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Research Article
Fucoidan and Fucoxanthin Ameliorate Cardiac Function of Aging Canine

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Abstract

Background and Objective: Aging process has been known to associated with cardiac abnormality. Fucoidan and fucoxanthin have been suggested as safe supplement. In this study, fucoidan in combination with fucoxanthin were used to enhance the impaired cardiac systolic and diastolic function in aging canine model. **Materials and Methods:** Aging canines were fed with fucoidan (125 mg kg^{-1}) and fucoxanthin (125 mg kg^{-1}) for 3 months. Echo cardiography was used to compute Tei index and related parameter such as E / E`, E` / A` and isovolumetric relaxation time (IVRT), isovolumetric contraction time (IVCT) was calculated using the Doppler model. T-Test was used to compare echo parameters. Data were displayed as Mean±SD. SPSS was used for statistical analysis. **Results:** IVRT and IVCT were shortened with 15.78±3.71 and 24.53±6.71%, respectively after fucoidan and fucoxanthin treatment, suggested that these two phytochemical compounds have cardioprotective effects in aging canine subjects with degenerative valve disease. **Conclusion:** After 3 consecutive months of Fucoidan and fucoxanthin usage, the cardiac function of aging canine was improved. Fucoidan and fucoxanthin may be considered as a potential treatment or supplement to aging canine subjects with abnormality in cardiac function.

Key words: Fucoidan, fucoxanthin, aging canine, cardiac functions, isovolumetric

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The heart has a crucial role in systemic circulation but early studies of heart failure have only focused on the cardiac systolic function as a potential pathogenetic mechanism of heart failure. Previous studies show that systolic function of the cardiac system is associated with severe abnormality of the heart[1-3]. Recently, gene therapy treatment for improving systolic function has been developed for patients with myocardial infarction[4]. However, the cardiac diastolic function has not been quantified and standardized so potential relevant assessment is often overlooked. In recent years, in some heart failure cases, the left ventricle still has systolic function, so the impact of diastolic function to heart failure is drawing more attention[5]. In human medicine, diastolic heart failure was widely defined in 1998 as a condition in which the left ventricle retains normal systolic function but has an abnormal diastolic function in patients with congestive heart failure[6].

Doppler echocardiography is currently the most widely used technique to assess both systolic and diastolic function. It provides information of anatomical and diastolic functions. It is noninvasive, safe and clinically easy to operate at reasonably price. Pulsed wave Doppler and tissue Doppler imaging are often used in clinical practice to calculate diastolic function. Traditional dog ultrasound routine examination program focuses on assessment of left ventricular systolic function. The main measurement of left ventricular diameter, septum and posterior wall thickness, diameter of the aorta and left atrium were recorded to provide systolic function parameters of the operation[7]. Assessment of left ventricular systolic function also includes measurement of left ventricular ejection fraction, shortening fraction, average muscle circumference shortening rate, stroke volume, injection time and other parameters.

Study has shown that the severity of diastolic dysfunction is associated with an increase in mean left ventricular mass index (LVMI) and mean LV volume index (LVVI). Ventricular hypertrophy may affect diastolic function, which in turn increases left atrial pressure and enlarges the left atrium[8]. Left ventricular diastolic function assessment has been a reliable indicator for cardiac dysfunction according to the European guidelines[9]. In the veterinary field, the clinical application of LVVI, LVMI and its ratio has not been evaluated.

Clinical diagnosis and treatment of diastolic heart failure in dogs are not fully explored to establish a consensus on these procedures. The purpose of this study is to compare the parameters of cardiac echocardiography examination, LVVI, LVMI and its ratio to the traditional diastolic function index items of aging canine subjects. These parameters can be used as indicators of canine with diastolic heart failure disease. Tei index can be integrated in the assessment of ventricular diastolic and systolic function of the cardiac echocardiography index, also known as myocardial performance index (MPI)[10,11]. In canine subjects, common symptoms are often in the ventricle (heart failure) such as ventricular volume increase and ventricular deformation (volume and mass increase). It is important to assess early systolic and diastolic function in order to prevent heart disease in the older canine population.

