RESEARCH ARTICLE Indian Journal of Animal Research, Volume 60 Issue 6: 1021-1029 (June 2026)

# Therapeutic Evaluation of Sacubitril-valsartan (Arni) in Canine Dilated Cardiomyopathy

K.S. Saikrishna[1], K. Jeyaraja[2], P. Selvaraj[3], S. Vairamuthu[4], Md. Shafiuzama[5]

10.18805/IJAR.B-5588


## ABSTRACT

**Background:** Canine dilated cardiomyopathy (DCM) is one of the commonly diagnosed acquired myocardial disease in dogs characterized by hypokinesis, eccentric myocardial hypertrophy. Activation of neurohumoral system (*i.e.*, sympathetic and RAAS) is crucial in the pathogenesis of DCM with deteriorative effect on the failing heart. In current protocol, ACE inhibitors are being employed to counter RAAS through blocking of ACE pathway, however aldosterone breakthrough due to alternate tissue pathway render the treatment less effective. The natriuretic peptides (*viz*., ANP, BNP) produced in response to cardiac wall stress have beneficial natriuretic, diuretic and suppressed cardiac sympathetic effects, however, will be cleaved by the enzyme neprilysin limiting their activity. With this background, this study was aimed to study the effect of Sacubitril-Valsartan, an ARNi drug in management of canine heart failure due to DCM, evaluating its effects on clinical, echocardiographic parameters as well as on RAAS and natriuretic peptides.

**Methods:** Dogs with confirmed DCM on echocardiography were randomly divided into two treatment groups, one with conventional protocol having ACEI (Group II) and another group with ARNi replacing ACEI in the protocol (Group III). Clinical signs, ECG, echocardiographic evaluation was done at monthly interval till 90$^{th}$ day of therapy. Radiographic evaluation as well as effect on RAAS by assessing plasma natriuretic peptides and urine aldosterone-creatinine ratio was done on 0$^{th}$ day and 90$^{th}$ days of therapy. Data was analysed using ANOVA and independent 't' test followed by LSD using IBM SPSS version 2.0.

**Result:** In the study, group III dogs showed early resolution of clinical signs compared to group II. Group III showed a significant decrease in left atrial diameter and LA/Ao ratio earlier by 30$^{th}$ day of therapy, while in Group II, the same was noticed on the 60$^{th}$ day. Cardiac internal diameters were found to be significantly decreased in group III while group II showed a non-significant decrease. Systolic function was found to be significantly increased by 30$^{th}$ day in group III while group III has a non significant increase in ejection fraction. Significant decline of Uald:cre ratio was noticed in group III indicating its effective inhibition of RAAS. In the study dogs treated with sacubitril-valsartan was found to have faster resolution of clinical signs, greater reduction in cardiac dimensions and improvement in systolic function compared to ACEI treated dogs.


**Key words:** Arni, Canine dilated cardiomyopathy, Heart failure, Sacubitril-Valsartan.

## INTRODUCTION

Dilated cardiomyopathy, an acquired myocardial disease is predominantly characterized by enlargement of the left cardiac chambers and myocardial hypokinesis with systolic failure that results in heart failure with a reduced ejection fraction (Yadav *et al*., 2023). Activation of sympathetic nervous system and Renin-Angiotensin-Aldosterone system (RAAS) pathway, though are compensatory mechanisms generated in response to the failing heart, will contribute to increased workload and contribute to disease progression (Borgarelli *et al*., 2001). In a study conducted by Gasparini *et al*. (2020), they observed that in dogs with dilated cardiomyopathy (DCM) a diffuse cardiac remodeling happens primarily contributed by macrophages and cardiomyocytes that results in cardiac dilation as well as dysfunction.

The neurohumoral activation in response to neurohormonal stimulation is well documented, particularly through the release of natriuretic peptides. These peptides play a significant role in promoting natriuresis, diuresis and exerting RAAS suppressive and sympathoinhibitory actions (Kuwahara, 2021). Neprilysin, a metallopeptidase will catalyze the degradation of these natriuretic peptides and render them less effective. Sacubitril inhibits the degradation of natriuretic peptides, thereby enhancing their levels and associated benefits. However, it also prevents the

[1]Department of Veterinary Medicine, College of Veterinary Science, Proddatur-516 360, Andhra Pradesh, India.
[2]Department of Veterinary Clinical Medicine, Madras Veterinary College, Chennai-600 001, Tamil Nadu, India.
[3]Veterinary University Peripheral Hospital, Tamil Nadu Veterinary and Animal Sciences University, Chennai-600 001, Tamil Nadu, India.
[4]Centralised Clinical Laboratory, Madras Veterinary College, Chennai-600 001, Tamil Nadu, India.
[5]Department of Veterinary Surgery and Radiology, Madras Veterinary College, Chennai-600 001, Tamil Nadu, India.

**Corresponding Author:** K.S. Saikrishna, Department of Veterinary Medicine, College of Veterinary Science, Proddatur-516 360, Andhra Pradesh, India. Email: vetdoc33@gmail.com

**How to cite this article:** Saikrishna, K.S., Jeyaraja, K., Selvaraj, P., Vairamuthu, S. and Shafiuzama, M. (2026). Therapeutic Evaluation of Sacubitril-valsartan (Arni) in Canine Dilated Cardiomyopathy. *Indian Journal of Animal Research*. **60(6):** 1021-1029. doi: 10.18805/IJAR.B-5588.

**Submitted:** 22-03-2025 **Accepted:** 23-05-2025 **Online:** 17-06-2025

degradation of angiotensin II, thereby increasing its levels and associated risks. Therefore, a novel combination of Angiotensin receptor and neprilysin inhibitor *i.e*., valsartan and sacubitril was developed to effectively inhibit neprilysin and block angiotensin -II at AT1 receptors.

The present study was designed to assess the efficacy of sacubitril-valsartan in the management of chronic heart failure due to DCM compared to the conventional protocol with ACEI.

