action of pimobendan would seem to be of little value. However, the vasodilator action may contribute to preload and afterload reduction. According to the owners, most dogs with overt signs of advanced heart

disease feel better and have improved activity tolerance within a few days of adding pimobendan to existing treatment.

2.10 Pimobendan in dogs

Pimobendan has been studied in dogs since the late 1980s. Pouleur *et al*. (1989) designed a study with the purpose to examine the time course of the changes in left ventricular inotropic state after intravenous pimobendan administration. In conscious dogs, cumulative doses of 1 and 2.5 mg of pimobendan significantly increased heart rate and the isovolumic indices of inotropic state and relaxation. The maximal effect, however, required 2 hrs to be present. The changes in cardiac index and capillary wedge pressure after the intravenous administration of 5 mg to patients with heart failure confirmed this slightly delayed action of pimobendan. Accordingly, the effects of pimobendan on left ventricular inotropic state in patients with moderate to severe heart failure were determined during cardiac catheterization 130-150 min after injection of 5 (n = 3) or 2.5 (n = 4) mg. After drug administration, heart rate increased slightly (+7 beats/min) while left ventricular end-diastolic and systolic pressure both decreased significantly (from 22.7 to 9.2 mm Hg, $p < 0.007$ and from 123 to 90 mm Hg, $p < 0.025$, respectively). The isovolumic index of contractility (dP/dt) increased by 19.6 +/- 14.7%. ($p < 0.02$) and the slope of the late systolic stress-volume relationship improved by 48% $P < 0.05$). It was thus concluded that pimobendan was a positive inotropic agent in a failing heart as well as a powerful veno- and arteriodilator.

Ichihara and Abiko (1991) examined the effect of pimobendan on myocardial mechanical function and energy metabolism in the dog heart and compared with that of dobutamine. He stated that although dobutamine and pimobendan increased the cardiac mechanical function, they did not disturb the myocardial energy and carbohydrate metabolism. Similarly, Goto and Hata (1997) examined the mechanoenergetic effect of pimobendan in failing dog hearts. To understand the effect of cardiotonic drugs with a calcium-sensitizing effect (Ca^{2+} sensitizers) on cardiac mechanoenergetics in the failing heart, they measured left ventricular (LV) contractility (E_{max}) and the relation between myocardial oxygen consumption (VO_2) and pressure-volume area (PVA; a

measure of LV total mechanical energy) before and during enhancement of contractility by infusion of dobutamine or pimobendan in six cross-circulated hearts isolated from pacing-induced heart failure (FL) dogs, and compared the results with those reported in normal hearts (NL, n = 12). They reasoned that pimobendan exerted a positive inotropic effect comparable to that of dobutamine in both NL and FL dogs. The O_2 cost of contractility with dobutamine was higher in FL dogs than in NL dogs and pimobendan had a relative O2-saving effect compared with dobutamine in FL dogs.

Nobuyuki *et al.* (1997) compared the effects of pimobendan (0.25 mg/kg I.V), a Ca^{++} sensitizer, with some phosphodiesterase-III inhibition effects, and amrinone (1 mg/kg plus 10 μg/kg/min I.V.), a PDE-III inhibitor, on left ventricular (LV) systolic and diastolic performance, both at rest and during exercise, in seven conscious dogs before and after pacing-induced congestive heart failure (CHF). Before CHF, under resting conditions, both pimobendan and amrinone caused a similar significant decrease in left ventricle size and end-systolic pressure, arterial elastance, and the time constant of LV relaxation. Similar results were obtained during exercise. Both agents also produced a similar increase in E_{ES}, the slope of the LV end-systolic pressure-volume relation (3.4 ± 1.5 *vs.*4.2 ± 1.1 mm Hg/ml; amrinone *vs.* pimobendan). After CHF, the vasodilatory effects of amrinone and pimobendan were preserved both at rest and during exercise; however, the inotropic actions were different. After CHF, pimobendan increased E_{ES} (3.9 ± 0.5 *vs.* 5.7 ± 0.4 mm Hg/ml, $P < .05$), decreased the time constant of LV relaxation, increased the maximum rate of LV filling (37 ± 19 ml/sec) ($P < .05$) and produced a downward shift of the early diastolic portion of LV pressure-volume loop. Pimobendan also augmented LV contractile performance during CHF exercise. In contrast, after CHF, amrinone no longer produced a positive inotropic effect. Amrinone improved LV relaxation and filling, both at rest and during exercise after CHF, but significantly less than pimobendan. Hence it was concluded that after CHF, the cardiac response to a PDE-III inhibitor was attenuated, but the response to Ca^{++} sensitizer was preserved. Thus, after CHF, pimobendan was proved to be more effective than amrinone in enhancing LV contractile state, LV relaxation and LV filling both at rest and during exercise.

Fuentes *et al*. (2002) conducted a double-blind, randomized, placebo-controlled study to examine the effect on heart failure class and survival of pimobendan, an oral calcium-sensitizing inodilator, in dogs with DCM. Pimobendan (0.3–0.6 mg/kg body weight/d) or placebo was administered to English Cocker Spaniels (CSs; n = 10) and Doberman Pinschers (DPs; n = 10) that had DCM in addition to background therapy of furosemide, enalapril, and digoxin. Addition of pimobendan to standard triple therapy was associated with a significant improvement in heart failure class, regardless of breed ($P < .02$, Mann-Whitney rank sum test). Overall, 8 of 10 animals in the pimobendan-treated group, and 1 of 10 animals in the placebo group improved their heart failure status by at least 1 modified New York Heart Association functional class after initial stabilization ($P= .005$, Fisher's exact test). Pimobendan had no significant effect on survival in the CSs ($P= 0.77$, log-rank test), but DPs treated with pimobendan had significantly longer survival times compared with placebo ($P < .02$, log-rank test), with a median survival time of 329 days in the pimobendan group compared with 50 days in the placebo group, and a hazard ratio of 3.4 (95% confidence interval 1.4–39.8). Pimobendan resulted in significant improvement in heart failure class when added to standard therapy in this group of dogs with DCM, and may have contributed to improved survival in DPs.

Smith *et al*. (2005) carried out a study with an objective of evaluating the clinical efficacy and safety of pimobendan by comparing it with ramipril over a six-month period in dogs with mild to moderate heart failure (HF) caused by myxomatous mitral valve disease (MMVD). This was a prospective randomised, single-blind, parallel-group trial. Client-owned dogs (n=43) with mild to moderate HF caused by MMVD were randomly assigned to one of two groups, which received either pimobendan (P dogs) or ramipril (R dogs) for six months. The outcome measures studied were: adverse HF outcome, defined as failure to complete the trial as a direct consequence of HF; maximum furosemide dose (mg/kg/day) administered during the study period; and any requirement for additional visits to the clinic as a direct consequence of HF. Treatment with pimobendan was well tolerated compared to treatment with ramipril. P dogs were 25 percent as likely as R dogs to have an adverse HF outcome (odds ratio 4.09, 95 per cent confidence interval 1.03 to 16.3, P=0.046).

Gordon *et al*. (2006) termed pimobendan as a silver bullet in heart failure therapy. They further stated pimobendan to be a novel agent with properties that were highly desirable in the clinical management of CHF secondary to both DCM and VHD in dogs. Review of available data suggested that pimobendan was safe, well tolerated, and lead to enhanced quality of life in dogs with CHF secondary to DCM or VHD when used in combination with furosemide or other conventional therapies (e.g., ACE inhibitors, digoxin). Pimobendan lead to a reduction in mortality from CHF associated with DCM.

Lombard *et al.* (2006) assessed the clinical efficacy of pimobendan versus benazepril for the treatment of acquired atrioventricular valvular disease in dogs. Seventy-six dogs with clinical acquired atrioventricular valvular disease were evaluated to determine the efficacy of pimobendan (n=41) versus benazepril hydrochloride (n=35) in a randomised, positive-controlled, multicentre study. The study was divided into 56-day and long-term evaluation periods. In a subgroup of dogs with concurrent furosemide treatment (pimobendan [n=31], benazepril [n=25]), the heart insufficiency score improved in favour of pimobendan (P=0.0011), equating to a superior overall efficacy rating (P<0.0001) at day 56. Long-term median survival (i.e., death or treatment failure) for dogs receiving pimobendan was 415 days versus 128 days for dogs not on pimobendan (P=0.0022).

Effects of pimobendan on mitral valve regurgitation in dogs were evaluated by Kanno *et al*. (2007). This study examined the effects of pimobendan on cardiac function, hemodynamics, and neurohormonal factors in dogs with mild mitral regurgitation (MR). The dogs were given 0.25 mg/kg of pimobendan orally every 12 hr for 4 weeks. With pimobendan, the heart rate and stroke volume did not change, but the systolic blood pressure gradually decreased and the degree of mitral valve regurgitation tended to decrease. Renal blood flow was significantly increased and the glomerular filtration rate was slightly increased at 2 and 4 weeks. Furthermore, over the 4-week period, the plasma nor-epinephrine concentration decreased significantly, the systolic index increased slightly, the left atrial diameter and the left ventricular diameters decreased significantly, and the heart size improved. Given these results, pimobendan appeared to be useful for treating MR in dogs. However, further long-term studies of pimobendan involving

a larger number of dogs with mild and moderate MR were needed to establish the safety of pimobendan and improvements in quality of life.

VHD continues to be an important cause of morbidity and mortality in geriatric dogs despite conventional therapy. Haggstrom *et al*. (2008) hypothesized that pimobendan in addition to conventional therapy would extend time to sudden cardiac death, euthanasia for cardiac reasons, or treatment failure when compared with conventional therapy plus benazepril in dogs with CHF attributable to VHD. Two hundred and sixty client-owned dogs in CHF caused by VHD were recruited from 28 centres in Europe, Canada, and Australia in their study. A prospective single-blinded study with dogs randomized to receive pimobendan (0.4–0.6 mg/kg/d) or benazepril hydrochloride (0.25–1.0 mg/kg/d) orally. The primary endpoint was a composite of cardiac death, euthanized for heart failure, or treatment failure. One hundred and twenty-four dogs were randomized to pimobendan and 128 to benazepril. One hundred and ninety dogs reached the primary endpoint; the median time was 188 days (267 days for pimobendan, 140 days for benazepril hazard ratio = 0.688, 95% confidence limits [CL] = 0.516–0.916, *P*= .0099). The benefit of pimobendan persisted after adjusting for all baseline variables. A longer time to reach the endpoint was also associated with being a Cavalier King Charles Spaniel, requiring a lower furosemide dose, and having a higher creatinine concentration. Increases in several indicators of cardiac enlargement (left atrial to aortic root ratio, vertebral heart scale, and percentage increase in left ventricular internal diameter in systole) were associated with a shorter time to endpoint, as was a worse tolerance for exercise. It was proved from the results that pimobendan plus conventional therapy prolonged time to sudden death, euthanasia for cardiac reasons, or treatment failure in dogs with CHF caused by VHD compared with benazepril plus conventional therapy.

Evaluation of pimobendan and N-terminal probrain natriuretic peptide in the treatment of pulmonary hypertension secondary to VHD in dogs was done by Atkinson *et.al* (2009). They speculated that pimobendan probably decreased the severity of pulmonary hypertension (PHT) measured echocardiographically and improved quality-of-life scores and changes in N-terminal probrain natriuretic peptide (NT-proBNP) concentrations would reflect improvement in severity of

PHT. Ten client-owned dogs with peak tricuspid regurgitant flow velocity (TRFV) ≥3.5 m/s were used for this prospective short-term, double-blinded, crossover design, with a long-term, open-label component study. Short term, dogs were randomly allocated to receive either placebo or pimobendan (0.18–0.3 mg/kg PO q12 hrs) for 14 days. After a 1-week washout, they received the alternative treatment for 14 days, followed by pimobendan open-label for 8 weeks. Short-term comparison revealed that peak TRFV decreased in all dogs on pimobendan compared with placebo from a median of 4.40 (range, 3.2–5.6) to 3.75 (range, 2.4–4.8) m/s ($P < .0001$). NT-proBNP concentration decreased after treatment with pimobendan from a median of 2,143 (range, 450–3,981) to 1,329 (range, 123–2,411) pmol/L ($P = .0009$). All dogs improved their quality-of-life score ($P = .006$). In the long-term comparisons, peak TRFV decreased in all dogs from a median of 4.28 (range, 3.5–5.7) to 3.52 (range, 2.4–5.0) m/s ($P < .0001$). No significant changes in NT-proBNP or quality-of-life scores were detected. Hence it was concluded that pimobendan lowered severity of measurable PHT, improved quality-of-life scores, and decreased NT-proBNP concentrations short-term. Long term, only the reduction in TRFV was maintained.

Ouellet *et al*. (2009) carried out a prospective, blinded, and controlled clinical trial to see if the addition of pimobendan to treatment decreases the regurgitant fraction (RF) in dogs with asymptomatic VHD. Twenty-four client-owned dogs affected by International Small Animal Cardiac Health Council class Ib VHD were used. Dogs were assigned to a pimobendan treatment group (n = 19) (0.2–0.3 mg/kg q12h) or a control group (n = 5). Echocardiographic evaluations were performed over a 6-month period. The addition of pimobendan to treatment did not decrease the RF of dogs affected by asymptomatic class 1b VHD over the study period ($P = .85$). There was a significant increase in the ejection fraction of the pimobendan treated dogs at 30 days (80.8 ± 1.42 versus 69.0 ± 2.76, corrected $P = .0064$), and a decrease in systolic left ventricular diameter (corrected $P = .011$) within the pimobendan group compared with baseline. However, this improvement in systolic function was not sustained over the 6-month trial period.

Caro *et al*. (2009) studied the effects of short-term treatment with pimobendan in dogs suffering from myxomatous valve disease (valvular heart

disease).The study involved twenty dogs with no previous treatment. Clinical history, complete physical examination including body weight and electrocardiographic study were made. Radiological study, including right lateral and dorso-ventral thoracic radiograph, to determine the degree of cardiomegaly according to the cardio-vertebral index and the degree of congestion-oedema in the lung was made. Finally, an echocardiographic study and a routine haematology and clinical chemistry were also performed. The echocardiographic examination was performed to compare the effects of the drug on heart chamber size and wall motion. The echocardiographic measurements included fractional shortening, left ventricular internal dimensions in systole and diastole, internal dimension of left atrium and dimension of aorta .Four weeks after the beginning of treatment, the initial tests were repeated in order to compare the final state of patients with the initial data. The results showed that pimobendan was well tolerated and could be administered effectively and safely in the treatment of congestive heart failure from myxomatous mitral valve disease of the dog as in general, most patients improved with regard to clinical symptoms.

There was a controversy related to the efficacy and safety of pimobendan, in controlling moderate to severe congestive heart failure (CHF) from VHD in dogs. Hence SoJeong *et al*. (2009) designed a study to check the clinical efficacy of pimobendan in dogs with chronic VHD in 20 dogs. Scores for quality of life, respiratory failure, circulatory failure and heart failure were evaluated along with radiographical and echocardiographical assessments for about 2 months period after the addition of pimobendan into the regular cardiac medications. This study proved clear evidence that pimobendan had beneficial therapeutic effects in dogs with advanced VHD, without particular adverse effects. However, further studies were essential to address the drug interaction with other cardiac therapeutics and to assess therapeutic effects in CHF from other type of heart diseases in dogs and other animals.

3. MATERIALS AND METHODS

3.1 Location

The entire study was carried out from February 2011 to July 2011, at a private veterinary clinic. Owned dogs were screened for valvular heart disease (VHD) and/or dilated cardiomyopathy (DCM). The study was conducted in three parts.

Part 1: Diagnosing VHD and/or DCM in dog patients.

Part 2: Evaluating the therapeutic efficacy of *Terminalia arjuna* in VHD and/or DCM in dog patients.

Part 3: Determining if the extract of *Terminalia arjuna* + enalapril + furosemide could substitute the modern contemporary treatment i.e. pimobendan+ enalapril + furosemide as an alternate therapy in treatment of VHD and/or DCM resulting into congestive heart failure (CHF) in dog patients.

3.2 Experimental animals

The study was conducted on owned dog patients (clinical patients), independent of their age, weight, sex and breed from private veterinary clinics. Selection criteria included only the ones who were diagnosed with VHD and/or DCM, prone to progression to CHF. These two cardiac ailments were selected because enough literature was available till date regarding the efficacy of pimobendan and arjuna in treating CHF once it has progressed. There were studies conducted with pimobendan to treat the stage of VHD/DCM before its advancement to CHF but not with arjuna. Hence evaluation of the efficacy of arjuna at an early stage of CHF was needed.Dogs with other exclusive cardiac ailments like systemic hypertension due to causes other than cardiac origin, congenital heart defects, pericardial disease, endocarditis, neoplasia were not included in the study.