Fucoidan has been identified first by Kylin[12]. Fucosylated polysaccharide, a sulfated phytochemical compound, is found in many species of brown seaweed[13,14]. The biological and chemical properties of fucoidan varying according to specific species of brown seaweed and extraction method[15]. Fucoidan has been well studied for their antitumor[16], antiviral[17], anti-inflammatory[18] and anticoagulant[19] activities. These biological activities are closely related to their molecular weight[20] and sulfate content[21]. Low molecular weight (LMW) fucoidan possesses higher bioactivities than that of high molecular weight (HMW) fucoidan[22]. In a recent study, LMW fucoidan, <30 kDa, was revealed to stimulate osteogenic differentiation *in vitro* and had an anabolic effect on bone mineralization *in vivo*[23]. Moreover, it also showed potential effects on bone biomaterial osteoconductive properties[24]. Low molecular weight (LMW) fucoidan possesses higher pharmacological attributes than high molecular weight (HMW) fucoidans[22]. Many studies have been conducted to show the cardioprotective effect of fucoidans[25,26]. In the current study, a combination of small molecular fucoidan and highly stable fucoxanthin was applied to treat aging dogs with cardiac function abnormality. The diastolic function, the diastolic blood flow and the velocity of the mitral annulus was examined to compare before and after treatment.

MATERIALS AND METHODS

Experimental design: This is the first study using aging canine with degenerative valve disease as model for examining the cardioprotective effect of fucoidan and fucoxanthin. About 10-14 years old (6males and 6female) dogs with degenerative valve disease were collected from Taipei City Board of You Kang Animal Hospital since December 2015 with owner agreement for experimental purpose only.

The subjects are classified into four grades based on their clinical symptoms according to the NYHA Heart Failure Classification Criteria[27]. Complete physical examination

including visual inspection, palpation, auscultation, observation of their movement and mental status was recorded. The heart rate, respiratory rate and body temperature were also measured before the treatment. All tests were conducted in a quiet environment to reduce the effect of stress on the values.

The subjects were fed with low molecular weight fucoidan (125 mg/5 kg) (Hi-Q Oligo-Fucoidanå) and highly stable fucoxanthin (HS-Fucox)(125 mg/5 kg) with alginate adjuvant therapy for 3 consecutive months. Fucoidan and fucoxanthin, derived from *Sargassum hemiphyllum* and were prepared by Hi-Q Marine Biotech International Ltd. (New Taipei City, Taiwan).

After detail medical history checkup, animal subjects were continuously monitored for cardiac electrocardiographic measurement. Echocardiography parameters before and after the administration of fucoidan and fucoxanthin are examined and compared to investigate which of these two small molecules could contribute to delaying the effects of cardiac aging.

The study recorded the dogs' basic information including: Age, sex, weight, eating habits, birth control, vaccination, the time of prevention of parasites *in vivo* and *in vitro*. Recent mental, appetite and urination status were also confirmed for movement intolerance, respiratory symptoms or mucosal tongue cyanosis. Frequency, time and severity of the degree examination have also been obtained. Medical and other treatments of disease changes during the study period were also recorded.

Experimental apparatus and equipment: The facilities used in this study were from the Hospital of Yukang Animal Care. They were maintained every year to ensure there liability of research data. The same instruments were used for both examinations before and after the experiment.

Animal blood pressure was measured by Pet MAP® blood pressure measurement device. Nihon kohden ECG 9020K® was used in this study to record the ECG. Medonic CA620-15® (Sweden) was used in the study to count CBC. Spotchem™SP-4410 (Japan) was used to measure the plasma biochemistry items in the study. Spotchem™SE-1510 (Japan) was used in this study to measure the Na^+, K^+ and Cl^-. GTR® Vet Rad system (USA) and KONICA REGIUS MODEL 110S® (Japan) were used in this study to obtain the lateral and ventral-dorsal chest images. Seven Esaotemylab class C® (Italy) and PA-122 probe Companion Animals, Cardio Phased Array -16 mm - 8-3 MHz were used in this study to obtain all the echocardiographic data.

The cardiac echocardiography parameters of each subject were repeated 3 times. Then, the relative values of Tei index, diastolic function, systolic function and other parameters of the dogs were compared after feeding for 3 months with fucoidan, fucoxanthin and alginin.

Cardiac echocardiography: The left ventricle (5 chambers) and the minor axis were scanned. Then, the left side of the testis was placed on the right side of the sternum. The probe was placed on the left side of the sternum position for the apical four-chamber, five-chamber and two-chamber cross-sectional scans. The parameters required for the study were measured using B mode, M mode, pulsed Doppler and tissue Doppler measurements.

