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*Case Report*

# Four case reports: Effects of fucoidan and fucoxanthin on the treatment of degenerative heart valve disease in dogs

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**ABSTRACT**: Degenerative heart valve disease (DHVD) is the most common heart disease in dogs, especially in small breed dogs weighing less than 20 kg. The incidence in dogs over 13 years old is as high as 85%, and more than 75% of these dogs have mitral valve problems. The valve, which includes the valve leaflet, chordae, and annulus, is mutinously degenerated due to the accumulation of abnormal collagen tissue and glycosaminoglycan, and nodules are formed at the edge of the valve as the disease progresses, eventually resulting in valve insufficiency. To improve the regular treatment of DHVD, especially medication, the natural supplements fucoidan and fucoxanthin were used in this case report. Four small-breed dogs were diagnosed with heart valve disease in the Yukang Veterinary Hospital, and the veterinarian used fucoidan and fucoxanthin as nutritional supplements for the adjuvant treatment of heart valve disease. In the first three cases, it was found that the cardiac function index, including the LA/Ao ratio, significantly improved after 3 to 6 months of continuous administration. Additionally, in the case of American College of Veterinary Internal Medicine (ACVIM) heart grading below Stage B2 after treatment, valve hypertrophy improved, thinned, and flattened, which either reduced the reflux condition caused by valve insufficiency or restored the complete valve closure function. However, in Case 4, the cardiac function index was not improved after 3 to 6 months of continuous administration without the supplement. To the best of our knowledge, this is the first documented clinical study showing that fucoidan and fucoxanthin can improve DHVD in different kinds of dogs.

**Keywords:** Degenerative heart valve disease, echocardiography, fucoidan, fucoxanthin.

**Abbreviations**: **CW,** continuous-wave Doppler; **DBP,** diastolic blood pressure; **DHVD,** degenerative heart valve disease, **DMVD,** degenerative mitral valve disease; **EDT,** early filling deceleration time, **MAP,** mean arterial pressure; **MR,** mitral regurgitation; **PW,** pulsed wave Doppler; **RVSP,** right ventricular systolic pressure; **SBP,** systolic blood pressure; **TDI,** tissue Doppler imaging; **TR,** tricuspid regurgitation; **VHS,** vertebral heart size.

## INTRODUCTION

Degenerative heart valve disease (DHVD), also known as myxomatous mitral valve disease (MMVD), is the most common heart disease (approximately 75%) in dogs, especially in small-breed dogs weighing less than 20 kg (Keene et al., 2019). Statistically, the foreign Cavalier King Charles Spaniel has the highest incidence of this disease, but in Taiwan, the Maltese breed has the highest incidence. The prevalence of MMVD increases markedly

with age in small-breed dogs, with up to 85% showing evidence of valve lesions by 13 years of age (Misbach et al., 2016). Although the major cause remains unknown, MMVD is characterized by changes in the cellular constituents and the intercellular matrix of the valve leaflets as well as the chordae tendineae, which involve both the collagen content and the alignment of collagen fibrils within the valve (Aupperle and Disatian, 2012).

According to the guidelines, the rate of progression of MMVD can be identified by age, progressive heart enlargement (of the left atrium and ventricle), resting heart rate, transmitral E-wave blood flow velocities, and serum N-terminal pro-B-type natriuretic peptide (NT-proBNP) concentrations (Keene et al., 2019). In addition, the volume overload caused by mitral valve insufficiency and eccentric left ventricular hypertrophy are important features of MMVD (Borgarelli and Buchanan, 2012).

Fucoidan is a sulfated fucose-rich polysaccharide that is present at high levels in brown seaweed and has been shown to have antioxidant and cardioprotective effects in animal experiments. A previous study showed that fucoidan not only reverses damage to the region induced by isoproterenol in a myocardial infarction rat model (Thomes et al., 2010) but it protects against damage induced by myocardial ischemia–reperfusion in rats (Li et al., 2011). Fucoxanthin is a red-orange carotenoid that is extracted from natural seaweed and exhibits potential cardioprotective effects *in vivo*. It has been reported that fucoxanthin and its metabolite fucoxanthinol result in significant decreases in triglyceride concentrations in jugular blood of rats (Matsumoto et al., 2010).

Moreover, the combination of fucoidan and fucoxanthin treatment has been reported to play a role in improving the ventricular rhythm and muscular function of the aging mouse model (Chang et al., 2019). However, the effects of combining fucoidan and fucoxanthin to improve DHVD are still unclear. Therefore, the purpose of this study was to investigate whether fucoidan and fucoxanthin affect DHVD.

## CASE PRESENTATION

### Case 1 (drugs with supplements)

A 12-year-old female Maltese weighing 3.4 kg was referred for a 1-year history of heart disease, cough, and weight loss. The dog's blood pressure was measured by the petMAP™ graphic II blood pressure measurement device. A physical examination showed that its systolic blood pressure (SBP) was 152 mmHg, its diastolic blood pressure (DBP) was 93 mmHg, and its mean arterial pressure (MAP) was 117 mmHg. In addition, the dog had tachycardia (heart rate (HR) = 150 beats/min) with an irregular heart rhythm, a grade IV/VI left apical systolic heart murmur consistent with the mitral insufficiency heart murmur pattern, a grade II/VI right apical systolic heart murmur with an irregular norm kinetic femoral arterial pulse, and suspected tricuspid regurgitation (Ljungvall et al., 2014). Respiratory auscultation revealed increased lung sounds. First-line examinations, including thoracic radiographs and echocardiography, were therefore scheduled.

