SHORT TERM EFFECTS OF SILDENAFIL IN TREATMENT OF PULMONARY HYPERTENSION IN DEGENERATIVE MITRAL VALVE DISEASE DOGS

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การศึกษาผลของยาซิลเดนาฟิลระยะสั้นในการรักษาภาวะความดันโลหิตปอดสูงในสุนัขที่เป็นโรคลิ้นหัวใจไมทรัลเสื่อม

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Field of Study	Veterinary Medicine
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การุณย์ แซ่ตั้ง : การศึกษาผลของยาซิลเดนาฟิลระยะสั้นในการรักษาภาวะความดันโลหิตปอดสูงในสุนัขที่เป็นโรคลิ้นหัวใจไมทรัลเสื่อม. (SHORT TERM EFFECTS OF SILDENAFIL IN TREATMENT OFPULMONARY HYPERTENSION IN DEGENERATIVE MITRAL VALVE DISEASE DOGS) อ.ที่ปรึกษาหลัก : รศ. ดร.สิริลักษณ์ สุรเชษฐพงษ์

โรคลิ้นหัวใจไมทรัลเสื่อมเป็นโรคหัวใจภายหลังกำเนิดที่พบได้บ่อยที่สุดในสุนัขพันธุ์เล็ก ภาวะความดันโลหิตปอดสูงเป็นภาวะแทรกซ้อนในโรคลิ้นหัวใจไมทรัลเสื่อมที่สามารถทำให้อาการแสดงทางคลินิกแย่ลง และระยะเวลาการอยู่รอดแย่ลงในสุนัขที่เป็นโรคลิ้นหัวใจไมทรัลเสื่อม การรักษาด้วยยามาตรฐานในโรคลิ้นหัวใจไมทรัลเสื่อมสามารถลดความดันโลหิตปอดได้เพียงเล็กน้อย ซึ่งการเพิ่มขนาดของยามาตรฐานเพื่อลดความดันโลหิตปอดไม่สามารถทำได้เนื่องจากอาจเกิดผลข้างเคียงจากยา ดังนั้นการเพิ่มยาชนิดอื่นจึงจำเป็น ซิลเดนาฟิลเป็นยาที่ถูกใช้เพื่อการรักษาภาวะความดันโลหิตปอดสูงที่เกิดจากโรคต่างๆ อย่างไรก็ตามผลของซิลเดนาฟิลในการรักษาภาวะความดันโลหิตปอดสูงจากโรคลิ้นหัวใจไมทรัลเสื่อมยังไม่มีการรายงาน ดังนั้นการศึกษานี้มีจุดประสงค์เพื่อประเมินผลของยาซิลเดนาฟิลเมื่อใช้ร่วมกับการรักษามาตรฐานในการรักษาสุนัขที่มีภาวะความดันโลหิตปอดสูงเนื่องมาจากโรคลิ้นหัวใจไมทรัลเสื่อม สุนัข 14 ตัว ได้รับการวินิจฉัยว่าเป็นโรคลิ้นหัวใจไมทรัลเสื่อมในระยะซีและมีภาวะความดันโลหิตปอดสูงได้รับการรักษาด้วยวิธีมาตรฐาน ถูกแบ่งแบบสุ่มออกเป็นสองกลุ่ม กลุ่มยาลวง 7 ตัว และกลุ่มซิลเดนาฟิล 7 ตัว ในวันที่ 0 สุนัขที่เข้าการศึกษาได้รับการตรวจร่างกาย ให้คะแนนอาการทางคลินิก ตรวจคลื่นไฟฟ้าหัวใจ วัดความดันโลหิต ตรวจเลือด ถ่ายภาพรังสีช่องอกและตรวจหัวใจด้วยคลื่นเสียงสะท้อนความถี่สูงเพื่อเป็นค่าพื้นฐาน สุนัขกลุ่มซิลเดนาฟิลได้รับการรักษามาตรฐานร่วมกับซิลเดนาฟิล ค่ากลางขนาดยา 1.79 (1.69-2.19) มิลลิกรัมต่อกิโลกรัมทุก 8 ชั่วโมงร่วมกับยามาตรฐานเป็นเวลา 1 สัปดาห์ ในขณะที่สุนัขในกลุ่มยาลวงได้รับยาลวงในรูปแบบเดียวกัน ในวันที่ 7 สุนัขที่เข้าร่วมการศึกษาได้รับการตรวจเช่นเดียวกับในวันที่ 0 ผลการรักษาพบว่าสุนัขกลุ่มที่ได้รับยาซิลเดนาฟิลมีความดันโลหิตปอดลดลงอย่างมีนัยสำคัญ (p=0.043) ในขณะที่ไม่พบการเปลี่ยนแปลงในกลุ่มยาลวง อย่างไรก็ตามไม่พบการเปลี่ยนแปลงของอาการและคะแนนรอยโรคที่ปอดภายหลังการรักษาด้วยซิลเดนาฟิล นอกจากนั้นการศึกษานี้พบความสัมพันธ์เชิงบวกระหว่างระดับความดันโลหิตปอดกับอัตราการเต้นของหัวใจและระยะการบีบตัวคงปริมาตรของหัวใจห้องล่างขวา(p=0.005 และ 0.014 ตามลำดับ) โดยสรุปยาซิลเดนาฟิลมีฤทธิ์ช่วยเสริมในการลดระดับความดันโลหิตปอดเมื่อใช้ร่วมกับยามาตรฐาน แต่ไม่มีผลต่อคะแนนอาการทางคลินิกและรอยโรคที่ปอด

สาขาวิชา	อายุรศาสตร์สัตวแพทย์	ลายมือชื่อนิสิต .....
ปีการศึกษา	2562	ลายมือชื่อ อ.ที่ปรึกษาหลัก .....

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KEYWORD: Degenerative mitral valve disease, Pulmonary Hypertension, Sildenafil, Treatment

Karun Saetang : SHORT TERM EFFECTS OF SILDENAFIL IN TREATMENT OFPULMONARY HYPERTENSION IN DEGENERATIVE MITRAL VALVE DISEASE DOGS. Advisor: Assoc. Prof. SIRILAK SURACHETPONG, D.V.M., Ph.D., D.T.B.V.M.

Degenerative mitral valve disease (DMVD) is the most common acquired cardiac disease in small breed dogs. Pulmonary hypertension (PH) is a common complication in DMVD that can worsen the clinical signs, cardiac function, and median survival time of DMVD dogs. Conventional therapy of DMVD can reduce systolic pulmonary arterial pressure (sPAP) minimally. Thus, additional therapy is required. Sildenafil is used to treatment of PH in various causes, but the effect of sildenafil in the treatment of PH secondary to DMVD is not published yet. Therefore, this study aimed to evaluate the effects of sildenafil in combination with conventional therapy in PH dogs caused by DMVD. Fourteen dogs were diagnosed with PH secondary to DMVD stage C and were being on conventional therapy. The recruited dogs were randomly assigned to the placebo (n=7) and sildenafil (n=7) groups. At day 0, the recruited dogs were performed a physical examination, clinical scores assessment, electrocardiography (ECG) measurement, systolic blood pressure measurement (SBP), blood collection, thoracic radiography examination, and echocardiography examination for baseline. Dogs in the sildenafil group received a combination of conventional therapy and sildenafil. The median dose of sildenafil was 1.79 (1.69-2.19) mg/kg every 8 hours for 1 week, while the placebo group received a placebo with the same regimen. At day 7, the recruited dogs were performed all examinations same as day 0. The results showed that the sildenafil group had a significant decrease in sPAP (p=0.043), while the sPAP did not change in the placebo group. However, clinical and lung scores did not improve after treatment with sildenafil. Additionally, this study found that sPAP had a positive correlation with heart rate and isovolumetric contraction time of right ventricle (p=0.005 and 0.014, respectively). In conclusion, sildenafil had a synergist effect to conventional therapy in reducing sPAP but effects of sildenafil on clinical and lung scores were not found.

Field of Study:	Veterinary Medicine	Student's Signature .....
Academic Year:	2019	Advisor's Signature .....

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CHAPTER I

INTRODUCTION

Importance and rationale

The most common acquired cardiovascular diseases in small and toy breed dogs is degenerative mitral valve disease or DMVD (Borgarelli and Haggstrom, 2010). The end-stage of DMVD dogs can lead to death by congestive heart failure. Pulmonary hypertension (PH) is a common complication in DMVD dogs that can worsen the clinical signs including dyspnea, syncope, exercise intolerance, and cough. Moreover, PH also decreases cardiac functions and median survival time (Pyle et al., 2004; Borgarelli et al., 2015). Pulmonary hypertension (PH) is a persistent increase in systolic pulmonary arterial pressure greater than 30 mmHg. The prevalence of DMVD dogs that develop PH ranged from 14 % to 53 % (Kellihan and Stepien, 2012).

At present, the standard protocol for the treatment of PH dogs caused by DMVD is still uncertain. Conventional therapy of DMVD, including pimobendan, furosemide, and angiotensin- converting enzyme inhibitor (ACEi), can reduce systolic pulmonary arterial pressure (sPAP) approximately 20 mmHg (Atkinson et al., 2009). There are combined effects of conventional therapy in decreasing sPAP as follows. The diuretic effect of furosemide can decrease left atrial pressure. The positive inotrope of phosphodiesterase-3 inhibitors (PDE-3i) can increase cardiac output by increasing

ventricular contraction. The vasodilation effect of ACEi and PDE-3i can decrease sPAP via a decrease in vascular resistance (Ohte et al., 1997; Kellihan and Stepien, 2012).

The additional therapy requires the treatment of PH dogs caused by DMVD because conventional therapy has a limitation to reduce sPAP by sided effects. Other drugs can decrease sPAP in human patients and dogs such as platelet-derived growth factor inhibitors, prostacyclin analogs, endothelin antagonists and phosphodiesterase-5 inhibitors (PDE-5i) (Stepien, 2009; Brown et al., 2010; Kellihan and Stepien, 2012; Arita et al., 2013). PDE-5 responses for vasoconstriction and is abundantly found in pulmonary vessels. PDE-5i, such as sildenafil, has selective vasodilation effect at pulmonary vessels (Kellihan and Stepien, 2012). The recent studies of PH caused by respiratory and cardiovascular diseases in dogs and rats found that sildenafil can decrease sPAP (Kellum and Stepien, 2007; Brown et al., 2010; Nakata et al., 2015; Murphy et al., 2017).

As mentioned above, DMVD dogs have a high prevalence in develop PH that can worse cardiac function, clinical signs and median survival time. Furthermore, conventional therapy has a limitation to reduce sPAP. Therefore, an additional drug like sildenafil that has a selective pulmonary vasodilation effect may improve the outcome. The combination effect of conventional therapy and sildenafil may help to improve clinical signs and reduce sPAP in PH dogs caused by DMVD.

Objective of the study

To evaluate the effects of sildenafil in combination with conventional therapy in PH dogs caused by DMVD stage C.

Keywords (Thai): โรคลิ้นหัวใจไมทรัลเสื่อม ภาวะความดันโลหิตปอดสูง ซิลเดนาฟิล การรักษา

Keywords (English): Degenerative mitral valve disease, Pulmonary Hypertension, Sildenafil, Treatment

Hypothesis

The combination of conventional therapy and sildenafil can improve the outcome including echocardiographic values, clinical scores, and estimated sPAP in PH dogs caused by DMVD.

CHAPTER II

LITERATURE REVIEWS

Pulmonary hypertension (PH) is a phenomenon that dogs had a persistent increase in systolic pulmonary artery pressure greater than 30 mmHg (Stepien, 2009; Kellihan and Stepien, 2012). There are many conditions and diseases that can induce PH in dogs. PH is classified into 6 groups as follow: group 1 pulmonary arterial hypertension (PAH), group 2 pulmonary hypertension due to left heart disease (LHD), group 3 pulmonary hypertension secondary to respiratory disease, hypoxia or both, group 4 pulmonary emboli, thrombi, or thromboemboli, group 5 parasitic disease, and group 6 pulmonary hypertension with multifactorial or unclear mechanisms (Reinero et al., 2020). Pulmonary hypertension can be divided into pulmonary arterial hypertension (PAH) or precapillary pulmonary hypertension and pulmonary venous hypertension (PVH) or postcapillary pulmonary hypertension (Kellihan and Stepien, 2012).

The development of pulmonary hypertension due to left heart disease or PVH is influenced by multi-factors including an increase in left atrial pressure, the effects of chronic neurohormonal activation to pulmonary vascular tone, and vascular remodeling (Kellihan and Stepien, 2012). The alteration of neurohormones in congestive heart failure dogs includes an increase in thromboxane A2, platelet-derived growth factor, and endothelin-1 and a decrease in natriuretic peptides and nitric oxide.

The consequences of the alteration of mediators consist of an increase in pulmonary vascular smooth muscle proliferation and an increase in pulmonary vasoconstriction (Stepien, 2009; Kellihan and Stepien, 2012; Arita et al., 2013). The common left heart disease that can cause PVH in dogs is degenerative mitral valve disease.

Degenerative mitral valve disease (DMVD) causes irregular in shape and thickening of the mitral valve, resulting in mitral valve regurgitation. The consequences of chronic mitral regurgitation include decreased cardiac output, left atrial volume overload, pulmonary vein congestion, increased pulmonary pressure, and pulmonary edema (Borgarelli and Haggstrom, 2010; Borgarelli and Buchanan, 2012). DMVD is the most common cause of pulmonary hypertension in dogs. The prevalence of DMVD dogs that develop PH ranged from 14 % to 53 % (Kellihan and Stepien, 2012). DMVD dogs affected with PH have awful consequences as follows. DMVD dogs affected with PH have clinical signs consisting of coughing, exercise intolerance, dyspnea, and syncope. DMVD dogs affected with PH will develop right ventricular remodeling including flattening of the interventricular septum, right ventricular enlargement, and tricuspid regurgitation (Pyle et al., 2004). Moreover, DMVD dogs with PH had shortened median survival time (456 days) compared to DMVD dogs without PH (758 days) (Borgarelli et al., 2015).