Left sternal apical long axis of each chamber scan: When scanning section on the left side of the sternum, the test dog was left lying, slightly lifting the front legs. The probe was placed in between the left rib apex in B-mode scanning. The sound beam was perpendicular to the long axis parallel to the long axis of the heart. The apex in the top of the screen and the heart at the bottom of the screen shown the long axis of four chamber cross-sectional images (Fig. 1). The endometrium, wall thickness, papillary muscle, chordal tendon, valve structure abnormal thickening, irregular-like, nodular hyperplasia, echo changes or valve prolapse and other changes were recorded. After that, blood Doppler model was used to observe whether there is spoiler, valve counter-current or thrombosis. The diastolic function indexes such as E / E`, E` / A`, isovolumetric relaxation time (IVRT) and isovolumetric contraction time (IVCT) were calculated using the Doppler model (Fig. 2).

Statistical analysis: T-test was used to compare two data of echo parameters. Data were displayed as Mean±SD. $p<0.05$ means there is statistically significant difference between before and after treatment data of the same subject. This was performed by SPSS Version 20.

RESULTS

Both IVRT and IVCT of aging subjects were shortened with 15.78±3.71 and 24.53±6.71% relative to before fucoidan and fucoxanthin treatment, respectively. These data suggesting both diastolic and systolic functions were improved after treatment with fucoidan and fucoxanthin. LVET was prolonged 7.1±1.4% compared to before treatment. Increase in E`and decrease in A` velocity suggesting

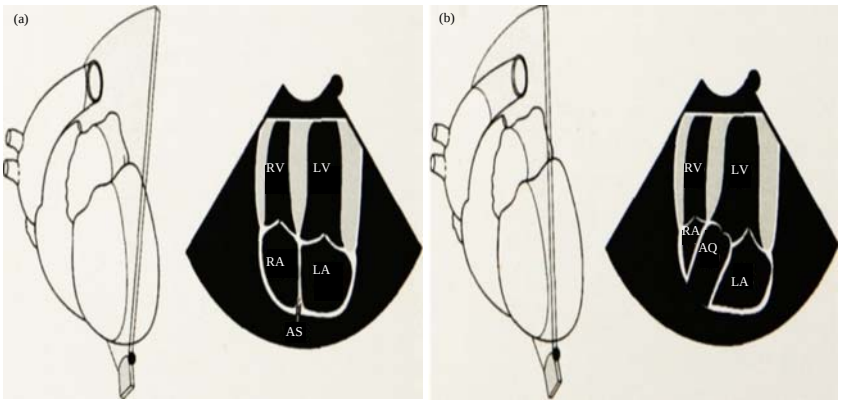

Fig. 1(a-b): Left caudal (apical) parasternal location, four-chamber views, (a) Four chamber view and (b), Five chamber (LV outflow) view

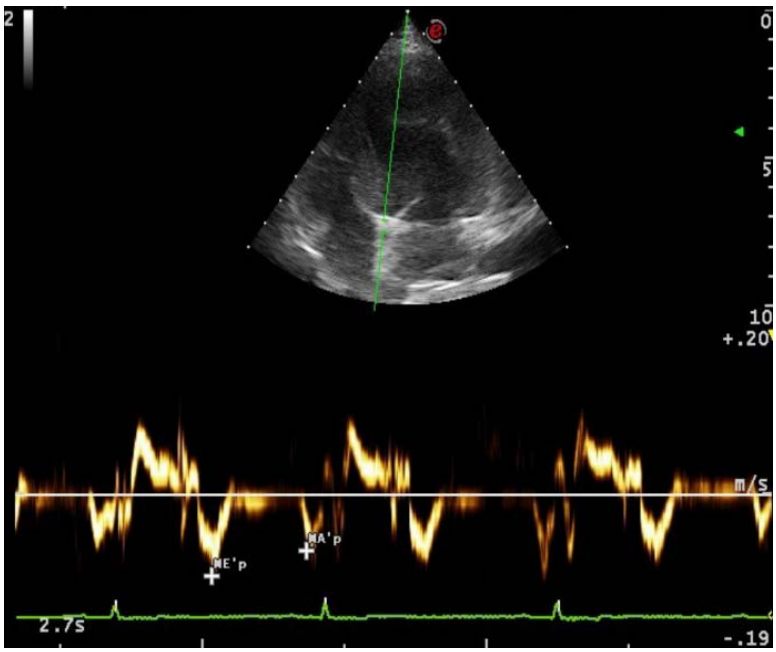

Fig. 2: Left parasternal four-chamber view with tissue Doppler. The cursor is placed at mitral valve annule for imaging tissue Doppler to measure the peak