Standard thoracic radiographs showed signs of mild pulmonary edema in the right posterior lobe. The left atrium, left atrial appendage, and left ventricle were significantly enlarged. The left atrium repressed the trachea and bronchi branches, and the vertebral heart size (VHS) index value was 11.8. Based on the physical examination, an acquired cardiac disease, likely DMVD, was suspected. Conventional echocardiography and a standard Doppler examination were performed on the awake dog, which was gently restrained and lying on its side, using Esaote's MyLab™ClassC® (Italy) equipped with a PA-122 probe cardio phased array (frequency range of 3–8 MHz) to obtain all of the echocardiographic data, as previously described (Fox, 2012; Boswood et al., 2016; Adams et al., 2017).

The left atrium to aorta (LA/Ao) ratio was measured on B-mode images acquired from a short axis five-chamber view of the right sternum wall. The LA/Ao ratio was 1.83. The hemodynamic parameters related to mitral and tricuspid valves were measured using a pulsed wave Doppler (PW), a continuous wave Doppler, (CW), and tissue Doppler imaging (TDI). Left ventricular pseudonormal diastolic dysfunction, right ventricular delay diastolic dysfunction, mitral regurgitation (MR) reflux with a flow rate of 5.66 m/s, tricuspid regurgitation (TR) reflux with a flow rate of 3.56 m/s, and right ventricular systolic pressure (RVSP) of 65.69 mmHg ($4(TR\ VMax)^2$ + RA pressure) suggested that this female Maltese dog also had moderate pulmonary hypertension.

The drug treatment for the dog included pimobendan (0.2 mg/kg), furosemide (1.5 mg/kg), and enalapril (0.5 mg/kg). The drugs were administered twice a day. The dog was also supplemented orally twice a day with low-molecular-weight (LMW) fucoidan (60 mg/kg; Hi-Q Oligo-Fucoidan, New Taipei City, Taiwan) and high-stability fucoxanthin (60 mg/kg; Hi-Q HS Fucoxanthin, New Taipei City, Taiwan).

After five months of treatment, the physical examination showed that the dog's SBP was 138 mmHg, its DBP was 85 mmHg, its MAP was 103 mmHg, and its HR was 125 beats/min. The blood biochemistry analysis showed that all of the data were within normal limits, except MCH, which was elevated from 23.4 to 25%. The standard thoracic radiographs showed that its VHS index had decreased from 11.8 to 10.4 and that left atrial dilatation had improved (Figure 1A and 1B). The echocardiographic data showed that the LA/Ao ratio had decreased from 1.83 to 1.37 (Figure 2A and 2B). The left ventricular diastolic function had improved from pseudonormal diastolic

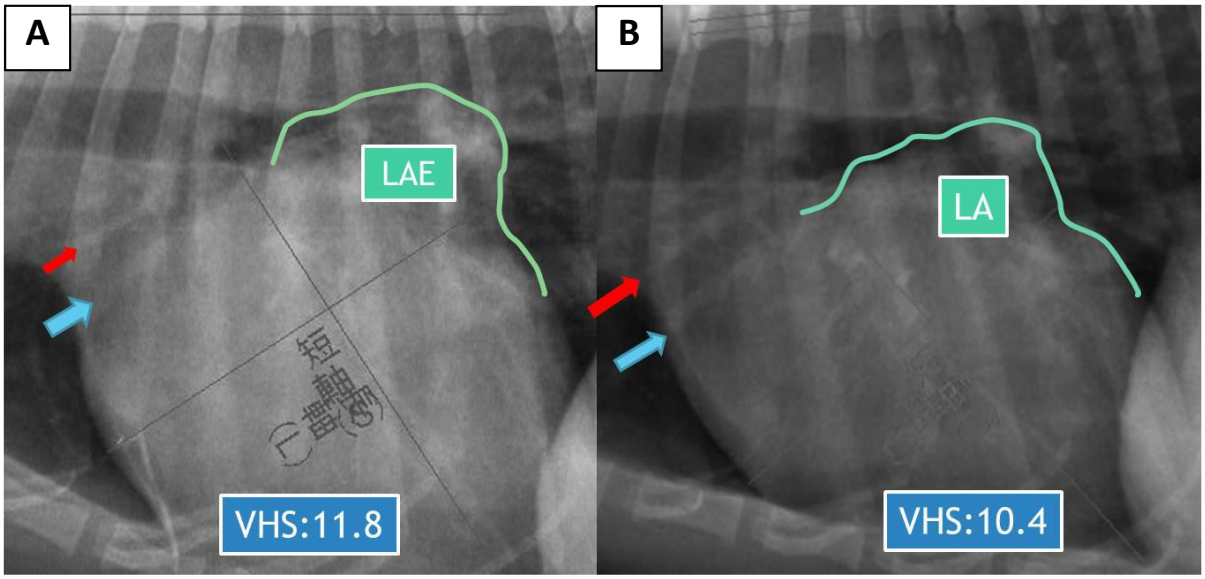


**Figure 1.** Effect of fucoidan and fucoxanthin on vertebral heart size (VHS) after five months of treatment: (A) before fucoidan and fucoxanthin treatment in case 1 and (B) after fucoidan and fucoxanthin treatment in case 1.