3.3 Diagnostic instruments used for diagnosis of DCM and VHD

- GE Genius 60 mobile X ray unit with a Kodak Point-of-care CR 140 processer for thoracic radiology (Fig.6).
- Mac 500 GE Medical systems Information technologies ECG machine for electrocardiography (Fig.7).
- 811-B Ultrasonic Doppler Flow Detector by Parks medical electronics, Inc, Beaverton, Oregon, USA for systolic blood pressure measurement (Fig.8).
- GE logiqbook XP Ultrasound using a cardiac probe 3S-RS 2-3.6 MHz Phase array for advanced cardiac imaging (Fig.9).

3.4 Diagnosis of VHD and/or DCM

3.4.1 Description of Patient and medical history

Age, breed, sex and weight were noted. Specific symptoms like coughing, weakness, exercise intolerance, ascites, cyanosis, syncope, weight loss, if any, were taken into consideration.

3.4.2 Physical examination

This included measuring body temperature, respiratory rate, examining mucous membranes, auscultation of lungs for detecting abnormal respiratory sounds, percussion (diagnosing ascites) and palpation (diagnosing organ enlargement). Cardiovascular examination included noting heart rate and rhythm, auscultation of abnormal heart sounds (arrhythmias, murmurs and their grades), noting pulse rate, quality and detecting pulse abnormalities was done as elucidated by Gompf (2008). The normal range of body temperature was considered as 99.5ºto 102.5º. The normal respiratory rate for dogs was taken as 10 to 34 breaths per minute. The normal color of mucous membranes was considered pink with capillary refill time (CRT) of less than 2 seconds. Normal heart rate values (beats per minute) were taken as 60 to 100 bpm in large sized dogs, 80 to 120 bpm in medium sized dogs and 90 to 140 bpm in small sized dogs. Normal pulse rate was taken as 70 bpm to 160 bpm depending upon the breed (Jones, 2006).

3.4.3 Thoracic radiology

The technique was carried out as described by O'Brien (2001) and Poteet (2008). All foreign objects like collars, leashes, bandages etc. were removed. The patient was restrained physically. The study included 3 views viz. left lateral, right lateral and ventrodorsal view. A balance of kVp and mAs was maintained and a high kVp-low mAs technique was opted for. An exposure chart was employed to optimize the radiographic result. For lateral thorax positioning, the animal was measured at widest point (12th or 13th rib). A cassette size was chosen that fitted the animal's thorax. It was made sure that the animal was in a "true" lateral position, not oblique. The front legs were pulled forward. The beam was centred at the caudal border of scapula. It was seen that there was elevation of the sternum so that the sternum and spine were the same distance above the cassette. The radiograph was taken at peak inspiration. For ventrodorsal view, the animal was measured at the tallest point (11th or 12th rib). A cassette size was chosen that fit the animal's thorax. The animal was placed in dorsal recumbency and it was made sure that the position was straight, not oblique. The thorax was included from the manubrium to the 13th rib and the exposure was taken at peak inspiration. For occasional dorsoventral view (in dyspnoeic patients) the animal was measured at the tallest point (11th or 12th rib) and a cassette size was chosen that fit the animal's thorax. The animal was placed in ventral recumbency and in a straight position. The thorax was included from the manubrium to the 13th rib and the exposure was taken at peak inspiration. Thoracic radiographic paradigm including the extrathoracic structures, pleural space, pulmonary parenchyma, mediastinum was examined in detail. Radiographic findings like focal or generalized enlargement of the cardiac silhouette, pulmonary venous distention suggestive of pulmonary congestion, interstitial or alveolar pulmonary oedema were recorded if present. Vertebral heart score (VHS) for each dog patient indicative of cardiac enlargement was noted according to Appendix 1. The normal VHS was taken as 9.7 ± 0.5 (Buchanan *et al*., 1995).

3.4.4 Electrocardiography

The procedure was followed as explained by Tilley and Smith (2008).The electrocardiogram was recorded in an area as quiet and as free of distraction as possible as noises from clinical activity and other animals would significantly affect rate and rhythm. The patients were restrained by having an assistant, hold them in right lateral recumbency on a blanket or heavy-duty mat on a non-metal table. Limbs were held perpendicular to the body. Each pair of limbs was held parallel and was not allowed to contact one another. The patients were held as still as possible during the electrocardiogram. When possible, panting was prevented. When dyspnoea or other factors prevented standard positioning, the electrocardiogram was recorded while the patient was standing. Contact gel was put on the skin at either distal or proximal elbow on both front and over the stifle on both back legs, making sure that the alcohol or gel contacted the skin and not hair. Alligator clips were used. Each electrode was wetted with 70 % isopropyl alcohol to ensure electrical contact. The six lead system (I, II, III, aVR, aVL, aVF) electrocardiogram was recorded for each patient and at least three to four complete complexes were recorded from each lead at 25 mm/s. The heart rate, heart rhythm, measurements for the waveforms and intervals of the PQRS complex, mean electric axis were interpreted in detail. A variety of rhythm disturbances and chamber enlargement/conduction abnormality patterns if any were diagnosed and compared with reference values as per Appendix 2.

3.4.5 Systolic blood pressure measurement

Systolic blood pressure was measured using an ultrasonic Doppler flow detector as described by Ware (2007). The Doppler method was used that causes change in frequency between emitted ultrasound and echoes reflected from moving blood cells, or vessel wall, to detect blood flow in a superficial artery and this frequency change (‘Doppler shift’) is converted into an audible signal. The cuff was placed distal to the elbow. The palmer common digital artery (forelimb) was selected as an effective location for pressure measurement. The probe was placed distal to the occluding cuff. A small area of hair was shaved over the artery where the probe was to be placed. Ultrasonic coupling gel was applied to the flat Doppler flow probe to achieve an air-free interface with the

skin. The probe was placed so that a clear flow signal was heard. Care was taken so that the probe wasn't placed perpendicular to the target artery and not held tightly as to occlude flow. The probe was held still to minimize noise. A low volume setting on the Doppler unit was used to minimize patient disturbance. The flow occluding cuff was attached to a sphygmomanometer and inflated to a pressure where flow stopped and no audible signals were heard. As the cuff was slowly deflated, by a few mm Hg/second, the return of blood cell motion produced characteristic flow signals during systole. The pressure at which pulsatile blood flow first recurred, indicated by brief 'swishing' sounds, was the systolic pressure. The cuff was completely deflated between measurements. Care was taken to avoid patient movement as it would interfere with measurement. The normal systolic blood pressure value was taken as 130-140 mm Hg. Values above the selected normal range were recorded as hypertension as per Ware (2007).

3.4.6 2-D and M mode Echocardiography

The procedure was followed according to Fuentes (2008) and Boon (2002).

3.4.6.a The echo table

The patients were positioned in lateral recumbency and approach was made from underneath, with the probe on the dependent chest wall. This minimized air artifact in the lung tissue between the probe and the heart. The echo table had a circular cut-out, and was covered with a comfortable and hygienic surface.

3.4.6.b Patient preparation

Most patients needed to be clipped for echocardiography. In some short-coated breeds, good acoustic contact was obtained with liberal use of alcohol and acoustic gel. Care was taken not to damage the transducer by alcohol.

3.4.6.c Echocardiographic measurements

The main purpose of including echocardiography in the study was to allow quantification of chamber dimensions, systolic performance and valve function.The following parameters were recorded.

i. Chamber dimensions

In 2D view, the left atrial diameter, aortic dimension, left atrium: aorta (LA: AO) and left ventricular wall thickness were assessed. Left ventricular diameter was typically measured in M- mode view. In M-mode, values were measured from “leading edge” to “leading edge”. Diastolic measurements were made at onset of QRS (LVDd). Systolic measurements made at peak excursion (LVDs). The values were then compared to the normal echocardiographic reference values related to body size in dogs, while taking individual readings as given in Boon *et al*. (1983) and Boon *et al*. (1998) as stated in Appendix 3. For standard reference values, Kienle and Thomas (2002) was referred.

ii. Systolic function parameters

Ejection fraction (EF), fractional shortening (FS) and E point to septal separation (EPSS) were the systolic function parameters assessed. Calculation of LV volumes allowed calculation of EF % and FS% as stated by (Fuentes, 2008; Moise and Fox 1999) are as follows;

$$EF\% = \frac{[\text{End-diastolic volume (EDV)} - \text{End-systolic volume (ESV)}]}{[\text{End-diastolic volume (EDV)}]} \times 100$$

LV fractional shortening (FS %) was measured using the following formula given by;

$$FS\% = \left\{[(LVIDd) - (LVIDs)] \div [(LVIDd)]\right\} \times 100$$

Where, (LVIDd) is left ventricular internal dimension at diastole and (LVIDs) is left ventricular dimension at systole.

The normal range for fractional shortening was considered as 25-35% and for ejection fraction; it was taken as 50-65 % (Fuentes, 2008; Moise and Fox 1999). EPSS was measured from an M-mode recording at the mitral valve level as the distance between the septum and peak opening of the anterior mitral valve leaflet. Normal reported mean values were taken in a range from 1-10 mm (Moise and Fox 1999; Boon, 1983)

iii. Valve function

Color doppler was used to make an assessment of the degree of MR by measuring the size of the regurgitant jets. This was evaluated in right parasternal long-axis view or left parasternal caudal four chamber view (Boon *et al*. 1998; Moise and Fox 1999).The jet size is often reported as the percentage of the left atrial area occupied by the jet and it was sufficient to assess this percentage by eye (Pederson, 2000). The mitral insufficiency was graded as mild, moderate, or severe (Pederson *et al*., 1999).

Mild- 10-30% left atrial area occupied by the jet

Moderate- 30-50% left atrial area occupied by the jet

Severe – More than 50 %

3.4.6.d Echocardiographic views

The following standard parasternal locations were used in a typical echocardiogram as per Thomas *et al.* (1993) and Boon (2002). M- mode measurement technique was used to assess the systolic function (ejection fraction, fractional shortening and EPSS).

1. Right parasternal long axis views (RPS LAx)

For the right parasternal long-axis left ventricular outflow tract view, the apical impulse was felt on the right side, the transducer was placed that the crystals pointed toward the lumbar spine, cable of the transducer extended toward the elbows and the reference mark was directed toward the neck. An angle of 45 degrees between the chest wall and the transducer was maintained. Efforts were taken to obtain a perfect imaging plane and a good quality image so

that the readings could be taken accurately. This view was used to assess the interventricular septum, aortic and left atrial size and compare the right and left ventricular chambers (Fig.10). By starting with a good left ventricular outflow view, the transducer was twisted so that the reference mark moved away toward the spine to obtain a right parasternal long-axis four-chamber view. Once the atrial septum was seen, the transducer was lifted and dropped slightly toward and away from the patient's thorax to create a longer wider left ventricular chamber and well defined atrial septum. The crystals were pointed more toward the thoracic spine in order to see more of the right atrium. This view was used to assess the interatrial septum, ventricular septum, mitral valves and compare the right and left ventricular walls.

2. Right parasternal short axis views (RPS SAx)

After a good right parasternal long-axis left ventricular outflow tract view was obtained, the transducer was twisted so that the reference mark moved away from the spine toward the patient's elbows. The transducer was dropped slightly to keep at least a 60 degree angle between the transducer and chest wall. The transducer was twisted until a round left ventricular chamber or aorta was seen and until the left ventricle was symmetrical & both papillary muscles were seen & aorta was imaged as a complete closed circle in the center of the image (Fig.11). At the level of papillary muscles, the left ventricular chamber, papillary muscles and comparison between the right and left ventricular wall was made. At the level of chordae tendinae, the chordae were assessed. At the level of mitral valve, mitral valve leaflets were assessed for their movement and presence of lesions. At the level of aortic valve, comparison between the size of left atrium and aorta, aortic cusps, left atrial wall and left auricle were assessed. At the level of pulmonary arteries, comparison between the diameter of aorta and pulmonary artery and pulmonic valves were assessed.

3. Left parasternal caudal views

For the left parasternal caudal four-chamber view (Fig. 12), the reference mark was directed toward the spine. The crystals were pointed toward the neck and an angle of about 30 degrees between the chest wall and the transducer was maintained. The liver was found near the last intercostal space, close to the sternum and then the transducer was moved forward until the liver disappeared and the heart was seen. The transducer was then lifted up toward the chest wall to elongate the heart and clear up the image. This view was used to assess the valve appearance and motion.

4. M- mode measurement technique

The M- mode was selected with the button on the ultrasound device. An appropriate M mode image was frozen showing the cardiac structures as they change during diastole and systole for measurements. The cursor was adjusted to view the structures under the cursor. For the left ventricle, right parasternal long axis (RPS LAx) left ventricular outflow tract view and right parasternal short axis view (RPS SAx) at the level of chordae tendinae (Fig.13a,b) were selected for measurements. M mode of the mitral valve in either right parasternal long axis or short axis view helped to measure end point to septal separation distance (EPSS) (Fig.14). End diastole coinciding with the greatest LV internal dimension (LVIDd) typically shortly after the onset of QRS complex and the systole coinciding with smallest LV internal dimension (LVIDs) was measured. Similarly, the left ventricular end-diastolic and end-systolic volumes were measured. From these left ventricular measurements, ejection fraction and fractional shortening was calculated.

3.5 Treatment used for DCM or VHD

The treatment for DCM or VHD aims at improving the heart function and controlling the signs of CHF. Most standard treatment involves the use of positive inotropes, ACE inhibitors and diuretics.

3.5.1 Positive inotropic drug

The standard drug used was pimobendan, which is categorized pharmacologically under the class inodilators (calcium sensitizer and phosphodiesterase III inhibitor) and is manufactured by Boehringer Ingelheim Vetmedica, Inc. under the proprietary name 'Vetmedin'. The amount of active ingredient is 1.25, 2.5, or 5 mg pimobendan per tablet and is available either as chewable tablets or capsules. It is supplied as either 50 chewable tablets per bottle or 100 capsules per bottle. Pimobendan (Vetmedin) was administered orally at a daily dose of 0.2-0.6 mg/ kg in dogs, q12 hrs, an hour before food.

3.5.2 Positive inotropic and cardiotonic herb

The test herb used was *Terminalia arjuna,* which is a pure herb product manufactured by Himalaya Herbal Healthcare under the proprietary name 'Arjuna'. It is available in the form of capsules and each capsule contains 250 mg of *Terminalia arjuna.* The dose was decided as total dose of 250 mg q12 hrs, according to the reviews on the available literature pertaining to safe dosage of *Terminalia arjuna* in dogs and on the basis of a short pilot study that was conducted in dogs to determine the dose at which an optimum response could be achieved and the possible side effects encountered with changes in dosage.

3.5.3 Drug used as an ACE inhibitor

Enalapril, an ACE inhibitor & hypotensive drug was used which is manufactured by Dr. Reddy's Laboratories, under the proprietary name 'Enam'. The amount of active ingredient is 2.5 mg, 5 mg, 10 mg, or 20 mg enalapril and it is available as 15 tablets per strip. It was administered orally at a daily dose of 0.25- 0.5 mg/kg, q12 hrs.

3.5.4 Drug used as a diuretic

Furosemide, a diuretic was used which is manufactured by Aventis Pharma Limited under the proprietary name 'Lasix'. The amount of active ingredient is either 20, 40 mg or 80 mg furosemide and it is available as 15

tablets per strip. It was administered orally at a daily dose of 1.1-4.4 mg/kg, q12 hrs.

3.6 Experimental design

Dogs were divided into two groups designated as 1 and 2, comprising of six dogs in each group. Group 1 represented dogs receiving only allopathic treatment which included pimobendan as an inotropic vasodilator drug, enalapril, an ACE inhibitor as a hypotensive drug and furosemide as a diuretic drug. Group 2 represented dogs recieving ayurvedic treatment in conjunction with allopathy which included *Terminalia arjuna* for inotropic effect, enalapril- an ACE inhibitor as a hypotensive drug and furosemide as a diuretic drug. All the dogs were under constant observation during the entire period of study.

Table 1: Treatment schedule for different groups of dogs

Group	Therapy	Treatment	Dose	Duration
1 n=6	Allopathy	Pimobendan	0.2-0.6 mg/ kg, q12 hrs, one hour before food	30 days
		+ Enalapril	0.25- 0.5 mg/kg, q12 hrs	
		+ Furosemide	1.1-4.4 mg/kg, q12 hrs	
2 n=6	Allopathy + Ayurvedic	*Terminalia arjuna*	Total dose 250 mg,q 12hrs	
		+ Enalapril	0.25- 0.5 mg/kg, q12 hrs	
		+ Furosemide	1.1-4.4 mg/kg, q12 hrs	

All the patients were subjected to a 30 day study after which they were clinically re-evaluated by detailed physical examination, thoracic radiology, electrocardiography, systolic blood pressure measurement and echocardiography to assess improvement in comparison to the basal values/parameters taken at

the start of the study. The data collected pre and post treatment were subjected to paired t-test according to Snedecor and Cochran (1994) and comparison in between the two treatments, pimobendan group and arjuna group was on the basis of percent change in the data per parameter, post treatment evaluation.