IVRT: Isovolumetric relaxation time, IVCT: Isovolumetric contraction time, LVET: Left ventricular ejection time, E`: E wave velocity, A`: A wave velocity, E/E`: The velocity ratio between E wave and mitral septal annular tissue E`, E`/A`: Ratio of mitral septal annular tissue velocity E` to A`. Mean±SD

improvement in diastolic function. E`/A` ratio increased (improved diastolic function in the case of the absolute value of the rising wave E` (such as a decline compared to the same time E` and A` row limit evidence of diastolic function deterioration). E / E` as the ratio of mitral diastolic blood flow and tissue E wave velocity loop of the base, the greater the value of the former representative of load (or and left atrial pressure) is higher, the worse diastolic function (Fig. 3).

The IVRT and IVCT and Tei index were the most significant $p<0.05$ improvements. The IVCT of systolic function and the Tei index of global myocardial performance showed significant improvement $p<0.05$ of cardiomyocyte potency, cardiac function and contraction (Fig. 3).

In this study, the subjects have age distribution between 10-14 years with varying degrees of degenerative myxoid valve disease and cardiovascular-related drug treatment time of 1.5-4 years. With fucoidan and fucoxanthin treatment, the

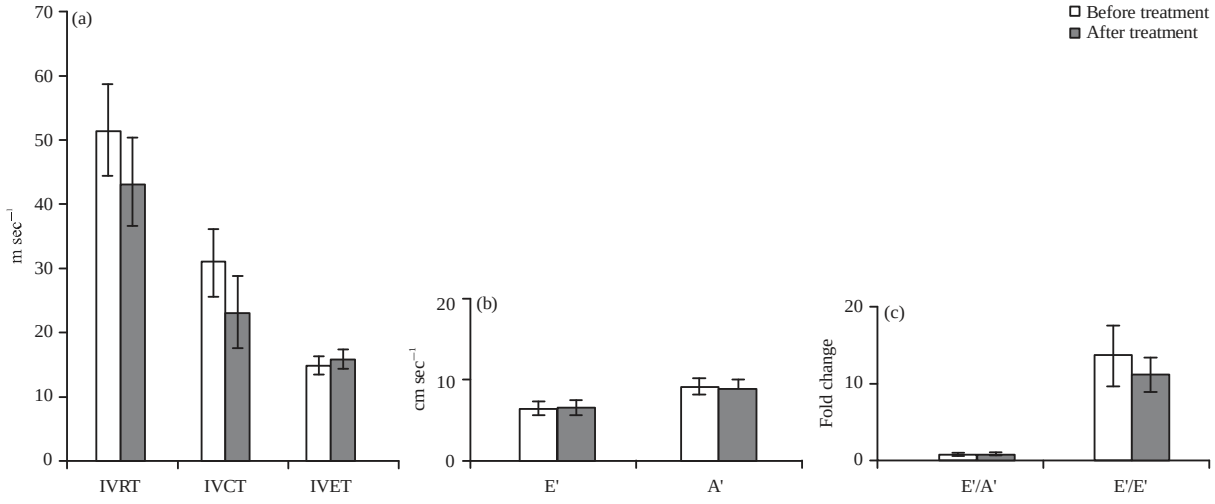

Fig. 3: Mitral annulus basal tissue velocity related parameters

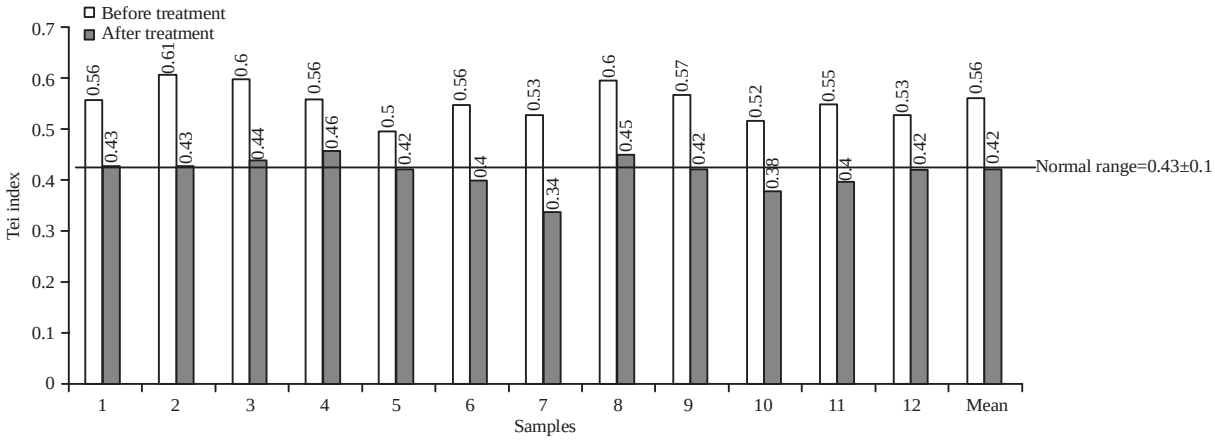

Fig. 4: Tei index before and after treatment of fucoidan and fucoxanthin

data and clinical symptoms, especially the performance of the Tei index showed a very significant improvement (Fig. 4).

DISCUSSION

In this study, shortened IVRT and IVCT of aging canines after 3 months treatment with fucoidan and fucoxanthin were recorded. These data suggest the synergistic effects of fucoidan and fucoxanthin to cardiac function. This is the first study to reveal fucoidan and fucoxanthin effects to aging canine with degenerative valve disease. Tei index with various parameters related to both cardiac systolic and diastolic function has been applied to evaluate the enhancement of cardiac function before and after 3 months treated with the phytochemical compounds in the same subjects. It was found that IVRT and IVCT were the most significant ameliorating parameters by fucoidan and fucoxanthin treatment. The cardioprotective effects of fucoidan from different species of algae have been reported previously[25,26,28,29].