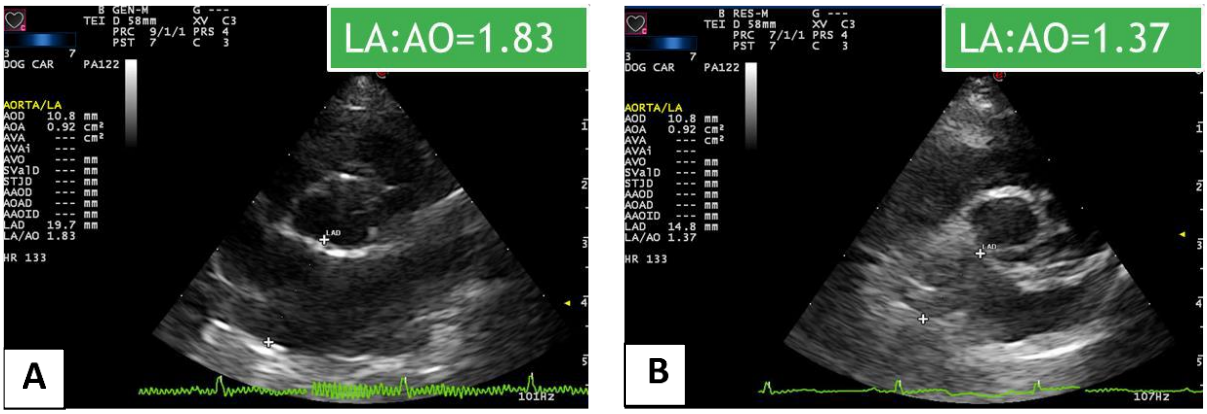


**Figure 2.** Effect of fucoidan and fucoxanthin on the left atrium to aorta ratio (LA/Ao ratio) after five months of treatment: (A) before and fucoxanthin treatment in case 1 and (B) after fucoidan treatment in case 1.

dysfunction to delayed diastolic dysfunction, and the mitral regurgitation had improved to physiological reflux (the reflux flow only in the early stage of contraction). Also, the tricuspid regurgitation flow rate had decreased from 3.56 to 2.88 m/s (Figure 3A and 3B).

### Case 2 (Drugs with supplements)

A 10-year-old neutered Miniature Pinscher weighing 4.8 kg was referred for a history of cough, loss of appetite, poor spirit, and decreased mobility. A physical examination showed that its SBP was 165 mmHg, its DBP was 115 mmHg, its MAP was 132 mmHg, and its HR was 150 beats/min. A grade III/VI left apical systolic heart murmur consistent with the mitral regurgitation heart murmur pattern was diagnosed (Ljungvall et al., 2014).

The standard thoracic radiographs showed signs of diffuse stroma in the lobe. This result suggested that the dog also had a mild to moderate degree of pulmonary fibrosis. The owner had adopted the dog when it was young, and the pulmonary disease history before the adoption was unknown. Its VHS index value was 10.6. The echocardiography results showed that the dog had mitral valve hypertrophy, mitral insufficiency, a moderate degree of mitral regurgitation (MR) (flow rate: 6.14 m/s), a mild