The gold standard for the diagnosis of pulmonary hypertension is the direct measurement of pulmonary arterial pressure by right heart catheterization (McLaughlin

et al., 2009). However, this invasive technique needs to be performed under an anesthetic condition in dogs (Kellihan and Stepien, 2012). Therefore, it is not practical to use this method in veterinary medicine because of the risk of anesthesia in cardiovascular disease dogs (Stepien, 2009). The indirect non-invasive measurement by echocardiography is used to measure the estimated sPAP in humans and dogs. Spectral Doppler echocardiography can be used for measuring the velocity of tricuspid regurgitant flow during systole (Soydan et al., 2015). The modified Bernoulli equation (pressure gradient = 4 x velocity^2) is used to convert the velocity of tricuspid regurgitant flow to estimated sPAP. The variation in magnitude of this method depends on an angle of interrogation beam to the tricuspid regurgitant jet (Boon, 2011; Kellihan and Stepien, 2012). The diagnosis and assessment of PH in dogs depend on tricuspid regurgitant velocity, right ventricular anatomical change, and right ventricular functional change according to the present guideline. The dogs that have tricuspid regurgitant velocity greater 3.4 meters/second with an anatomical or functional alteration of the right ventricle have a high probability of pulmonary hypertension (Reinero et al., 2020). Other echocardiographic parameters that have relationships with pulmonary hypertension include right ventricular Tei index, tricuspid annulus plane systolic excursion to aortic ratio (TAPSE:Ao), acceleration time to ejection time ratio (AT:ET ratio) of the pulmonary artery flow profile, and right pulmonary artery distensibility index (RPAD) (Schober and Baade, 2006; Paradies et al., 2014; Visser et al., 2016; Caivano et al., 2018). Unfortunately, echocardiography alone can not differentiate

between pulmonary arterial hypertension and pulmonary venous hypertension and also can not classify groups of PH. An assessment with other tools is needed to rule in or out of groups of pulmonary hypertension. Physical examination, thoracic imaging, detection of *Dirofilaria* or *Angiostrongylus*, and blood examination are used together with echocardiography for diagnosis of the specific cause of PH (Reinero et al., 2020).

The common clinical presentation of dogs affected with PH consisted of syncope, coughing, and exercise intolerance (Bach et al., 2006; Kellum and Stepien, 2007; Murphy et al., 2017). One of the aims of treatment is a decrease in clinical signs. Clinical signs of dogs affected with PH have been assessed by the clinical score, which can be used for clinical responsive after treatment (Haggstrom et al., 2008; Arita et al., 2013).

The treatment of PH in DMVD dogs is still uncertain, but the goal of treatment aims to decrease left atrial pressure, improve the systolic and diastolic function of the left ventricle, and dilate pulmonary vessels (Kellihan and Stepien, 2012). At present, conventional therapy of DMVD, including pimobendan, furosemide, and angiotensin-converting enzyme inhibitor (ACEi) can reduce systolic pulmonary arterial pressure (sPAP) approximately 20 mmHg (Atkinson et al., 2009). The combined effects of conventional therapy in decreasing sPAP as follow. The diuretic effect of furosemide can decrease left atrial pressure. The positive inotrope of phosphodiesterase-3 inhibitors (PDE-3i) increases the ejection of blood via an increase in ventricular

contraction resulting in a return of blood flow from the left atrium during diastole. The vasodilation effect of ACEi and PDE-3i can decrease sPAP via a decrease in vascular resistance (Ohte et al., 1997; Kellihan and Stepien, 2012).

Many drugs show effective treatment of pulmonary hypertension in human patients and dogs such as platelet-derived growth factor inhibitors (PDGFi), prostacyclin analogs, endothelin antagonists and phosphodiesterase-5 inhibitors (PDE-5i) (Stepien, 2009; Brown et al., 2010; Kellihan and Stepien, 2012; Arita et al., 2013). In human patients, prostacyclin analogs such as selexipag and epoprostenol show an improvement of hemodynamic via a pulmonary vasodilation effect (Oudiz, 2007; Sitbon et al., 2015). An expensive cost and the administration routes, inhalation and intravenous injection, are the limitation in the long-term use of prostacyclin analogs (Oudiz, 2007; Kellihan and Stepien, 2012). Endothelin antagonists including sitaxentan and bosentan can decrease sPAP by inhibiting cellular proliferation and pulmonary vasoconstriction (Channick et al., 2001; Ooi et al., 2002; Rubin et al., 2002). An expensive cost and a risk of hepatotoxicity are the limitations of endothelin antagonists (Stepien, 2009). Imatinib, a PDGFi, has an inhibiting lung vascular remodeling effect in PH dogs caused by heartworm disease and DMVD. An expensive cost of imatinib is the main limitation in long-term use (Arita et al., 2013). Unluckily, the financial issue is the main limitation of these drugs in veterinary medicine. Sildenafil may be a good choice

for treatment PH in dogs because of its benefit in treatment, low cost, and the possibility to use in the long-term.

Phosphodiesterase-5 (PDE-5), an enzyme abundantly found in pulmonary vessels, responses for vasoconstriction. PDE-5 also upregulates in heart failure patients (Kellihan and Stepien, 2012). Phosphodiesterase-5 inhibitors (PDE-5i) have a target pulmonary vasodilation effect that is one of the goals in the treatment of pulmonary hypertension. Sildenafil is one of the PDE-5i that has been used for a long time in human and veterinary medicine. Previous studies show some benefits of sildenafil in the treatment of pulmonary hypertension caused by various diseases in dogs and rats. A previous retrospective study of PDE5i effects found that the clinical scores had improved in dogs affected with PH caused by respiratory and cardiovascular diseases (Kellum and Stepien, 2007). Additionally, nicorandil, pimobendan, and sildenafil are used as a single and combination treatment in rats induced PH by monocrotaline injection. The results found an improvement in echocardiographic values and a decrease in sPAP. The better result also shows in a combination of sildenafil, pimobendan, and nicorandil. (Nakata et al., 2015). Moreover, the retrospective study of dogs affected with PH caused by respiratory diseases showed that sildenafil and combination use of sildenafil and pimobendan could decrease sPAP. The result between the single-use of sildenafil and a combination of sildenafil and pimobendan was not different (Murphy et al., 2017). Furthermore, a previous retrospective study

showed that sildenafil can improve quality of life and decrease sPAP in PH dogs caused by DMVD. However, the study has some limitations, such as the variation of medical use in each dog, no control group, and a small sample size (Brown et al., 2010). Unfortunately, the prospective, randomized, and placebo-control study of sildenafil in treatment PH dogs secondary to DMVD is not established yet.

CHAPTER III

MATERIALS AND METHODS

The design of the study was a single-blinded, prospective, randomized, placebo-control study. The owner had been informed of the process and had signed the consent form. The protocol of this study was approved by the Chulalongkorn University Animal Care and Use Committee (Protocol No. 1831077). The study was performed at the Small Animal Teaching Hospital, Faculty of Veterinary Science, Chulalongkorn University.

Inclusion criteria

PH dogs caused by DMVD stage C (chronic) were recruited to the study. The inclusion criteria were: 1) Toy or small breed dogs, weight less than 15 kg, age older than 6 years. 2) PH secondary to DMVD determined by echocardiographic evidence of tricuspid regurgitant flow velocity greater than 2.7 m/s or estimated sPAP greater than 30 mmHg (Kellihan and Stepien, 2012) 3) DMVD stage C defined by radiographic evidence of current or history of pulmonary edema and cardiomegaly (VHS greater than 10.7, (Buchanan and Bucheler, 1995)), and echocardiographic evidence of mitral valve regurgitation, mitral valve thickening, left atrial enlargement [LA:Ao > 1.5, measuring in the short-axis view, (Hansson et al., 2002)], and left ventricular enlargement [LVIDd > 1.7, (Keene et al., 2019)], and 4) being on conventional therapy consisting of furosemide, pimobendan, and ACEi at least for 2 weeks before the study.

Exclusion criteria

Recruited dogs were excluded from the study if they had any of the following conditions: 1) other acquired or congenital cardiac diseases and other systemic diseases consisting of severe or chronic respiratory disease, acute or chronic kidney disease, severe or chronic gastrointestinal disease, and severe or chronic liver diseases. 2) positive result to heartworm antigen test 3) creatinine greater than 1.4 mg/dL, alkaline phosphatase (ALP) (10-150 IU/L) or alanine aminotransferase (ALT) (5-60 IU/L) greater than 3x of the upper limit, and abnormal of other blood profile values consisting of red blood cell (RBC) (5.5-8.5 $x10^6/mm^3$), white blood cell (WBC) (6-17 $x10^3/mm^3$), platelet (200-500 $x10^3/mm^3$), albumin (2.6-4.3 g/dL), and total protein (5.1-7.8 g/dL) (Macintire et al., 2012b). 4) blood pressure less than 100 mmHg or greater than 180 mmHg (Macintire et al., 2012a) 5) the requirement of cardiovascular drugs besides conventional therapy.

Dogs

At day 0, recruited dogs were performed a physical examination, clinical score assessment, systemic blood pressure measurement, electrocardiography (ECG), blood collection, thoracic radiography, and echocardiography by an investigator. The dog infomation consisting of age, breed, and sex was recorded.

Clinical evaluation

Physical findings including body condition score, weight, heart rate (HR), heart sound, respiratory rate (RR), lung sound, and respiratory patterns were recorded. Electrocardiography was performed on the right lateral recumbency position for 3 minutes. Dogs were performed systolic blood pressure by using the Doppler device from the median artery of thoracic limbs in the lateral recumbency position.

Thoracic radiography

Right lateral and ventrodorsal views of thoracic radiography were performed. The radiographic examination consisted of lung patterns and vertebral heart scale (VHS) (Buchanan, 2000). The lung score of the recent study was evaluated by using the method of (Kellihan et al., 2015). The lung fields were divided into 4 quadrants as figure 1. The score was given in each quadrant. Score 0 was no lung infiltration. Score 1,2,3, and 4 were lung infiltration <25%, 25-50%, 50-75%, and >75% respectively.

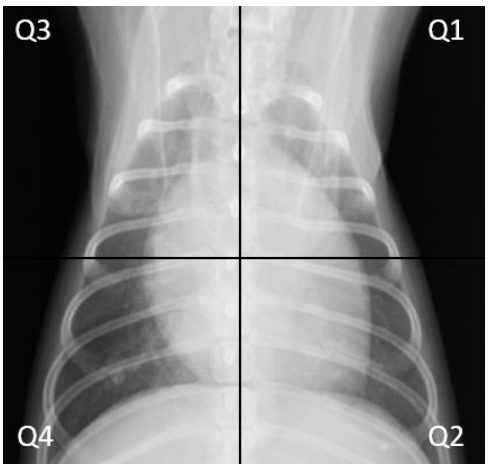

Figure 1. The thoracic radiograph was divided into 4 quadrants. The vertical line was divided at the middle of the trachea at the thoracic inlet. The horizontal line was perpendicular to the vertical line at the level of the center of the carina.

Blood profile analysis

Blood samples were collected from the saphenous vein, kept in heparin and EDTA tubes, and proceeded for measurements immediately. Blood tests included complete blood count, alanine aminotransferase, alkaline phosphatase, creatinine, blood urea nitrogen, albumin, total protein, and heartworm antigen test (Snap4Dx, IDEXX).

Echocardiography

The echocardiographic values were obtained by using the ultrasound machine (Mindray, M9, Shenzhen, China) with 2-4 and 4-12 MHz phased array transducers. Echocardiographic examination was performed in dogs with an un-sedated condition.

M-mode echocardiography was done on the right parasternal long-axis view (Boon, 2011) M-mode echocardiographic values included left ventricular internal diastolic diameter (LVIDd), left ventricular internal systolic diameter (LVIDs), left ventricular free wall thickness during diastole (LVWd), left ventricular free wall thickness during systole (LVWs), ventricular septal thickness during diastole (VSd) , and ventricular septal thickness during systole (VSs). The ratio of the left atrial to aorta diameters (LA:Ao) was performed on the right parasternal short-axis views at the mitral valve level (Boon, 2011).

Peak tricuspid regurgitant flow velocity (PTRV) was achieved by measuring the velocity of regurgitant flow at the tricuspid valve during systole on the left apical four-chamber view by spectral flow Doppler echocardiography. The spectral Doppler cursor aligns parallel to the tricuspid regurgitant flow direction (figure 2). The velocity was converted to the estimated sPAP by the modified Bernoulli equation (pressure gradient = 4x the peak of tricuspid regurgitant velocity^2) (Boon, 2011; Kellihan and Stepien, 2012).

Ejection time (ET) was attained by measuring the time at the starting to the end of the pulmonary artery flow profile that received from the right parasternal short axis at the pulmonic valve level. Acceleration time (AT) was achieved by measuring the time at the starting to the peak of the pulmonary artery flow profile (figure 3). The ratio of AT to ET (AT:ET) was calculated (Schober and Baade, 2006).

Right ventricular ejection time (RVET), isovolumic relaxation time (IVRT), and isovolumic contraction time (IVCT) were measured by tissue Doppler echocardiography on the left apical four-chamber view (Morita et al., 2016). Tei index was calculated by the equation Tei index = (IVCT + IVRT)/RVET (Paradies et al., 2014).

Tricuspid annular plane systolic excursion to the aortic diameter ratio (TAPSE:Ao) was achieved by M-mode echocardiography in the left apical four-chamber view. The movement of the lateral aspect of the tricuspid annulus was recorded (figure 4). The distance of the lateral aspect of the tricuspid annulus movement between systole and diastole was measured (Pariaut et al., 2012).

The right pulmonary artery distensibility index (RPAD) was obtained by two-dimensional echocardiography as figure 5. The minimum diastolic (RPA_D) and maximum systolic (RPA_S) internal diameter of the right pulmonary artery were measured on the right parasternal short-axis view at the same location. The equation was RPAD index = (RPAS-RPAD)/RPAS x 100 (Visser et al., 2016).

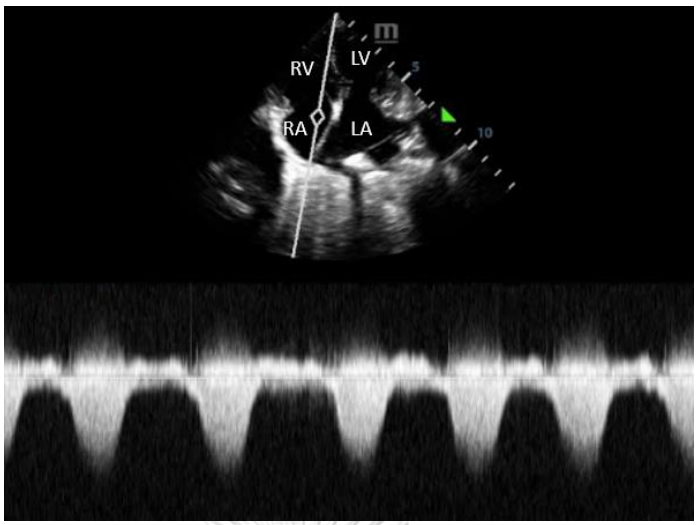

Figure 2. Peak tricuspid regurgitant flow velocity (PTRV) was achieved in the left apical four chambers view by spectral flow Doppler echocardiography. RV = right ventricle, LV = left ventricle, RA = right atrium, LA = left atrium

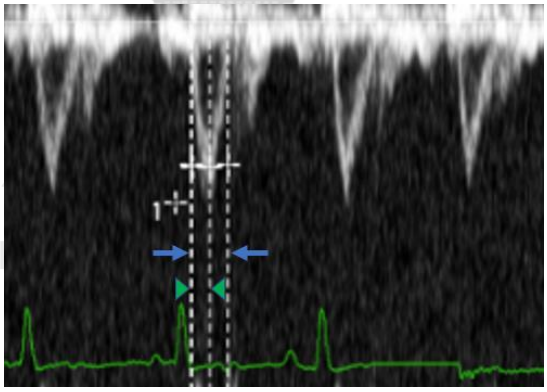

Figure 3. Ejection time (ET) and acceleration time (AT) were assessed by spectral flow Doppler echocardiography. Ejection time was measured at the beginning to the end of the pulmonary flow (arrows). Acceleration time was measured at the beginning to the peak of the pulmonary flow (arrowheads).