In normal circumstances, the length of IVRT versus IVCT depends on myocardial performance. IVRT represents for an interval of cardiac cycle. It starts from the end of contraction to the atrioventricular valve opening. It can lead to slowing down of relaxation of the heart rate and reducing ventricular pressure. Additionally, lower atrial pressure also caused by atrioventricular valve opening. Many studies have been used isovolumetric relaxation time as an indicator of diastolic dysfunction in heart failure subjects[30-33]. In a recent study, IVRT was significantly increased but not E/A ratio in mice with left ventricular (LV) diastolic dysfunction induced by high-fat, high-sucrose diet, which suggested the importance of IVRT in cardiac diastolic function[34]. In this study, after oral feeding with fucoidan and fucoxanthin, the IVRT was significantly $p<0.05$ shortened which

demonstrating the effect of these extracts in rescuing the diastolic function of aging canines.

A more in-depth study of left ventricular diastolic dysfunction can be interpreted as an abnormal expansion of the myocardium. Two major abnormalities are diastolic and impaired relaxation ability (common delay). They could be caused by development of hypertension which could results in heart failure. Consequently, left ventricular myocardium compensatory mechanism would involve in these physiological disorders. Human medical research shown that more than half of hypertensive patients with diastolic dysfunction. It confirmed that ventricular diastolic abnormalities in the elderly population of heart failure and sudden death are of the independent cause. In heart failure patients, assessment of left ventricular systolic and diastolic function revealed normal systolic function but diastolic dysfunction. Thus, additional research and analysis should be focused on left ventricular diastolic function[8,35].

IVCT is an interval of cardiac cycle. It starts in early systole during ventricular contraction. IVCT can be used to access for left ventricular performance. Therefore, it is critical to measure this parameter in aging canine. Left ventricle injection time (LVET) shows a negative correlation with the rhythm. LVET is reciprocal to Tei index, so the greater the LVET value, the worsen the myocardial performance[36-38]. Vogel *et al.*[39] has reported that isovolumetric contraction can be used as novel indicator of acute changes of right ventricular function. From the present study, fucoidan and fucoxanthin could be used as supplement for aging subject with prolonged IVRT and IVCT. Fucoidan and fucoxanthin can be used in further study to investigate the underlying mechanism of cardioprotective effect in other animal models with different cardiac abnormality. This study also set standard concentration for combination of fucoidan and fucoxanthin at 125 mg/5 kg of each compound. However, this study also remains potential caveats such as *in vitro* study with small sample size.