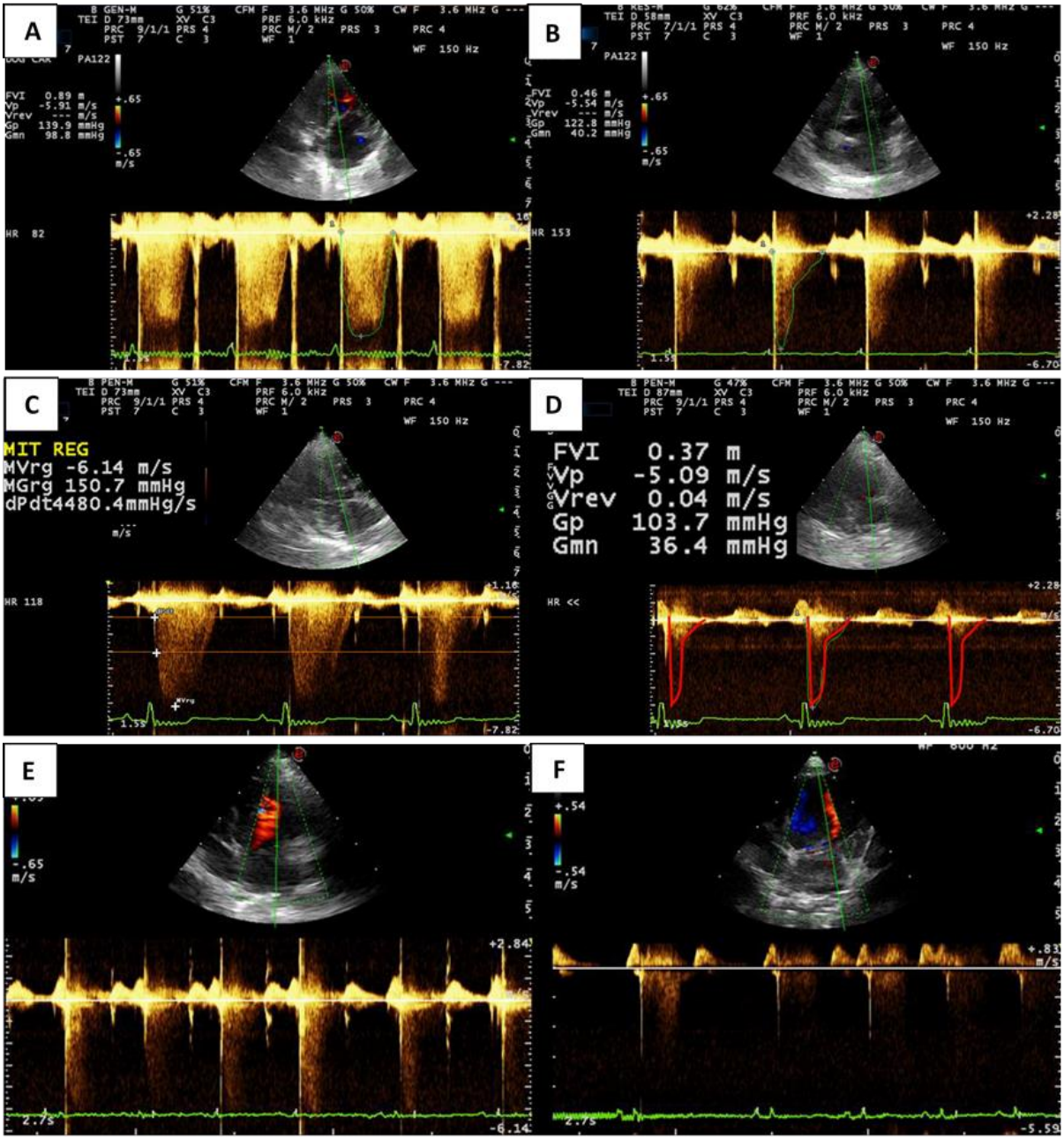


**Figure 3.** Effect of fucoidan and fucoxanthin on mitral insufficiency causing blood to flow from the left ventricle to the left atrium: (A) before fucoidan and fucoxanthin treatment in Case 1; (B) after fucoidan and fucoxanthin treatment in Case 1; (C) before fucoidan and fucoxanthin treatment in Case 2; (D) after fucoidan and fucoxanthin treatment in Case 2; (E) before fucoidan and fucoxanthin treatment in Case 3; and (F) after fucoidan and fucoxanthin treatment in Case 3.

degree of tricuspid valve regurgitation (TR) (flow rate: 2.13 m/s), and left and right ventricular delayed diastolic dysfunction. Pulmonary artery systolic pressure reached 23.14 mmHg. The 2D echocardiographic results showed the valve thickness (D1: 4.0 mm, D2: 3.7 mm) (Figure 4).

The drug treatment for the dog included pimobendan (0.2 mg/kg), furosemide (1.5 mg/kg), and enalapril (0.5 mg/kg). The drugs were administered twice a day. The dog was also administered orally twice a day with low-molecular-weight fucoidan (60 mg/kg) and fucoxanthin (60 mg/kg).

After 5.5 months of treatment, physical examination

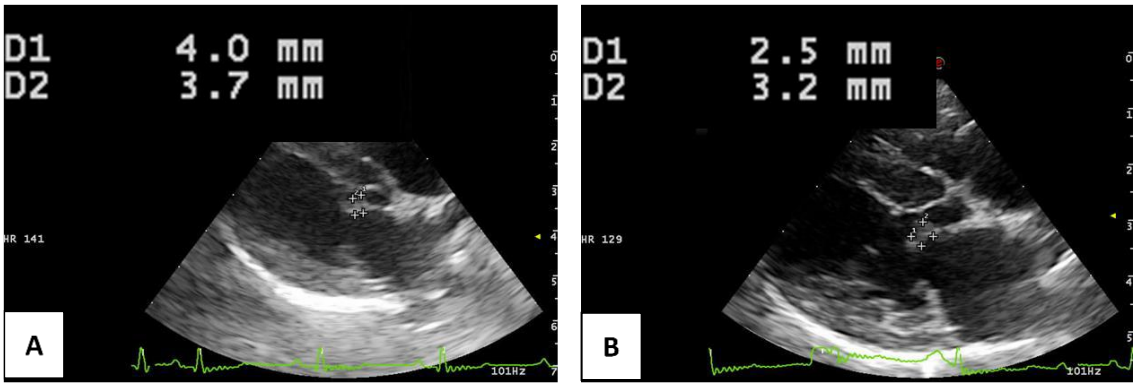


**Figure 4.** Effect of fucoidan and fucoxanthin on valve thickness: (A) before fucoidan treatment in case 2 and (B) after fucoidan treatment in case 2.

showed that its SBP was 127 mmHg, its DBP was 79 mmHg, its MAP was 103 mmHg, and its HR was 96 beats/min. The blood biochemistry analysis showed that all of the data were within normal limits, except MPV, which was elevated from 9.5 to 13.4 fl. No significant changes were shown on the standard thoracic radiographs after treatment. The echocardiographic data showed that mitral regurgitation was therapeutically improved to physiological reflux (the reflux flow only in the early stage of contraction), and tricuspid regurgitation had significantly improved (Figure 3C and 3D). The thickness of the anterior mitral valve had reduced from 4 mm and 3.7 mm to 3.2 mm and 2.5 mm (Figure 4).

## Case 3 (drugs with supplements)

An 11-year-old castrated female Chihuahua weighing 4.8 kg was referred for a 3-year history of heart disease, degenerative heart valve disease, MR, TR, and delayed diastolic dysfunction. A physical examination showed that its SBP was 124 mmHg and its HR was 69–140 beats/min (sinus arrhythmia). The dog was diagnosed with a grade III/VI left apical systolic heart murmur consistent with the mitral regurgitation heart murmur pattern and a grade I/VI right apical systolic heart murmur consistent with the tricuspid regurgitation heart murmur pattern.