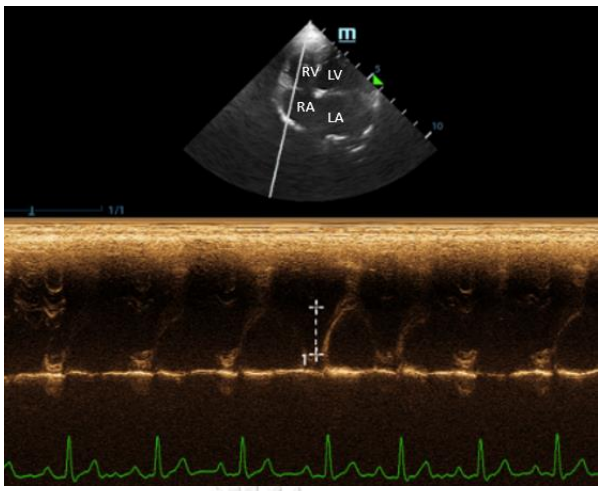

Figure 4. Tricuspid annular plane systolic excursion to the aortic diameter ratio (TAPSE:Ao) was assessed by M-mode echocardiography. The distance of the lateral aspect of the tricuspid annulus between systole and diastole was measured.

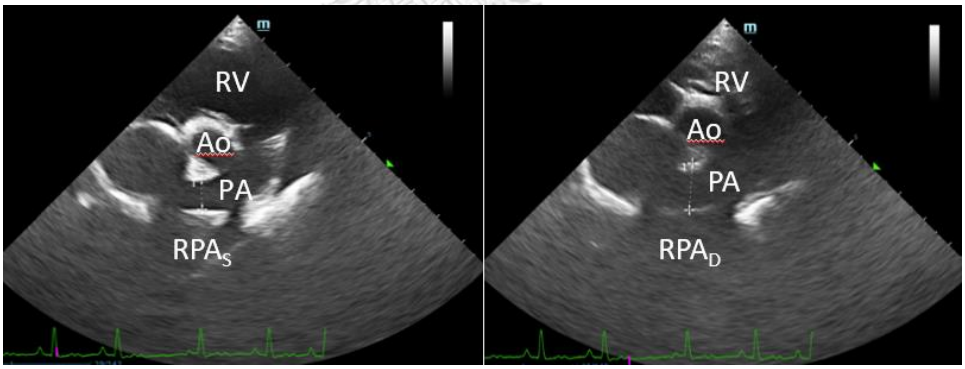

Figure 5. The minimum diastolic internal diameter of the right pulmonary artery (RPA_S) and the maximum systolic internal diameter of the right pulmonary artery (RPA_D) were obtained by two-dimensional echocardiography. The measurements were done at the same location of the right pulmonary artery. The left picture showed the measurement of RPA_S during systole. The right picture showed the measurement of RPA_D during diastole

Clinical scores

Each owner was interviewed at day 0 (before the treatement) and day 7 (after the treatment) by an investigator. The clinical scores included coughing, exercise intolerance, dyspnea, syncope, and appetite (Table 1). All scores of each dogs were summarized to total score.

Table 1. Scoring protocol for clinical variables

Variable	Score	Clinical presentation
Cough	0	None
	1	Few times a week
	2	Few times a day
	3	Frequently during the day
Exercise intolerance	0	Dogs had ability to fully exercise.
	1	Dog was active. Ability to run was reduced.
	2	Dogs was less active than normal. Avoided long walk
	3	Dogs was inactive and only got up to eat, drink, urinate, or defecate.
Dyspnea	0	Dogs was able to rest. Resting respiratory rate <25
	1	tpm
	2	Dogs was able to rest. Resting respiratory rate >25 tpm
		Dogs was restlessness and had respiratory effort
Syncope	0	None
	1	2-6 times/week
	2	Everyday, <3 times/day
	3	>3 times/day
Appetite	0	Increased
	1	Normal
	2	Decreased (>2/3 of normal)
	3	Markedly decreased (<2/3 of normal)

Modified from (Haggstrom et al., 2008; Arita et al., 2013), tpm = time per minute

Sample size

This study was recruited 7 dogs for each group. The sample size was calculated by using a formula; sample size = $\frac{(\sigma_1^2+\sigma_2^2)\left(Z_{1-\alpha/2}+Z_{1-\beta}\right)^2}{\Delta^2}$ (Rosner, 2010). The mean and standard deviation were from the recent study that investigated the effects of sildenafil in dogs with PH caused by acute pulmonary embolism (Dias-Junior et al., 2005). The sample size was calculated at 5% for the level of significance and 80% for the power of the study.

Experiment

The study was designed as a single-blinded study. Besides conventional therapy (ACEi, furosemide and pimobendane), the owners were blinded to the type of the treatment drug (sildenafil or placebo). Sildenafil and placebo were prescribed without labels. Fourteen recruited dogs were randomly divided into sildenafil and placebo groups with a 1:1 ratio to maintain a similar sample size in both groups. All dogs in both groups had been being treated with conventional therapy at least for 2 week before received the treatment and dosing of the conventional drugs had not adjusted during the study. The sildenafil group received a combination of sildenafil (1-3 mg/kg tid) and conventional therapy. The placebo group received a combination of placebo (tid) and conventional therapy. The placebo was vitamin b complex that divided in pieces as a sildenafil. Both groups received the treatment for 7 days. At day 7, dogs were performed a physical examination, electrocardiography (ECG), systolic blood

pressure (SBP) measurement, blood collection, clinical score assessment, thoracic radiography, and echocardiography by the same investigator.

Statistical analyses

Data collected at day 0 and day 7 were analyzed with a statistical software (SPSS statistics version 22, IBM Corporation, USA). The data distribution was tested by the Kolmogorov-Smirnov test. All data presented as the median and interquartile range (IQR). Comparing data between groups were tested by the Mann-Whitney U test. Comparing data between days 0 and 7 were tested by the Wilcoxon signed-rank test. For all analyses, a p-value < 0.05 was considered significant. The relationship between sPAP and echocardiographic values was tested by the Spearman's correlation.

Conceptual framework

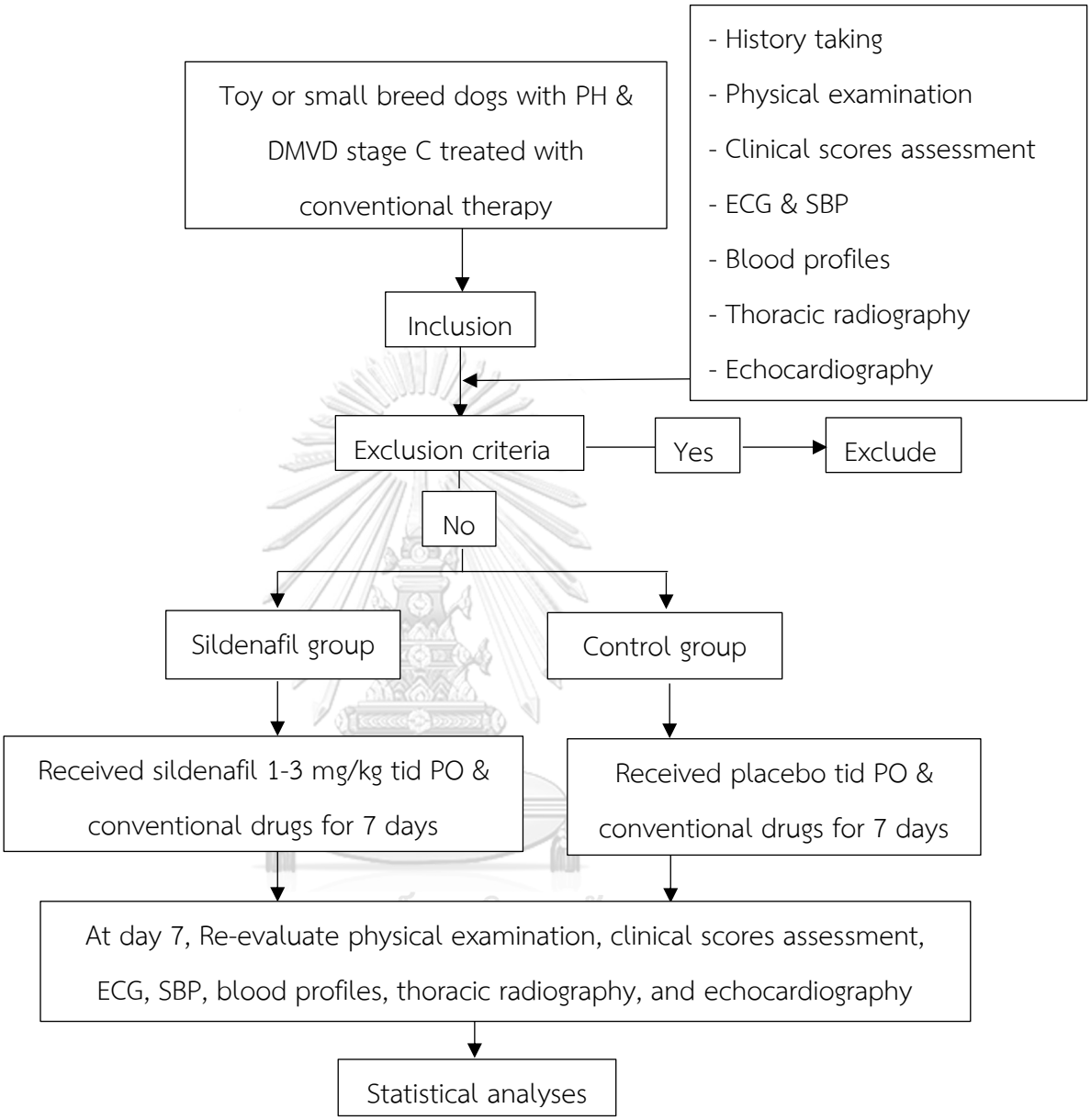

Figure 6. The diagram of the study procedure (PH = pulmonary hypertension, DMVD = degenerative mitral valve disease, ECG = electrocardiography, SBP = systolic blood pressure)

CHAPTER IV

RESULT

Seventeen dogs were recruited to study. Two dogs were excluded due to absence at day 7 and one dog was excluded because of concurrent disease of seizures. Dogs were divided into two groups: the sildenafil and placebo groups. Fourteen dogs were recruited and were randomly assigned to both groups. The dog information in both groups on day 0 was similar and shown in Table 2. All dogs received triple therapy including furosemide, pimobendan, and ACEi. Dogs in the sildenafil group received the median dose of sildenafil 1.79 (1.69-2.19) mg/kg every 8 hours for 1 week. Dogs in the placebo and sildenafil groups received the following convention therapy including the median dose of furosemide 3.60 (2.84-4.08) and 3.30 (3.10-3.60) mg/kg/day, the median dose of pimobendan 0.60 (0.42-0.84) and 0.70 (0.59-0.76) mg/kg/day, and the median dose of ramipril 0.16 (0.14-0.21) and 0.13 (0.12-0.15) mg/kg/day respectively. In addition, dose of all conventional drugs were not different between placebo and sildenafil groups.

Table 2. The information of recruited dogs at day 0 in the placebo and sildenafil groups

Parameter	Placebo	Sildenafil
Number of dogs	7	7
Breed	2 Poodles, 2 Chihuahuas, 1 Miniature Pinscher, 1 Jack Russel, and 1 Shih-tzu	2 Poodles, 3 Chihuahuas, 1 Pommeranian 1 Shih-tzu,
Sex	4 Males, 3 Females	3 Males, 4 Females
Age (y)	12 (9.5-12.5)	11 (10.0-11.5)
Body condition score (0-5)	3.0 (3.0-3.5)	3.0 (2.0-4.0)
Body weight (kg)	5.10 (4.41-5.38)	4.10 (4.01-5.42)

All values presented as median and interquartile range (IQR).

The vital signs, lung sound, respiratory pattern, systemic blood pressure and electrocardiography of the placebo and sildenafil at day 0 and day 7 are shown in Table 3. The vital signs (temperature, heart rate and respiratory rate) and systemic blood pressure of the sildenafil and placebo groups were not different at day 0 and day 7. In addition, the vital signs and systemic blood pressure were not different between day 0 and day 7 in the sildenafil and placebo groups.

Table 3. The vital signs, electrocardiography, and systemic blood pressure of in the placebo and sildenafil groups at day 0 and day7

Parameter	Placebo Day 0	Sildenafil Day 0	Placebo Day 7	Sildenafil Day 7
Temp.(°F)	101 (100.5-101.0)	101 (100.2-101.1)	100.4 (100.2-101.0)	100.0 (100.0-101.2)
HR (bpm)	137 (122-170)	144 (126-179)	143 (136-168)	150 (112-169)
RR (bpm)	56 (40-66)	42 (29-57)	45 (38-57)	45 (27-60)
Lung sound	Normal (1/7) Increased (3/7) Crackled (3/7)	Normal (4/7) Increased (3/7)	Normal (3/7) Increased (2/7) Crackled (2/7)	Normal (4/7) Increased (3/7)
Respiratory patterns	Normal (2/7) Tachypnea (3/7) Dyspnea (1/7) Panting (1/7)	Normal (2/7) Tachypnea (1/7) Dyspnea (1/7) Panting (3/7)	Normal (4/7) Tachypnea (2/7) Dyspnea (0/7) Panting (1/7)	Normal (3/7) Tachypnea (2/7) Panting (2/7)
SBP (mmHg)	126 (111-143)	133 (123-148)	126 (122-137)	137 (130-156)
ECG	RSA (1/7) SR (2/7) ST (4/7)	SR (4/7) ST (3/7)	SR (4/7) ST (3/7)	SR (4/7) ST (3/7)

All values presented as median and IQR. Temp. = Body temperature, RSA = respiratory sinus arrhythmia, SR = sinus rhythm, ST = sinus tachycardia , HR = heart rate, RR = respiratory rate, ECG = electrocardiography, SBP = systolic blood pressure

The blood profile values of the placebo and sildenafil groups at day 0 and day 7 are shown in Table 4. The blood profile values of the sildenafil and placebo groups were the same at day 0. There was no difference in blood profile values in the sildenafil and placebo groups between day 0 and day 7 (Table 4).