## MATERIALS AND METHODS

### Study design

This study was conducted as a prospective randomized study at Madras Veterinary College teaching Hospital, Chennai over a period of two years from 2021 to 2022. The study was approved by the university advisory committee.

Client owned dogs presented with chronic heart failure of class 3a (ISAHC) were taken up for the study. Twelve healthy dogs formed group I for the purpose of establishing reference values.

Fifty-two diseased dogs were randomly allotted to treatment groups II and III. Group II dogs were treated with Tab.Pimobendan @ 0.25 mg/kg BID orally, Tab. Enalapril @ 0.5 mg/kg BID orally, Tab. Furosemide + spironolactone @ 2 mg/kg BID and cardiac supplement BID orally. In Group III dogs were treated with Tab.Pimobendan @0.25 mg/kg BID orally, Tab. Sacubitril/valsartan @ 5 mg/kg BID orally, Tab. Furosemide + spironolactone @ 2 mg/kg BID and cardiac supplement BID orally.

Post inclusion removal criteria from the treatment groups was poor owner compliance, any adverse events, comorbid conditions and incomplete data during trial. The final design of study was group I with twelve number of healthy dogs, group II twelve diseased dogs and group III twelve diseased dogs. Clinical evaluation was done on 0th day and were compared during the therapy on $30^{th}$, $60^{th}$ and $90^{th}$ days.

### ECG examination

Electrocardiographic recording was done with patient in right lateral recumbency using a standard 4 lead electrocardiogram (Vesta 30 Li). Recordings were done at 25 mm/s, 50 mm/s paper speed and 10 mm = 1 mv voltage calibration. All the readings were recorded on thermosensitive paper and were evaluated for rate, rhythm and amplitude on days 0, 30, 60 and 90.

### Radiographic evaluation

Thoracic radiographs were taken to evaluate the presence of cardiomegaly using a digital X-ray (Konicka) on day 0 and day 90. All the images were taken on right lateral recumbency during inspiration. Vertebral heart score (VHS) measurement was done as described by Buchanan and Bucheler (1995). Radiographs were also evaluated for the presence of pulmonary edema, pleural effusions and their response to therapy.

### Echocardiographic examination

Echocardiographic evaluation was done using Aeroscan CD 25 ultrasound system with a phased array probe (2.0 - 6.0 Hz) to obtain 2-D, M mode, color Doppler, spectral Doppler and Tissue Doppler images as suggested by Boon (2011). Therapeutic evaluation was done on $30^{th}$, $60^{th}$, $90^{th}$ days and were compared to 0th day values.

### Natriuretic peptides

Plasma sample was collected from the healthy dogs as well as diseased dogs on $0^{th}$, $90^{th}$ days and were stored at -80°C. Batch analysis of samples was done to evaluate atrial natriuretic peptide (ANP) and NT pro BNP levels. Sandwich ELISA was performed to measure ANP and NT-proBNP levels using kits obtained from Krishgen® Biosystems, as per the manufacturer's instructions.

### Urine aldosterone:creatinine ratio

Urine sample was collected from the patient by catheterization and transferred to an Eppendorf tube. 5 ml of urine sample was immediately processed for urine creatinine level using fully automated biochemical analyzer (A-15 Biosystem Random access analyzer, Biosystems, Barcelona, Spain). Rest of the sample was immediately stored at -80°C for batch evaluation on $0^{th}$ day and $90^{th}$ day. Urine aldosterone was estimated by Radio Immuno Assay technique using Genesys gamma -1 machine as per the manufacturer protocol.

### Adverse effect monitoring

All the dogs in therapeutic groups were evaluated for adverse events using systolic blood pressure evaluation, BUN and creatinine levels.

### BUN, creatinine

Two ml of the sample was collected by phlebotomy in a clot activator, allowed to clot and centrifuged to separate the serum extruded. Separated serum was analysed using a fully automated biochemical analyzer (A-15 Biosystem Random access analyzer, Biosystems, Barcelona, Spain) to measure BUN and creatinine on $0^{th}$, $30^{th}$, $60^{th}$ and $90^{th}$ days of therapy.

### Therapeutic evaluation

Therapeutic evaluation was done at monthly interval till 90 days of therapy. Dogs that were regularly presented for the evaluation was taken up for comparative evaluation between the groups.

### Statistical methods

The statistical differences among different groups were tested using ANOVA and independent t test followed by "Least Significant Difference" posthoc analysis in IBM SPSS software version 20.

## RESULTS AND DISCUSSION

Exercise intolerance, lethargy, dyspnea and ascites were the most common signs recorded in DCM affected dogs.

Group II dogs showed complete resolution by the 90th day of therapy, except for one case where ascites was still persistent. On the other hand, in Group III, complete resolution of all the signs was noticed by the 60th day of therapy (Table 1).

Electrocardiography revealed significant ($p<0.05$) increase of PR interval by 90th day in both therapeutic groups. Group III showed a significant decrease in QRS duration by 90th day when compared with 0th day (Table 2).

**Radiographic evaluation**

VHS (vertebral heart size) was significantly higher ($p<0.01$) in Groups II (12.33±0.17) and III (12.25±0.15) (Fig 1a) on day 0 compared to healthy dogs. By day 90, VHS significantly decreased ($p<0.01$) to 11.50±0.29 in Group II and 10.96± 0.21 in Group III. Pre-therapy radiographs showed pulmonary edema in 11 dogs (Group II) and 10 dogs (Group III), with 2 in each group having pleural effusions (Fig 1b). By day 90, 7 dogs in Group II and 9 in Group III recovered from pulmonary edema, while mild cases persisted in 4 (Group II) and 1 (Group III). No pleural effusions were observed in either group by day 90.