4. RESULTS AND DISCUSSIONS

Among the vast majority of heart disease cases in dogs, some 95 percent are considered as "acquired" heart disease. Typically they result from normal wear and tear, but can also be caused by injury or infection. They're most often seen in middle-aged and older dogs. The two most common types of acquired heart diseases are chronic valvular heart disease (VHD) and dilated cardiomyopathy (DCM). Diagnosis of DCM and VHD expected to progress to congestive heart failure (CHF) is done on basis of a good medical history of the patient along with clinical presentation, detailed physical examination and diagnostic techniques like thoracic radiology, electrocardiography, Doppler blood pressure measurement and 2-D and M mode echocardiography (Fuentes, 2008). There are now cardiac neurohormones and biomarkers like cardiac troponin I and T, ANP and BNP's that aid in determining myocyte injury, ischemia, and necrosis. Other special diagnostic techniques like holter monitoring and cardiac event recorder implants, continuous in-hospital electrocardiographic monitoring, non selective and selective angiography are gaining popularity overseas. Newer cardiac imaging techniques include computed tomography, magnetic resonance imaging and nuclear cardiology are also being employed (Sleeper, 2008). Standard treatment of these cardiac ailments involves the use of diuretics, positive inotropes, and ACE inhibitors (Ware, 2007).

Pimobendan is a novel cardiac pharmaceutical, termed an "inodilator" because it possesses both, positive inotropic and balanced peripheral vasodilatation properties (Gordon and Kittleson , 2008).Unlike historical positive inotropes (e.g. digoxin, milrinone) which function by increasing intracellular calcium concentrations, resulting in increased cardiac energy and oxygen requirements, pimobendan acts as a positive inotrope principally by enhancing the affinity of myocardial troponin C to existing intracellular calcium (Pouleur *et al*., 1989). The result is improved contractility without additional increased myocardial oxygen or energy requirement. Peripherally, pimobendan is a phosphodiesterase III (PDE III) inhibitor, resulting in balanced peripheral vasodilatation through increased efflux of intracellular calcium from vascular smooth muscle. Additional properties include reversal of desensitisation of

baroreceptors, improved cardiac relaxation (lusitropy), reduced platelet aggregation, and an anti-inflammatory effect mediated through favourable cytokine modulation (Gordon and Kittleson , 2008). Based on pimobendan's pharmacodynamic profile, it appears to be ideally suited in the treatment of heart failure associated with valvular heart disease and dilated cardiomyopathy (Kleeman *et al*. 1998). In spite of being a proven life saving drug in treatment of heart failure, it's not widely used in India by the veterinarians. The reasons being difficulty in procuring this drug in time as it has to be shipped from the UK, USA and Canada only on the order of a licensed veterinarian and also it being expensive for pet owners in India. Hence, in India, use of digoxin continues to persist in spite of pimobendan being the clinically preferred drug to treat the early as well as advanced stages of CHF in dogs.This study was hence planned with an objective for providing an easily available, affordable and effective remedy that could substitute the modern contemporary treatment i.e. pimobendan + enalapril + furosemide in treatment of VHD and/or DCM leading to CHF in dogs.

Ayurveda, an ancient system of Indian medicine has recommended number of drugs from plants for medicinal use. A single plant may be associated with a wide spectrum of activity without any concrete pharmacological basis. Most of the plants have a number of chemical constituents, some of which may be medicinally active. One such ayurvedic wonder drug is *Terminalia arjuna.* Apart from having medicinal effects on the digestive (Kirtikar and Basu 1935; Bakhru 2008), respiratory (Kumar *et al.* 1987), integumentary (Samy *et al.* 1998), urinary (Colabawalla, 1951), reproductive (Bakhru, 2008) and nervous (Nadkarni 1976) systems, it possesses a prime effect on the cardiovascular system. Its bark possesses cardiotonic properties (Ghoshal, 1909), chronotropic property (Chopra *et al*. 1958) and positive inotropic activity (Radhakrishnan *et al*., 1993).The extract of the bark is known to increase coronary flow (Bhatia *et al*., 1998) and has anti-hypertensive properties (Srivastava *et al*., 1992). Its effect on aortic prostaglandins and coronary flow (Bhatia *et al*., 1998), anti-oxidant properties (Dwivedi *et al*. 1988), hypolipidemic action (Shaila *et al.* 1998), left ventricular mass lowering effect (Dwivedi and Jauhari, 1997), have been documented. It is widely used in human medicine to treat angina pectoris, congestive cardiac failure, ischemic heart disease, cardiomyopathy (Dwivedi, 1998), and hypercholesterolemia (Khanna *et al*.,

1996). Very few studies have been carried out in dog models to validate arjuna's positive inotropic action (Gupta *et al.,* 1976), hypotensive property (Bhatia *et al.,* 2000) and its use as a cardiotonic in congestive heart failure (Rao *et al*., 2009). The potential of arjuna in veterinary cardiology has not been explored in spite of it possessing these versatile properties. *Terminalia arjuna* was therefore selected for the study as a positive inotropic and hypotensive herb, to determine its efficacy in VHD and/or DCM before their progression to CHF in clinical dog patients, so as to assess the possibility of replacement of pimobendan with arjuna.

4.1 Experimental animals

The entire study was carried out at a private veterinary clinic from February, 2011 to July, 2011. Owned dogs were screened for valvular heart disease (VHD) and/or dilated cardiomyopathy (DCM). Selection of patients was done independent of their age, weight, sex and breed. Selection criteria included only the ones diagnosed with VHD and/or DCM. These two cardiac ailments were selected because enough literature was available till date regarding the efficacy of pimobendan and arjuna in treating CHF once it has progressed. There were studies conducted with pimobendan to treat the stage of VHD/DCM before its advancement to CHF but not with arjuna. Hence to evaluate the efficacy of arjuna at an early stage of CHF was needed. Dogs with other exclusive cardiac ailments like systemic hypertension due to causes other than cardiac origin, congenital heart defects, pericardial disease, endocarditis, neoplasia were not included in the study. Total of 35 dogs were selected after screening for VHD and/or DCM using medical history, detailed physical examination, thoracic radiology, Doppler blood pressure measurement, electrocardiography and 2-D and M mode echocardiography. Out of these 35 dogs, 15 were put under group 1 and were administered pimobendan, enalapril and furosemide. While 20 of them were put under group 2 and were administered *Terminalia arjuna*, enalapril and furosemide. Non compliance included failure to administer drugs at proper intervals and at accurate dosages by owners due to which one patient from group 1 and three from group 2 had to be eliminated from the study. one patient from group 1 and two from group 2 that could not be administered the medicines due to their difficult behaviour on a regular basis were eliminated from the study.

Three patients in group 1 and two from group 2, to whom the coated capsules of pimobendan and *Terminalia arjuna* were opened and just the powder was fed by the owner, for easy administration were removed from the study. Finally the data of six patients out of ten patients from group 1 and six out of the thirteen under group 2 were finally included in the present work on the basis of the optimum responses achieved on post-treatment evaluation parameters at the end of the study.

Daily compliance was assured telephonically and through personal visits by the research worker during the study. All the patients were constantly observed by their owners and research worker for any slight deviation in their normal behaviour, appetite loss, mannerisms, general activity level and daily routine parameters like urine output and defecation output to assess the digestibility tolerance of drugs during the entire period of study. All the patients were subjected to a 30 day study after which they were clinically re-evaluated by detailed physical examination, thoracic radiology, electrocardiography, systolic blood pressure measurement and echocardiography to assess improvement in comparison to the basal values/parameters taken at the start of the study. The data collected pre and post treatment were subjected to paired t-test according to Snedecor and Cochran (1994) and comparison in between the two treatments, pimobendan and arjuna was done on the basis of percent change in the data per parameter, post treatment evaluation.

4.2 Drugs used for treatment of VHD and/or DCM resulting in CHF in clinical dog patients

The information regarding the dosage and dose regime of the drugs used was according to Plumb (2008). The standard drug used was pimobendan, which is categorized pharmacologically under the class Inodilators (calcium sensitizer and phosphodiesterase III inhibitor) and is manufactured by Boehringer Ingelheim Vetmedica, Inc. under the proprietary name Vetmedin. According to the body weight of the patient and ease of administration 1.25, 2.5, or 5 mg pimobendan available either as chewable tablets or capsules was used. It was given at the dose rate of 0.2-0.6 mg/ kg, q12 hrs, an hour before food (Fuentes, 2004). The test herb used was *Terminalia arjuna,* which is a pure herb product

manufactured by Himalaya Herbal Healthcare under the proprietary name Arjuna. A 250 mg capsule of *Terminalia arjuna* was administered twice daily (q12hrs) as per the available literature pertaining to safe dosage of *Terminalia arjuna* in dogs (Rao *et al*., 2009) and on the basis of a short pilot study conducted in dogs to determine the dose at which an optimum response could be achieved and the possible side effects encountered with changes in dosage. Enalapril, an ACE inhibitor and hypotensive drug was used which is manufactured by Dr. Reddy's Laboratories, under the proprietary name Enam. According to the body weight of the patient either 2.5 mg, 5 mg, 10 mg, or 20 mg tablets were administered at a dose rate of 0.25- 0.5 mg/kg, q12 hrs for each patient. Furosemide, a diuretic was used which is manufactured by Aventis Pharma Limited under the proprietary name Lasix. According to the body weight of the patient, either 20, 40 mg or 80 mg furosemide was used at the dose rate of 1.1-4.4 mg/kg, q12 hrs.

4.3 Description of patient and medical history

The description of each dog is given in Appendix 4 with respect to the study group the patient belongs to, name of the patient, name of the owner, breed of the patient, age of the patient, sex of the patient and weight of the patient. The patients in group 1 were in the range of 11 to 15 yrs with an average (mean ± S.E) of 12.6 ± 0.58 yrs. The body weights before treatment were in a range from 6 to 39 kg with an average (mean ± S.E) of 18.4 ± 5.84 and after treatment were in a range from 7 to 39.4 kg with an average (mean ± S.E) of 18.8 ± 5.9. The patients in group 2 were in the range of 7 to 11 yrs with an average (mean ± S.E) of 9 ± 0.56 yrs. The body weights before treatment were in a range from 11 to 40 kg with an average (mean ± S.E) of 31.1 ± 4.33 and after treatment were in a range from 10 to 38.6 kg with an average (mean ± S.E) of 30.7 ± 4.33.

The medical history is illustrated in Appendix 5 with respect to the specific symptom or symptoms exhibited by each patient because of which they were screened for a complete cardiac checkup that included detailed physical examination, thoracic radiology, Doppler blood pressure measurement, electrocardiography and 2-D and M mode echocardiography. The three main symptoms exhibited by the dog patients were coughing, exercise intolerance and

weakness or lethargy suggestive of a cardiac disease (Abbot, 2008). These were assessed pre and post treatment.

In group 1, patient 2 and 5 exhibited a subtle, dry and unproductive cough that wasn't time related. Patient 3 and 4 exhibited occasional nocturnal, subtle, dry and unproductive cough. Patient 2 and 6 showed signs of weakness. Patient 3, 4 and 5 were presented with the complaint of exercise intolerance. Post treatment with pimobendan, furosemide and enalapril for 30 days, in patient 2 and 5, the subtle, dry and unproductive cough reduced considerably; while in patient 3 and 4 nocturnal coughing completely subsided. In patient 2 and 6, symptom of weakness resolved and in patient 3, 4 and 5, improved exercise tolerance and willingness to go for a walk was noted. These observations are in accordance with those documented by Lombard *et al.* (2006) and Caro *et al*. (2009). Lombard *et al.* (2006) assessed the clinical efficacy of pimobendan versus benazepril for the treatment of acquired atrioventricular valvular disease in dogs and proved that pimobendan provided a significant advantage over benazepril in improving the clinical symptoms and quality of life in affected dogs. Caro *et al*. (2009) conducted a study in dogs, suffering from congestive heart failure caused by myxomatous valve disease (valvular heart disease). Post short–term treatment with pimobendan, they observed that the clinical symptoms like depressed demeanour, exercise intolerance, coughing, nocturnal dyspnoea, increased respiratory effort resolved. It also proved efficient in improving the quality of life in dogs suffering from heart failure as well as improved survivability (Fuentes *et al*. 2002; Smith *et al*. 2005; Gordon *et al*. 2006; Haggstrom *et al*. 2008; SoJeong *et al*. 2009).

In group 2, patient 1 and 5 were presented with a nocturnal, subtle, dry and unproductive cough. Patient 3 exhibited slightly moist and productive cough and 6 exhibited subtle, dry and unproductive cough that was time unrelated and worsened with exercise. Patient 1, 4 and 5 showed signs of weakness. Patient 2, 3, 4 and 6 were presented with the complaint of exercise intolerance. Post the treatment with *Terminalia arjuna*, furosemide and enalapril for 30 days, in patient 3 and 6, the cough reduced considerably; while in patient 1 and 5, nocturnal coughing completely subsided. In patient 1, 4 and 5, symptom of lethargy resolved and in patient 2, 3, 4 and 6, improved exercise tolerance and willingness

to go for a walk was noted. These observations are in agreement with Rao *et al.* (2009), who carried out a similar study to evaluate the efficacy of *Terminalia arjuna* in dogs suffering from congestive heart failure, using digoxin as a standard drug. They observed signs of clinical improvement in as early as 15 days to 30 days of treatment.

Hence, the integrated therapy of *Terminalia arjuna,* furosemide and enalapril proved to equally resolve the clinical manifestations of cardiac insufficiency like coughing, exercise intolerance and weakness to a considerable extent just like the combination of pimobendan, furosemide and enalapril. This proves strong potential of *Terminalia arjuna*, a positive inotrope that is known to improve contractility of the cardiac muscles, thus alleviating signs of weakness and exercise intolerance. However in this group, since furosemide and enalapril were also given along with arjuna, the decongestant effect could not be attributed directly to its action (Kumar *et al*., 1987) and further isolated studies in such patients using only arjuna without diuretics and ACE inhibitors are recommended.

4.4 Physical examination

The physical examination of all dog patients during the study was carried out as elucidated by Gompf (2008).The table in Appendix 6 denotes the information about parameters like rectal temperature, color of mucous membranes and capillary refill time (CRT), respiratory rate, abnormal lung sounds heard on lung auscultation, percussion and palpation findings, pre and post treatment. The detected abnormal lung sounds and breathing pattern were the probable reasons of VHD and/or DCM, which needed further investigations (Oyama 2008; Olsen *et al*. 2009). The range of body temperature of patients in group 1 pre treatment was 100.7 ºC to 102.5 ºC with an average (mean ± S.E) of 101.4 ± 0.291 ºC and the range post treatment was 101.5 ºC to 102.4 ºC with an average (mean ± S.E) of 102 ±0.144 ºC, which was within normal range. The average (mean ± S.E) body temperature of patients in group 2, pre treatment was 101.9 ± 0.186 ºC and post treatment was 101.7 ± 0.180, which was within normal range.

In group 1, patient 1,3,4 and 6 exhibited normal pink mucous membranes with CRT less than 2 sec which was within normal range as noted by Jones (2006).Patient 2 presented pale pink mucous membranes with normal CRT, while patient 5 exhibited hyperemic mucous membranes with normal CRT. The respiration rate in all patients was within normal range as stated by Jones (2006), except in patient 2 and 5, who displayed tachypnoea. The range for the respiratory rate in group 1, pre-treatment was 27 to 51 breaths/min with an average (mean ± S.E) of 35 ± 3.882 breaths/ min and the range post treatment was 31 to 36 breaths/min with an average (mean ± S.E) of 33 ± 0.730 breaths/ min. On lung auscultation, all patients exhibited varied degree and severity of pulmonary oedema and congestion, diagnosed by detection of crackles and wheezes, except patient 1 and 6, in whom abnormal lung sounds were not detectable. Patient 2 and 5 were detected with hepatomegaly that was diagnosed on palpation. Post treatment with pimobendan, furosemide and enalapril for 30 days, there was no change in the colour of the mucous membranes and CRT in the patients. Also the patients with normal respiratory rate showed no change in breathing pattern. But the breathing pattern regularised and normalised post treatment in patient 2 and 5 with tachypnoea that was detected before treatment. These observations in regards to the respiration rate and pattern were in harmony with those noted by Lombard *et al.* (2006) and Caro *et al*. (2009) who inferred that the regularization of respiration rate and pattern was due to reduction in the severity of pulmonary oedema that was secondary to DCM and VHD .

In group 2, patient 2 and 5 exhibited normal pink mucous membranes with CRT less than 2 sec which was within normal range as noted by Jones (2006).Patient 1,4 and 6 presented pale pink mucous membranes with normal CRT, while patient 3 exhibited hyperemic mucous membranes with prolonged CRT (3 sec). The respiration rate in all patients was within normal range as stated by Jones (2006), except in patient 3 and 6, who displayed tachypnoea. The range of respiratory rate in group 2, pre treatment was 27 to 49 breaths/min with an average (mean ± S.E) of 35.833 ± 3.572 breaths/ min and the range post treatment was 31 to 35 breaths/min with an average (mean ± S.E) of 32.8 ± 0.65 breaths/min. On lung auscultation, all patients exhibited varied degree and severity of pulmonary oedema and congestion, diagnosed by detection of

crackles and wheezes, except patient 2 and 4, in whom abnormal lung sound were not detectable. Patient 6 was detected with hepatomegaly that was diagnosed on palpation. Post the treatment with *Terminalia arjuna*, furosemide and enalapril for 30 days, there was no change in the colour of the mucous membranes and CRT, except patient 3 exhibited normal CRT. Also the patients with normal respiratory rate showed no change in breathing pattern. But the breathing pattern regularised and normalised post treatment in patient 3 and 6 with tachypnoea that was detected before treatment.