Table 4. The blood profile values of the placebo and sildenafil groups at day 0 and day 7

Parameter	Placebo Day 0	Sildenafil Day 0	Placebo Day 7	Sildenafil Day 7
RBC (10^6/mm^3)	6.25 (6.01-6.76)	6.24 (5.70-6.58)	6.67 (6.28-6.78)	6.37 (5.83-6.58)
WBC (10^3/mm^3)	12.87 (9.74-13.35)	10.49 (8.11-12.77)	11.17 (9.73-11.82)	8.52 (8.00-11.58)
Platelets (10^3/mm^3)	278 (223-435)	398 (356-440)	290 (245-498)	288 (241-428)
Creatinine (mg/dL)	1.00 (0.75-1.20)	1.00 (0.95-1.25)	1.20 (0.79-1.20)	1.10 (0.95-1.10)
BUN (mg/dL)	30.6 (23.95-38.65)	35.40 (29.20-36.90)	31.50 (23.45-45.05)	36.10 (27.35-40.00)
ALT (IU/L)	54 (44-106)	75 (61-109)	53 (49-103)	72 (55-103)
ALP (IU/L)	86 (49-108)	105 (76-293)	89 (47-109)	78 (76-252)
Total Protein (g/dL)	6.20 (5.90-6.55)	6.60 (5.90-7.00)	6.30 (6.15-6.75)	6.70 (6.40-7.10)
Albumin (g/dL)	3.20 (2.75-3.40)	3.10 (2.70-3.35)	3.30 (2.95-3.40)	3.20 (2.80-3.40)
Globulin (g/dL)	3.20 (2.80-3.35)	3.20 (2.95-3.60)	3.30 (3.10-3.35)	3.60 (3.20-3.75)

All values presented as median and interquartile range (IQR), RBC = red blood cell, WBC = white blood cell, BUN = blood urea nitrogen, ALT = alanine aminotransferase, ALP = alkaline phosphatase

The vertebral heart score and lung score of the placebo and sildenafil groups at day 0 and day 7 are shown in Table 5. The thoracic radiography of all dogs showed pulmonary edema and cardiomegaly. The lung scores of quadrant 1 and quadrant 3 of the sildenafil group at day 0 were significantly lower than the placebo group (p-

value = 0.024 and 0.024, respectively). The average lung score of the sildenafil group at day 7 tended to increase but not significant (p = 0.157).

Table 5. The vertebral heart score and lung score of the placebo and sildenafil groups at day 0 and day 7

Parameter	Placebo Day 0	Sildenafil Day 0	Placebo Day 7	Sildenafil Day 7
VHS	11.50 (11.35-12.60)	11.70 (11.50-12.75)	11.70 (11.20-12.60)	11.80 (11.60-12.85)
Q1	1.0 (0.0-1.0)*	0*	1.0 (0.0-1.5)$^{+}$	0^{+}
Q2	1.0 (1.0-2.0)	1.0 (1.0-1.5)	1.0 (1.0-2.0)	2.0 (1.0-2.0)
Q3	1.0 (0.0-1.0)*	0*	1.0 (0.0-1.5)$^{+}$	0^{+}
Q4	1.0 (1.0-1.5)	1.0 (1.0-1.5)	2.0 (1.0-2.0)	2.0 (1.0-2.0)
Average LS	1.0 (0.5-1.5)	0.5 (0.5-0.63)	1.0 (0.5-1.88)	0.75 (0.5-0.75)

*p-value<0.05, Compared between placebo and sildenafil at day 0. $^{+}$p-value<0.05, Compared between placebo and sildenafil at day 7. All values presented as median and IQR. VHS = vertebral heart score, Q1 = quadrant 1, Q2 = quadrant 2, Q3 = quadrant 3, Q4 = quadrant 4, LS = lung score

The clinical scores of the placebo and sildenafil groups at day 0 and day 7 are presented in Table 6. Thirteen dogs (92%) were presented with coughing, seven (50%) with exercise intolerance, two (14%) with dyspnea, one (7%) with inappetence, and none with syncope. The clinical scores of both groups were not different at day 0. However, the total score of sildenafil group was lower than placbo group at day 7.

Table 6. The clinical scores of the placebo and sildenafil groups at day 0 and day 7

Parameter	Placebo Day 0	Sildenafil Day 0	Placebo Day 7	Sildenafil Day 7
Inappetite[1]	1.0 (1.0-1.0)	1.0 (1.0-1.0)	1.0 (1.0-1.0)	1.0 (1.0-1.0)
Exercise intolerance[2]	1.0 (0-1.5)	0 (0-1.0)	1.0 (0.5-1.5)	0 (0-1.0)
Coughing[3]	2 (1.5-2.0)	2 (1.5-2.0)	2.0 (1.0-2.5)	1.0 (0.5-2.0)
Dyspnea[4]	0 (0-0)	0 (0-0)	0 (0-0.5)	0 (0-0)
Syncope[5]	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)
Total score	4.0 (3.0-4.5)	3.0 (3.0-3.5)	4.0 (3.0-4.5)*	2.0 (2.0-3.0)*

*p-value<0.05, Compared between placebo and sildenafil at day 7. All values presented as median and IQR, [1]Inappetite score – increased appetite = 0 point, normal = 1 point, can eat >2/3 of normal = 2 points, can eat < 2/3 of normal = 3 points, [2]Exercise intolerance score – none= 0 point, Dog is active and ability to run is reduced =1 point, Dogs is less active than normal and avoid long walk = 2 points., Dogs is inactive and only get up to eat, drink, urinate, or defecate = 3 points, [3]Coughing score –none = 0 point, few times a week = 1 point, few times a day = 2 points, frequently during the day = 3 points, [4]Dyspnea –Dogs is able to rest and resting respiratory rate <25 times/min = 0 point, Dogs is able to rest and resting respiratory rate > 25 times/min = 1 point, Dogs is restlessness and respiratory effort = 2 points, [5]Syncope –none = 0 point, 2-6 times/week = 1 point, everyday <3 times/day = 2 points, Severe >3 times/day = 3 points

The echocardiographic values were shown in Table 7. All dogs had an intermediate to high probability of PH based on the classification of the American College of Veterinary Medicine (ACVIM) 2020 (Reinero et al., 2020). At day 0, the interventricular septal thickness during systole (IVSs) in the placebo group was higher than the sildenafil group; however, the value was still in the normal range (0.43-0.79) (p=0.047). At day 7, the LA and LA:Ao of the placebo group were significantly decreased when compared to those at day 0 (p= 0.028 and 0.018, respectively). The LA and LA:Ao

of the sildenafil group were not different at day 0 and day 7. At day 7, the tricuspid regurgitant flow velocity and the estimated sPAP of the sildenafil group were significantly decreased when compared to day 0 (p = 0.043 and 0.043, respectively). The estimated sPAP was decreased by approximately 7 mmHg. The other echocardiographic parameters were different between before and after treatment with sildenafil. Additionally, the sPAP positively correlated with HR (r=0.7, p= 0.005) and IVCT (r=0.63, p = 0.014) (Table 8).

Table 7. The echocardiographic values of the placebo and sildenafil groups at day 0 and day 7

Parameter	Placebo Day 0	Sildenafil Day 0	Placebo Day 7	Sildenafil Day 7
VSd	0.44 (0.41-0.50)	0.43 (0.40-0.49)	0.43 (0.37-0.49)	0.48 (0.40-0.51)
LVIDd	1.99 (1.89-2.09)	1.92 (1.79-2.04)	1.96 (1.77-2.04)	1.87 (1.74-2.05)
LVWd	0.34 (0.33-0.42)	0.34 (0.32-0.38)	0.41 (0.37-0.47)	0.37 (0.35-0.40)
VSs	0.76^{+} (0.66-0.77)	0.62^{+} (0.58-0.66)	0.67 (0.59-0.80)	0.62 (0.58-0.68)
LVIDs	0.98 (0.83-1.04)	1.06 (0.82-1.19)	1.02 (0.91-1.06)	1.08 (0.66-1.16)
LVWs	0.62 (0.58-0.73)	0.69 (0.56-0.76)	0.60 (0.58-0.68)	0.71 (0.58-0.78)
LA	1.68* (1.43-1.82)	1.57 (1.46-1.93)	1.50* (1.15-1.68)	1.63 (1.49-1.80)
Ao	0.63 (0.59-0.69)	0.66 (0.58-0.81)	0.63 (0.62-0.75)	0.76 (0.61-0.79)
LA:Ao	2.40* (2.29-2.61)	2.66 (2.13-2.74)	2.05* (1.97-2.12)	2.23 (2.06-2.51)
FS (%)	51 (47-57)	48 (41-54)	48 (42-54)	48 (43-60)
RPAD (%)	29 (24-33)	21 (13-25)	19 (13-24)	15 (9-16)
TR (m/s)	3.28 (3.15-3.56)	3.69$^{\#}$ (3.30-3.98)	3.51 (3.10-3.62)	3.43$^{\#}$ (3.20-3.68)
sPAP (mmHg)	43.03 (39.79-50.98)	54.46$^{\#}$ (43.66-63.30)	49.51 (38.60-52.46)	47.13$^{\#}$ (41.08-54.15)
AT (msec)	47 (39-56)	44 (31-61)	47 (36-52)	50 (47-61)
ET (msec)	111 (92-121)	97 (78-127)	108 (81-120)	126 (98-142)
AT:ET	0.48 (0.41-0.53)	0.46 (0.41-0.50)	0.44 (0.43-0.46)	0.46 (0.42-0.51)
IVCT(msec)	41 (40-55)	53 (42-56)	43 (38-48)	50 (32-76)
IVRT(msec)	52 (39-61)	39 (33-56)	56 (46-66)	61 (54-72)
RVET(msec)	117 (88-143)	117 (110-126)	116 (97-131)	131 (118-136)
Tei index	0.74 (0.68-1.19)	0.76 (0.69-0.80)	0.79 (0.79-0.97)	0.78 (0.58-1.02)
TAPSE:Ao	1.11 (0.74-1.23)	1.27 (0.99-1.39)	1.27 (1.13-1.40)	0.97 (0.93-1.24)

$^{+}$p-value<0.05, compared between placebo and sildenafil at day 0, *p-value < 0.05, compared between day 0 and day 7 of placebo group, $^{\#}$p-value<0.05, compared between day 0 and day 7 of sildenafil group. All values presented as median and IQR. VSd = ventricular septal thickness during diastole, LVIDd = left ventricular internal diastolic diameter, LVWd = left ventricular free wall thickness during diastole, VSs = ventricular septal thickness during systole, LVIDs = left ventricular internal systolic diameter, LVWs = left ventricular free wall thickness during systole, LA = left atrial diameter, Ao = aortic diameter, LA:Ao = ratio of left atrium to aorta, FS = fraction shortening, RPAD = Right pulmonary artery distensibility index, TR = Tricuspid regurgitant flow velocity, ,sPAP = systolic pulmonary artery pressure, AT = acceleration time, ET = ejection time, AT:ET = ratio of acceleration time to ejection time, IVCT = isovolumetric contraction time, IVRT = isovolumetric relaxation time, RVET = right ventricular ejection time, TAPSE:Ao = ratio of tricuspid annular plane systolic excursion to aortic diameter.

Table 8. The correlation between estimated systolic pulmonary artery pressure and parameters of echocardiography

Parameter	r	P=value
HR	0.70*	0.005
VSd	0.15	0.593
LVIDd	-0.30	0.284
LVWd	-0.03	0.904
VSs	0.20	0.477
LVIDs	-0.20	0.482
LVWs	0.002	0.994
LA	0.20	0.473
Ao	0.06	0.834
LA:Ao	0.12	0.675
FS	0.14	0.626
RPAD	0.12	0.675
AT	-0.13	0.658
ET	0.07	0.805
AT:ET	-0.34	0.226
IVCT	0.63*	0.014
IVRT	-0.33	0.242
RVET	-0.53	0.050
Tei index	0.43	0.124
TAPSE:Ao	-0.20	0.480

*p-value < 0.05, VSd = ventricular septal thickness during diastole, LVIDd = left ventricular internal diastolic diameter, LVWd = left ventricular free wall thickness during diastole, VSs = ventricular septal thickness during systole, LVIDs = left ventricular internal systolic diameter, LVWs = left ventricular free wall thickness during systole, LA = left atrial diameter, Ao = aortic diameter, LA:Ao = ratio of left atrium to aorta, RPAD = Right pulmonary artery distensibility index, TR = Tricuspid regurgitant flow velocity, ,sPAP = systolic pulmonary artery pressure, AT = acceleration time, ET = ejection time, AT:ET = ratio of acceleration time to ejection time, IVCT = isovolumetric contraction time, IVRT = isovolumetric relaxation time, RVET = right ventricular ejection time, TAPSE:Ao = ratio of tricuspid annular plane systolic excursion to aortic diameter.

CHAPTER V

DISCUSSION

This study demonstrates the effects of sildenafil in combination with conventional therapy to clinical scores, radiographic lung score, echocardiographic values, and estimated sPAP in dogs affected with PH secondary to DMVD.

Although, previous studies showed that sildenafil could improve the clinical scores in PH dogs secondary to respiratory disease (Bach et al., 2006; Kellum and Stepien, 2007; Kellihan et al., 2015), the clinical scores of dogs affected with PH secondary to DMVD in the present study were not different between before and after treatment with sildenafil. These findings may be observed due to different effects of sildenafil. PH secondary to respiratory disease is caused by increased pulmonary vascular resistance (PVR) resulting from vasoconstriction and vascular remodelling, whereas PH secondary to left-sided heart disease is caused by increased pulmonary venous pressure due to an increse in left atrial pressure (Poser and Guglielmini, 2016). Sildenafil can reduce pulmonary artery pressure but not pulmonary capillary wedge pressure, i.e. estimated left atrial pressure in human patients affected with PAH (Michelakis et al., 2002). The common clinical presentations of DMVD dogs with PH in the present study were coughing, exercise intolerance, dyspnea, and inappetite. None of the dogs in the present study had syncope. This result is different from previous studies that found syncope, coughing and exercise intolerance were the common

clinical presenting in PH dogs (Bach et al., 2006; Kellum and Stepien, 2007; Murphy et al., 2017).

The dogs in the present study had rapid respiratory rate [median 51 (IQR 31-65) breath per minute] and pulmonary edema assessed by radiography at day 0. At day 7, after treatment with sildenafil, respiratory rate and lung scores were not different from those at day 0. This result is different from a previous study that found the improvement of the lung score after a median of 3.5 days post sildenafil treatment. However, most of the dogs in that study were affected by PH secondary to respiratory disease (Kellihan et al., 2015). The average lung score in dogs treated with sildenafil in this study tended to increase but did not reach statistical significance. This result is consistent with the recommendation of the ACVIM that sildenafil should be prescribed carefully in dogs with pulmonary edema due to left heart disease because sildenafil had a potential risk to induce the progression of pulmonary edema (Reinero et al., 2020). Sildenafil dilates the pulmonary artery and reduces afterload to the heart resulting in increased blood flow to the pulmonary system that may contribute pulmonary edema in left-sided heart disease (Kellihan and Stepien, 2012; Reinero et al., 2020).