**Echocardiography**

**LA/Ao ratio**

The mean±SE value of left atrial diameter and left atrium-to-aorta ratio was found to be significantly increased ($p<0.01$) in DCM-affected dogs on the 0th day in both Group II and Group III, compared to healthy dogs. Upon therapy, dogs in Group III showed a significant decrease in left atrial diameter and LA/Ao ratio earlier by 30th day of therapy (Fig 2), while in Group II, the same was noticed on the 60th day (Table 3).

**M-mode evaluation**

Dogs with DCM showed a significant increase ($p<0.01$) in LVIDd (left ventricular internal diameter in diastole), LVIDs (left ventricular internal diameter in systole), EPSS (E-point septal separation) and a significant decrease of fractional shortening (FS) on 0th day when compared with healthy ones (Fig 3). On 90th day, Group II dogs showed no significant difference in LVIDd, LVIDs and EPSS (Table 4, Fig 4). In contrast Group III had a significant decrease in LVIDd, LVIDs and EPSS by the 60th and 90th days ($p<0.01$) of therapy and a significant increase in FS by 30th day (Fig 4).

**Modified Simpson disc summation method**

A significant increase ($p<0.01$) was noticed in end-diastolic volume (EDV) and end-systolic volume (ESV) on the 0th day in both Group II and Group III, along with a significant decrease ($p<0.01$) in ejection fraction (EF). Dogs treated in Group III showed a significant decrease in EDV and ESV ($p<0.01$) by the 60th day and a significant increase ($p<0.05$) in EF was noticed early by the 30th day. No significant difference in EDV, ESV and EF was observed in dogs treated in Group II until the 90th day of therapy (Table 5).

**Aortic flow velocity**

Aortic velocity was significantly lower ($p<0.01$) in dogs with DCM compared to healthy dogs. Aortic velocity was significantly increased by the 60th day in Group II, while in Group III, this increase was noticed as early as the 30th day of therapy.

**Natriuretic peptides**

Pretherapeutic evaluation of natriuretic peptides, ANP and NT pro BNP, revealed a significant increase ($p<0.01$) in both parameters in DCM-affected dogs compared to healthy dogs.

On 90th day of therapy significantly decreased ($p<0.01$) ANP levels was noticed in both the groups. Of the two therapeutic groups, pronounced decline ($p<0.05$) was observed in Group III compared to Group II. NT pro BNP also significantly decreased ($p<0.01$) by 90th day compared to pretherapeutic levels in both the groups (Table 6).

**Urine aldosterone:creatinine ratio**

Dogs with DCM exhibited a significantly increased ($p<0.05$) urine aldosterone creatinine ratio (Uald:cre ratio) compared to healthy dogs. Notably, Group III showed a significant reduction ($p<0.05$) in the Uald: Cre ratio after therapy, indicating marked improvement (Table 7). In contrast,

**Table 1:** Comparison of clinical signs during therapy in group II and group III.

| Clinical sign | Group II (n=12) | | | | Group III (n=12) | | | |
|---|---|---|---|---|---|---|---|---|
| | 0th day | 30th day | 60th day | 90th day | 0th day | 30th day | 60th day | 90th day |
| Exercise intolerance | 12 | 7 | 2 | 0 | 11 | 3 | 0 | 0 |
| Lethargy | 12 | 3 | 1 | 0 | 11 | 0 | 0 | 0 |
| Dyspnoea | 11 | 2 | 1 | 0 | 11 | 3 | 0 | 0 |
| Ascites | 10 | 4 | 4 | 1 | 7 | 1 | 0 | 0 |
| Cough | 6 | 2 | 0 | 0 | 7 | 1 | 0 | 0 |
| Inappetence | 2 | 3 | 1 | 0 | 3 | 0 | 0 | 0 |
| Sleeping difficulty | 5 | 0 | 0 | 0 | 3 | 0 | 0 | 0 |
| Anorexia | 5 | 0 | 0 | 0 | 3 | 1 | 0 | 0 |
| Edema | 1 | 0 | 1 | 0 | 1 | 0 | 0 | 0 |
| Syncope | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |

**Table 2:** Mean±S.E values comparison of electrocardiographic parameters within and between the therapeutic groups II and III.

| | P amplitude (mv) | | | P duration (sec) | | | PR interval (sec) | | | R amplitude (mv) | | | QRS duration (sec) | | | T amp (mv) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Group II (n=12) | Group III (n=12) | p-value | Group II (n=12) | Group III (n=12) | p-value | Group II (n=12) | Group III (n=12) | p-value | Group II (n=12) | Group III (n=12) | p-value | Group II (n=12) | Group III (n=12) | p-value | Group II (n=12) | Group III (n=12) | p-value |
| 0th day | 0.154$^{aq}$± 0.21 | 0.150$^{aq}$ ± 0.03 | 0.921 | 0.035$^{aq}$± 0.00 | 0.383 $^{aq}$± 0.007 | 0.714 | 0.075 $^{aq}$± 0.008 | 0.069$^{aq}$± 0.12 | 0.707 | 1.76$^{aq}$± 0.20 | 1.85$^{aq}$± 0.167 | 0.728 | 0.04$^{aq}$± 0.003 | 0.04$^{aq}$± 0.002 | 0.843 | 0.20$^{aq}$± 0.02 | 0.30$^{aq}$± 0.05 | 0.102 |
| 90th day | 0.118$^{aq}$± 0.11 | 0.112$^{aq}$± 0.16 | 0.771 | 0.043$^{aq}$± 0.00 | 0.383 $^{aq}$± 0.003 | 0.193 | 0.11 $^{bq}$± 0.01 | 0.10 $^{bq}$± 0.008 | 0.813 | 1.55 $^{aq}$± 0.157 | 1.60 $^{aq}$± 0.139 | 0.814 | 0.04 $^{aq}$± 0.00 | 0.037 $^{bq}$± 0.001 | 0.177 | 0.18$^{aq}$± 0.02 | 0.24 $^{aq}$± 0.05 | 0.330 |
| p-value | 0.237 | 0.258 | | 0.116 | 1.00 | | 0.01 | 0.02 | | 0.339 | 0.071 | | 0.490 | 0.046 | | 0.667 | 0.320 | |

In the present study, the prominent clinical signs were exercise intolerance and lethargy (94.23%) followed by dyspnea (80.76%), ascites.