These observations in regards to the respiration rate and pattern were in accordance with those stated by Rao *et al.* (2009) who documented reduction in pulmonary oedema and congestion, that occurred after concurrent treatment with *Terminalia arjuna*, furosemide and enalapril in dogs suffering from congestive heart failure. This in turn led to improvement in breathing rate and pattern in those suffering from tachypnoea.

The decongestant action of *Terminalia arjuna* was proven by Kumar *et al.* (1987) while its mild diuretic effect was documented by Colabawalla (1951). However in this group, since furosemide and enalapril were also given along with arjuna, the decongestant and diuretic effect could not be attributed directly to arjuna's action and further isolated studies in such patients using only arjuna without diuretics and ACE inhibitors should be conducted. The reduction in pulmonary oedema and congestion decreased the intensity of the crackles and occasional wheezes along with ameliorating the tachypnoeic breathing pattern of the dog patients.

The table in Appendix 7 reveals data about the cardiovascular parameters like heart rate and rhythm, murmurs heard on auscultation and their grades, pulse rate, rhythm and quality, pre and post treatment. The murmurs heard on auscultation were located and graded according to Appendix 8. The technique was followed as stated by Gompf (2008). The presence of murmurs, abnormal heart rhythm and pulse abnormalities was probably suggestive of VHD and/or DCM, which needed further investigations (Haggstrom *et al.* 2004; Oyama, 2008; Atkins *et al*. 2009).The heart rate and pulse rate was within normal range for all the dog patients in both the groups as elucidated by Jones (2006).

The range of heart rate in group 1 pre and post treatment was 82 to 130 bpm and 92 to 126 bpm respectively with corresponding average (mean ± S.E) of 105.667 ± 8.188 bpm and 108.667 ± 5.719 bpm. The range of pulse rate pre and post treatment was 76 to 127 bpm and 81 to 129 bpm with corresponding average (mean ± S.E) of 100.5 ± 9.266 bpm and 103.167 ± 7.791 bpm. The range of heart rate in group 2 pre and post treatment was 98 to 123 bpm and 103 to 120 bpm with a corresponding average (mean ± S.E) of 107 ± 3.958 bpm and 110.5 ± 2.907 bpm. The range of pulse rate pre and post treatment was 82 to 120 bpm and 91 to 118 bpm with corresponding average (mean ± S.E) of 100.833 ± 5.689 bpm and 107.667 ± 3.844 bpm.The heart rhythm, type of murmur and grade, pulse rhythm and quality did not alter post the treatment with either pimobendan or *Terminalia arjuna,* that was added to furosemide and enalapril in the patient groups. All patients exhibited a systolic left apical murmur of various grades symbolizing severity of mitral regurgitation. The other murmurs were noted but not included in the study. Patient 3 and 5 from group 1 and patient 3 and 4 from group 2, who suffered from arrhythmia, did not show any improvement in their heart rhythm post the treatment. These observations could not be compared as there were no such data available in the literature referred. However, pimobendan and arjuna both had no effect on the heart rate and rhythm, pulse rate and rhythm and change in grade of murmur.

The table no. 2, 3 and 4 provide statistical interpretation of the pre and post values of both treatments for all the parameters studied by thoracic radiology, electrocardiography, systolic B.P measurement and 2-D & M mode echocardiography.

4.5 Thoracic radiology

The table in Appendix 9 shows the vertebral heart score (VHS) values of all the dogs in group 1 and group 2. The range of VHS in group 1 pre and post treatment was 10.2 to 10.5 with an average (mean ± S.E) of 10.383± 0.048. The range of VHS in group 2 pre and post treatment was 10.2 to10.8 with an average (mean ± S.E) of 10.417±0.087. According to Buchanan *et al*. (1995), the VHS of the dogs in both groups had increased considering the breed variation in the present study. There was absolutely no difference in the average values of both

Table no. 2 Group 1: paired T -test results at 5 % significance level

Parameter	Pre	Post	$T_{Calculated}$	T_{Table}	D.F
VHS	10.383± 0.048	10.383± 0.048	0	0	5
HR (bpm) (ECG)	104.333 ±7.219	107.5 ± 4.884	0.616	2.570	5
P wave width (sec)	0.063 ± 0.003	0.063 ± 0.003	0	0	5
P wave height (mV)	0.25 ± 0.043	0.25 ± 0.043	0	0	5
PR interval (sec)	0.107 ± 0.008	0.107 ± 0.008	0	0	5
QRS width (sec)	0.043 ± 0.003	0.043 ± 0.003	0	0	5
QRS height (mV)	1.6 ± 0.312	1.6 ± 0.312	0	0	5
QT segment (sec)	0.213 ± 0.008	0.213 ± 0.008	0	0	5
MEA (degree)	80 ± 6.325	80 ± 6.325	0	0	5
Systolic B.P (mm Hg)	170 ± 10.801	130.8333 ± 3.005	4.0754	2.5705	5

Table no. 2 Group 1: (contd.)

Parameter	Pre	Post	$T_{Calculated}$	T_{Table}	D.F
HR(ECHO) (bpm)	110.333±7.33	111.667±6.020	0.4	2.570	5
LA (cm)	3.103 ± 0.281	3.122 ± 0.280	5.965	2.570	5
AO (cm)	1.835 ± 0.152	1.828 ± 0.144	0.5	2.570	5
LA:AO	1.687 ± 0.031	1.697 ± 0.025	0.759	2.570	5
LVIDd (cm)	3.953 ± 0.287	3.79 ± 0.286	9.572	2.570	5
LVIDs (cm)	3.135 ± 0.267	2.9 ± 0.251	11.140	2.570	5
FS (%)	20.952±1.495	23.695 ±1.420	12.241	2.570	5
EDV (ml)	41.088±3.244	39.412±3.238	40.454	2.570	5
ESV (ml)	23.493 ± 2.45	21.135 ±2.337	17.244	2.570	5
EF (%)	43.16 ± 2.477	46.685 ±2.530	36.399	2.570	5
EPSS (cm)	0.488 ±0.056	0.51 ± 0.060	4.539	2.570	5
IVSd (cm)	0.798 ± 0.081	0.865 ± 0.089	6.523	2.570	5
IVSs (cm)	1.152 ± 0.097	1.213 ± 0.099	4.646	2.570	5
LVPWd (cm)	0.712 ± 0.082	0.76 ± 0.077	3.145	2.570	5
LVPWs (cm)	1.037 ± 0.088	1.092 ± 0.088	7.651	2.570	5

Table no. 3 Group 2: paired T -test results at 5 % significance level

Parameter	Pre	Post	$T_{Calculated}$	T_{Table}	D.F
VHS	10.417±0.087	10.417±0.087	0	0	5
HR (bpm) (ECG)	107 ± 1.732	106.5 ± 5.018	0.0980	2.5705	5
P wave width (sec)	0.057± 0.003	0.057 ± 0.003	0	0	5
P wave height (mV)	0.317± 0.065	0.317 ± 0.065	0	0	5
PR interval (sec)	0.113 ± 0.007	0.113 ± 0.007	0	0	5
QRS width (sec)	0.047± 0.007	0.047± 0.007	0	0	5
QRS height (mV)	1.517 ± 0.250	1.517 ± 0.250	0	0	5
QT segment (sec)	0.22 ± 0.009	0.22 ± 0.009	0	0	5
MEA (degree)	85 ± 5.000	85 ± 5.000	0	0	5
Systolic B.P (mm Hg)	160.8333 ± 6.509	140.8333 ± 5.069	7.7459	2.5705	5

Table no. 3 Group 2: (contd.)

Parameter	Pre	Post	$T_{Calculated}$	T_{Table}	D.F
HR(ECHO) (bpm)	111.167±4.512	110.5 ±3.704	0.147	2.570	5
LA (cm)	3.742± 0.167	3.79 ± 0.164	8.043	2.570	5
AO (cm)	2.373 ± 0.113	2.418 ± 0.116	5.891	2.570	5
LA:AO	1.575 ± 0.017	1.567 ± 0.021	1.274	2.570	5
LVIDd (cm)	4.52 ± 0.213	4.42 ± 0.214	5.319	2.570	5
LVIDs (cm)	3.518 ± 0.152	3.392 ± 0.156	9.683	2.570	5
FS (%)	22.043 ± 0.995	23.178 ± 0.931	8.460	2.570	5
EDV (ml)	42.28 ± 2.667	40.53 ± 2.631	10.32	2.570	5
ESV (ml)	22.79 ± 1.250	21.39 ± 1.255	5.125	2.570	5
EF (%)	44.915 ± 1.900	46.993 ± 1.762	6.651	2.570	5
EPSS (cm)	0.617 ± 0.043	0.642 ± 0.040	5.838	2.570	5
IVSd (cm)	0.98 ± 0.072	1.023 ± 0.075	6.5	2.570	5
IVSs (cm)	1.358 ± 0.072	1.403 ± 0.074	10.509	2.570	5
LVPWd (cm)	0.822 ± 0.048	0.863 ± 0.048	8.730	2.570	5
LVPWs (cm)	1.212 ± 0.055	1.258 ± 0.061	5.533	2.570	5

Table no. 4 % Change in between the 2 treatments

Sr.no	Parameter	% Change in pimobendan group (1)	% Change in arjuna group (2)
1.	VHS	0	0
2.	HR (ECG) (bpm)	3.035 increase	0.467 decrease
3.	P wave width (sec) & height (mV), PR interval (sec),QRS width(sec) & height (mV), QT segment (sec), MEA(degree)	0	0
4.	Systolic B.P (mm Hg)	23.039 decrease	12.435 decrease
5.	HR (ECHO) (bpm)	1.209 increase	0.599 decrease
6.	LA (cm)	0.6123 increase	1.282 increase
7.	AO (cm)	0.3814 decrease	1.896 increase
8.	LA:AO	0.5927 increase	0.507 decrease
9.	LVIDd (cm)	4.123 decrease	2.212 decrease

Table no. 4 (contd.)

Sr.no	Parameter	% Change in pimobendan group (1)	% Change in arjuna group (2)
10.	LVIDs (cm)	7.496 decrease	3.581 decrease
11.	FS (%)	13.091 increase	5.149 increase
12.	EDV (ml)	4.079 decrease	4.13 decrease
13.	ESV (ml)	10.037 decrease	6.143 decrease
14.	EF (%)	8.167 increase	4.626 increase
15.	EPSS (cm)	4.508 increase	4.051 increase
16.	IVSd (cm)	8.395 increase	4.387 increase
17.	IVSs (cm)	5.295 increase	3.313 increase
18.	LVPWd (cm)	6.741 increase	4.987 increase
19.	LVPWs (cm)	5.303 increase	3.795 increase

groups before and after treatment. Both the drugs, arjuna and pimobendan did not have any effect on the VHS. These observations about pimobendan were not in accordance with Lombard *et al.* (2006) and Caro *et al*. (2009), who reported reduction in the VHS with use of pimobendan in asymptomatic and early stages of VHD and DCM. In present study, the selected dogs were in middle to advanced stage of VHD and DCM, from where the reversal of pathological remodeling would be impossible. Also it is unlikely that in the current study, in such a short treatment time (30 days), the pathological remodeling would revert back to normalcy. The observations about arjuna were in harmony with Rao *et al.* (2009) who reported no change in the VHS post the use of arjuna in treating CHF.

The table in Appendix 10 displays the qualitative assessment of the pulmonary patterns in the thoracic radiographs. This table gives us the data about the severity of pulmonary oedema and congestion before and after treatment. In group 1 and 2, the dogs exhibited mild to moderate pulmonary oedema and congestion on the thoracic radiographs. Post the treatment with in both groups, the dogs showed considerable improvement and the pulmonary oedema and congestion reduced in severity. The observations regarding pimobendan were in agreement with Lombard *et al.* (2006) and Caro *et al*. (2009), who demonstrated reduction in pulmonary oedema and congestion after pimobendan administration. Atkinson *et al*. (2009) also stated that the pulmonary hypertension leading to pulmonary congestion was significantly reduced after administration of pimobendan in dog VHD patients. The observations regarding arjuna were in harmony with Rao *et al.* (2009) who documented reduction in pulmonary oedema and congestion on radiographs in dogs suffering from CHF post treatment with arjuna, furosemide and enalapril. Hence, the integrated therapy of *Terminalia arjuna,* furosemide and enalapril proved to equally resolve the pulmonary oedema and congestion just like pimobendan, furosemide and enalapril.

The radiographs pre and post treatment of patient 5 from group 1 and patient 2 from group 2 are shown in Fig no. 15, 16 and 17, 18 respectively. In Fig. no. 15, the arrow indicates alveolar pulmonary pattern mixed with interstitial pulmonary pattern indicating moderate pulmonary oedema and congestion

respectively in patient pre treatment. This pulmonary pattern was observed to improve after treatment in the pimobendan group, as seen in Fig.no.16. In Fig. no. 17, the arrows indicate alveolar pulmonary pattern mixed with interstitial pulmonary pattern indicating severe pulmonary oedema and congestion respectively in patient pre treatment. This pulmonary pattern was observed to improve after treatment in the arjuna group, as seen in Fig.no.17.

4.6 Electrocardiography

The Appendix 11 gives a view of the electrocardiography readings of the dog patients in both the groups.

The pre treatment range of the heart rate in group 1 was 87 to 131 bpm with an average (mean ± S.E) of 104.333 ± 7.219 bpm while the post treatment range was 92 to 120 bpm with an average (mean ± S.E) of 107.5 ± 4.884 bpm. The pre treatment range of the heart rate in group 2 was 102 to 113 bpm with an average (mean ± S.E) of 107 ± 1.732 bpm while the post treatment range was 88 to 120 bpm with an average (mean ± S.E) of 106.5 ± 5.018. While comparing the two treatment groups, no statistically significant difference was observed in heart rate in either group. This indicated that both drugs did not bring about any change in heart rate at the given dose. However, during comparison between the two drugs, a meagre rise in the heart rate was noted in the pimobendan treated group while a slight fall was noted in the arjuna treated group. The variation in breed and nature of the dogs used in this study by the author were the probable reason for this mild deviation in the heart rate. No comparable data were available regarding the effect of pimobendan and arjuna on the heart rate of clinical cardiac patients in the literature referred.

The heart rhythm was noted in all dog patients. Patient 3 and 5 from group 1 exhibited atrial fibrillation diagnostic of VHD and DCM while others exhibited sinus arrhythmia. In group 2, patient 3 and 4 showed atrial fibrillation, while others exhibited sinus arrhythmia. This arrhythmic pattern and atrial fibrillation on the electrocardiogram probably suggestive of VHD and/or DCM (Abbot, 2008; Meurs, 2009) was unaffected post treatment in both the groups. This indicated that pimobendan and arjuna had no effect on heart rhythm. These

observations were in accordance with Lombard *et al.* (2006) and Caro *et al*. (2009) who documented that pimobendan had no effect on arrhythmia and atrial fibrillation and Rao *et al.* (2009) who stated that arjuna had no effect on electrocardiographic findings including the heart rhythm.

The P wave width in group 1, both, pre and post treatment ranged from 0.06-0.08 sec with an average (mean ± S.E) of 0.063 ± 0.003 sec. In group 2, the P wave width, pre and post treatment ranged from 0.04-0.06 sec with an average (mean ± S.E) of 0.057± 0.003 sec. The averages of both the groups were indicative of left atrial enlargement as elucidated by Tilley and Smith (2008). Hence it was certain that all the dogs in the study had a left atrial enlargement probably suggestive of VHD and/or DCM (Harpster, 1983; Meurs, 2009). As there were no differences in the averages pre and post treatment, in both groups, pimobendan and arjuna both did not alter the P wave width measurements in the affected dogs. The height of P wave, in group 1 ranged from 0.1 to 0.4 mV with an average (mean ± S.E) of 0.25 ± 0.043 mV**,** before and after treatment. The height of P wave, in group 2 ranged from 0.1 to 0.5 mV with an average (mean ± S.E) of 0.317± 0.065 mV, before and after treatment. The averages of both the groups, pre and post treatment were within normal limits as elucidated by Tilley and Smith (2008). As there were no differences in the averages pre and post treatment, in both groups, pimobendan and arjuna both did not alter the P wave height measurements in the affected dogs.

These observations were in harmony with Lombard *et al.* (2006) and Caro *et al*. (2009) who documented that pimobendan had no effect on electrocardiogram findings and Rao *et al.* (2009) who stated that arjuna did not alter the electrocardiographic measurements in cardiac patients. As the selected dogs were in middle to advanced stage of VHD and DCM, from where the reversal of pathological remodeling would be impossible and hence it is impractical to expect reduction in the electrocardiogram measurements indicative of chamber enlargements.