The echocardiographic values of DMVD dogs with PH in the placebo and the sildenafil groups were not different at day 7. The left ventricular dimension was not different between before and after treatment with sildenafil for 7 days. This result was

similar to a previous study demonstrating that the long-term treatment (160 days) of sildenafil did not affect the left ventricular dimension in dogs with DMVD stage B (Kijtawornrat et al., 2017).

The left atrium diameter (LA) and left atrial to aortic diameter ratio (LA:Ao) of all dogs in this study were greater than normal (LA:Ao >1.5, measuring in the short-axis view) (Hansson et al., 2002). The LA and LA:Ao in the placebo group were significantly decreased at day 7, while the LA and LA:Ao in the sildenafil group was not different between before and after treatment. The decrease of LA:Ao may indicate the decrease of blood return from the lung to the left atrium. A chronic elevation of pulmonary arterial increases afterload to the heart leading to a decrease in blood flow to the left atrium (MacIver et al., 2016).

At day 0, dogs had increased fractional shortening [median 50% (IQR 45-58%)]. An increase in fractional shortening in dogs with mitral regurgitation may be due to an increase in preload in the left ventricle (Melenovsky, 2013). A few studies investigating the effect of sildenafil on fractional shortening have been published. An improvement of fractional shortening has been found in mice induced myocardial infarction that received sildenafil (Salloum et al., 2008). Another experiment in rats induced aortic regurgitation demonstrated that sildenafil inhibited left ventricular remodelling and improved fractional shortening (Eskesen et al., 2015). However, an effect of sildenafil in increasing fractional shortening was not found in dogs affected with PH secondary

to DMVD in the present study. A similar result was shown in a previous study that sildenafil did not affect FS in DMVD dogs stage B (Kijtawornrat et al., 2017).

The median of the RPAD index was lower than 30 percent in the present study. This result is consistent with a previous study that the RPAD index can be used as an indicator of PH in case of an absence of tricuspid regurgitation (Visser et al., 2016). The present study showed that treatment with sildenafil did not affect the RPAD index. This result is in contrast to a previous study showing that dogs affected with PH secondary to respiratory disease had an increase of the RPAD after treatment with sildenafil and tadalafil (Jaffey et al., 2019).

The median acceleration time of the pulmonary artery flow assessed by spectral Doppler echocardiography of dogs in the present study was lower than 58 msec [median 45 msec (IQR 33-59 msec)]. This result is similar to a previous study that the acceleration time was shortened in PH dogs (Schober and Baade, 2006). Previous studies showing that the acceleration and ejection time were increased after treatment with sildenafil in dogs affected by PH secondary to respiratory and cardiovascular diseases (Kellum and Stepien, 2007; Nakamura et al., 2011). It has been suggested that an increase in acceleration and ejection time occur secondary to a decrease in pulmonary artery pressure (Schober and Baade, 2006; Kellum and Stepien, 2007). In the present study, the acceleration time and ejection time were not different between before and after treatment with sildenafil. This finding may be due to a small reduction

of pulmonary artery pressure after treatment with sildenafil that may not be enough to reflect any changes in the pulmonary artery flow profile.

Tei index is used to evaluate global myocardial function in the right ventricle (Boon, 2011). Tei index can be obtained by several methods including conventional pulse-waved Doppler, dual pulse-waved Doppler, and tissue Doppler echocardiography (Morita et al., 2016). Tei index assessed by tissue Doppler imaging was increased in the PH dogs secondary to DMVD in the present study. Additionally, a previous study found a positive correlation between right ventricular Tei index and sPAP and a strong correlation with survival time in DMVD dogs (Nakamura et al., 2016). Previous studies found that sildenafil had effects in improvement of Tei index in PH induced-rats and PH human patients secondary to a hypoxia condition (Reichenberger et al., 2007; Yoshiyuki et al., 2016). However, the difference of Tei index between before and after treatment with sildenafil in the present study was not found.

Tricuspid annulus plane systolic excursion to the aortic diameter ratio (TAPSE:Ao) is used to evaluate right ventricular systolic function. The lower limit value (0.65) was used to rule out pulmonary hypertension. TAPSE has low sensitivity but high specificity to rule out pulmonary hypertension (Pariaut et al., 2012; Caivano et al., 2018). In the present study, TAPSE:Ao was increased, but had no relationship with sPAP. This result is different from a previous study demonstrating that TAPSE:Ao had a negative relationship with sPAP (Caivano et al., 2018). This study showed that TAPSE:Ao

was not different between dogs treated with placebo and sildenafil. This result is similar to previous studies showing that there was no improvement of TAPSE in PH induced-rats and PH patients treated with sildenafil (Hussain et al., 2016; Yoshiyuki et al., 2016).

The estimated systolic pulmonary artery pressure (sPAP) in this study was decreased after treatment with sildenafil. The sPAP of the sildenafil group was significantly decreased after the treatment with sildenafil while the sPAP in the placebo group tended to increase. This finding is similar to previous studies of sildenafil treatment in PH dogs caused by respiratory and various cardiac diseases (Bach et al., 2006; Brown et al., 2010). The left-sided heart failure causes pulmonary venous hypertension secondary to an increased left atrial pressure and reactive pulmonary arterial vasoconstriction. Additionally, increased thickness of pulmonary vascular wall or the pulmonary vascular remodeling influenced by increased pulmonary artery pressure can worsen and increase the progression of PH (Kellihan and Stepien, 2012). By these mechanisms, sildenafil may help by decreasing pulmonary artery pressure and vascular remodeling.

The present study found a positive relationship between heart rate and estimated sPAP. This finding was similar to a previous study demonstrating that dogs with higher pulmonary pressure had a higher heart rate (Tidholm et al., 2015). The rationale of this relationship may be as follow. The dogs affected with PH has a

reduction in stroke volume secondary to an increase in afterload. Therefore, the heart rate is increased to maintain cardiac output. The study in human patients affected with pulmonary arterial hypertension showed a significant change of heart rate after 6 minutes walk test (Provencher et al., 2006). In the present study, the estimated sPAP correlated positively with isovolumic contraction time. This finding may occur because the heart takes a longer time to generate pressure in the isovolumic contraction phase when pulmonary arterial pressure is rising (Feher, 2017).

The target pulmonary vasodilating effect of sildenafil decreases the pulmonary pressure with minimal effects on systemic blood pressure (Michelakis et al., 2002; Lepore et al., 2005a; Silva et al., 2014). However, some studies found that systemic blood pressure tended to decrease, but still within normal range after treatment with sildenafil (Bach et al., 2006; Kellum and Stepien, 2007; Brown et al., 2010). The present study showed that systolic blood pressure in dogs affected with PH secondary to DMVD was not changed after treatment with sildenafil.

Sildenafil has minimal systemic effects because it has a selective vasodilation effect in organs that have abundant phosphodiesterase-5 expression, such as lungs (Michelakis et al., 2002; Lepore et al., 2005b; Kellihan and Stepien, 2012). The adverse effects of sildenafil have rarely been reported in dogs (Silva et al., 2014). The present study showed no difference in complete blood counts and blood chemistry profile values between the placebo and sildenafil groups at day 7 and between day 0 and 7

of the sildenafil groups. In humans, one of the major side effects of sildenafil is cardiac arrhythmia (Kloner, 2000). The presence of cardiac arrhythmia was not found in the present study after treatment with sildenafil. These results suggest that it is safe to use sildenafil at least short-term in dogs affected with PH secondary to DMVD.

There are several limitations in this study. First, the study investigated the short-term effect of sildenafil in treatment dogs affected with PH secondary to DMVD. Thus, the result cannot be extrapolated for using in the long-term treatment and managing the dogs affected with PH from other causes. Second, the pulmonary arterial pressure was estimated by measuring the tricuspid regurgitant velocity assessed by echocardiography. This technique has several factors affecting the estimated pulmonary arterial pressure including poor resolution of images due to pulmonary pathology, the alignment of the cursor to tricuspid jet while measuring, and the right ventricular systolic function (Kellihan and Stepien, 2012). Third, sildefil tablets were divided by the owner. This variation in dose received might affect to the result. Finally, the number of recruited dogs in this study was low.

In conclusion, sildenafil has a synergist effect to conventional therapy in reducing estimated pulmonary arterial pressure. Sildenafil does not affect the systemic blood pressure and blood profile values. There was no short-term effects of sildenafil on clinical and lung scores of dogs in this study. The long term effect of sildenafil should be investigated for more information.

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APPENDIX

Appendix A: the p value of the information of recruited dogs at day 0 in the placebo and sildenafil groups

Parameters	Placebo	Sildenafil	P value
Number of dogs	7	7	-
Breed	2 Poodles, 2 Chihuahuas, 1 Miniature Pinscher, 1 Jack Russel, and 1 Shih-tzu	2 Poodles, 3 Chihuahuas, 1 Pommeranian 1 Shih-tzu,	-
Sex	4 Males, 3 Females	3 Males, 4 Females	-
Age (y)	12 (9.5-12.5)	11 (10.0-11.5)	0.473
Body condition score (0-5)	3.0 (3.0-3.5)	3.0 (2.0-4.0)	0.786
Body weight (kg)	5.10 (4.41-5.38)	4.10 (4.01-5.42)	0.306

All values presented as median and interquartile range (IQR). The data between groups were compared by Man-Whitney U test.

Appendix B: the p value of the vital signs, electrocardiography, and systemic blood pressure in the placebo and sildenafil groups at day 0

Parameters	Placebo (day 0)	Sildenafil (day 0)	P value
Temp.(F)	101 (100.5-101.0)	101 (100.2-101.1)	0.842
HR (bpm)	137 (122-170)	144 (126-179)	0.749
RR (bpm)	56 (40-66)	42 (29-57)	0.370
Lung sound	Normal (1/7) Increased (3/7) Crackled (3/7)	Normal (4/7) Increased (3/7)	-
Respiratory patterns	Normal (2/7) Tachypnea (3/7) Dyspnea (1/7) Panting (1/7)	Normal (2/7) Tachypnea (1/7) Dyspnea (1/7) Panting (3/7)	-
SBP (mmHg)	126 (111-143)	133 (123-148)	0.406
ECG	RSA (1/7) SR (2/7) ST (4/7)	SR (4/7) ST (3/7)	-

All values presented as median and interquartile range (IQR). The data between groups were compared by Man-Whitney U test. Temp. = Body temperature, RSA = respiratory sinus arrhythmia, SR = sinus rhythm, ST = sinus tachycardia , HR = heart rate, RR = respiratory rate, ECG = electrocardiography, SBP = systolic blood pressure

Appendix C: the p value of the vital signs, electrocardiography, and systemic blood pressure in the placebo group at day 0 and day 7

Parameters	Placebo (day 0)	Placebo (day 7)	P value
Temp.(F)	101 (100.5-101.0)	100.4 (100.2-101.0)	0.109
HR (bpm)	137 (122-170)	143 (136-168)	0.128
RR (bpm)	56 (40-66)	45 (38-57)	0.553
Lung sound	Normal (1/7) Increased (3/7) Crackled (3/7)	Normal (3/7) Increased (2/7) Crackled (2/7)	-
Respiratory patterns	Normal (2/7) Tachypnea (3/7) Dyspnea (1/7) Panting (1/7)	Normal (4/7) Tachypnea (2/7) Dyspnea (0/7) Panting (1/7)	-
SBP (mmHg)	126 (111-143)	126 (122-137)	0.752
ECG	RSA (1/7) SR (2/7) ST (4/7)	SR (4/7) ST (3/7)	-

All values presented as median and interquartile range (IQR). The data between day 0 and day 7 were compared by Wilcoxan signed ranks test. Temp. = Body temperature, RSA = respiratory sinus arrhythmia, SR = sinus rhythm, ST = sinus tachycardia , HR = heart rate, RR = respiratory rate, ECG = electrocardiography, SBP = systolic blood pressure

Appendix D: the p value of the vital signs, electrocardiography, and systemic blood pressure in the sildenafil group at day 0 and day 7

Parameters	Sildenafil (day 0)	Sildenafil (day 7)	P value
Temp.(°F)	101 (100.2-101.1)	100.0 (100.0-101.2)	0.833
HR (bpm)	144 (126-179)	150 (112-169)	0.136
RR (bpm)	42 (29-57)	45 (27-60)	1.000
Lung sound	Normal (4/7) Increased (3/7)	Normal (4/7) Increased (3/7)	-
Respiratory patterns	Normal (2/7) Tachypnea (1/7) Dyspnea (1/7) Panting (3/7)	Normal (3/7) Tachypnea (2/7) Panting (2/7)	-
SBP (mmHg)	133 (123-148)	137 (130-156)	1.000
ECG	SR (4/7) ST (3/7)	SR (4/7) ST (3/7)	-

All values presented as median and interquartile range (IQR). The data between day 0 and day 7 were compared by Wilcoxan signed ranks test. Temp. = Body temperature, RSA = respiratory sinus arrhythmia, SR = sinus rhythm, ST = sinus tachycardia , HR = heart rate, RR = respiratory rate, ECG = electrocardiography, SBP = systolic blood pressure

Appendix E: the p value of the vital signs, electrocardiography, and systemic blood pressure in the placebo and sildenafil groups at day 7

Parameters	Placebo (day 7)	Sildenafil (day 7)	P value
Temp.(°F)	100.4 (100.2-101.0)	100.0 (100.0-101.2)	0.371
HR (bpm)	143 (136-168)	150 (112-169)	0.565
RR (bpm)	45 (38-57)	45 (27-60)	0.798
Lung sound	Normal (3/7) Increased (2/7) Crackled (2/7)	Normal (4/7) Increased (3/7)	-
Respiratory patterns	Normal (4/7) Tachypnea (2/7) Dyspnea (0/7) Panting (1/7)	Normal (3/7) Tachypnea (2/7) Panting (2/7)	-
SBP (mmHg)	126 (122-137)	137 (130-156)	0.225
ECG	SR (4/7) ST (3/7)	SR (4/7) ST (3/7)	-

All values presented as median and interquartile range (IQR). The data between groups were compared by Man-Whitney U test. Temp. = Body temperature, RSA = respiratory sinus arrhythmia, SR = sinus rhythm, ST = sinus tachycardia , HR = heart rate, RR = respiratory rate, ECG = electrocardiography, SBP = systolic blood pressure

Appendix F: the p value of blood profile values in the placebo and sildenafil groups at day 0