**Table 3:** Mean±S.E values comparison of left atrium aorta ratio within and between treatment groups II, III.

| | Aorta diameter (cm) | | | Left atrium diameter (cm) | | | LA:Ao ratio | | |
|---|---|---|---|---|---|---|---|---|---|
| | Group II (n=12) | Group III (n=12) | p-value | Group II (n=12) | Group III (n=12) | p-value | Group II (n=12) | Group III (n=12) | p-value |
| 0th day | 2.07$^{aq}$±0.07 | 2.11$^{aq}$±0.10 | .505 | 4.83$^{aq}$±0.34 | 4.63$^{aq}$±0.29 | .554 | 2.35$^{aq}$±0.16 | 2.22$^{aq}$±0.15 | .697 |
| 30th day | 1.94$^{aq}$±0.10 | 2.25$^{aq}$±0.10 | .870 | 4.29$^{abq}$±0.40 | 3.78$^{bq}$±0.28 | .454 | 2.34$^{aq}$±0.28 | 1.73$^{bq}$±0.16 | .057 |
| 60th day | 2.07$^{aq}$±0.11 | 2.32$^{aq}$±0.11 | .812 | 3.62$^{bq}$±0.30 | 3.23$^{bq}$±0.17 | .095 | 1.83$^{aq}$±0.19 | 1.45$^{bcq}$±0.12 | .084 |
| 90th day | 2.04$^{aq}$±0.09 | 2.37$^{aq}$±0.13 | .309 | 3.67$^{bq}$±0.50 | 3.12$^{br}$±0.19 | .009 | 1.83$^{aq}$±0.27 | 1.34$^{cr}$±0.09 | .005 |
| p-value | 0.712 | .391 | | 0.109 | 0.00 | | 0.181 | 0.00 | |

**Table 4:** Mean±S.E values comparison of M -mode echocardiographic parameters within and between treatment groups II, III.

| | LVIDd (cm) | | | LVIDs (cm) | | | FS (%) | | | EPSS (cm) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Group II (n=12) | Group III (n=12) | p-value | Group II (n=12) | Group III (n=12) | p-value | Group II (n=12) | Group III (n=12) | p-value | Group II (n=12) | Group III (n=12) | p-value |
| 0th day | 6.15$^{aq}$ ±0.24 | 6.01 $^{aq}$0.20 | .447 | 5.11$^{aq}$ ±0.20 | 5.11$^{aq}$±0.18 | .887 | 16.77$^{aq}$±0.94 | 14.25±1.05$^{aq}$ | .510 | 1.66 $^{aq}$±0.14 | 1.80 $^{aq}$±0.88 | .052 |
| 30th day | 6.02$^{aq}$±0.29 | 5.83$^{acq}$±0.17 | .052 | 4.78$^{aq}$±0.28 | 4.71$^{acq}$±0.16 | .138 | 20.83$^{aq}$±1.74 | 19.20 $^{bq}$±1.25 | .096 | 1.65 $^{aq}$±0.14 | 1.85 $^{aq}$±0.16 | .688 |
| 60th day | 5.82$^{aq}$±0.28 | 5.39$^{bcq}$±0.14 | .192 | 4.63$^{aq}$±0.31 | 4.32$^{bcq}$±0.13 | .052 | 20.65$^{aq}$±1.95 | 20.14$^{bcq}$±1.34 | .195 | 1.75 $^{aq}$±0.17 | 1.42 $^{bq}$±0.08 | .111 |
| 90th day | 5.68$^{aq}$±0.27 | 5.12$^{bq}$±0.21 | .603 | 4.51$^{aq}$±0.28 | 3.95$^{bq}$±0.21 | .408 | 21.28$^{aq}$±1.54 | 23.89$^{cq}$±1.88 | .546 | 1.41 $^{aq}$±0.12 | 1.14 $^{bq}$±0.08 | .112 |
| p-value | 0.62 | 0.005 | | 0.434 | 0.00 | | .173 | 0.00 | | 0.397 | 0.00 | |

**Table 5:** Mean±S.E values comparison of b-mode-simpsons method echocardiographic parameters within and between treatment groups II, III.

| | EDV (ml) | | | ESV (ml) | | | SV (ml) | | | EF (%) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Group II (n=12) | Group III (n=12) | p-value | Group II (n=12) | Group III (n=12) | p-value | Group II (n=12) | Group III (n=12) | p-value | Group II (n=12) | Group III (n=12) | p-value |
| 0th day | 193.17$^{aq}$ ±18.70 | 181.70$^{aq}$±14.5 | .520 | 126.52$^{aq}$±11.97 | 125.99$^{aq}$±10.45 | .882 | 66.65±7.48$^{aq}$ | 52.82±6.78$^{aq}$ | .732 | 34.22$^{aq}$±1.72 | 29.29$^{aq}$±2.02 | .482 |
| 30th day | 186.53$^{aq}$±19.67 | 170.65$^{aq}$±10.80 | .054 | 111.57$^{aq}$±15.44 | 103.05$^{acq}$±9.54 | .193 | 74.96$^{aq}$±7.94 | 65.35$^{aq}$±5.07 | .174 | 41.44$^{aq}$±3.04 | 38.53$^{bq}$ ±2.21 | .107 |
| 60th day | 167.37$^{aq}$±17.22 | 139.05$^{bq}$±7.26 | .106 | 102.73$^{aq}$±15.26 | 83.09$^{bcr}$±4.74 | .014 | 64.64$^{aq}$±4.48 | 55.32$^{aq}$±5.17 | .943 | 41.76$^{aq}$± 3.48 | 39.61$^{bcq}$±2.34 | .134 |
| 90th day | 161.62$^{aq}$±18.40 | 124.60$^{bq}$±9.92 | .150 | 97.79$^{aq}$±14.34 | 69.84$^{bq}$±8.94 | .136 | 63.83$^{aq}$±4.56 | 56.34$^{aq}$±5.21 | .503 | 41.88$^{aq}$±2.66 | 46.58$^{cq}$±3.21 | .596 |
| p-value | .575 | .002 | | .514 | 0.00 | | .587 | .424 | | .164 | 0.00 | |