CONCLUSION

It is concluded that examination of the effects of small molecular fucoidan combined with high-stability fucoxanthin to cardiac function after 3 months treatment shown improvement in of aging dogs heart by shorter IVRT and IVCT value. Tei index and parameters related to diastolic and systolic function were improved quantitatively.

SIGNIFICANCE STATEMENT

This study discovers the cardioprotective effects of fucoidan and fucoxanthin extracted from brown algae to the aging canine with degenerative valve disease. The present results can be beneficial for the treatment of aging subject with impaired cardiac function. This study will help the researcher to reveal the critical roles of marine product supplement to cardiac function. Therefore, marine product such as fucoidan and fucoxanthin may be applied to cardiac function abnormality study.

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REFERENCES

1. Vanhoutte, L., H. Esfahani, O. Feron, J.L. Balligand, G. Bernard and S. Moniotte, 2014. 0331: Left ventricular systolic function assessment in a mouse model of cardiac hypertrophy using an 11.7T MRI scanner and comparison with *ex-vivo* measurements. Arch. Cardiovasc. Dis. Suppl., 6: 62-62.
2. Hirose, A., Y. Maeno, T. Horinouchi, Y. Kozuma, D. Hori, K. Suda and O. Iwata, 2015. OP21.09: Assessment of fetal cardiac systolic function using value of annular M-mode displacement versus tissue Doppler velocities. Ultrasound Obstet. Gynecol., 46: 119-119.
3. Kraigher-Krainer, E., A.M. Shah, D.K. Gupta, A. Santos and B. Claggett *et al*., 2014. Impaired systolic function by strain imaging in heart failure with preserved ejection fraction. J. Am. Coll. Cardiol., 63: 447-456.
4. Fattah, C., K. Nather, C.S. McCarroll, M.P. Hortigon-Vinagre and V. Zamora *et al*., 2016. Gene therapy with angiotensin-(1-9) preserves left ventricular systolic function after myocardial infarction. J. Am. Coll. Cardiol., 68: 2652-2666.
5. Chi-Hung, C., 2012. Evaluation of left ventricular diastolic function in maltese dogs. Department of Veterinary Medicine, Pingtung University of Science and Technology, pp: 1-76.
6. Paulus, W.J., D. Brutsaert, T. Gillebert, F. Rademarkers, S. Sys and A. Leite-Moreira, 1998. How to diagnose diastolic heart failure. European Study Group on diastolic heart failure. Eur. Heart J., 19: 990-1003.
7. Kienle, R.D. and W.P. Thomas, 2002. Echocardiography: Mitral Valve Dysplasia. In: Small Animal Diagnostic Ultrasound, Nyland, T.G. and J.S. Mattoon (Eds.). 2nd Edn., W. B. Saunders, Philadelphia, ISBN: 9780721677880, pp: 393-394.
8. Redfield, M.M., S.J. Jacobsen, J.C. Burnett Jr., D.W. Mahoney, K.R. Bailey and R.J. Rodeheffer, 2003. Burden of systolic and diastolic ventricular dysfunction in the community: Appreciating the scope of the heart failure epidemic. J. Am. Med. Assoc., 289: 194-202.