In group 1, the PR segment ranged from 0.08-0.12 sec with an average (mean ± S.E) of 0.107 ± 0.008 sec, pre and post treatment. In group 2, the PR segment ranged from 0.08-0.12 sec with an average (mean ± S.E) of 0.113 ±

0.007, pre and post treatment. The averages of both the groups, pre and post treatment were within normal limits as elucidated by Tilley and Smith (2008). As there were no differences in the averages pre and post treatment, in both groups, pimobendan and arjuna both did not alter the PR segment measurements in the affected dogs. The QRS width, in group 1, ranged from 0.04- 0.06 sec with an average (mean ± S.E) of 0.043 ± 0.003 sec, before and after treatment. The QRS width, in group 2, ranged from 0.04- 0.08 sec with an average (mean ± S.E) of 0.047± 0.007 sec, before and after treatment. Hence there was no electrocardiographic evidence of left ventricular enlargement. The values were within normal range as documented by Tilley and Smith (2008). The QRS height, in group 1, ranged from 0.6- 2.3 mV with an average (mean ± S.E) of 1.6 ± 0.312 mV, before and after treatment. The QRS height, in group 2, ranged from 0.6-2.3 mV with an average (mean ± S.E) of 1.517 ± 0.250 mV, before and after treatment. The values were within normal range as documented by Tilley and Smith (2008). As there were no differences in the averages pre and post treatment, in both groups, pimobendan and arjuna both did not alter the QRS width and height in the dog patients.

The QT segment, in group 1, ranged from 0.20-0.24 sec with an average (mean ± S.E) of 0.213 ± 0.008 sec, before and after treatment. The QT segment, in group 2, ranged from 0.20-0.24 sec with an average (mean ± S.E) of 0.22 ± 0.009 sec, before and after treatment. The values were within normal range as documented by Tilley and Smith (2008). As there were no differences in the averages pre and post treatment, in both groups, pimobendan and arjuna both did not alter the QT segment measurements in the dog patients. ST slurring was noted in patient 3 and 6 of group 1 and patient 3 of group 2. This was indicative of left ventricular enlargement or myocardial ischaemia as stated by Tilley and Smith (2008). This ST segment pattern did not change post the administration of pimobendan and arjuna at their therapeutic doses. Patient 5 from group 1 and patient 2 from group 2 exhibited tall T waves indicative of either hyperkalaemia or myocardial infarction as documented by Tilley and Smith (2008). This T wave pattern did not change post the administration of pimobendan and arjuna at their therapeutic doses.

The mean electrical axis (MEA) in group 1 ranged from 60-90º with an average (mean ± S.E) of 80 ± 6.325º, pre and post treatment, while the MEA in group 2, ranged from 60-90º with an average (mean ± S.E) of 85 ± 5.000º, pre and post treatment. The values were within normal range as stated by Tilley and Smith (2008). MEA did not change post the administration of pimobendan and arjuna at their therapeutic doses. Patient 2 and 5 in group 1 and patient 6 from group 2 exhibited low voltage PQRS pattern indicative of a respiratory disease. This pattern did not alter post the administration of pimobendan and arjuna at their therapeutic doses.

The figures, 19 and 20, represent the pre and post ECG findings of patient 6 from group 1 respectively and figures 21 and 22 represent the pre and post ECG findings of patient 1 from group 2 respectively. There was no change noted in the ECG readings by both the drugs. Hence the overall interpretation of the electrocardiogram suggested that there was an evidence of left atrial enlargement in all dog patients from group 1 and 2. There was no significant difference in the values of the electrocardiogram measurements in both groups after treatment suggesting that pimobendan and arjuna had no impact on electrocardiographic values. These observations were in harmony with Lombard *et al.* (2006) and Caro *et al*. (2009) who documented that pimobendan had no effect on electrocardiogram findings and Rao *et al.* (2009) who stated that arjuna did not affect the electrocardiographic measurements in dog cardiac patients.

4.7 Systolic blood pressure (B.P) measurement

Vasodilators help to reduce the preload and after load in dog patients suffering from DCM and VHD. Systolic B.P was measured using an ultrasonic Doppler flow detector in the dog patients and the values are given in Appendix 12.

The recorded B.P, pre treatment in group 1, was in the range 145-220 mm Hg with an average (mean ± S.E) of 170 ± 10.801 mm Hg and in group 2, was in the range 145-190 mm Hg with an average (mean ± S.E) of 160.833 ± 6.509 mm Hg, which was indicative of hypertension as elucidated by Ware (2007). The post treatment B.P in group 1 was in the range 120-140 mm Hg with

an average (mean ± S.E) of 130.833 ± 3.005 mm Hg and in group 2 was in the range 130-165 mm Hg with an average (mean ± S.E) of 140.833 ± 5.069 mm Hg which was within normal limits (Ware, 2007). While comparing the data statistically, there was a significant reduction in the B.P in dog patients treated with both, pimobendan and in arjuna ($P < 0.05$). While comparing the efficacy of these two drugs, pimobendan brought about 23.039% reduction in the systolic B.P as against 12.435% reduction by arjuna. The observations regarding the performance of pimobendan were in harmony with views expressed by Gordon and Kittleson (2008) who classified pimobendan as an inodilator (i.e. positive inotrope and balanced systemic arterial and venous dilator) and Fugimoto (1994) who proved that pimobendan had a potent vasorelaxant property by acting directly on vascular smooth muscles and that the vasorelaxant effect was mediated through cyclic AMP-dependent mechanisms using murine model. The observations aligned with Fugimoto and Matsuda (1990) who stated that pimobendan through phosphodiesterase III and V inhibition, promotes arteriolar and venous dilation, thus reducing afterload and preload respectively. The observations regarding the performance of arjuna were in total agreement with Colabawalla (1951), Singh *et al*. (1982), Srivastava *et al*. (1992), Takahashi *et al*. (1997), Bhatia *et al*. (2000) and Nammi *et al*. (2003) who proved the strong anti-hypertensive potential of *Terminalia arjuna* in different animal models including dogs. The present study also supported arjuna's vasodilator properties.

Systemic arterial blood pressure is the product of cardiac output and the total peripheral resistance and hence anti-hypertensive drugs should aim at reducing cardiac output, total peripheral resistance, or both in cardiac patients. Pimobendan and arjuna, both significantly reduced the systolic blood pressure in VHD and DCM patients suffering from hypertension in the present study. However the treatment with pimobendan was found to be superior as compared with arjuna as far as the anti-hypertensive property was concerned.

4.8 2-D and M mode echocardiography

The procedure was followed according to Fuentes (2008) and Boon (2002). The echocardiographic values obtained by the right parasternal long axis (RPS LAx) and short axis (RPS SAx) views using the M mode have been

illustrated in Appendix 13. The Appendix 14 shows the degree of mitral regurgitation found on the left parasternal (LPS) caudal four-chamber view as categorised by Pederson *et al*. (1999).

Both the drugs, namely pimobendan and arjuna are known to possess chronotropic property. In the present study, the heart rate, in group 1, pre and post treatment, ranged from 88-131 bpm and 95-133 bpm respectively with a corresponding average (mean ± S.E) of 110.333±7.333 bpm and 111.667±6.020 bpm. The heart rate, in group 2, pre and post treatment, ranged from 97-127 bpm and 94-118 bpm respectively with a corresponding average (mean ± S.E) of 111.167± 4.512 bpm and 110.5 ± 3.704 bpm. The values were in normal range in both the groups as stated by Kienle and Thomas (2002).While comparing the two treatment groups, there was no significant difference observed in heart rate in either group receiving these drugs. However when the two treatments were compared, arjuna brought about 0.599% reduction in heart rate as compared to 1.209% increase in the pimobendan group.

The reduction in heart rate in dogs, post treatment with pimobendan was noted by Caro et al. (2009), but the present study recorded a marginal rise (1.209 %) in the pimobendan treatment group. The probable reason for this disagreement with Caro *et al*. (2009) could be the presence of two cases of atrial fibrillation within the pimobendan group and the variation in breed and nature of the dogs used in this study by the author. While the observations of the present study were in harmony with Pouleur *et al*. (1989) who also observed slight rise in the heart rate of dogs post administering pimobendan. While in case of arjuna, marginal reduction in the heart rate (0.599%) was noted and these findings were in harmony with Chopra *et al.* (1958) who proved chronotropic activity of aqueous extract of *Terminalia arjuna* in an isolated frog heart.

The dimensions of the left atrial size (LA), aorta (AO) and LA: AO ratio in before treatment pimobendan group ranged from 2.35-4.07 cm, 1.53-2.34 cm and 1.61-1.81 respectively with corresponding averages (mean ± S.E) of 3.103 ± 0.281 cm, 1.835 ± 0.152 cm and 1.687 ±0.031. The post treatment dimensions of LA, AO and LA: AO ranged from 2.37-4.08 cm, 1.45-2.31 cm and 1.63-1.77 respectively with corresponding averages (mean ± S.E) of 3.122 ± 0.280 cm, 1.828 ± 0.144 cm and 1.697±0.025. In group 2, the dimensions of the left atrial

size (LA), aorta (AO) and LA: AO ratio in before treatment pimobendan group ranged from 2.95-4.03 cm, 1.88-2.26 cm and 1.5-1.62 respectively with corresponding averages (mean ± S.E) of 3.742± 0.167 cm, 2.373 ± 0.113 cm and 1.575 ±0.017. The post treatment dimensions of LA, AO and LA: AO ranged from 3.01-4.07 cm, 1.92-2.74 cm and 1.48-1.63 respectively with corresponding averages (mean ± S.E) of 3.79 ± 0.164 cm, 2.418 ± 0.116 cm and 1.567 ±0.021.

In the present study, the mean LA :AO ratio in both pimobendan and arjuna treated groups was more than 1.3 indicating echocardiographic evidence of left atrial enlargement in all dogs according to Kienle and Thomas (2002) suggestive of either VHD and/or DCM. It was observed that there was no significance difference in before and after treatment values of all these three parameters, i.e. LA, AO and LA: AO ratio in both the groups. This indicated that none of the drugs was effective enough in decreasing the LA, AO and LA: AO values. However, while comparing LA: AO ratio in pimobendan treated group, a increase of 0.5927 % was observed after treatment, whereas in arjuna treated group, a decrease of 0.507 % in the LA: AO ratio was noted. Though this change was insignificant, Arjuna proved its inotropic property that resulted in improving atrial myocardial contractility which probably contributed to this minor reduction in LA: AO ratio in the present study. This observation denoting arjuna's inotropic activity was in agreement with Chopra *et al*. (1958), Ghoshal (1909) and Gupta *et al.* (1976) who demonstrated cardiotonic and stimulant actions of arjuna in isolated frog and rabbit heart, Radhakrishnan *et al*. (1993), Karamsetty *et al.* (1995) and Oberoi and Liu (2007) who studied arjuna's inotropic activity on murine model and Rao *et al*. (2009) who proved that arjuna improved left ventricular ejection fraction in clinical dog patients. However, no such parallel data was available in the literature of pimobendan for comparison.

Many herbs claim to improve the myocardial contractility (inotropic effect) and arjuna is one of them. Pimobendan is also known to improve the myocardial contractility by channelizing the calcium uptake, being a phosphodiesterase III inhibitor. In the present study, the improvement in myocardial contractility was studied by 2 D and M mode echocardiography. The measurements of the left ventricular internal dimension diastole (LVIDd), left ventricular internal dimension systole (LVIDs) and fractional shortening (FS) in

group 1, before treatment ranged from 3.12-4.88 cm, 2.49-4.16 cm and 14.75-24.27 % respectively with corresponding averages (mean ± S.E) of 3.953 ±0.287 cm, 3.135 ±0.267 cm and 20.952 ±1.495 %. The post treatment measurements of LVIDd, LVIDs and FS ranged from 2.94-4.65 cm, 2.30-3.83 cm and 17.63-26.52 % with corresponding averages (mean ± S.E) of 3.79±0.286 cm, 2.9±0.0251 cm and 23.695±1.420 %. In group 2, measurements of LVIDd, LVIDs and FS before treatment ranged from 3.54-5.02 cm, 2.85-3.88 cm and 18.56-24.60 % respectively with corresponding averages (mean ± S.E) of 4.52 ±0.213 cm, 3.518 ±0.152 cm and 22.043 ±0.995 %. The post treatment measurements of LVIDd, LVIDs and FS ranged from 3.46-4.92 cm, 2.74-3.77 cm and 20.10-25.88 % with corresponding averages (mean ± S.E) of 4.42 ±0.241 cm, 3.392 ±0.156 cm and 23.178 ±0.931 %. In the pre treatment group, considering the mean values of internal diameters of the left ventricles in all dogs during systole and diastole were found to be significantly high ($P<0.05$) to those reported in normal dogs of same weight group by Boon *et al* (1983) indicating that all the dogs in the study had left ventricular enlargement. At the same time FS% values in the pre treatment group for both the drugs were lower than the standard value as stated by Fuentes, (2008) and Moise and Fox (1999) that indicated enlargement of left ventricles that resulted in the reduction of myocardial contractility which is commonly a complication of DCM alone or along with VHD.

After analysis of the data, it was observed that there was a significant reduction in systolic and diastolic left ventricular diameters with a marked significant improvement in FS % in both groups ($P < 0.05$), after treatment. Between the two treatments, pimobendan brought about improvement by 4.123 % reduction in LVIDd and 7.496 % reduction in LVIDs with corresponding 13.091 % improvement in FS% as against the 2.212 % reduction in LVIDd, 3.581 % reduction in LVIDs with corresponding 5.149% increase in FS% by Arjuna. The observations regarding improvement with pimobendan were in harmony with Caro *et al*. (2009) and Lombard (2006) who conducted study on VHD affected dogs to evaluate the short term effects of pimobendan. They observed a marked reduction in the LVIDd and LVIDs with a highly significant increase in the FS%. Nobuyuki *et al.* (1997) also stated that pimobendan significantly enhanced LV systolic and diastolic performances in CHF affected dogs as in comparison to amrinone. Ouellet *et al.* (2009) reported significant decrease in the LVIDd and

LVIDs in asymptomatic VHD affected dogs post treatment at 30 days. In case of Arjuna, the observations were in accordance with Rao *et al*. (2009) who also documented highly significant decrease in LVIDd and LVIDs along with highly significant increase in FS% in CHF affected dogs in a two month long study with arjuna and digoxin. In the present day study, the treatment with pimobendan was found to be superior as compared with arjuna as far as the inotropic property was concerned. However, no parallel data was available in the referred literature indicating direct comparison of these two drugs.

The echocardiographic measurements of end diastolic volume (EDV), end systolic volume (ESV) and ejection fraction (EF) in group 1, pre treatment ranged from 32.43-52.22 ml, 17.69-31.11 ml and 33.69-48.96 % respectively with corresponding averages (mean ± S.E) of 41.088 ±3.244 ml, 23.493 ±2.259 ml and 43.16 ±2.477 %. The post treatment measurements of EDV, ESV and EF ranged from 30.92-50.61 ml, 15.49-28.46 ml and 36.99-52.64 % respectively with corresponding averages (mean ± S.E) of 39.412±3.238 ml, 21.135±2.337 ml and 46.685±2.530 %. In group 2, measurements of EDV, ESV and EF pre treatment ranged from 33.62-50.97 ml, 19.07-26.52 ml and 37.45-49.03 & respectively with corresponding averages (mean ± S.E) of 42.28 ±2.667 ml, 22.79-1.250 ml and 44.951 ±1.900 %. The post treatment measurements of EDV, ESV and EF ranged from 32.14-49.29 ml, 17.46-24.38 ml and 40.89-51.04 % respectively with corresponding averages (mean ± S.E) of 40.53 ±2.631 ml, 21.39 ±1.255 ml and 46.993 ±1.762 %. EF% in both groups was lower than the recommended levels by Fuentes, (2008) and Moise and Fox (1999) indicating reduction in cardiac output as a result of diminished LV contractility due to either DCM or DCM and VHD (O'Grady and Horne, 1998).

After analysis of the data, it was observed that there was a significant reduction in end systolic and diastolic volumes with a marked significant improvement in EF % in both groups ($P < 0.05$), after treatment. Between the two treatments, pimobendan brought about improvement by 4.079 % reduction in EDV and 10.037 % reduction in ESV with corresponding 8.167 % improvement in EF% as against the 4.13 % reduction in EDV, 6.143 % reduction in ESV with corresponding 4.626 % increase in EF% by Arjuna. Hence both pimobendan and arjuna were capable of improving the EF% post treatment.