Group II did not exhibit any significant change compared to their pre-therapeutic levels (67.30%) and cough (46.15%). These were consistent with reports of Jeyaraja *et al*. (2015), Kumar *et al*. (2018); Harmon *et al*. (2017) and Dutton and López Alvarez (2018). Superior resolution of clinical signs was seen in group III due to the beneficial effects of the valsartan which promotes higher levels of Ang1-7 peptide and other alternative angiotensin peptides, such as Ang1-5, through increased pooling of angiotensin II (ATII). These alternative peptides have potential natriuretic, vasodilatory and cardioprotective effects (Larouche Lebel *et al*., 2021). In addition to valsartan, sacubitril through facilitation of sustained natriuretic peptides action contribute towards inhibition of cardiac remodeling and fibrosis (Braunwald, 2015). Early recovery from clinical signs in heart failure patients using sacubitril/valsartan, compared to enalapril, has also been reported by McMurray *et al*. (2014).

Proinflammatory response was seen in DCM demonstrated by neutrophilic leukocytosis, was thought to be due to increased secretion of proinflammatory mediators from the failing myocardium (Domanjko Petric *et al*., 2018) and diminished anti-inflammatory effects of the endothelium (Cunningham *et al*., 2012). This results in a state of aseptic inflammation, further exacerbated by increased angiotensin II through its action on the AT1 receptor (Di Raimondo *et al*., 2012). Dogs treated in group III had earlier suppression of this inflammatory response by 30$^{th}$ day compared to 60$^{th}$ day in group II. This earlier response highlights the effective AT1 receptor blockade by valsartan when compared with reduced angiotensin II by ACE inhibitor (Di Raimondo *et al*., 2012). The therapeutic efficacy of sacubitril/valsartan in controlling inflammation in patients with chronic heart failure was also reported by Goncalves *et al*. (2020) and Bolla *et al*. (2022). Di Raimondo *et al*. (2012) also highlighted the univocal evidence supporting the anti-inflammatory role of ARBs on the AT1 receptor.

Pulmonary edema in dilated cardiomyopathy occurs as a consequence of left ventricular systolic dysfunction and elevated left atrial pressure increasing pulmonary venous hydrostatic pressure (Saini, 2021). Sympathetic activation further contributes by constriction of splanchnic vessels, causing an increased circulation to the pulmonary vasculature and precipitation of pulmonary edema (Ettinger, 2017). Better clearance of pulmonary edema was seen in group III compared to group II, likely due to to the protective effect of sacubitril/valsartan on the natriuretic peptides and the increased Ang1-7, which promote vasodilation, natriuresis

**Table 6:** Natriuretic peptides within and between treatment groups II, III.

| | ANP (pg/ml) | | | | NT-pro BNP (pmol/l) | | | |
|---|---|---|---|---|---|---|---|---|
| | Group I (n=12) | Group II (n=12) | Group III (n=12) | p-value | Group I (n=12) | Group II (n=12) | Group III (n=12) | p-value |
| 0$^{th}$ day | 32.47$^{a}$±2.90 | 152.68$^{aq}$±7.26 | 158.19$^{aq}$±9.17 | 0.00 | 696.91$^{a}$±30.90 | 2130.30$^{aq}$±132.50 | 2098.47$^{aq}$±122.83 | 0.00 |
| 90$^{th}$ day | - | 53.00$^{bq}$±5.29 | 69.87$^{br}$±4.57 | 0.024 | - | 1109.62$^{bq}$±64.90 | 1043.20$^{bq}$± 50.42 | 0.428 |
| p-value | | 0.000 | 0.000 | | | 0.000 | 0.000 | |

**Table 7:** Comparison of urine aldosterone:creatinine ratio between and within therapeutic groups.

| | Uald:creatinine (pg/mg) | | | |
|---|---|---|---|---|
| | Group I (n=12) | Group II (n=12) | Group III (n=12) | p-value |
| 0$^{th}$ day | 0.06 $^{a}$±0.01 | 0.14$^{aq}$±0.04 | 0.12$^{aq}$±0.01 | 0.043 |
| 90$^{th}$ day | - | 0.10$^{aq}$±0.03 | 0.04$^{bq}$±0.01 | 0.113 |
| p-value | | 0.553 | 0.006 | |

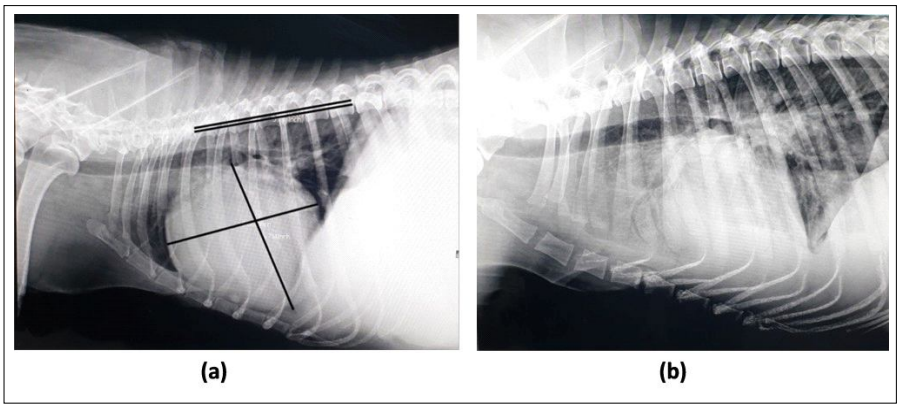


**Fig 1a:** Cardiomegaly recorded in a dog with DCM (VHS= 14.0) and 1b pleural effusion and pulmonary edema.

and diuresis (Lee and Daniels, 2016; Larouche Lebel *et al*., 2021).