The standard thoracic radiographs showed no significant abnormalities. The echocardiography results showed that the dog had a mild degree of mitral regurgitation (MR) (flow rate: 5.25 m/s) and a mild degree of tricuspid regurgitation (TR) (flow rate: 2.84 m/s). Its pulmonary artery systolic pressure reached 37.2 mmHg. The left and right ventricles had delayed diastolic dysfunction, and the E-wave deceleration time (early filling deceleration time, EDT) was prolonged to 200 ms in the tricuspid valve. The initial diagnosis was that tricuspid hypertrophy caused the diastolic tricuspid opening to become smaller.

The drugs of pimobendan (0.2 mg/kg), furosemide (1.5 mg/kg), and enalapril (0.5 mg/kg) were administered twice a day. The dog was also fed twice a day with low-molecular-weight fucoidan (60 mg/kg) and fucoxanthin (60 mg/kg).

After 4 months of treatment, the physical examination showed that the dog's SBP was stable between 120 and 125 mmHg. The blood biochemistry analysis showed that the values of HGB, PCV, RBC, MCV, RDW, and PLT returned from abnormal after nutrient supplementation and drug treatment. All of the data were within normal limits, except MCHC, which reduced from 39.5 to 38.5 g/dL.

No significant changes were shown on the standard thoracic radiographs after treatment. The echocardiographic data showed that mitral regurgitation had improved and converted to physiological reflux (reflux flow only in the early stage of contraction). Fucoidan and fucoxanthin improve that mitral insufficiency causing blood to flow from the left ventricle to the left atrium significantly (Figure 3E and 3F), and tricuspid regurgitation had significantly improved (Figure 5). The deceleration time of the EDT had improved from 200 to 108 ms.

## Case 4 (Drugs only)

A 14-year-old neutered male Maltese weighing 5.6 kg was referred for a 1-year history of heart disease, cough, and poor spirit. A physical examination showed that its SBP was 153 mmHg, its DBP was 98 mmHg, and its MAP was 116 mmHg. The dog's blood pressure (157 bpm) was measured by a petMAP™ graphic II blood pressure measurement device. A grade IV/VI left apical systolic heart murmur consistent with the mitral insufficiency heart murmur pattern was diagnosed.

The standard thoracic radiographs showed signs of mild pulmonary edema around the heart. The left atrium, left atrial appendage, and left ventricle were significantly enlarged. The left atrium repressed the trachea and bronchi branches, and the VHS index value was 10.6.

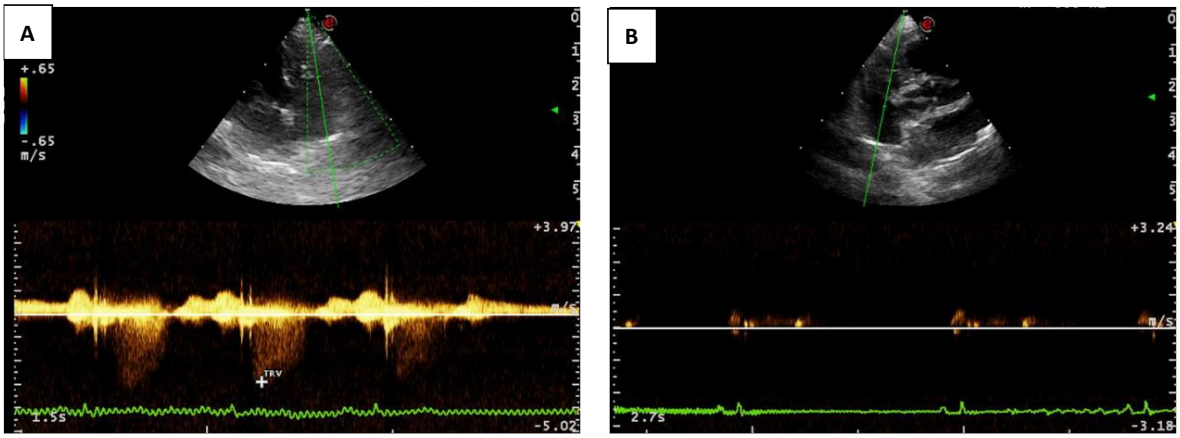


**Figure 5.** Effect of fucoidan and fucoxanthin on tricuspid regurgitation: (A) before fucoidan and fucoxanthin treatment in case 3 and (B) after fucoidan and fucoxanthin treatment in case 3.