Parameters	Placebo (day 0)	Sildenafil (day 0)	P- value
RBC (10^6/mm^3)	6.25 (6.01-6.76)	6.24 (5.70-6.58)	0.565
WBC (10^3/mm^3)	12.87 (9.74-13.35)	10.49 (8.11-12.77)	0.482
Platelets (10^3/mm^3)	278 (223-435)	398 (356-440)	0.224
Creatinine (mg/dL)	1.00 (0.75-1.20)	1.00 (0.95-1.25)	0.700
BUN (mg/dL)	30.6 (23.95-38.65)	35.40 (29.20-36.90)	0.565
ALT (IU/L)	54 (44-106)	75 (61-109)	0.337
ALP (IU/L)	86 (49-108)	105 (76-293)	0.180
Total Protein (g/dL)	6.20 (5.90-6.55)	6.60 (5.90-7.00)	0.798
Albumin (g/dL)	3.20 (2.75-3.40)	3.10 (2.70-3.35)	0.797
Globulin (g/dL)	3.20 (2.80-3.35)	3.20 (2.95-3.60)	0.701

All values presented as median and interquartile range (IQR), The data between groups were compared by Man-Whitney U test. RBC = red blood cell, WBC = white blood cell, BUN = blood urea nitrogen, ALT = alanine aminotransferase, ALP = alkaline phosphatase

Appendix G: the p value of the blood profile values in the placebo group at day 0 and day 7

Parameters	Placebo (day 0)	Placebo (day 7)	P value
RBC (10^6/mm^3)	6.25 (6.01-6.76)	6.67 (6.28-6.78)	0.463
WBC (10^3/mm^3)	12.87 (9.74-13.35)	11.17 (9.73-11.82)	0.090
Platelets (10^3/mm^3)	278 (223-435)	290 (245-498)	0.400
Creatinine (mg/dL)	1.00 (0.75-1.20)	1.20 (0.79-1.20)	0.891
BUN (mg/dL)	30.6 (23.95-38.65)	31.50 (23.45-45.05)	0.600
ALT (IU/L)	54 (44-106)	53 (49-103)	0.753
ALP (IU/L)	86 (49-108)	89 (47-109)	0.917
Total Protein (g/dL)	6.20 (5.90-6.55)	6.30 (6.15-6.75)	0.168
Albumin (g/dL)	3.20 (2.75-3.40)	3.30 (2.95-3.40)	0.414
Globulin (g/dL)	3.20 (2.80-3.35)	3.30 (3.10-3.35)	0.223

All values presented as median and interquartile range (IQR), The data between day 0 and day 7 were compared by Wilcoxan signed ranks test. RBC = red blood cell, WBC = white blood cell, BUN = blood urea nitrogen, ALT = alanine aminotransferase, ALP = alkaline phosphatase

Appendix H: the p value of the blood profile values in the sildenafil group at day 0 and day 7

Parameters	Sildenafil (day 0)	Sildenafil (day 7)	P value
RBC (10^6/mm^3)	6.24 (5.70-6.58)	6.37 (5.83-6.58)	0.352
WBC (10^3/mm^3)	10.49 (8.11-12.77)	8.52 (8.00-11.58)	0.866
Platelets (10^3/mm^3)	398 (356-440)	288 (241-428)	0.176
Creatinine (mg/dL)	1.00 (0.95-1.25)	1.10 (0.95-1.10)	0.726
BUN (mg/dL)	35.40 (29.20-36.90)	36.10 (27.35-40.00)	0.310
ALT (IU/L)	75 (61-109)	72 (55-103)	0.271
ALP (IU/L)	105 (76-293)	78 (76-252)	0.063
Total Protein (g/dL)	6.60 (5.90-7.00)	6.70 (6.40-7.10)	0.093
Albumin (g/dL)	3.10 (2.70-3.35)	3.20 (2.80-3.40)	0.453
Globulin (g/dL)	3.20 (2.95-3.60)	3.60 (3.20-3.75)	0.058

All values presented as median and interquartile range (IQR), The data between day 0 and day 7 were compared by Wilcoxan signed ranks test. RBC = red blood cell, WBC = white blood cell, BUN = blood urea nitrogen, ALT = alanine aminotransferase, ALP = alkaline phosphatase

Appendix I: the p value of blood profile values in the placebo and sildenafil groups at day 7

Parameters	Placebo (day 7)	Sildenafil (day 7)	P- value
RBC (10^6/mm^3)	6.67 (6.28-6.78)	6.37 (5.83-6.58)	0.406
WBC (10^3/mm^3)	11.17 (9.73-11.82)	8.52 (8.00-11.58)	0.383
Platelets (10^3/mm^3)	290 (245-498)	288 (241-428)	0.655
Creatinine (mg/dL)	1.20 (0.79-1.20)	1.10 (0.95-1.10)	0.649
BUN (mg/dL)	31.50 (23.45-45.05)	36.10 (27.35-40.00)	0.565
ALT (IU/L)	53 (49-103)	72 (55-103)	0.848
ALP (IU/L)	89 (47-109)	78 (76-252)	0.277
Total Protein (g/dL)	6.30 (6.15-6.75)	6.70 (6.40-7.10)	0.405
Albumin (g/dL)	3.30 (2.95-3.40)	3.20 (2.80-3.40)	0.846
Globulin (g/dL)	3.30 (3.10-3.35)	3.60 (3.20-3.75)	0.335

All values presented as median and interquartile range (IQR), The data between groups were compared by Man-Whitney U test. RBC = red blood cell, WBC = white blood cell, BUN = blood urea nitrogen, ALT = alanine aminotransferase, ALP = alkaline phosphatase

Appendix J: the p value of the vertebral heart score and lung score in the placebo and sildenafil groups at day 0

Parameters	Placebo (day 0)	Sildenafil (day 0)	P value
VHS	11.50 (11.35-12.60)	11.70 (11.50-12.75)	0.602
Quadrant 1	1.0 (0.0-1.0)	0	0.024
Quadrant 2	1.0 (1.0-2.0)	1.0 (1.0-1.5)	0.580
Quadrant 3	1.0 (0.0-1.0)	0	0.024
Quadrant 4	1.0 (1.0-1.5)	1.0 (1.0-1.5)	1.000
Average LS	1.0 (0.5-1.5)	0.5 (0.5-0.63)	0.274

All values presented as median and IQR, The data between groups were compared by Man-Whitney U test. VHS = vertebral heart score, LS = lung score

Appendix K: the p value of the vertebral heart score and lung score in the placebo group at day 0 and day 7

Parameters	Placebo (day 0)	Placebo (day 7)	P value
VHS	11.50 (11.35-12.60)	11.70 (11.20-12.60)	0.705
Quadrant 1	1.0 (0.0-1.0)	1.0 (0.0-1.5)	1.000
Quadrant 2	1.0 (1.0-2.0)	1.0 (1.0-2.0)	1.000
Quadrant 3	1.0 (0.0-1.0)	1.0 (0.0-1.5)	0.157
Quadrant 4	1.0 (1.0-1.5)	2.0 (1.0-2.0)	1.000
Average LS	1.0 (0.5-1.5)	1.0 (0.5-1.88)	0.535

All values presented as median and IQR, The data between day 0 and day 7 were compared by Wilcoxan signed ranks test. VHS = vertebral heart score, LS = lung score

Appendix L: the p value of the vertebral heart score and lung score in the sildenafil group at day 0 and day 7

Parameters	Sildenafil (day 0)	Sildenafil (day 7)	P value
VHS	11.70 (11.50-12.75)	11.80 (11.60-12.85)	0.713
Quadrant 1	0	0	1.000
Quadrant 2	1.0 (1.0-1.5)	2.0 (1.0-2.0)	1.000
Quadrant 3	0	0	1.000
Quadrant 4	1.0 (1.0-1.5)	2.0 (1.0-2.0)	0.157
Average LS	0.5 (0.5-0.63)	0.75 (0.5-0.75)	0.157

All values presented as median and IQR, The data between day 0 and day 7 were compared by Wilcoxan signed ranks test. VHS = vertebral heart score, Q1 = quadrant 1, Q2 = quadrant 2, Q3 = quadrant 3, Q4 = quadrant 4, LS = lung score

Appendix M: the p value of the vertebral heart score and lung score in the placebo and sildenafil groups at day 7

Parameters	Placebo (day 7)	Sildenafil (day 7)	P value
VHS	11.70	11.80	0.438
Quadrant 1	1.0 (0.0-1.5)	0	0.025
Quadrant 2	1.0 (1.0-2.0)	2.0 (1.0-2.0)	0.580
Quadrant 3	1.0 (0.0-1.5)	0	0.025
Quadrant 4	2.0 (1.0-2.0)	2.0 (1.0-2.0)	1.000
Average LS	1.0 (0.5-1.88)	0.75 (0.5-0.75)	0.315

All values presented as median and IQR, The data between groups were compared by Man-Whitney U test. VHS = vertebral heart score, LS = lung score

Appendix N: the p value of the clinical scores in the placebo and sildenafil groups at day 0

Parameters	Placebo (day 0)	Sildenafil (day 0)	P value
Inappetite[1]	1.0 (1.0-1.0)	1.0 (1.0-1.0)	0.317
Exercise intolerance[2]	1.0 (0-1.5)	0 (0-1.0)	0.362
Coughing[3]	2 (1.5-2.0)	2 (1.5-2.0)	0.600
Dyspnea[4]	0 (0-0)	0 (0-0)	1.000
Syncope[5]	0 (0-0)	0 (0-0)	1.000
Total score	4.0 (3.0-4.5)	3.0 (3.0-3.5)	0.196

All values presented as median and IQR, The data between groups were compared by Man-Whitney U test., [1]Inappetite score – increased appetite = 0 point, normal = 1 point, can eat >2/3 of normal = 2 points, can eat < 2/3 of normal = 3 points, [2]Exercise intolerance score – none= 0 point, Dog is active and ability to run is reduced =1 point, Dogs is less active than normal and avoid long walk = 2 points., Dogs is inactive and only get up to eat, drink, urinate, or defecate = 3 points, [3]Coughing score –none = 0 point, few times a week = 1 point, few times a day = 2 points, frequently during the day = 3 points, [4]Dyspnea –Dogs is able to rest and resting respiratory rate <25 times/min = 0 point, Dogs is able to rest and resting respiratory rate > 25 times/min = 1 point, Dogs is restlessness and respiratory effort = 2 points, [5]Syncope –none = 0 point, 2-6 times/week = 1 point, everyday <3 times/day = 2 points, Severe >3 times/day = 3 points

Appendix O: the p value of the clinical scores in the placebo group at day 0 and day 7

Parameters	Placebo (day 0)	Placebo (day 7)	P value
Inappetite[1]	1.0 (1.0-1.0)	1.0 (1.0-1.0)	1.000
Exercise intolerance[2]	1.0 (0-1.5)	1.0 (0.5-1.5)	0.317
Coughing[3]	2 (1.5-2.0)	2.0 (1.0-2.5)	1.000
Dyspnea[4]	0 (0-0)	0 (0-0.5)	0.317
Syncope[5]	0 (0-0)	0 (0-0)	1.000
Total score	4.0 (3.0-4.5)	4.0 (3.0-4.5)	0.458

All values presented as median and IQR. The data between day 0 and day 7 were compared by Wilcoxan signed ranks test, [1]Inappetite score – increased appetite = 0 point, normal = 1 point, can eat >2/3 of normal = 2 points, can eat < 2/3 of normal = 3 points, [2]Exercise intolerance score – none= 0 point, Dog is active and ability to run is reduced =1 point, Dogs is less active than normal and avoid long walk = 2 points., Dogs is inactive and only get up to eat, drink, urinate, or defecate = 3 points, [3]Coughing score –none = 0 point, few times a week = 1 point, few times a day = 2 points, frequently during the day = 3 points, [4]Dyspnea –Dogs is able to rest and resting respiratory rate <25 times/min = 0 point, Dogs is able to rest and resting respiratory rate > 25 times/min = 1 point, Dogs is restlessness and respiratory effort = 2 points, [5]Syncope –none = 0 point, 2-6 times/week = 1 point, everyday <3 times/day = 2 points, Severe >3 times/day = 3 points

Appendix P: the p value of the clinical scores of the sildenafil group at day 0 and day 7

Parameters	Sildenafil (day 0)	Sildenafil (day 7)	P value
Inappetite[1]	1.0 (1.0-1.0)	1.0 (1.0-1.0)	0.157
Exercise intolerance[2]	0 (0-1.0)	0 (0-1.0)	1.000
Coughing[3]	2 (1.5-2.0)	1.0 (0.5-2.0)	0.180
Dyspnea[4]	0 (0-0)	0 (0-0)	0.317
Syncope[5]	0 (0-0)	0 (0-0)	1.000
Total score	3.0 (3.0-3.5)	2.0 (2.0-3.0)	0.063

All values presented as median and IQR. The data between day 0 and day 7 were compared by Wilcoxan signed ranks test. [1]Inappetite score – increased appetite = 0 point, normal = 1 point, can eat >2/3 of normal = 2 points, can eat < 2/3 of normal = 3 points, [2]Exercise intolerance score – none= 0 point, Dog is active and ability to run is reduced =1 point, Dogs is less active than normal and avoid long walk = 2 points., Dogs is inactive and only get up to eat, drink, urinate, or defecate = 3 points, [3]Coughing score –none = 0 point, few times a week = 1 point, few times a day = 2 points, frequently during the day = 3 points, [4]Dyspnea –Dogs is able to rest and resting respiratory rate <25 times/min = 0 point, Dogs is able to rest and resting respiratory rate > 25 times/min = 1 point, Dogs is restlessness and respiratory effort = 2 points, [5]Syncope –none = 0 point, 2-6 times/week = 1 point, everyday <3 times/day = 2 points, Severe >3 times/day = 3 points

Appendix Q: the p value of the clinical scores in the placebo and sildenafil groups at day 7

Parameters	Placebo (day 7)	Sildenafil (day 7)	P value
Inappetite[1]	1.0 (1.0-1.0)	1.0 (1.0-1.0)	0.317
Exercise intolerance[2]	1.0 (0.5-1.5)	0 (0-1.0)	0.164
Coughing[3]	2.0 (1.0-2.5)	1.0 (0.5-2.0)	0.203
Dyspnea[4]	0 (0-0.5)	0 (0-0)	0.141
Syncope[5]	0 (0-0)	0 (0-0)	1.000
Total score	4.0 (3.0-4.5)	2.0 (2.0-3.0)	0.007