The observations regarding improvement with pimobendan were in harmony with Caro et al. (2009) and Lombard (2006) who conducted study on VHD affected dogs to evaluate the short term effects of pimobendan. They observed a highly significant increase in the EF%. Nobuyuki et al. (1997) also stated that pimobendan significantly enhanced LV systolic and diastolic performances in CHF affected dogs as in comparison to amrinone. Ouellet *et al.* (2009) reported significant increase in EF% in asymptomatic VHD affected dogs at 30 days post treatment with pimobendan while Pouleur *et al.* (1989) proved pimobendan's inotropic effect in dogs with heart failure. Also Gordon *et al*. (2006) stated that pimobendan was recommended in VHD and/or DCM affected dogs due to its ability to improve the quality of life by increasing the myocardial contractility and efficacy in heart failure patients. In case of Arjuna, the observations were in accordance with Rao *et al*. (2009) who also documented highly significant increase in EF% in CHF affected dogs in a two month long study with arjuna and digoxin. Animal studies denoting arjuna's inotropic activity such as Chopra *et al*. (1958), Ghoshal (1909) and Gupta *et al.* (1976) who demonstrated cardiotonic and stimulant actions of arjuna in isolated frog and rabbit heart, Radhakrishnan *et al*. (1993), Karamsetty *et al.* (1995) and Oberoi and Liu (2007) who studied arjuna's inotropic activity on murine model supported the inotropic potential of arjuna. However, in the present day study, the treatment with pimobendan was found to be superior as compared with arjuna as far as the inotropic property was concerned. However, no parallel data was available in the referred literature indicating direct comparison of these two drugs.

The end point to septal separation (EPSS) values in pre and post treatment in group 1 ranged from 0.36-0.69 cm and 0.37-0.73 cm respectively with their corresponding average (mean ± S.E) of 0.488 ±0.056 cm and 0.51±0.060 cm. The EPSS values in pre and post treatment in group 2 ranged from 0.41-0.71 cm and 0.45-0.73 cm respectively with their corresponding average (mean ± S.E) of 0.617-0.043 cm and 0.642± 0.040 cm. The EPSS values in both groups, before and after treatment were in normal range (Moise and Fox 1999; Boon, 1983). After analysis of the data, it was observed that there was a significant increase ($P<0.05$) in the EPSS values before and after

treatment in both the groups, however the increase was observed more in the pimobendan group (4.508 %) as against arjuna (4.051 %).

The EPSS values generally depend upon the EF and indicate the distance between ventricular wall and these both are inversely related to each other. EPSS value also gives a rough assessment of the LV size (Moise and Fox 1999). In the present case, EPSS values were within normal limits in spite of increased EF % post treatment in both groups, EPSS also has increased which could be attributed either to the large sized LV or to the probable observer error during recording of observations as opined by Moise and Fox (1999). However there was no comparable data available regarding the effect of these two individual drugs on EPSS in the literature referred.

The echocardiographic measurements of inter-ventricular septum at diastole (IVSd) and inter-ventricular septum at systole (IVSs) in group 1, pre treatment ranged from 0.61-1.06 cm and 0.92-1.49 cm respectively with corresponding averages (mean ± S.E) of 0.798 ±0.081 cm and 1.152 ±0.097 cm. The post treatment values of IVSd and IVSs ranged from 0.66-1.14 cm and 0.94-1.53 cm respectively with corresponding averages (mean ± S.E) of 0.865±0.089 cm and 1.213±0.099 cm. In group 2, pre treatment measurements of IVSd and IVss ranged from 0.99-1.12 cm and 1.03-1.52 cm respectively with corresponding averages (mean ± S.E) of 0.98 ±0.072 cm and 1.358 ±0.072 cm. The post treatment values of IVSd and IVSs ranged from 0.66-1.18 cm and 1.06-1.56 cm respectively with corresponding averages (mean ± S.E) of 1.023± 0.075 cm and 1.403± 0.074 cm. The means of IVSd and IVSs on the body weight basis, pre treatment values in both groups were found to be lower than those quoted by (Boon *et al*. 1983).

After analysis of the data, it was observed that there was a significant increase ($P<0.05$) in the IVSd and IVSs values, post treatment in both the groups. While comparing the two treatments, it was found that pimobendan had superior inotropic activity thereby improving the myocardial contractility (increase in IVSd and IVSs) by 8.395 % as against 4.387% by arjuna during diastole and 5.295% as against 3.313% (arjuna) during systole. This increase in their measurements indicated improvement in the myocardial contractility, there by

strengthening the views expressed by Pouleur *et al.* (1989), Nobuyuki *et al.* (1997), Lombard (2006), Caro *et al*. (2009) and Ouellet *et al.* (2009) who in their different works proved the inotropic effect of pimobendan in CHF affected dogs. The improvement seen with arjuna in the present study was in accordance with the observations of Rao *et al.* (2009) who evaluated the efficacy of arjuna in dogs suffering from CHF and reported a significant increase in the IVSd and IVSs measurements post the treatment with arjuna proving its inotropic effect.

The echocardiographic measurements of left ventricular posterior wall thickness at diastole (LVPWd) and left ventricular posterior wall thickness at systole (LVPWs) in group 1, pre treatment ranged from 0.60-0.85 cm and 0.83-1.33 cm respectively with corresponding averages (mean ± S.E) of 0.712 ±0.082 cm and 1.037 ±0.088 cm. The post treatment values of LVPWd and LVPWs ranged from 0.56-1.06 cm and 0.88-1.38 cm respectively with corresponding averages (mean ± S.E) of 0.76±0.077 cm and 1.092±0.088 cm. In group 2, pre treatment measurements of LVPWd and LVPWS ranged from 0.62-0.96 cm and 0.96-1.34 cm respectively with corresponding averages (mean ± S.E) of 0.822 ±0.048 cm and 1.212 ±0.055 cm. The post treatment values of LVPWd and LVPWs ranged from 0.66-0.99 cm and 0.98-1.38 cm respectively with corresponding averages (mean ± S.E) of 0.863± 0.048 cm and 1.258± 0.061 cm. The values of LVPWd and LVPWs on the body weight basis, before the treatment in both the groups were lower than the recommended range by Boon *et al*. (1983) indicating the thinning of the LVPW in all dogs in the study.

After analysis of the data, it was observed that there was a significant increase ($P<0.05$) in the LVPWd and LVPWs values, post treatment in both the groups, thereby again strengthening the assumption that arjuna and pimobendan, both possessed inotropic properties. While comparing the two treatments for the inotropic effect, it was found that pimobendan brought about improvement in myocardial contractility contractility (increase in LVPWd and LVPWs) by 6.741 % as compared to 4.987% by arjuna during diastole and 5.303% as against 3.795 % (arjuna) during systole. This increase in their measurements indicated improvement in the myocardial contractility, there by strengthening the views expressed by Pouleur *et al.* (1989), Nobuyuki *et al.* (1997), Lombard (2006), Caro *et al*. (2009) and Ouellet *et al.* (2009) who in their

different works proved the inotropic effect of pimobendan in CHF affected dogs. The improvement seen with arjuna in the present study was in agreement with the observations of Rao *et al.* (2009) who evaluated the efficacy of arjuna in dogs suffering from CHF and reported a significant increase in the LVPWd and LVPWs measurements post the treatment with arjuna proving its inotropic effect.

The gradation of mitral regurgitation in the dogs, pre and post treatment in both the groups is given in Appendix 14. Right parasternal long axis view or left parasternal caudal four chamber view was followed. During the spectral doppler echocardiography, the regurge was evaluated in all four valves i.e. mitral, tricuspid, aortic and pulmonic in all dogs belonging to both the groups. Among these four valves, the mitral regurge in the dogs of both groups was graded according to Pederson *et al*. (2000). It was observed that there was no improvement in the gradation of mitral regurge, pre and post treatment in both the groups, indicating that both drugs had no effect on the regurge severity. The probable reason being the permanent deformity of the mitral valve as seen in VHD and DCM due to which the alteration in gradation is almost impossible. It was hence, concluded that both these drugs could not bring about improvement in the pathological remodelling of valves but their use definitely helped in imparting improvement in clinical signs in the form of exercise tolerance, reduced degree of cough and respiratory distress and reversion of lethargy. The excess volume overload created due to the mitral regurgitation was successfully combated due to the inotropic effect of pimobendan and arjuna and induced diuresis using furosemide. However, the diuretic property of arjuna as claimed by by Colabawalla (1951) couldn't be evaluated separately due to simultaneous use of furosemide and vasodilation effect of enalapril. Hence a separate detailed study is recommended in the similar manner excluding furosemide and enalapril. Fig.no.23 represents mitral regurge graded moderate in patient 1 of group 1, pre treatment that showed no improvement post treatment with pimobendan as in Fig.no.24. Fig.no. 25 represents mitral regurge graded severe in patient 5 of group 2, pre treatment that showed no improvement post treatment with arjuna as in Fig.no.26.

Between the two treatments, it was found that both the drugs successfully brought about the desired effect of increased myocardial contractility, improved EF % and FS%, lowering of LVIDd and LVIDs along with reducing the systolic B.P in dogs suffering from hypertension. Both drugs were easy to administer, no refusal was noted in any of the dog patients and the current form of the drug was suitable for administration. Considering the cost/dose for pimobendan (approximately 100 Rs/ tablet or capsule) and arjuna (approximately 1.5 Rs. /capsule) and ease of availability, it is concluded that arjuna may be recommended in cases of VHD and/or DCM in dogs at the total dose of 250 mg BID for the owners who cannot afford the cost of pimobendan and make it available for their pets. Pimobendan is available in a purified form with a target specific action as against arjuna, that comprises of a number of glycosides and other chemical constituents (Dwivedi and Udupa 1989) having a combined effect to impart the desired inotropic and anti-hypertensive effect.

5. SUMMARY AND CONCLUSIONS

In the present study, the extract of *Terminalia arjuna* was evaluated for its efficacy in treating valvular heart disease (VHD) and dilated cardiomyopathy (DCM)) in dogs, that are expected to progress to congestive heart failure. Its efficacy was compared to pimobendan, a benzimidazole pyridazinone derivative and a phosphodiesterase (PDE) III inhibitor classified as an inodilator. This novel drug is being used overseas to resolve the symptoms of VHD and DCM affected dogs. The aim of the study was to evaluate and compare the efficacy of arjuna to pimobendan, that is costly and difficult to procure so that an effective, economic and safe remedy could be recommended for treating these cardiac diseases in dogs.

Aged dogs exhibiting clinical manifestations of cardiac disease, like exercise intolerance, cough and lethargy were screened for VHD and/or DCM using diagnostic techniques like thoracic radiology, electrocardiography, systolic blood pressure (B.P) measurement and 2 D and M mode echocardiography along with detailed physical examination, primarily cardiac auscultation. Post diagnosis of VHD and/or DCM, the dogs was divided into two groups with six in each group. Group 1 was administered pimobendan along with furosemide and enalapril, while, group 2 was treated with arjuna along with furosemide and enalapril. The dogs were subjected to a 30 day clinical trial after which they were re-evaluated on the same parameters taken at the start of the study, to see the post treatment response in both the groups.

The clinical signs were minimised with improved exercise capacity, reduced frequency and intensity of cough and decreased risk of clinical deterioration of heart failure in dogs of both treatment groups. No change in the pulse quality and rhythm, heart rhythm and mitral murmur grade was noted, however, the breathing pattern of tachypnoeic patients improved and the audible abnormal lung sounds diminished in intensity on auscultation in both the groups. An appreciable reduction in pulmonary oedema and congestion was noted on the

thoracic radiographs in both groups but neither of the treatments had an effect on the vertebral heart score of the dogs. The quantitative and qualitative changes in the electrocardiogram were non significant in both the groups after treatment. Both the drugs significantly decreased the systolic B.P in hypertensive dogs, thus proving their anti-hypertensive property. However pimobendan proved its superiority over arjuna by giving almost a double response to that of arjuna, in lowering the systolic B.P. During the 2 D and M mode echocardiography, both the drugs brought about an improvement in myocardial contractility, thus proving their inotropic property. Both drugs brought about significant reduction in the left ventricular internal dimensions at systole and diastole, post treatment. Significant improvement was noted in the fractional shortening (FS %) and ejection fraction (EF %) as compared to the pre treatment values in both the groups. However, pimobendan's inotropic effect on the VHD and/or DCM patients was observed to be almost twice that of arjuna. Both the drugs brought about significant increase in the interventricular septum dimensions and left ventricular posterior wall thickness at systole and diastole in both groups indicating considerable improvement in the myocardial contractility thus enhancing left ventricular systolic and diastolic performances. Both drugs showed no effect on the intensity of mitral regurgitation in the dogs.

Hence, it is concluded that:

1) 2 D and M mode echocardiography was found to be the most ideal out of all the diagnostics used in the study for confirmatory diagnosis of VHD and DCM.

2) *Terminalia arjuna*, just like pimobendan, has anti-hypertensive effect that can be put to use in dogs, while treating clinical cardiac patients.

3) *Terminalia arjuna* seems to possess inotropic and cardiotonic properties that help in improving the myocardial contractility in VHD and/or DCM patients similar to pimobendan and thus it can also be termed as an inodilator. However, further research to elucidate its mechanism of action at molecular level is suggested.

4) The combination of *Terminalia arjuna*, furosemide and enalapril is found to be effective in therapeutic management of VHD and/or DCM in dogs.

5) When the cost, ease of availability and desired effective response in cardiac failure dogs is concerned, *Terminalia arjuna* can be definitely recommended specially in countries where the availability of pimobendan is questionable or where there is intolerance for pimobendan in dog patients.

6) Despite its high cost and difficulties experienced in procurement, the superiority of pimobendan as an inodilator cannot be overlooked.

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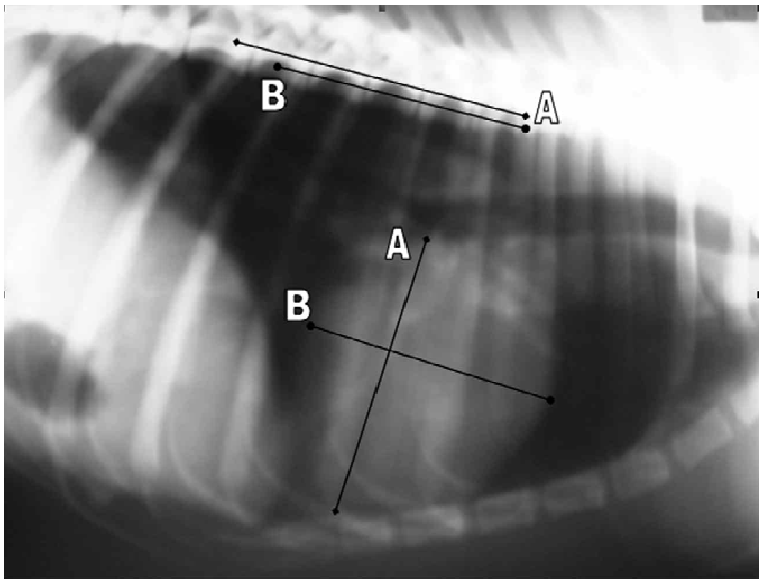
APPENDIX 1: Calculation of VHS score in dogs

The method for measuring the size of the canine heart in lateral radiographs given below is as elucidated by (Buchanan *et al*., 1995).The VHS method measures heart size in two dimensions on the lateral view of thoracic radiographs.

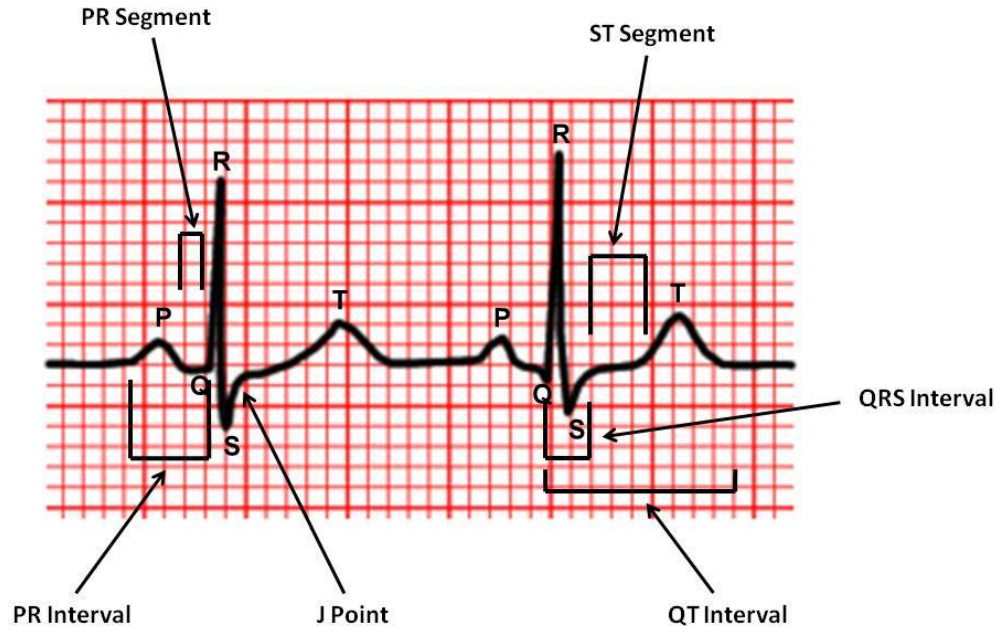


In the figure above, the long axis measurement of the cardiac silhouette(A) encompasses 5.2 thoracic vertebrae; the short axis measurement(B) encompasses 4.7 thoracic vertebrae. Hence, VHS = A+B = 5.2 + 4.7 = 9.9v

- The long axis is measured from the ventral border of the left mainstem bronchus (cranio-ventral border of the carina) to the cardiac apex (the most distal point on the ventral contour of the cardiac radiographic image).
- The short axis is measured at the widest point of the cardiac image on a line perpendicular to the long axis.