Altered mitral valve geometry in DCM causes regurgitation of blood into the atrium and increased left atrial volume overload resulted in significantly increased left atrial aorta ratio (Janus *et al*., 2016). In this study, Group III showed a significant decrease in LA/Ao, while Group II had a non-significant decrease. These results concur with Newhard *et al*. (2018), who reported reduced left atrial diameter in dogs treated with sacubitril/valsartan for myxomatous mitral valve disease. Sun *et al*. (2022) suggested that angiotensin receptor-neprilysin inhibitors (ARNi) are more effective in left atrial reverse remodeling compared to ACE inhibitors or ARBs alone.

M-mode parameters showed non-significant improvement in group II while group III dogs showed significant improvement of systolic functional parameters. Modified Simpson disc evaluation revealed significant decrease in EDV and increase in EF in group III while no significance in group II. The E/Ea ratio, an indicator for left ventricular filling pressure was elevated in DCM dogs. Sacubitril/valsartan significantly reduced filling pressure while the enalapril group has no significant effect.

Group III's superiority was due to the inclusion of ARNi (sacubitril/valsartan), which effectively addresses the pathophysiology of heart failure. In contrast, enalapril in Group II is an ACE inhibitor that blocks angiotensin II production *via* the ACE pathway but does not affect the local tissue pathway, where angiotensin II is produced by chymase. This limitation can lead to aldosterone breakthrough (ABT) in Group II. Lantis *et al*. (2015) showed that ACE inhibitors fail to prevent aldosterone breakthrough in dogs with activated RAAS. Ames *et al*. (2017) reported that 30% of dogs treated with ACE inhibitors like enalapril/benazepril for congestive heart failure had incomplete RAAS blockade. In contrast, valsartan blocks the AT1 receptor of angiotensin II, which is responsible for the harmful effects like fibrosis (Webb and de Gasparo, 2001). In process valsartan allows continued angiotensin II production, leading to the formation of Ang1-9, Ang1-7 and Ang1-5, which are vasodilatory, natriuretic and cardioprotective in nature (Larouche Lebel *et al*., 2021). It also enhances natriuretic peptides, which inhibit renin secretion and block aldosterone, disrupting the hormonal cascade involved in heart failure (Newhard *et al*., 2018). Additionally, ANP and BNP protect the heart by inhibiting angiotensin-II effects on myocytes and reducing norepinephrine-induced growth of cardiac cells (D' Elia *et al*., 2017). ANP also limits cell proliferation and collagen synthesis, offering antifibrotic benefits (Volpe, 2014). As a result, the combination of sacubitril and valsartan led to significant improvements in fractional shortening and reductions in left atrial and left ventricular diameters in Group III, compared to Group II treated with enalapril.

The urine aldosterone-to-creatinine ratio was significantly reduced by ARNi, while enalapril had no effect, with similar findings reported by Mochel *et al*. (2019). Sacubitril/valsartan's superiority over enalapril lies in its