All values presented as median and IQR, The data between groups were compared by Man-Whitney U test., [1]Inappetite score – increased appetite = 0 point, normal = 1 point, can eat >2/3 of normal = 2 points, can eat < 2/3 of normal = 3 points, [2]Exercise intolerance score – none= 0 point, Dog is active and ability to run is reduced =1 point, Dogs is less active than normal and avoid long walk = 2 points., Dogs is inactive and only get up to eat, drink, urinate, or defecate = 3 points, [3]Coughing score –none = 0 point, few times a week = 1 point, few times a day = 2 points, frequently during the day = 3 points, [4]Dyspnea –Dogs is able to rest and resting respiratory rate <25 times/min = 0 point, Dogs is able to rest and resting respiratory rate > 25 times/min = 1 point, Dogs is restlessness and respiratory effort = 2 points, [5]Syncope –none = 0 point, 2-6 times/week = 1 point, everyday <3 times/day = 2 points, Severe >3 times/day = 3 points

Appendix R: the p value of the echocardiographic values in the placebo and sildenafil groups at day 0

Parameters	Placebo (day 0)	Sildenafil (day 0)	P value
VSd	0.44 (0.41-0.50)	0.43 (0.40-0.49)	0.749
LVIDd	1.99 (1.89-2.09)	1.92 (1.79-2.04)	0.482
LVWd	0.34 (0.33-0.42)	0.34 (0.32-0.38)	0.653
VSs	0.76 (0.66-0.77)	0.62 (0.58-0.66)	0.047
LVIDs	0.98 (0.83-1.04)	1.06 (0.82-1.19)	0.749
LVWs	0.62 (0.58-0.73)	0.69 (0.56-0.76)	0.798
LA	1.68 (1.43-1.82)	1.57 (1.46-1.93)	0.609
Ao	0.63 (0.59-0.69)	0.66 (0.58-0.81)	0.608
LA:Ao	2.40 (2.29-2.61)	2.66 (2.13-2.74)	0.848
FS (%)	51 (47-57)	48 (41-54)	0.565
RPAD (%)	29 (24-33)	21 (13-25)	0.142
TR (m/s)	3.28 (3.15-3.56)	3.69 (3.30-3.98)	0.179
sPAP (mmHg)	43.03 (39.79-50.98)	54.46 (43.66-63.30)	0.179
AT (msec)	47 (39-56)	44 (31-61)	0.949
ET (msec)	111 (92-121)	97 (78-127)	0.898
AT:ET	0.48 (0.41-0.53)	0.46 (0.41-0.50)	0.749
IVCT (msec)	41 (40-55)	53 (42-56)	0.798
IVRT (msec)	52 (39-61)	39 (33-56)	0.749
RVET (msec)	117 (88-143)	117 (110-126)	0.898
Tei index	0.74 (0.68-1.19)	0.76 (0.69-0.80)	0.949
TAPSE:Ao	1.11 (0.74-1.23)	1.27 (0.99-1.39)	0.337

All values presented as median and IQR. The data between groups were compared by Man-Whitney U test. VSd = ventricular septal thickness during diastole, LVIDd = left ventricular internal diastolic diameter, LVWd = left ventricular free wall thickness during diastole, VSs = ventricular septal thickness during systole, LVIDs = left ventricular internal systolic diameter, LVWs = left ventricular free wall thickness during systole, LA = left atrial diameter, Ao = aortic diameter, LA:Ao = ratio of left atrium to aorta, FS = fraction shortening, RPAD = Right pulmonary artery distensibility index, TR = Tricuspid regurgitant flow velocity, ,sPAP = systolic pulmonary artery pressure, AT = acceleration time, ET = ejection time, AT:ET = ratio of acceleration time to ejection time, IVCT = isovolumetric contraction time, IVRT = isovolumetric relaxation time, RVET = right ventricular ejection time, TAPSE:Ao = ratio of tricuspid annular plane systolic excursion to aortic diameter.

Appendix S: the p value of the echocardiographic values in the placebo at day 0 and day 7

Parameters	Placebo (day0)	Placebo (day 7)	P value
VSd	0.44 (0.41-0.50)	0.43 (0.37-0.49)	0.310
LVIDd	1.99 (1.89-2.09)	1.96 (1.77-2.04)	0.237
LVWd	0.34 (0.33-0.42)	0.41 (0.37-0.47)	0.671
VSs	0.76^{+} (0.66-0.77)	0.67 (0.59-0.80)	1.000
LVIDs	0.98 (0.83-1.04)	1.02 (0.91-1.06)	0.612
LVWs	0.62 (0.58-0.73)	0.60 (0.58-0.68)	0.866
LA	1.68 (1.43-1.82)	1.50 (1.15-1.68)	0.028
Ao	0.63 (0.59-0.69)	0.63 (0.62-0.75)	0.246
LA:Ao	2.40 (2.29-2.61)	2.05 (1.97-2.12)	0.018
FS (%)	51 (47-57)	48 (42-54)	0.128
RPAD (%)	29 (24-33)	19 (13-24)	0.128
TR (m/s)	3.28 (3.15-3.56)	3.51 (3.10-3.62)	0.866
sPAP (mmHg)	43.03 (39.79-50.98)	49.51 (38.60-52.46)	0.866
AT (msec)	47 (39-56)	47 (36-52)	0.599
ET (msec)	111 (92-121)	108 (81-120)	0.799
AT:ET	0.48 (0.41-0.53)	0.44 (0.43-0.46)	0.799
IVCT (msec)	41 (40-55)	43 (38-48)	1.000
IVRT (msec)	52 (39-61)	56 (46-66)	0.249
RVET (msec)	117 (88-143)	116 (97-131)	0.735
Tei index	0.74 (0.68-1.19)	0.79 (0.79-0.97)	0.612
TAPSE:Ao	1.11 (0.74-1.23)	1.27 (1.13-1.40)	0.176

All values presented as median and IQR. The data between day 0 and day 7 were compared by Wilcoxan signed ranks test. VSd = ventricular septal thickness during diastole, LVIDd = left ventricular internal diastolic diameter, LVWd = left ventricular free wall thickness during diastole, VSs = ventricular septal thickness during systole, LVIDs = left ventricular internal systolic diameter, LVWs = left ventricular free wall thickness during systole, LA = left atrial diameter, Ao = aortic diameter, LA:Ao = ratio of left atrium to aorta, FS = fraction shortening, RPAD = Right pulmonary artery distensibility index, TR = Tricuspid regurgitant flow velocity, ,sPAP = systolic pulmonary artery pressure, AT = acceleration time, ET = ejection time, AT:ET = ratio of acceleration time to ejection time, IVCT = isovolumetric contraction time, IVRT = isovolumetric relaxation time, RVET = right ventricular ejection time, TAPSE:Ao = ratio of tricuspid annular plane systolic excursion to aortic diameter.

Appendix T: the p value of the echocardiographic values in the sildenafil at day 0 and day 7

Parameters	Sildenafil day 0	Sildenafil day 7	P value
VSd	0.43 (0.40-0.49)	0.48 (0.40-0.51)	0.553
LVIDd	1.92 (1.79-2.04)	1.87 (1.74-2.05)	0.672
LVWd	0.34 (0.32-0.38)	0.37 (0.35-0.40)	0.528
VSs	0.62+ (0.58-0.66)	0.62 (0.58-0.68)	0.917
LVIDs	1.06 (0.82-1.19)	1.08 (0.66-1.16)	1.000
LVWs	0.69 (0.56-0.76)	0.71 (0.58-0.78)	0.686
LA	1.57 (1.46-1.93)	1.63 (1.49-1.80)	0.497
Ao	0.66 (0.58-0.81)	0.76 (0.61-0.79)	0.498
LA:Ao	2.66 (2.13-2.74)	2.23 (2.06-2.51)	0.310
FS (%)	48 (41-54)	48 (43-60)	0.735
RPAD (%)	21 (13-25)	15 (9-16)	0.063
TR (m/s)	3.69 (3.30-3.98)	3.43 (3.20-3.68)	0.043
sPAP (mmHg)	54.46 (43.66-63.30)	47.13 (41.08-54.15)	0.043
AT (msec)	44 (31-61)	50 (47-61)	0.176
ET (msec)	97 (78-127)	126 (98-142)	0.173
AT:ET	0.46 (0.41-0.50)	0.46 (0.42-0.51)	0.600
IVCT(msec)	53 (42-56)	50 (32-76)	0.600
IVRT(msec)	39 (33-56)	61 (54-72)	0.116
RVET(msec)	117 (110-126)	131 (118-136)	0.176
Tei index	0.76 (0.69-0.80)	0.78 (0.58-1.02)	1.000
TAPSE:Ao	1.27 (0.99-1.39)	0.97 (0.93-1.24)	0.236

All values presented as median and IQR. The data between day 0 and day 7 were compared by Wilcoxan signed ranks test. VSd = ventricular septal thickness during diastole, LVIDd = left ventricular internal diastolic diameter, LVWd = left ventricular free wall thickness during diastole, VSs = ventricular septal thickness during systole, LVIDs = left ventricular internal systolic diameter, LVWs = left ventricular free wall thickness during systole, LA = left atrial diameter, Ao = aortic diameter, LA:Ao = ratio of left atrium to aorta, FS = fraction shortening, RPAD = Right pulmonary artery distensibility index, TR = Tricuspid regurgitant flow velocity, ,sPAP = systolic pulmonary artery pressure, AT = acceleration time, ET = ejection time, AT:ET = ratio of acceleration time to ejection time, IVCT = isovolumetric contraction time, IVRT = isovolumetric relaxation time, RVET = right ventricular ejection time, TAPSE:Ao = ratio of tricuspid annular plane systolic excursion to aortic diameter.

Appendix U: the p value of the echocardiographic values in the placebo and sildenafil groups at day 7

Parameters	Placebo (day 7)	Sildenafil (day 7)	P value
VSd	0.43 (0.37-0.49)	0.48 (0.40-0.51)	0.609
LVIDd	1.96 (1.77-2.04)	1.87 (1.74-2.05)	0.798
LVWd	0.41 (0.37-0.47)	0.37 (0.35-0.40)	0.276
VSs	0.67 (0.59-0.80)	0.62 (0.58-0.68)	0.565
LVIDs	1.02 (0.91-1.06)	1.08 (0.66-1.16)	0.949
LVWs	0.60 (0.58-0.68)	0.71 (0.58-0.78)	0.482
LA	1.50* (1.15-1.68)	1.63 (1.49-1.80)	0.337
Ao	0.63 (0.62-0.75)	0.76 (0.61-0.79)	0.564
LA:Ao	2.05* (1.97-2.12)	2.23 (2.06-2.51)	0.338
FS (%)	48 (42-54)	48 (43-60)	0.655
RPAD (%)	19 (13-24)	15 (9-16)	0.085
TR (m/s)	3.51 (3.10-3.62)	3.43# (3.20-3.68)	0.749
sPAP (mmHg)	49.51 (38.60-52.46)	47.13# (41.08-54.15)	0.749
AT (msec)	47 (36-52)	50 (47-61)	0.275
ET (msec)	108 (81-120)	126 (98-142)	0.276
AT:ET	0.44 (0.43-0.46)	0.46 (0.42-0.51)	0.565
IVCT (msec)	43 (38-48)	50 (32-76)	0.608
IVRT (msec)	56 (46-66)	61 (54-72)	0.563
RVET (msec)	116 (97-131)	131 (118-136)	0.338
Tei index	0.79 (0.79-0.97)	0.78 (0.58-1.02)	0.522
TAPSE:Ao	1.27 (1.13-1.40)	0.97 (0.93-1.24)	0.949

All values presented as median and IQR. The data between groups were compared by Man-Whitney U test. VSd = ventricular septal thickness during diastole, LVIDd = left ventricular internal diastolic diameter, LVWd = left ventricular free wall thickness during diastole, VSs = ventricular septal thickness during systole, LVIDs = left ventricular internal systolic diameter, LVWs = left ventricular free wall thickness during systole, LA = left atrial diameter, Ao = aortic diameter, LA:Ao = ratio of left atrium to aorta, FS = fraction shortening, RPAD = Right pulmonary artery distensibility index, TR = Tricuspid regurgitant flow velocity, ,sPAP = systolic pulmonary artery pressure, AT = acceleration time, ET = ejection time, AT:ET = ratio of acceleration time to ejection time, IVCT = isovolumetric contraction time, IVRT = isovolumetric relaxation time, RVET = right ventricular ejection time, TAPSE:Ao = ratio of tricuspid annular plane systolic excursion to aortic diameter.

Appendix V: data of signalments of the control group

No.	Name	Age	Breed	Sex	Weight
1	โดโด้	15	Poodle	Male	4.7
2	Roxy	11	Chihuahua	Female	4.12
3	Ben	12	MP	Male	5.26
4	ข้าวหอม	8	Poodle	Female	5.1
5	moji	13	Jack russel	Female	7.3
6	หมั่นโถว	7	Chihuahua	Male	4.06
7	โกโก้	12	Shih Tzu	Male	5.5

Appendix W: data of signalments data of the sildenafil group

No.	Name	Age	Breed	Sex	Weight
1	คาซึ	10	Shih Tzu	Female	5.8
2	เปรี้ยว	11	Chihuahua	Female	3.98
3	มะยม	12	Chihuahua	Male	3.5
4	ไข่ขาว	12	Poodle	Male	4.04
5	baby	10	poodle	Female	7.3
6	ข้าวหอม	11	Chihuahua	Female	5.04
7	ริกิ	10	Pommeranian	Male	4.1

Appendix X: data of clinical evaluation of the placebo group at day 0 and day 7

No.	Name	HR		RR		SBP		ECG	
		D 0	D 7	D 0	D 7	D 0	D 7	D 0	D 7
1	โดโด้	166	179	66	57	126	126	ST	SR
2	Roxy	174	196	66	90	136	131	ST	SR
3	Ben	127	135	32	45	112	113	SR	SR
4	ข้าวหอม	114	136	48	44	150	142	SR	SR
5	moji	117	135	56	32	107	121	RSA	SR
6	หมั่นโถว	137	143	24	18	158	153	ST	ST
7	โกโก้	177	157	75	57	109	122	ST	ST

ST = sinus tachycardia, SR = sinus rhythm, RSA = respiratory sinus arrhythmia, HR = heart rate, RR = respiratory rate, SBP = systemic blood pressure, ECG = electrocardiography

Appendix Y: data of clinical evaluation of the sildenafil group at day 0 and day 7

No.	Name	HR		RR		SBP		ECG	
		D 0	D 7	D 0	D 7	D 0	D 7	D 0	D 7
1	คาซึ	193	174	54	66	122	177	SR	ST
2	เปรี้ยว	193	188	60	75	168	167	ST	ST
3	มะยม	164	164	72	54	163	137	ST	ST
4	ไข่ขาว	98	98	28	24	133	108	SR	SR
5	baby	144	150	42	24	120	144	SR	SR
6	ข้าวหอม	120	106	24	30	124	133	SR	SR
7	ริกิ	131	117	30	45	133	127	ST	SR

ST = sinus tachycardia, SR = sinus rhythm, RSA = respiratory sinus arrhythmia, HR = heart rate, RR = respiratory rate, SBP = systemic blood pressure, ECG = electrocardiography