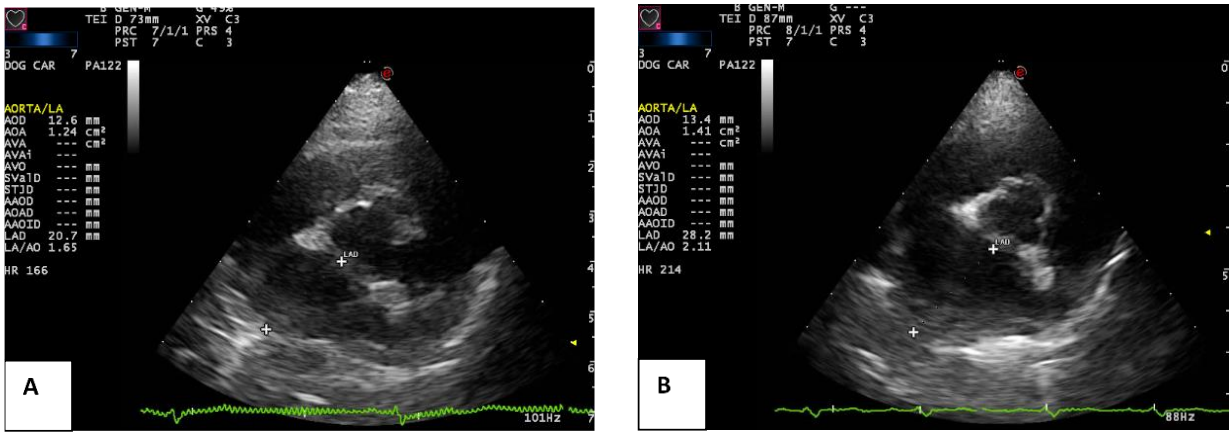


**Figure 6.** The left atrium to aorta ratio (LA/Ao ratio) after nine months of drug treatment: (A) before drug treatment in case 4 and (B) after drug treatment in case 4.

Echocardiography was performed using Esaote's MyLab™ClassC® (Italy) equipped with a PA-122 probe cardio phased array (frequency range of 3–8 MHz). The LA/Ao ratio was measured on B-mode images acquired from a short axis five-chamber view of the right sternum wall. The LA/Ao ratio was 1.65. The hemodynamic parameters relating to mitral and tricuspid valves were measured using a pulsed wave Doppler (PW), a continuous wave Doppler (CW), and tissue Doppler imaging (TDI). This male Maltese had left ventricular pseudonormal diastolic dysfunction, right ventricular delay diastolic dysfunction, mitral regurgitation (MR) reflux with a flow rate of 5.84 m/s along with a dense complete high echo pattern, and a mild degree of tricuspid regurgitation (TR) reflux with a flow rate of 1.22 m/s along with a low-density incomplete echo pattern.

The drug treatment included pimobendan (0.2 mg/kg), furosemide (1.5 mg/kg), and enalapril (0.5 mg/kg). The drugs were administered twice a day. The results were checked after 6 and 9 months of treatment. The physical examination showed that its SBP was 161 mmHg, its DBP was 101 mmHg, its MAP was 119 mmHg, and its HR was 153 beats/min (3.5cm/Tail/PetMAP). The blood biochemistry analysis showed that the values of MCH, MCHC, RDWa, and PLT were still abnormal after drug treatment. Specifically, MCH was elevated from 25.8 to 26.1%, MCHC was elevated from 37.8 g/dL to 38.4 g/dL, RDWa was elevated from 58.9 to 60.8 fl, and PLT was elevated from 705 x 1000 to 870 x 1000 ul. The standard thoracic radiographs showed that the VHS index value had increased from 10.6 to 11.1 after 6 months and then increased to 11.6 after 9 months. The echocardiographic data showed that the LA/Ao ratio had increased from 1.65 to 1.71 after 6 months and then increased to 2.11 after 9 months (Figure 6A and 6B). The left ventricular diastolic function was still maintained in a pseudonormal diastolic

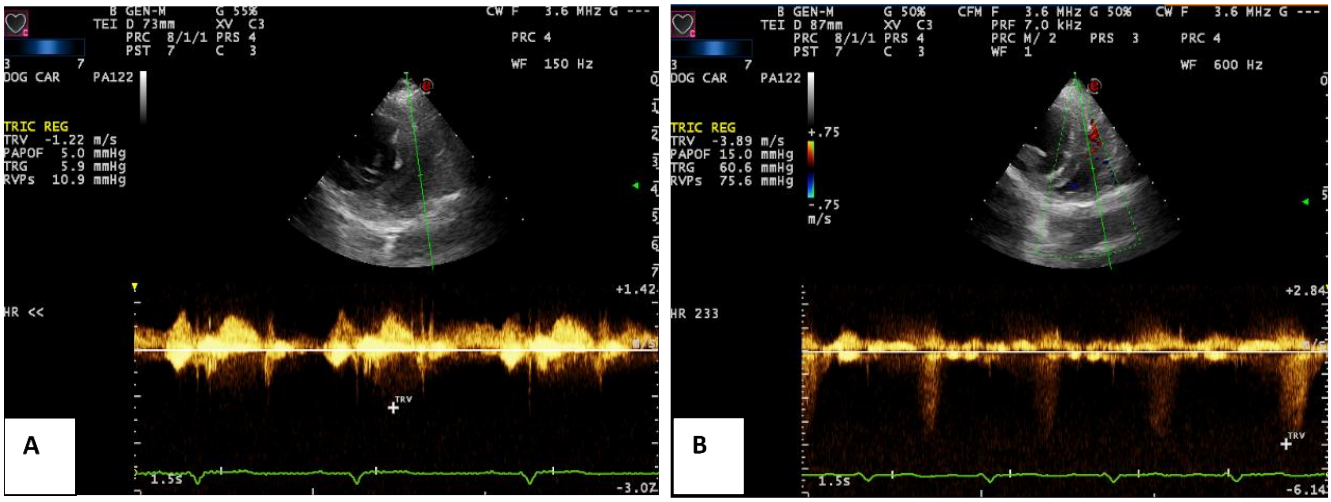


**Figure 7.** The wave area of tricuspid regurgitation: (A) before drug treatment in case 4 and (B) after drug treatment in case 4.

dysfunction. Mitral regurgitation still had a high-density (brightness) full-wave appearance. The blood flow velocity of the tricuspid regurgitation had deteriorated from 1.22 to 3.89 m/s (the pressure gradient was calculated according to the simple Bernoulli equation) (Figure 7A and 7B). The pulmonary artery systolic pressure had reached 75 mmHg.