The two measurements are then compared to the vertebrae starting at the cranial edge of T4, and a result, expressed in units of vertebral lengths, is obtained for each axis. The sum of the measurements on both axes constitutes the VHS. The normal VHS was taken as 9.7 ± 0.5.

APPENDIX 2: Normal ECG values in canines



Measurements of waves and segments

- **PR interval**- beginning of P wave to the beginning of the QRS
- **QRS interval**- beginning of the first deflection to the end of the final deflection of QRS complex
- **ST interval**- end of QRS interval to the onset of the T wave
- **QT interval**- beginning of QRS complex to the end of the T wave
- **J Point -** The junction between the QRS complex and the ST segment

APPENDIX -2: Normal ECG values in canines (Contd.)

Sr. no.	Parameter	Normal range
1	Heart rate (HR)	Puppy: 70-220 bpm
		Toy breeds: 70-180 bpm
		Standard: 70-160 bpm
		Giant breeds: 60-140 bpm
2	Rhythm	Sinus rhythm
		Sinus arrhythmia
		Wandering pacemaker
3	P wave	
	Height	Maximum: 0.4 mV
	Width	Maximum: 0.04 s (Giant breeds 0.05 s)
4	PR interval	0.06-0.13 s
5	QRS complex	
	Height	Large breeds: 3.0 mV maximum
		Small breeds: 2.5 mV maximum
	Width	Large breeds: 0.06 s maximum
		Small breeds: 0.05 s maximum
6	ST segment	
	Depression	No more than 0.2 mV
	Elevation	No more than 0.15 mV
7	QT interval	0.15-0.25 s at normal HR
8	T wave	May be positive, negative, or biphasic, amplitude range ± 0.05-1.0 mV in any lead, not more than ¼ of R wave amplitude
9	Mean electrical axis	+40 to + 100

APPENDIX-3 :Normal echocardiographic indices in canines											
Gr no.	**Pat.**	**Wt.**	**LVIDd (cm)**	**LVIDs (cm)**	**EPSS (cm)**	**IVSd (cm)**	**IVSs (cm)**	**LVPWd (cm)**	**LVPWs (cm)**	**LA (cm)**	**AO (cm)**
I	1	34	3.3-4.47	1.95-2.97	0.1-1	1.06-1.19	1.61-1.76	0.85-0.96	1.37-1.51	2.22-3.75	1.76-3.13
	2	13	2.41-3.58	1.33-2.3	0.1-1	0.79-0.90	1.19-1.31	0.63-0.72	1.04-1.15	1.42-2.95	1.12-2.5
	3	6.8	1.78-2.96	0.9-1.93	0.1-1	0.66-0.81	0.99-1.15	0.52-0.65	0.87-1.03	0.86-2.4	0.68-2.06
	4	6	1.65-2.83	0.8-1.83	0.1-1	0.63-0.79	0.95-1.13	0.5-0.64	0.84-1.01	0.74-2.28	0.58-1.96
	5	39	3.43-4.60	2.04-3.06	0.1-1	1.10-1.26	1.68-1.86	0.89-1.02	1.43-1.60	22.33-3.87	1.85-3.32
	6	12	2.34-3.51	1.28-2.31	0.1-1	0.77-0.88	1.16-1.29	0.62-0.71	1.01-1.13	1.36-2.89	1.07-2.45
II	1	36	3.35-4.52	1.99-3.01	0.1-1	1.07-1.21	1.64-1.79	0.86-0.98	1.39-1.54	2.26-3.79	1.79-3.16
	2	33	3.28-4.45	1.93-2.95	0.1-1	1.05-1.18	1.59-1.74	0.84-0.95	1.36-1.50	2.2-3.73	1.74-3.11
	3	11	2.23-3.4	1.21-2.23	0.1-1	0.75-0.87	1.12-1.25	0.6-0.7	0.98-1.1	1.26-2.79	0.99-2.37
	4	29	3.16-4.32	1.85-2.87	0.1-1	1.01-1.12	1.53-1.65	0.81-0.91	1.31-1.43	2.09-3.62	1.65-3.03
	5	38	3.41-4.58	2.02-3.05	0.1-1	1.09-1.25	1.67-1.84	0.88-1.01	1.42-1.58	2.32-3.85	1.83-3.2
	6	40	3.45-4.63	2.05-3.08	0.1-1	1.11-1.27	1.7-1.88	0.89-1.03	1.44-1.61	2.35-3.89	1.86-3.24

APPENDIX-4: Description of patients in the study

Group no.	Pat. no.	Patient name	Owner name	Breed	Age (yrs)	Sex	Weight (kg)	
							Pre	Post
I	1	Classic	Shilpi Barai	Golden Retriever	11.5	M	34	35
	2	Dusty	Megha Dhavan	Lhasa Apso	11	M	13	12.5
	3	Moti	Manju Sawhney	Miniature Pinscher	13	F	6.8	7
	4	Magic	Manju Sawhney	Jack Russel Terrier	15	F	6	6.3
	5	Tyson	Neha Raheja	Labrador Retriever	12	M	39	39.4
	6	Frisky	Arrshie Singh	Spitz	13	F	12	12.7
Mean ± S.E					**12.6 ± 0.58**		**18.4 ± 5.84**	**18.8 ± 5.9**
II	1	Pebbles	Anisha Ahuja	Golden Retriever	11	F	36	34.5
	2	Stoopie	Sameer Pakwasa	Labrador Retriever	9.5	M	33	34
	3	Mutley	Roshni Khan	Pug	9	M	11	10
	4	Georgie	Natasha Sinha	Basset Hound	7	M	29	30
	5	Russell	Zayed Khan	Golden Retriever	9.5	M	38	37.4
	6	Maximus	Nalin Patel	Labrador Retriever	8	M	40	38.6
Mean ± S.E					**9 ± 0.56**		**31.1 ± 4.33**	**30.7 ± 4.33**

APPENDIX-5: Medical history of patients in the study

Group no.	Patient no.	Patient name	Cough & type		Exercise intolerance		Weakness/ lethargy	
			Pre	Post	Pre	Post	Pre	Post
I	1	Classic	---	---	decreased	improved considerably	---	---
	2	Dusty	subtle, dry, unproductive	reduced considerably	---	---	present	reduced considerably
	3	Moti	nocturnal, subtle, dry, unproductive	completely subsided	decreased	improved considerably	---	---
	4	Magic	nocturnal, subtle, dry, unproductive	completely subsided	decreased	improved considerably	---	---
	5	Tyson	subtle, dry, unproductive	reduced considerably	decreased	improved considerably	---	---
	6	Frisky	---	---	---	---	present	reduced considerably

APPENDIX-5: Medical history of patients in the study (Contd.)

Group no.	Patient no.	Patient name	Cough & type		Exercise intolerance		Weakness/ lethargy	
			Pre	Post	Pre	Post	Pre	Post
II	1	Pebbles	nocturnal, subtle, dry, unproductive	completely subsided	---	---	present	reduced considerably
	2	Stoopie	---	---	decreased	improved considerably	---	---
	3	Mutley	mildly moist and productive	reduced considerably	decreased	improved considerably	---	---
	4	Georgie	---	---	decreased	improved considerably	present	reduced considerably
	5	Russell	nocturnal, subtle, dry, unproductive	completely subsided	---	---	present	reduced considerably
	6	Maximus	subtle, dry, unproductive	reduced considerably	decreased	improved considerably	---	---

APPENDIX- 6 Physical examination of patients

Gr. no.	Pat no.	Temp (ºC)		Mucous membr. colour & CRT (sec)		Respiratory rate (breaths/min)		Lung Auscultation		Percussion & Palpation	
		Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post
I	1	102.1	101.8	pink, < 2 sec	pink, < 2 sec	32	34	---	---	---	---
	2	101.1	102.2	pale pink, < 2 sec	pale pink, < 2 sec	42, tachy-pnoea	32	fine crackles, occasional wheezes	reduced intensity of crackles	hepato-megaly	hepato-megaly
	3	102.5	102.3	pink, < 2 sec	pink, < 2 sec	28	31	occasional fine crackles	reduced intensity of crackles	---	---
	4	100.7	101.8	pink, < 2 sec	pink, < 2 sec	30	33	occasional fine crackles	reduced intensity of crackles	---	---
	5	100.9	101.5	Hyperemic, < 2 sec	Hyperemic < 2 sec	51, tachy-pnoea	36	fine crackles, occasional wheezes	reduced intensity of crackles	hepato-megaly	hepato-megaly
	6	101.3	102.4	pink, < 2 sec	pink, < 2 sec	27	32	---	---	---	---
Mean ± S.E		**101.4 ± 0.291**	**102 ± 0.144**	---	---	**35 ± 3.882**	**33 ± 0.730**	---	---	---	---

APPENDIX- 6 Physical examination of patients											
Gr. no.	**Pat no.**	**Temp (ºC)**		**Mucous membr colour & CRT(sec)**		**Respiratory rate (breaths/min)**		**Lung Auscultation**		**Percussion & Palpation**	
		Pre	**Post**	**Pre**	**Post**	**Pre**	**Post**	**Pre**	**Post**	**Pre**	**Post**
II	1	102.1	101.6	pale pink, < 2 sec	pale pink, < 2 sec	27	33	fine crackles, occasional wheezes	reduced intensity of crackles	---	---
	2	101.2	101.7	pink, < 2 sec	pink, < 2 sec	29	31	---	---	---	---
	3	102.4	102	hyperemic, < 3 sec	hyperemic, < 2 sec	49, tachy-pnoea	34	fine crackles, occasional wheezes	reduced intensity of crackles	---	---
	4	101.6	102.5	pale pink, < 2 sec	pale pink, < 2 sec	32	33	---	---	---	---
	5	102	101.3	pink, < 2 sec	pink, < 2 sec	34	31	occasional fine crackles	reduced intensity of crackles	---	---
	6	102.3	101.4	pale pink, < 2 sec	pale pink, < 2 sec	44, tachy-pnoea	35	fine crackles, occasional wheezes	reduced intensity of crackles	hepato-megaly	hepato megaly
Mean ± S.E		**101.9 ± 0.186**	**101.7 ± 0.180**	---	---	**35.833 ± 3.572**	**32.8 ± 0.65**	---	---	---	---

APPENDIX- 7 : Cardiovascular (CVS) examination of patients									
Gr. no.	**Pat. no.**	**Heart rate and rhythm**		**Murmur/ abnormal heart sounds**		**Pulse rate and rhythm**		**Pulse quality**	
		Pre	**Post**	**Pre**	**Post**	**Pre**	**Post**	**Pre**	**Post**
I	1	130, regular	124, regular	systolic : left apical grade III/VI left basilar gradeIII/VI	systolic : left apical grade III/VI left basilar gradeIII/VI	127, regular	122, regular	Hypo-kinetic	Hypo-kinetic
	2	112, regular	108, regular	Systolic: left apical grade V/VI left basilar grade III/VI , right apical grade II/VI	Systolic: left apical grade V/VI left basilar grade III/VI , right apical grade II/VI	110, regular	105, regular	Hypo-kinetic	hypo-kinetic
	3	96, arrhythmia	106, arrhythmia	systolic left apical grade V/VI,left basilar grade III/VI, right apical grade III/VI	systolic left apical grade V/VI,left basilar grade III/VI, right apical grade III/VI	76, irr-regular	81, irr-regular	pulse deficit	pulse deficit
	4	88, regular	92, regular	systolic left apical grade V/VI, right apical grade III/VI	systolic left apical grade V/VI, right apical grade III/VI	86, regular	89, regular	Hypo-kinetic	Hypo-kinetic
	5	126, arrhythmia	126, arrhythmia	systolic left apical grade IV/VI, left basilar grade II/VI right apical grade III/VI	systolic left apical grade IV/VI, left basilar grade II/VI right apical grade III/VI	124, irr-regular	129, irr-regular	pulse alterans	pulse alterans
	6	82, regular	96, regular	systolic left apical grade III/VI right apical grade II/VI	systolic left apical grade III/VI right apical grade II/VI	80, regular	93, regular	Hypo-kinetic	Hypo-kinetic
	Mean ± S.E	**105.66 ± 8.188**	**108.66 ± 5.719**			**100.5 ± 9.266**	**103.17 ± 7.79**		

APPENDIX- 7 : Cardiovascular (CVS) examination of patients

Gr. no.	Pat. no.	Heart rate and rhythm		Murmur/ abnormal heart sounds		Pulse rate and rhythm		Pulse quality	
		Pre	Post	Pre	Post	Pre	Post	Pre	Post
II	1	123, regular	117, regular	systolic left apical grade III/VI,left basilar grade III/VI	systolic left apical grade III/VI,left basilar grade III/VI	120, regular	115, regular	pulse alterans	pulse alterans
	2	103, regular	108, regular	systolic left apical grade IV/VI, left basilar grade II/VI	systolic left apical grade IV/VI, left basilar grade II/VI	100, regular	106, regular	pulse alterans	pulse alterans
	3	99, arrhythmia	103, arrhythmia	systolic left apical grade V/VI,left basilar grade III/VI, right apical grade II/VI	systolic left apical grade V/VI,left basilar grade III/VI, right apical grade II/VI	82, irr-regular	91,irr-regular	pulse deficit	pulse deficit
	4	98, arrhythmia	103, arrhythmia	systolic left apical grade IV/VI, left basilar grade IV/VI	systolic left apical grade IV/VI, left basilar grade IV/VI	89, irr-egular	107, irr-egular	pulse deficit	pulse deficit
	5	114, regular	120, regular	systolic left apical grade IV/VI, left basilar grade II/VI , right apical grade II/VI	systolic left apical grade IV/VI, left basilar grade II/VI , right apical grade II/VI	111, regular	118, regular	Hypo-kinetic	Hypo-kinetic
	6	105, regular	112, regular	systolic left apical grade IV/VI,right apical grade II/VI	systolic left apical grade IV/VI,right apical grade II/VI	103, regular	109, regular	pulse alterans	pulse alterans
	Mean ± S.E	**107 ± 3.95**	**110.5 ± 2.90**			**100.83 ± 5.68**	**107.66 ± 3.84**		

APPENDIX 8: Grades of heart murmurs

Area of auscultation	Structure
Left side, fifth intercostal space at the costochondral junction	Mitral valve
Left side, fourth intercostal space just above costochondral junction	Aortic valve
Left side, between second and fourth intercostal space just above the sternum	Pulmonic valve
Right side, third to fifth intercostal space near costochondral junction	Tricuspid valve

Grade	Diagnosis
I/VI	It can only be heard after listening for several minutesand sounds like a prolonged first heart sound
II/VI	It is very soft, but can be heard immediately
III/VI	It is low to moderate in intensity
IV/VI	It is very loud, but a thrill cannot be palpated on the thorax
V/VI	It is very loud, and a thrill can be palpated on the thorax
VI/VI	It can be heard without the use of a stethoscope or with the stethoscope slightly off the thoracic wall.