Appendix Z: data of blood values of the placebo group at day 0 and day 7

Name	โดโด้		Roxy		Ben		ข้าวหอม		Moji		หมั่นโถว		โกโก้	
Parameters	D 0	D 7	D 0	D 7	D 0	D 7	D 0	D 7	D 0	D 7	D 0	D 7	D 0	D 7
RBC (10^6/mm^3)	6.42	6.87	5.78	6.31	5.56	6.16	7.1	6.93	7.32	6.68	6.25	6.93	6.23	6.67
WBC (10^3/mm^3)	12.8	11.9	13.7	11.7	7.2	8.3	12.9	11.1	9.1	9.1	10.3	11.1	21.8	15.4
Platelets (10^3/mm^3)	204	246	579	693	278	421	223	244	223	217	290	244	717	574
Creatinine (mg/dL)	1	1.2	1.1	1.2	1.5	1.2	0.8	0.9	1.3	1.3	0.67	0.9	0.7	0.6
BUN (mg/dL)	30.6	70.7	21.1	31.5	69.1	56.3	26.8	26.4	31.1	20.5	16.6	26.4	46.2	33.8
ALT (IU/L)	54	53	108	90	37	47	28	20	103	123	50	20	108	115
ALP (IU/L)	86	149	130	89	101	111	46	42	39	37	51	42	115	106
Total Protein (g/dL)	5.2	6.1	6.8	6.7	6.2	6.3	8	7.9	6.3	6.8	5.8	7.9	6	6.2
Albumin (g/dL)	2.7	3.1	3.4	3.4	3.2	3.3	3.4	3.4	3.7	3.6	2.5	3.4	2.8	2.8
Globulin (g/dL)	2.5	3	3.4	3.3	3	3	4.6	4.5	2.6	3.2	3.3	4.5	3.2	3.4

RBC = red blood cell, WBC = white blood cell, BUN = blood urea nitrogen, ALT = alanine aminotransferase, ALP = alkaline phosphatase

Appendix AA: data of blood values of the sildenafil group at day 0 and day 7

Name	คาซี		เปรี้ยว		มะยม		ไข่ขาว		baby		ข้าวหอม		ริกิ	
Parameter	D 0	D 7	D 0	D 7	D 0	D 7	D 0	D 7	D 0	D 7	D 0	D 7	D 0	D 7
RBC (10^6/mm^3)	5.52	5.56	5.88	6.38	6.55	6.78	6.61	6.1	4.99	4.95	7.05	7.13	6.24	6.37
WBC (10^3/mm^3)	11.8	7.8	10.1	8.5	5.7	8.1	6.0	6.9	14.0	15.6	10.4	10.9	13.7	12.1
Platelets (10^3/mm^3)	599	506	398	226	439	420	228	256	441	212	383	436	328	288
Creatinine (mg/dL)	0.9	1	1.2	1.1	1	1.1	1	1.1	1.3	0.9	1.7	1.2	0.6	0.7
BUN (mg/dL)	35.8	32.5	23.2	22.2	35.2	38	35.4	36.1	38	42	61.2	70.2	20.6	21.4
ALT (IU/L)	112	107	55	36	66	72	75	72	106	98	134	118	29	37
ALP (IU/L)	240	219	105	75	73	67	62	78	345	284	369	337	79	76
Total Protein (g/dL)	5.9	6.5	5.9	6.3	6.6	6.7	6.8	7	4.9	6	7.2	7.2	7.5	7.3
Albumin (g/dL)	2.7	2.8	3.1	3.2	3.5	3.5	3.2	3.4	2.6	2.8	3.6	3.4	2.7	2.6
Globulin (g/dL)	3.2	3.7	2.8	3.1	3.1	3.2	3.6	3.6	2.3	3.2	3.6	3.8	4.8	4.7

RBC = red blood cell, WBC = white blood cell, BUN = blood urea nitrogen, ALT = alanine aminotransferase, ALP = alkaline phosphatase

Appendix AB: data of vertebral heart score and lung score of the placebo group at day 0 and day 7

Name	โดโด้		Roxy		Ben		ข้าวหอม		**Moji**		หมั่นโถว		โกโก้	
Parameter	D 0	D 7	D 0	D 7	D 0	D 7	D 0	D 7	D 0	D 7	D 0	D 7	D 0	D 7
VHS	13	12.7	11.2	11.7	11.5	11.2	12.5	12.5	11.2	11.2	12.7	12.7	11.5	11.2
Q1	3	2	1	2	0	0	1	1	0	0	1	1	0	0
Q2	2	2	2	2	1	1	3	3	1	1	1	1	0	0
Q3	3	2	1	2	0	0	1	1	0	0	1	1	0	0
Q4	2	2	1	2	1	1	2	2	1	1	1	1	1	2

VHS = vertebral heart score, Q1 = quadrant 1, Q2 = quadrant 2, Q3 = quadrant 3, Q4 = quadrant 4

Appendix AC: data of vertebral heart score and lung score of the sildenafil group at day 0 and day 7

Name	คาซี		เปรี้ยว		มะยม		ไข่ขาว		baby		ข้าวหอม		ริกิ	
Parameter	D 0	D 7	D 0	D 7	D 0	D 7	D 0	D 7	D 0	D 7	D 0	D 7	D 0	D 7
VHS	11.5	10.7	13	13	12.5	12.7	11.7	11.7	11.2	11.5	11.5	11.8	13	13
Q1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Q2	1	1	1	1	1	1	2	2	2	2	1	1	0	0
Q3	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Q4	1	2	1	2	1	1	1	1	2	2	1	1	2	2

VHS = vertebral heart score, Q1 = quadrant 1, Q2 = quadrant 2, Q3 = quadrant 3, Q4 = quadrant 4

Appendix AD: data of clinical scores of the placebo group at day 0 and day 7

Name	โดโด้		Roxy		Ben		ข้าวหอม		**Moji**		หมั่นโถว		โกโก้	
Parameter	D 0	D 7	D 0	D 7	D 0	D 7	D 0	D 7	D 0	D 7	D 0	D 7	D 0	D 7
Inappetite	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Exercise intolerance	1	1	2	2	0	1	0	0	1	1	0	0	2	2
Coughing	2	1	2	3	1	2	3	3	1	1	2	2	2	1
Dyspnea	0	0	0	1	1	1	0	0	0	0	0	0	0	0
Syncope	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Total	4	3	5	7	3	5	4	4	3	3	3	3	5	4

Appendix AE: data of clinical scores of the sildenafil group at day 0 and day 7

Name	คาซี		เปรี้ยว		มะยม		ไข่ขาว		baby		ข้าวหอม		ริกิ	
Parameter	D 0	D 7	D 0	D 7	D 0	D 7	D 0	D 7	D 0	D 7	D 0	D 7	D 0	D 7
Inappetite	1	0	1	1	1	1	1	1	2	1	1	1	1	1
Exercise intolerance	0	0	1	1	1	1	0	0	1	1	0	0	0	0
Coughing	2	2	2	0	1	1	2	1	0	0	2	2	2	2
Dyspnea	0	0	0	0	0	0	0	0	1	0	0	0	0	0
Syncope	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Total	3	2	4	2	3	3	3	2	4	2	3	3	3	3

Appendix AF: data of echocardiographic values of the placebo group at day 0 and day 7

Name	โดโด้		Roxy		Ben		ข้าวหอม		**Moji**		หมั่นโถว		โกโก้	
Parameter	D 0	D 7	D 0	D 7	D 0	D 7	D 0	D 7	D 0	D 7	D 0	D 7	D 0	D 7
VSd	0.27	0.54	0.6	0.53	0.4	0.44	0.45	0.4	0.44	0.34	0.54	0.43	0.41	0.32
LVIDd	2.13	1.81	1.99	2	1.79	1.65	2.52	2.4	1.89	2.08	1.88	1.73	2.04	1.96
LVWd	0.32	0.45	0.5	0.48	0.34	0.54	0.33	0.41	0.43	0.34	0.41	0.39	0.33	0.25
VSs	0.69	0.57	0.86	0.85	0.78	0.81	0.63	0.67	0.76	0.78	0.76	0.55	0.5	0.61
LVIDs	1.04	1.02	0.9	0.94	0.72	0.65	1.48	1.45	0.75	1.08	1.04	1.03	0.98	0.88
LVWs	0.52	0.59	0.76	0.78	0.69	0.72	0.56	0.57	0.85	0.6	0.62	0.63	0.6	0.51
LA	1.78	1.65	2.06	1.9	1.24	1.08	1.68	1.7	1.85	1.5	1.35	1.08	1.51	1.22
Ao	0.64	0.76	0.59	0.62	0.56	0.54	0.73	0.82	0.76	0.73	0.59	0.61	0.63	0.63
LA:Ao	2.78	2.17	3.49	3.06	2.21	2.00	2.30	2.07	2.43	2.05	2.28	1.77	2.39	1.93
FS (%)	51	44	55	53	60	61	41	40	60	48	45	40	52	55
RPAD (%)	28	34	37	14	27	25	35	19	20	11	31	21	4	9
TR (m/s)	4.14	3.76	3.83	3.72	3.28	3.52	2.91	3.51	3.19	3.01	3.28	3.08	3.11	3.12
sPAP (mmHg)	68	56	58	55	43	49	34	49	40	36	43	38	38	39
AT (msec)	28	32	50	23	61	50	65	54	47	47	43	61	35	40
ET (msec)	100	72	111	53	122	117	133	108	83	122	119	126	62	90
AT:ET	0.28	0.44	0.45	0.43	0.50	0.43	0.48	0.50	0.57	0.39	0.36	0.48	0.56	0.44
IVCT (msec)	72	36	67	67	28	39	40	47	43	43	40	49	41	32
IVRT (msec)	31	36	19	61	56	56	65	83	46	70	65	38	52	54
RVET(msec)	92	116	64	72	117	122	165	140	159	143	83	86	126	108
Tei index	1.12	0.62	1.34	1.78	0.72	0.78	0.64	0.92	0.55	0.79	1.26	1.01	0.74	0.79
TAPSE:Ao	0.61	0.81	0.75	0.90	1.26	1.32	0.73	0.93	1.36	1.16	1.20	1.43	1.71	1.00

VSd = ventricular septal thickness during diastole, LVIDd = left ventricular internaldiastolic diameter, LVWd = left ventricular free wall thickness during diastole, VSs = ventricular septal thickness during systole, LVIDs = left ventricular internal systolic diameter, LVWs = left ventricular free wall thickness during systole, LA = left atrial diameter, Ao = aortic diameter, LA: Ao = ratio of left atrium to aorta, FS = fraction shortening, RPAD = Right pulmonary artery distensibility index, TR = Tricuspid regurgitant flow velocity, ,sPAP = systolic pulmonary artery pressure, AT = acceleration time, ET = ejection time, AT: ET = ratio of acceleration time to ejection time, IVCT = isovolumetric contraction time, IVRT = isovolumetric relaxation time, RVET = right ventricular ejection time, TAPSE:Ao = ratio of tricuspid annular plane systolic excursion to aortic diameter.

Appendix AG: data of echocardiographic values of the sildenafil group at day 0 and day 7

Name	คาซี		เปรี้ยว		มะยม		ไข่ขาว		baby		ข้าวหอม		ริกิ	
Parameter	D 0	D 7	D 0	D 7	D 0	D 7	D 0	D 7	D 0	D 7	D 0	D 7	D 0	D 7
VSd	0.52	0.43	0.43	0.48	0.37	0.33	0.39	0.52	0.56	0.59	0.45	0.5	0.4	0.37
LVIDd	1.92	1.87	1.76	1.78	2.25	2.3	2.06	2.1	1.34	1.69	1.81	1.54	2.01	2
LVWd	0.25	0.34	0.34	0.35	0.4	0.4	0.34	0.41	0.47	0.4	0.35	0.37	0.3	0.27
VSs	0.67	0.72	0.62	0.62	0.67	0.64	0.6	0.59	0.64	0.56	0.56	0.9	0.44	0.4
LVIDs	1.23	1.13	0.69	0.67	1.15	1.19	1.06	1.08	0.27	0.53	0.95	0.65	1.3	1.32
LVWs	0.37	0.3	0.79	0.82	0.72	0.73	0.69	0.69	1.05	0.86	0.65	0.71	0.47	0.47
LA	1.57	1.72	2.06	1.63	2.17	1.87	1.80	1.95	1.36	1.53	1.54	1.11	1.38	1.45
Ao	0.85	0.77	0.56	0.53	0.79	0.84	0.66	0.76	0.82	0.81	0.58	0.63	0.57	0.59
LA:Ao	1.85	2.23	3.68	3.08	2.75	2.23	2.73	2.57	1.66	1.89	2.66	1.76	2.42	2.46
FS (%)	36	40	61	62	49	48	49	49	80	69	48	58	35	34
RPAD (%)	27	19	33	16	18	7	9	15	3	8	21	15	22	10
TR (m/s)	4.27	4.12	3.9	3.55	3.69	3.3	2.93	2.91	4.05	3.8	3.3	3.43	3.3	3.1
sPAP (mmHg)	73	68	61	50	54	44	34	34	66	58	44	47	44	39
AT (msec)	67	56	44	31	22	44	33	50	29	50	54	79	68	65
ET (msec)	128	106	83	53	56	89	72	144	97	126	126	180	140	140
AT:ET	0.52	0.52	0.53	0.59	0.39	0.49	0.46	0.34	0.30	0.40	0.43	0.44	0.49	0.46
IVCT (msec)	53	50	44	25	28	28	39	83	63	83	58	68	54	36
IVRT (msec)	81	61	39	61	33	47	61	72	50	79	29	29	32	72
RVET(msec)	86	142	117	119	108	131	122	139	148	115	130	133	112	117
Tei index	1.56	0.78	0.71	0.37	0.56	0.43	0.82	1.12	0.76	1.40	0.67	0.73	0.77	0.92
TAPSE:Ao	0.46	0.42	1.43	2.07	1.35	0.94	1.01	0.97	0.98	0.91	1.27	1.11	1.43	1.38

VSd = ventricular septal thickness during diastole, LVIDd = left ventricular internaldiastolic diameter, LVWd = left ventricular free wall thickness during diastole, VSs = ventricular septal thickness during systole, LVIDs = left ventricular internal systolic diameter, LVWs = left ventricular free wall thickness during systole, LA = left atrial diameter, Ao = aortic diameter, LA: Ao = ratio of left atrium to aorta, FS = fraction shortening, RPAD = Right pulmonary artery distensibility index, TR = Tricuspid regurgitant flow velocity, ,sPAP = systolic pulmonary artery pressure, AT = acceleration time, ET = ejection time, AT: ET = ratio of acceleration time to ejection time, IVCT = isovolumetric contraction time, IVRT = isovolumetric relaxation time, RVET = right ventricular ejection time, TAPSE:Ao = ratio of tricuspid annular plane systolic excursion to aortic diameter.

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