## DISCUSSION

The traditional treatment strategy for degenerative heart valve disease (DHVD), focuses on excessive or inappropriate neurohormone compensation to inhibit or regulate the reduction of the cardiac load in order to increase tissue blood perfusion, prolong the lives of animals, and improve quality of life. The initiating factors of DHVD are currently unclear, but the pathological phenomenon of DHVD appears to be associated with genetic factors or aging, and certain abnormalities of extracellular matrix components have been suggested to elevate the susceptibility of small-breed dogs to DHVD (Lee et al., 2019). Clinically, valve deformation, valve hypertrophy, valve prolapse, and valve insufficiency are considered important indicators to improve DHVD in dogs (Borgarelli and Buchanan, 2012).

The current study focused on four cases of DHVD in elderly small-breed dogs. A physical examination, serum biochemical laboratory diagnosis (including hematology test, liver function test), chest X-ray, and cardiac ultrasound were carried out by an experienced veterinarian. Based on the pulmonary artery systolic pressure value, Case 1 was diagnosed with a high degree of DHVD and poor prognosis before fucoidan and fucoxanthin treatment. However, intervention with nutritional supplements reversed the results, not only improving the prognosis of Case 1, but also prolonging the survival rate. In contrast, Case 4 was not treated with nutritional supplements, and there was no improvement in heart valve indicators.

According to ACVIM's guidelines for treatment of heart disease and heart failure at all stages, certain drugs, such as angiotensin converting enzyme inhibitors (ACEIs), pimobendan and furosemide, are often used, except for in the first stage (Keene et al., 2019). The confirmed heart disease stages in this study were Stage C (Case 1) and Stage B1 (Case 2 and Case 3) based on the ACVIM heart disease classification (Atkins et al., 2009; Dickson et al., 2017; Menciotti et al., 2018). Therefore, Cases 1 to 3 used the same medication combined with fucoidan and fucoxanthin (as a nutritional supplement). After 4 to 6 months of continuous treatment, significant improvement was revealed in the heart valve appearance and hemodynamic parameters. To verify that the supplements are indeed effective, Case 4 (Stage C) only received medicinal treatment. The results, in this case, were similar to the progression of general heart valve disease, with the condition deteriorating over time. In this condition, due to continuous mitral regurgitation, the left atrial pressure and volume continue to rise. Left heart failure causes secondary pulmonary hypertension. The regurgitation velocity of tricuspid insufficiency also increases, which may aggravate the congestive heart failure and increase the risk of death.

Fucoidan has been reported to have cardioprotective effects, and a previous study showed that low-molecular-weight fucoidan (LMWF) prevented intimal lesion and myofibroblastic parenchymal remodelling in a heterotopic cardiac transplantation rat model (Alkhatib et al., 2006). In addition, a previous study showed that low-molecular-weight fucoidan and high-stability fucoxanthin improved the heart function of an aging mouse (Chang et al., 2019). The authors found significant improvements in cardiac

morphology and muscular function after the aging mice were fed with fucoidan alone or fucoidan supplemented with fucoxanthin. Also, both of them showed the potential to reverse QT interval prolongation, action potential impairment, cardiac hypertrophy, and cardiac fibrosis decline, as well as decreasing the concentration of reactive oxygen species.

Compared with the case of co-administered supplements, medication alone does not seem to significantly improve the symptoms caused by the DHVD. This proves that fucoidan and fucoxanthin have the potential to improve the function of heart and relieve the symptoms of DHVD in dogs. However, due to the limited number of cases, the findings are not statistically significant. It is therefore necessary to continue to collect more cases to better understand the effects of fucoidan and fucoxanthin on DHVD, with the hope that this nutritional supplement could help in the treatment of DHVD in the future.

## Ethical approval and consent to participate

The present case report does not include experimental data and all further investigations were performed as routine diagnostics during the clinical outbreak. Therefore, animal ethics committee and consent to participate in approval were not necessary. The study was intended to improve the welfare of animals, and only important diagnostic procedures were conducted.

## COMPETING INTERESTS

The authors declare that they have no competing interests.

## REFERENCES


Adams, D. S., Marolf, A. J., Valdés-Martínez, A., Randall, E. K., & Bachand, A. M. (2017). Associations between thoracic radiographic changes and severity of pulmonary arterial hypertension diagnosed in 60 dogs via Doppler echocardiography: A retrospective study. *Veterinary radiology & ultrasound: the official journal of the American College of Veterinary Radiology and the International Veterinary Radiology Association*, 58(4), 454-462.

Alkhatib, B., Freguin-Bouilland, C., Lallemand, F., Henry, J. P., Litzler, P.-Y., Marie, J. P., Richard, V., Thuillez, C., &Plissonnier, D. (2006). Low molecular weight fucan prevents transplant coronaropathy in rat cardiac allograft model. *Transplant Immunology*, 16(1), 14-19.