APPENDIX 9: Vertebral heart score (VHS) readings of patients

Parameter	Group no.	Name	Patient No.	Pre	Post
Vertebral heart score	I	Classic	1	10.5	10.5
		Dusty	2	10.5	10.5
		Moti	3	10.4	10.4
		Magic	4	10.3	10.3
		Tyson	5	10.4	10.4
		Frisky	6	10.2	10.2
	Mean ± S.E			**10.383 ± 0.048**	**10.383 ± 0.048**
	Range			**10.2-10.5**	**10.2-10.5**
	II	Pebbles	1	10.3	10.3
		Stoopie	2	10.5	10.5
		Mutley	3	10.8	10.8
		Georgie	4	10.2	10.2
		Russell	5	10.4	10.4
		Maximus	6	10.3	10.3
	Mean ± S.E			**10.417 ±0.087**	**10.417± 0.087**
	Range			**10.2-10.8**	**10.2-10.8**

Pre- Pre treatment, **Post**- Post treatment

APPENDIX- 10 Qualitative assessment of thoracic radiographs of patients

Parameter	Group no.	Name	Patient	Pre-treatment	Post-treatment
Pulmonary oedema	I	Classic	1	+	+
		Dusty	2	+++	++
		Moti	3	++	+
		Magic	4	++	+
		Tyson	5	+++	+++
		Frisky	6	+	+
	II	Pebbles	1	++	+
		Stoopie	2	+	+
		Mutley	3	+++	++
		Georgie	4	+	+
		Russell	5	++	++
		Maximus	6	+++	+++
Pulmonary Congestion	I	Classic	1	+	+
		Dusty	2	++	+
		Moti	3	+	+
		Magic	4	+	+
		Tyson	5	++	+
		Frisky	6	+	+
	II	Pebbles	1	++	+
		Stoopie	2	+	+
		Mutley	3	++	++
		Georgie	4	+	+
		Russell	5	+	+
		Maximus	6	++	+

+ - Mild**; ++-** Moderate**; +++ -** Severe

APPENDIX- 11 ECG (electrocardiography) readings of patients													
PRE TREATMENT													
Gr. no.	**Pat. no.**	**HR (bpm)**	**Rhythm**	**P wave**		**PR seg. (sec)**	**QRS complex**		**ST seg. (sec)**	**QT seg. (sec)**	**T wave (mV)**	**MEA (degree)**	**ECG interpretation**
				Wd (sec)	**Ht (mV)**		**Wd (sec)**	**Ht (mV)**					
I	1	120	Sinus arrhythmia	0.08	0.2	0.12	0.06	2.3	0.12	0.24	0.2	90	LAE
	2	104	Sinus arrhythmia	0.06	0.1	0.12	0.04	0.6	0.12	0.2	0.1	90	LAE, low voltage PQRS
	3	93	Atrial fibrillation	0.06	0.4	0.08	0.04	2.3	0.12	0.2	0.1	60	ST slurring 0.4 mV, LAE,
	4	87	Sinus arrhythmia	0.06	0.2	0.08	0.04	1.8	0.04	0.2	0.2	60	LAE
	5	131	Atrial fibrillation	0.06	0.3	0.12	0.04	0.7	0.08	0.24	0.4	90	LAE, low voltage PQRS,
	6	91	Sinus arrhythmia	0.06	0.3	0.12	0.04	1.9	0.08	0.2	0.2	90	ST depression 0.3 mV, LAE,
Mean ± S.E		**104.333 ± 7.219**		**0.063 ± 0.003**	**0.25 ± 0.043**	**0.107 ± 0.008**	**0.043 ± 0.003**	**1.6 ± 0.312**	**0.093 ± 0.013**	**0.213 ± 0.008**	**0.2 ± 0.045**	**80 ± 6.325**	
Range		**87-131**		**0.06-0.08**	**0.1-0.4**	**0.08-0.12**	**0.04-0.06**	**0.6-2.3**	**0.04-0.12**	**0.20-0.24**	**0.1-0.4**	**60-90**	

APPENDIX- 11 ECG (electrocardiography) readings of patients

PRE TREATMENT

Gr. no.	Pat. no.	HR (bpm)	Rhythm	P wave		PR seg. (sec)	QRS complex		ST seg. (sec)	QT seg. (sec)	T wave (mV)	MEA (degree)	ECG interpretation
				Wd (sec)	Ht (mV)		Wd (sec)	Ht (mV)					
II	1	111	Sinus arrhythmia	0.04	0.5	0.12	0.08	1.7	0.08	0.24	0.4	90	LAE
	2	113	Sinus arrhythmia	0.06	0.3	0.12	0.04	1.2	0.08	0.24	0.5	60	LAE, Tall T
	3	102	Atrial fibrillation	0.06	0.3	0.08	0.04	1.3	0.08	0.2	0.3	90	ST slurring 0.3 mV, LAE
	4	107	Atrial fibrillation	0.06	0.5	0.12	0.04	2.3	0.12	0.24	0.3	90	LAE, RAE
	5	105	Sinus arrhythmia	0.06	0.2	0.12	0.04	2	0.12	0.2	0.1	90	LAE
	6	104	Sinus arrhythmia	0.06	0.1	0.12	0.04	0.6	0.08	0.2	0.3	90	LAE, low voltage PQRS
Mean ± S.E		**107 ± 1.732**		**0.057± 0.003**	**0.317± 0.065**	**0.113 ± 0.007**	**0.047± 0.007**	**1.517 ± 0.250**	**0.093 ± 0.008**	**0.22 ± 0.009**	**0.317 ± 0.054**	**85 ± 5.000**	
Range		**102-113**		**0.04-0.06**	**0.1-0.5**	**0.08-0.12**	**0.04-0.08**	**0.6-2.3**	**0.08-0.12**	**0.20-0.24**	**0.1-0.5**	**60-90**	

APPENDIX- 11 ECG (electrocardiography) readings of patients													
POST TREATMENT													
Gr. no.	**Pat. no.**	**HR (bpm)**	**Rhythm**	**P wave**		**PR seg. (sec)**	**QRS complex**		**ST seg. (sec)**	**QT seg. (sec)**	**T wave (mV)**	**MEA (degree)**	**ECG interpretation**
				Wd (sec)	**Ht (mV)**		**Wd (sec)**	**Ht (mV)**					
I	1	108	Sinus arrhythmia	0.08	0.2	0.12	0.06	2.3	0.12	0.24	0.2	90	LAE
	2	120	Sinus arrhythmia	0.06	0.1	0.12	0.04	0.6	0.12	0.2	0.1	90	LAE
	3	110	Atrial fibrillation	0.06	0.4	0.08	0.04	2.3	0.12	0.2	0.1	60	ST slurring 0.4 mV, LAE
	4	92	Sinus arrhythmia	0.06	0.2	0.08	0.04	1.8	0.04	0.2	0.2	60	LAE
	5	120	Atrial fibrillation	0.06	0.3	0.12	0.04	0.7	0.08	0.24	0.4	90	LAE,Tall T
	6	95	Sinus arrhythmia	0.06	0.3	0.12	0.04	1.9	0.08	0.2	0.2	90	ST slurring 0.3 mV, LAE
Mean ± S.E		**107.5 ± 4.884**		**0.063 ± 0.003**	**0.25 ± 0.04**	**0.107 ± 0.008**	**0.04± 0.003**	**1.6 ± 0.312**	**0.093 ±0.013**	**0.213 ± 0.008**	**0.2 ± 0.045**	**80 ± 6.325**	
Range		**92-120**		**0.06-0.08**	**0.1-0.4**	**0.08-0.12**	**0.04-0.06**	**0.6-2.3**	**0.04-0.12**	**0.20-0.24**	**0.1-0.4**	**60-90**	

APPENDIX- 11 ECG (electrocardiography) readings of patients													
POST TREATMENT													
Gr. no.	**Pat. no.**	**HR (bpm)**	**Rhythm**	**P wave**		**PR seg. (sec)**	**QRS complex**		**ST seg. (sec)**	**QT seg. (sec)**	**T wave (mV)**	**MEA (degree)**	**ECG interpretation**
				Wd (sec)	**Ht (mV)**		**Wd (sec)**	**Ht (mV)**					
II	1	118	Sinus arrhythmia	0.04	0.5	0.12	0.08	1.7	0.08	0.24	0.4	90	LAE
	2	104	Sinus arrhythmia	0.06	0.3	0.12	0.04	1.2	0.08	0.24	0.5	60	LAE, Tall T
	3	98	Atrial fibrillation	0.06	0.3	0.08	0.04	1.3	0.08	0.2	0.3	90	ST slurring 0.3 mV, LAE
	4	88	Atrial fibrillation	0.06	0.5	0.12	0.04	2.3	0.12	0.24	0.3	90	LAE, RAE
	5	120	Sinus arrhythmia	0.06	0.2	0.12	0.04	2	0.12	0.2	0.1	90	LAE
	6	111	Sinus arrhythmia	0.06	0.1	0.12	0.04	0.6	0.08	0.2	0.3	90	LAE, low voltage PQRS
Mean ± S.E		**106.5 ± 5.018**		**0.057± 0.003**	**0.317 ± 0.065**	**0.113 ± 0.007**	**0.047 ± 0.007**	**1.517 ± 0.250**	**0.093 ± 0.008**	**0.22 ± 0.009**	**0.317 ± 0.054**	**85 ± 5.000**	
Range		**88-120**		**0.04-0.06**	**0.1-0.5**	**0.08-0.12**	**0.04-0.08**	**0.6-2.3**	**0.08-0.12**	**0.20-0.24**	**0.1-0.5**	**60-90**	

APPENDIX-12 Systolic B.P readings of patients

Parameter	Group no.	Patient no.	Pre-treatment	Post-treatment
Systolic B.P	I	1	155	125
		2	175	135
		3	160	140
		4	165	130
		5	220	135
		6	145	120
	Mean ± S.E		**170 ± 10.801**	**130.833 ± 3.005**
	Range		**145-220**	**120-140**
	II	1	150	135
		2	165	135
		3	160	140
		4	190	165
		5	155	140
		6	145	130
	Mean ± S.E		**160.833 ± 6.509**	**140.833 ± 5.069**
	Range		**145-190**	**130-165**

APPENDIX- 13: Echocardiography readings of patients

PRE TREATMENT

Gr. no.	Pat no.	HR (bpm)	LA (cm)	AO (cm)	LA : AO	LVIDd (cm)	LVIDs (cm)	FS (%)	EDV (ml)	ESV (ml)	EF (%)	EPSS (cm)	IVSd (cm)	IVSs (cm)	LVPWd (cm)	LVPWs (cm)
I	**1**	122	3.76	2.22	1.69	4.53	3.61	20.3	52.22	30.86	40.9	0.63	1.02	1.4	0.85	1.28
	2	105	3.02	1.67	1.81	4.16	3.15	24.27	44.14	22.63	48.73	0.42	0.68	1.06	0.71	1.01
	3	124	2.46	1.53	1.61	3.24	2.49	23.15	33.28	17.69	46.84	0.36	0.63	0.96	0.54	0.83
	4	88	2.35	1.42	1.65	3.12	2.52	19.23	32.43	19.51	39.84	0.39	0.61	0.92	0.53	0.89
	5	131	4.07	2.34	1.74	4.88	4.16	14.75	46.92	31.11	33.69	0.69	1.06	1.49	1.04	1.33
	6	92	2.96	1.83	1.62	3.79	2.88	24.01	37.54	19.16	48.96	0.44	0.79	1.08	0.60	0.88
Mean ± S.E		110.3 ±7.33	3.10± 0.281	1.83± 0.152	1.687 ±0.031	3.953 ±0.28	3.135 ±0.26	20.95 ±1.49	41.088 ±3.244	23.49 ±2.25	43.16 ±2.47	0.488 ±0.05	0.798 ±0.08	1.152 ±0.097	0.712 ±0.08	1.037 ±0.08
Range		88-131	2.35-4.07	1.53-2.34	1.61-1.81	3.12-4.88	2.49-4.16	14.75 - 24.27	32.43-52.22	17.69-31.11	33.69 - 48.96	0.36-0.69	0.61-1.06	0.92-1.49	0.60-0.85	0.83-1.33
II	1	113	4.03	2.68	1.50	4.58	3.73	18.56	42.4	26.52	37.45	0.62	1.04	1.39	0.83	1.22
	2	120	3.86	2.44	1.58	5.02	3.88	22.71	40.88	21.86	46.52	0.65	1.02	1.41	0.86	1.27
	3	97	2.95	1.88	1.57	3.54	2.85	19.49	33.62	19.86	40.93	0.41	0.63	1.03	0.62	0.96
	4	127	3.67	2.26	1.62	4.43	3.34	24.60	37.42	19.07	49.03	0.67	0.99	1.32	0.77	1.18
	5	104	3.93	2.45	1.60	4.69	3.60	23.24	48.39	23.65	47.14	0.71	1.08	1.48	0.89	1.34
	6	106	4.01	2.53	1.58	4.86	3.71	23.66	50.97	25.78	48.42	0.64	1.12	1.52	0.96	1.30
Mean ± S.E		111.16± 4.5	3.74± 0.167	2.37 ± 0.11	1.575 ±0.017	4.52 ±0.21	3.518 ±0.15	22.04 ±0.99	42.28 ±2.667	22.79-1.250	44.95 ±1.90	0.617 ±0.04	0.98 ±0.07	1.35 ±0.072	0.822 ±0.04	1.212 ±0.05
Range		97-127	2.95-4.03	1.88-2.26	1.50-1.62	3.54-5.02	2.85-3.88	18.56-24.60	33.62-50.97	19.07-26.52	37.45-49.03	0.41-0.71	0.99-1.12	1.03-1.52	0.62-0.96	0.96-1.34

APPENDIX-13																
POST TREATMENT																
Group no.	**Pat no.**	**HR (bpm)**	**LA (cm)**	**AO (cm)**	**LA : AO**	**LVIDd (cm)**	**LVIDs (cm)**	**FS (%)**	**EDV (ml)**	**ESV (ml)**	**EF (%)**	**EPSS (cm)**	**IVSd (cm)**	**IVSs (cm)**	**LVPWd (cm)**	**LVPWs (cm)**
I	1	113	3.78	2.19	1.72	4.42	3.36	23.98	50.61	28.02	44.63	0.66	1.13	1.49	0.88	1.33
	2	111	3.04	1.71	1.77	4.03	2.97	26.30	42.44	20.31	52.14	0.44	0.74	1.16	0.76	1.08
	3	133	2.49	1.51	1.65	3.08	2.28	25.97	31.58	15.49	50.63	0.37	0.69	1.04	0.56	0.88
	4	95	2.37	1.45	1.63	2.94	2.30	21.77	30.92	17.6	43.08	0.41	0.66	0.94	0.58	0.92
	5	122	4.08	2.31	1.76	4.65	3.83	17.63	45.17	28.46	36.99	0.73	1.14	1.53	1.06	1.38
	6	96	2.97	1.8	1.65	3.62	2.66	26.52	35.75	16.93	52.64	0.45	0.83	1.12	0.72	0.96
Mean ± S.E		111.6 ±6.02	3.122 ± 0.280	1.828 ± 0.14	1.697± 0.025	3.79±0 .286	2.9±0. 0251	23.695 ±1.420	39.412± 3.238	21.135 ±2.337	46.685 ±2.530	0.51± 0.060	0.865 ±0.08	1.213± 0.099	0.76±0. 077	1.092± 0.088
Range		95-133	2.37-4.08	1.45-2.31	1.63-1.77	2.94-4.65	2.30-3.83	17.63-26.52	30.92-50.61	15.49-28.46	36.99-52.64	0.37-0.73	0.66-1.14	0.94-1.53	0.56-1.06	0.88-1.38
II	1	116	4.07	2.74	1.48	4.53	3.62	20.1	41.25	24.38	40.89	0.64	1.08	1.43	0.86	1.26
	2	106	3.89	2.51	1.55	4.92	3.77	23.37	38.62	20.18	47.75	0.68	1.08	1.47	0.92	1.35
	3	94	3.01	1.92	1.56	3.46	2.74	20.81	32.14	18.50	42.44	0.45	0.66	1.06	0.66	0.98
	4	115	3.74	2.29	1.63	4.25	3.15	25.88	35.66	17.46	51.04	0.68	1.04	1.37	0.81	1.22
	5	118	3.98	2.47	1.61	4.57	3.47	24.07	46.24	23.50	49.18	0.73	1.10	1.53	0.94	1.38
	6	114	4.05	2.58	1.57	4.79	3.60	24.84	49.29	24.32	50.66	0.67	1.18	1.56	0.99	1.36
Mean ± S.E		110.5 ± 3.70	3.79 ± 0.164	2.418 ± 0.11	1.567 ±0.021	4.42 ±0.241	3.392 ±0.156	23.178 ±0.931	40.53 ±2.631	21.39 ±1.255	46.993 ±1.762	0.642 ± 0.04	1.023 ± 0.07	1.403± 0.074	0.863± 0.048	1.258± 0.061
Range		94-118	3.01-4.07	1.92-2.74	1.48-1.63	3.46-4.92	2.74-3.77	20.10-25.88	32.14-49.29	17.46-24.38	40.89-51.04	0.45-0.73	0.66-1.18	1.06-1.56	0.66-0.99	0.98-1.38

APPENDIX 14: Grading of mitral regurge in patients

Parameter	Group no.	Name	Patient	Pre treatment	Post treatment
Mitral Valve Regurge	I	Classic	1	++	++
		Dusty	2	+++	+++
		Moti	3	+++	+++
		Magic	4	+++	+++
		Tyson	5	+++	+++
		Frisky	6	++	++
	II	Pebbles	1	++	++
		Stoopie	2	+++	+++
		Mutley	3	+++	+++
		Georgie	4	++	++
		Russell	5	+++	+++
		Maximus	6	+++	+++

Mild (+) 10-30 %, Moderate (++) 30-50%, Severe (+++) above 50%

VITA

Dr. Vaidehi Vinay Paranjape was born on twenty-fourth February, 1987 in Mumbai, Maharashtra .She completed her secondary school certificate exam from V.N Sule Guruji Vidyalaya, Dadar, in year 2002 and higher certificate exam from Ramnivas Ruia college, in year 2004 under Maharashtra board, Mumbai. Being interested in Veterinary Science since the tender age of four, she joined Bombay Veterinary College, Mumbai in year 2004 to fulfil her dream of serving animals and completed her graduation in year 2009 with the grade point of 8.15 on 10 scale, first class with distinction. She joined the Department of Pharmacology and Toxicology, Bombay Veterinary College in 2009 and worked on clinical pharmacology in veterinary cardiology for her thesis research. She has attended prestigious conferences conducted by WSAVA and NAVC (Orlando, Florida).She has also been a part of various animal rescue camps and NSS camps. Her hobbies include dancing, reading, travelling and photography apart from volunteering for NGO's associated with animal welfare.