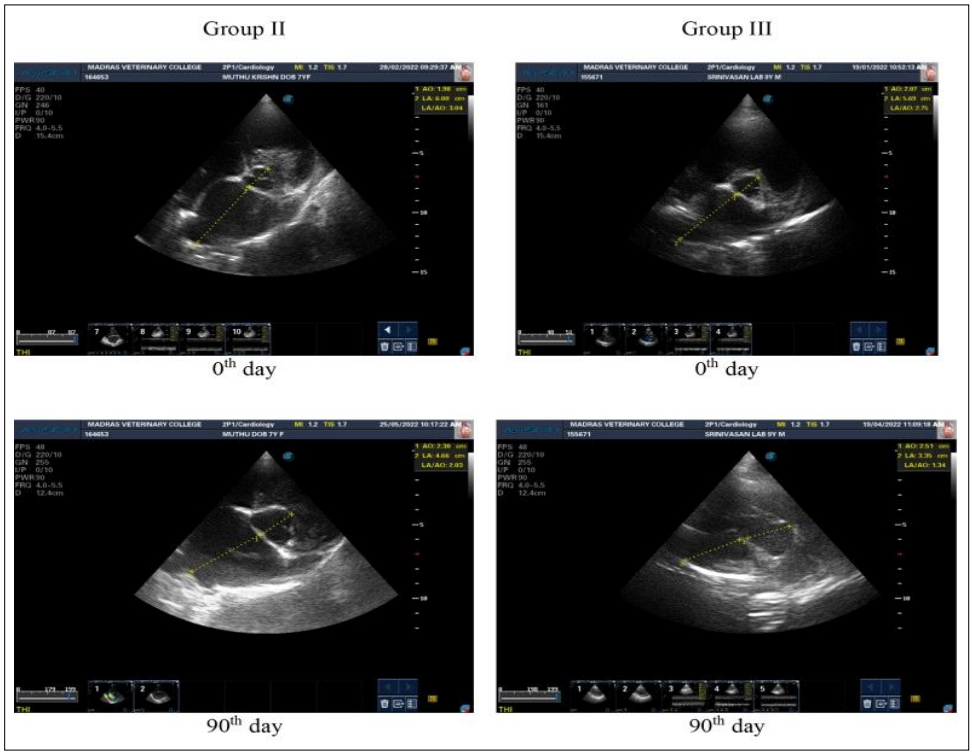


**Fig 2:** Comparison of 0th day and 90th day LA/Ao in group II and group III dogs.

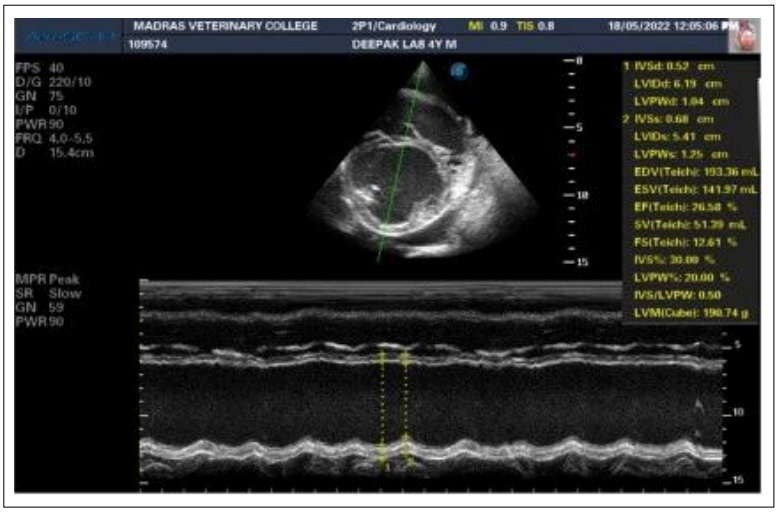


**Fig 3:** M-mode echocardiographic measurement of canine DCM.

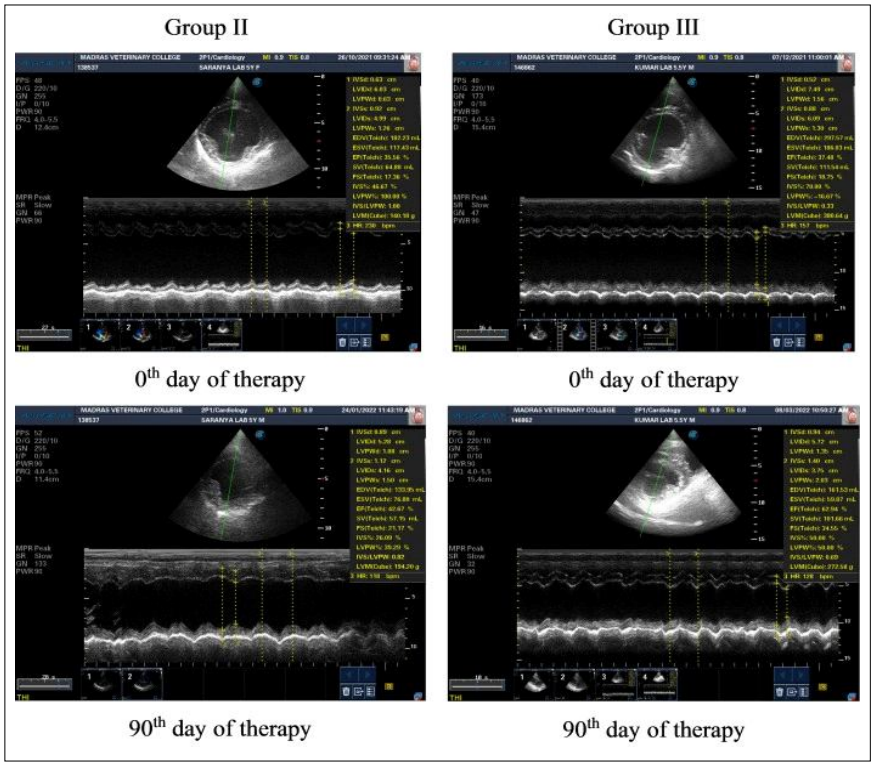


**Fig 4:** Pre therapy and 90th day therapeutic comparison of M - mode findings in treated dogs.

effective inhibition of RAAS by blocking AT1 receptors, preventing angiotensin II from binding and reducing aldosterone synthesis from both plasma and tissue pathways (Webb and de Gasparo, 2001). Additionally, sacubitril plays a key role in further reducing aldosterone levels.

## CONCLUSION

Dogs treated with Sacubitril- valsartan showed earlier resolution of clinical signs, better reduction in cardiac dimensions and substantial improvement in cardiac function compared to conventional treatment with ACE inhibitor. Therefore, sacubitril-valsartan can be added to the regular treatment protocol for patients with dilated cardiomyopathy replacing ACE inhibitors. Further it is advocated to have a multicenter blind fold study for further validation of sacubitril-valsartan (ARNi).

### Conflict of interest

All authors declared that there is no conflict of interest.

## REFERENCES


Ames, M.K., Atkins, C.E., Eriksson, A. and Hess, A.M. (2017). Aldosterone breakthrough in dogs with naturally occurring myxomatous mitral valve disease. *J. Vet. Cardiol*. **19(3):** 218-227.

Bolla, G.B., Fedele, A., Faggiano, A., Sala, C, Santangelo, G. and Carugo, S. (2022). Effects of Sacubitril/Valsartan on biomarkers of fibrosis and inflammation in patients with heart failure with reduced ejection fraction. *BMC Cardiovascular Disorders*. **22(1):** 1-8.

Boon, J.A. (2011). Veterinary Echocardiography. Second edition. John Wiley and Sons. United Kingdom.

Borgarelli, M., Tarducci, A., Tidholm, A. and Haggstrom, J. (2001). Canine idiopathic dilated cardiomyopathy. Part II: pathophysiology and therapy. *Vet. J*. **162(3):** 182-195.

Braunwald, E. (2015). The path to an angiotensin receptor antagonist-neprilysin inhibitor in the treatment of heart failure. *J. Am. Coll. Cardiol*. **65(10):** 1029-1041.

Buchanan, J.W. and Bucheler, J. (1995). Vertebral scale system to measure canine heart size in radiographs. *J. Am. Vet. Med. Assoc*. **206:** 194-194.

Cunningham, S.M., Rush, J.E. and Freeman, L.M. (2012). Systemic inflammation and endothelial dysfunction in dogs with congestive heart failure. *J. Vet. Intern. Med*. **26(3):** 547-557.