Atkins, C., Bonagura, J., Ettinger, S., Fox, P., Gordon, S., Haggstrom, J., Hamlin, R., Keene, B., Luis-Fuentes, V., &Stepien, R. (2009). Guidelines for the diagnosis and treatment of canine chronic valvular heart disease. *Journal of Veterinary Internal Medicine*, 23(6), 1142-1150.

Aupperle, H., &Disatian, S. (2012). Pathology, protein expression and signaling in myxomatous mitral valve degeneration: comparison of dogs and humans. *Journal of Veterinary Cardiology: The Official Journal of the European Society of Veterinary Cardiology*, 14(1), 59-71.

Borgarelli, M., & Buchanan, J. W. (2012). Historical review, epidemiology and natural history of degenerative mitral valve disease. *Journal of Veterinary Cardiology*, 14(1), 93-101.

Boswood, A., Häggström, J., Gordon, S. G., Wess, G., Stepien, R. L., Oyama, M. A., Keene, B. W., Bonagura, J., MacDonald, K. A., Patteson, M., Smith, S., Fox, P. R., Sanderson, K., Woolley, R., Szatmári, V., Menaut, P., Church, W. M., O'Sullivan, M. L., Jaudon, J. P., Kresken, J. G., Rush, J., Barrett, K. A., Rosenthal, S. L., Saunders, A. B., Ljungvall, I., Deinert, M., Bomassi, E., Estrada, A. H., Fernandez Del Palacio, M. J., Moise, N. S., Abbott, J. A., Fujii, Y., Spier, A., Luethy, M. W., Santilli, R. A., Uechi, M., Tidholm, A., & Watson, P. (2016). Effect of Pimobendan in Dogs with Preclinical Myxomatous Mitral Valve Disease and Cardiomegaly: The EPIC Study-A Randomized Clinical Trial. *Journal of veterinary internal medicine*, 30(6), 1765-1779.

Chang, P. M., Li, K. L., & Lin, Y. C. (2019). Fucoidan-fucoxanthin ameliorated cardiac function via IRS1/GRB2/ SOS1, GSK3β/CREB pathways and metabolic pathways in senescent mice. *Mar Drugs*, 17(1).

Dickson, D., Caivano, D., Matos, J. N., Summerfield, N., & Rishniw, M. (2017). Two-dimensional echocardiographic estimates of left atrial function in healthy dogs and dogs with myxomatous mitral valve disease. *Journal of Veterinary Cardiology: The Official Journal of the European Society of Veterinary Cardiology*, 19(6), 469-479.

Fox, P. R. (2012). Pathology of myxomatous mitral valve disease in the dog. *Journal of Veterinary Cardiology: The Official Journal of the European Society of Veterinary Cardiology*, 14(1), 103-126.

Keene, B. W., Atkins, C. E., Bonagura, J. D., Fox, P. R., Häggström, J., Fuentes, V. L., Oyama, M. A., Rush, J. E., Stepien, R., & Uechi, M. (2019). ACVIM consensus guidelines for the diagnosis and treatment of myxomatous mitral valve disease in dogs. *Journal of Veterinary Internal Medicine*, 33(3), 1127-1140.

Lee, C. M., Song, D. W., Ro, W. B., Kang, M. H., & Park, H. M. (2019). Genome-wide association study of degenerative mitral valve disease in Maltese dogs. *Journal of Veterinary Science*, 20(1), 63-71.

Li, C., Gao, Y., Xing, Y., Zhu, H., Shen, J., & Tian, J. (2011). Fucoidan, a sulfated polysaccharide from brown algae, against myocardial ischemia–reperfusion injury in rats via regulating the inflammation response. *Food and Chemical Toxicology*, 49(9), 2090-2095.

Ljungvall, I., Rishniw, M., Porciello, F., Ferasin, L., & Ohad, D. G. (2014). Murmur intensity in small-breed dogs with myxomatous mitral valve disease reflects disease severity. *The Journal of Small Animal Practice*, 55(11), 545-550.

Matsumoto, M., Hosokawa, M., Matsukawa, N., Hagio, M., Shinoki, A., Nishimukai, M., Miyashita, K., Yajima, T., & Hara, H. (2010). Suppressive effects of the marine carotenoids, fucoxanthin and fucoxanthinol on triglyceride absorption in lymph duct-cannulated rats. *European Journal of Nutrition*, 49(4), 243-249.

Menciotti, G., Borgarelli, M., Aherne, M., Camacho, P., Häggström, J., Ljungvall, I., Lahmers, S. M., &Abbott, J. A. (2018). Comparison of the mitral valve morphologies of

Cavalier King Charles Spaniels and dogs of other breeds using 3D transthoracic echocardiography. *Journal of Veterinary Internal Medicine*, 32(5), 1564-1569.

Misbach, C., Pey, P., Gouni, V., Trehiou-Sechi, E., Petit, A. M., Pouchelon, J., &Chetboul, V. (2016). Antemortem diagnosis of a left auricular appendage herniation through a partial pericardial defect in a dog with degenerative mitral valve disease. *Schweizer Archiv fur Tierheilkunde*, 158(5), 351-356.

Thomes, P., Rajendran, M., Pasanban, B., & Rengasamy, R. (2010). Cardioprotective activity of Cladosiphon okamuranus fucoidan against isoproterenol induced myocardial infarction in rats. *Phytomedicine: International Journal of Phytotherapy and Phytopharmacology*, 18(1), 52-57.