D' Elia, E., Iacovoni, A., Vaduganathan, M., Lorini, F.L, Perlini, S. and Senni, M. (2017). Neprilysin inhibition in heart failure: mechanisms and substrates beyond modulating natriuretic peptides. *Eur. J. Heart Fail*. **19(6):** 710-717.

Di Raimondo, D., Tuttolomondo, A., Butta, C., Miceli, S., Licata, G. and Pinto, A. (2012). Effects of ACE-inhibitors and angiotensin receptor blockers on inflammation. *Curr. Pharm. Des*. **18(28):** 4385-4413.

Domanjko Petriè, A., Lukman, T., Verk, T.B. and Nemec, S.A. (2018). Systemic inflammation in dogs with advanced-stage heart failure. *Acta Vet. Scand*. **60(1):** 1-9.

Dutton, E. and López Alvarez, J. (2018). An update on canine cardiomyopathies-is it all in the genes?. *J. Small Anim. Pract*. **59(8):** 455-464.

Ettinger, S.J., Feldman, E.C. and Cote, E. (2017). Textbook of Veterinary Internal Medicine-Inkling E-Book. 8th Edn. Elsevier Health Sciences. pp. 2868-2869.

Gasparini, S., Fonfara, S., Kitz, S., Hetzel, U. and Kipar, A. (2020). Canine dilated cardiomyopathy: Diffuse remodeling, focal lesions and the involvement of macrophages and new vessel formation. *Veterinary Pathology*. **57(3):** 397-408.

Goncalves, A.V., Pereira-da-Silva, T., Galrinho, A., Rio, P., Branco, L.M., Soares, R., Moreira, R.I. and Ferreira, R.C. (2020). C-reactive protein reduction with sacubitril-valsartan treatment in heart failure patients. *Am. J. Cardiovasc. Dis*. **10(3):** 174-181.

Harmon, M.W., Leach, S.B. and Lamb, K.E. (2017). Dilated cardiomyopathy in standard schnauzers: Retrospective study of 15 cases. *J. Am. Anim. Hosp. Assoc*. **53(1):** 38-44.

Janus, I., Noszczyk-Nowak, A., Nowak, M., Ciaputa, R., Kandefer-Gola, M. and Pasławska, U. (2016). A comparison of the histopathologic pattern of the left atrium in canine dilated cardiomyopathy and chronic mitral valve disease. *BMC Vet. Res*. **12(1):** 1-7.

Jeyaraja, K., Arun, A., Hamsa, Y.S., Sesh, P.S.L. and Nambi, A.P. (2015). Diagnostic evaluation of dilated cardiomyopathy in Labrador Retrievers. *Int. J. Adv. Res*. **3(12):** 628-655.

Kumar, A., Dey, S., Saxena, A.C. and Mahajan, S. (2018). Evaluation of add on effect of carvedilol on standard protocol of digoxin, frusemide and benazepril in the management of dilated cardiomyopathy in dogs. *Indian Journal of Animal Research*. **52(4):** 583-590. doi: 10.18805/ijar.B-3377.

Kuwahara, K. (2021). The natriuretic peptide system in heart failure: Diagnostic and therapeutic implications. *Pharmacology and Therapeutics*. **227:** 107863.

Lantis, A.C., Ames, M.K., Atkins, C.E., DeFrancesco, T.C., Keene, B.W. and Were, S.R. (2015). Aldosterone breakthrough with benazepril in furosemide activated renin angiotensin aldosterone system in normal dogs. *J. Vet. Pharmacol. Ther*. **38(1):** 65-73.

Larouche Lebel, E., Loughran, K.A., Huh, T. and Oyama, M.A. (2021). Effect of angiotensin receptor blockers and angiotensin converting enzyme 2 on plasma equilibrium angiotensin peptide concentrations in dogs with heart disease. *J. Vet. Intern. Med*. **35(1):** 22-32.

Lee, N.S. and Daniels, L.B. (2016). Current understanding of the compensatory actions of cardiac natriuretic peptides in cardiac failure: A clinical perspective. *Card. Fail. Rev*. **2(1):** 14.

McMurray, J.J., Packer, M., Desai, A.S., Gong, J., Lefkowitz, M.P., Rizkala, A.R. and Zile, M.R. (2014). Angiotensin-neprilysin inhibition versus enalapril in heart failure. *N. Engl J. Med*. **371:** 993-1004.

Mochel, J. (2019). A review of relevant therapeutic targets for the management of congestive heart failure in dogs: Present and future directions. *Preprints*. pp. 2019030082.

Newhard, D.K., Jung, S., Winter, R.L. and Duran, S.H. (2018). A prospective, randomized, double blind, placebo controlled pilot study of sacubitril/valsartan (Entresto) in dogs with cardiomegaly secondary to myxomatous mitral valve disease. *J. Vet. Intern. Med*. **32(5):** 1555-1563.

Saini, N., Uppal, S.K. and Anand, A. (2021). Radiographic assessment of dogs with congestive heart failure. *Indian Journal of Animal Research*. **57(7):** 922-927. doi: 10.18805/IJAR.B-4352.

Sun, Y., Song, S., Zhang, Y., Mo, W., Zhang, X., Wang, N., Xia, Y., Tse, G. and Liu,Y. (2022). Effect of angiotensin receptor neprilysin inhibitors on left atrial remodeling and prognosis in heart failure. *ESC Heart Fail*. **9(1):** 667-675.

Volpe, M. (2014). Natriuretic peptides and cardio-renal disease. *Int. J. Cardiol*. **176(3):** 630-639.

Webb, R.L. and de Gasparo, M. (2001). Role of the angiotensin II receptor blocker valsartan in heart failure. *Exp. Clin. Cardiol*. **6(4):** 215-221.

Yadav, A., Kumar, T., Sindhu, N., Agnihotri, D., Jajoria, C. and Sharma, M. (2023). Characterization of cardiac diseases in dogs prevalent in Indian Conditions. *Indian Journal of Animal Research.* **57(5):** 644-649. doi: 10.18805/IJAR.B-4544.