9. Paulus, W.J., C. Tschope, J.E. Sanderson, C. Rusconi and F.A. Flachskampf *et al*., 2007. How to diagnose diastolic heart failure: A consensus statement on the diagnosis of heart failure with normal left ventricular ejection fraction by the Heart Failure and Echocardiography Associations of the European Society of Cardiology. Eur. Heart J., 28: 2539-2550.
10. Pellett, A.A., W.G. Tolar, D.G. Merwin and E.K. Kerut, 2004. The Tei index: Methodology and disease state values. Echocardiography, 21: 669-672.
11. Tei, C., K.S. Dujardin, D.O. Hodge, K.R. Bailey, M.D. McGoon, A.J. Tajik and J.B. Seward, 1996. Doppler echocardiographic index for assessment of global right ventricular function. J. Am. Soc. Echocardiogr., 9: 838-847.
12. Kylin, H., 1913. Zur biochemie der meeresalgen. Biol. Chem., 83: 171-197.
13. Ponce, N.M.A., C.A. Pujol, E.B. Damonte, M.L. Flores and C.A. Stortz, 2003. Fucoidans from the brown seaweed *Adenocystis utricularis*: Extraction methods, antiviral activity and structural studies. Carbohydr. Res., 338: 153-165.
14. Zhuang, C., H. Itoh, T. Mizuno and H. Ito, 1995. Antitumor active fucoidan from the brown seaweed, *Umitoranoo* (*Sargassum thunbergii*). Biosci. Biotechnol. Biochem., 59: 563-567.
15. Fitton, J.H., 2011. Therapies from fucoidan; Multifunctional marine polymers. Mar. Drugs, 9: 1731-1760.
16. Hsu, H.Y., T.Y. Lin, P.A. Hwang, L.M. Tseng, R.H. Chen, S.M. Tsao and J. Hsu, 2012. Fucoidan induces changes in the epithelial to mesenchymal transition and decreases metastasis by enhancing ubiquitin-dependent TGFβ receptor degradation in breast cancer. Carcinogenesis, 34: 874-884.
17. Tengdelius, M., C.J. Lee, M. Grenegard, M. Griffith, P. Pahlsson and P. Konradsson, 2014. Synthesis and biological evaluation of fucoidan-mimetic glycopolymers through cyanoxyl-mediated free-radical polymerization. Biomacromolecules, 15: 2359-2368.
18. Hwang, P.A., S.Y. Chien, Y.L. Chan, M.K. Lu, C.H. Wu, Z.L. Kong and C.J. Wu, 2011. Inhibition of Lipopolysaccharide (LPS)-induced inflammatory responses by *Sargassum hemiphyllum* sulfated polysaccharide extract in RAW 264.7 macrophage cells. J. Agric. Food Chem., 59: 2062-2068.
19. Irhimeh, M.R., J.H. Fitton and R.M. Lowenthal, 2009. Pilot clinical study to evaluate the anticoagulant activity of fucoidan. Blood Coagul. Fibrinol., 20: 607-610.
20. Yang, C., D. Chung, I.S. Shin, H.Y. Lee and J.C. Kim *et al*., 2008. Effects of molecular weight and hydrolysis conditions on anticancer activity of fucoidans from sporophyll of Undaria pinnatifida. Int. J. Biol. Macromol., 43: 433-437.
21. Soeda, S., S. Sakaguchi, H. Shimeno and A. Nagamatsu, 1992. Fibrinolytic and anticoagulant activities of highly sulfated fucoidan. Biochem. Pharmacol., 43: 1853-1858.
22. Park, S.B., K.R. Chun, J.K. Kim, K. Suk, Y.M. Jung and W.H. Lee, 2010. The differential effect of high and low molecular weight fucoidans on the severity of collagen-induced arthritis in mice. Phytother. Res., 24: 1384-1391.
23. Hwang, P.A., Y.L. Hung, N.N. Phan, P.M. Chang, K.L. Li and Y.C. Lin, 2016. The *in vitro* and *in vivo* effects of the low molecular weight fucoidan on the bone osteogenic differentiation properties. Cytotechnology, 68: 1349-1359.
24. Changotade, S.I.T., G. Korb, J. Bassil, B. Barroukh and C. Willig *et al*., 2008. Potential effects of a low-molecular-weight fucoidan extracted from brown algae on bone biomaterial osteoconductive properties. J. Biomed. Mater. Res. Part A, 87: 666-675.
25. Hao, S., 2009. Methods of treatment of cardiovascular and cerebrovascular diseases with fucoidan. Patents No. US 20090170801 A1. https://www.google.com/patents/US20090170801.
26. Thomes, P., M. Rajendran, B. Pasanban and R. Rengasamy, 2010. Cardioprotective activity of *Cladosiphon okamuranus* fucoidan against isoproterenol induced myocardial infarction in rats. Phytomedicine, 18: 52-57.
27. Wolk, M.J., S.R. Bailey, J.U. Doherty, P.S. Douglas and R.C. Hendel *et al*., 2014. ACCF/AHA/ASE/ASNC/HFSA/HRS/SCAI/SCCT/SCMR/STS 2013. Multimodality appropriate use criteria for the detection and risk assessment of stable ischemic heart disease. J. Am. Coll. Cardiol., 63: 380-406.
28. Zhang, S.M., Z.P. Xie, M.L. Xu and L.F. Shi, 2015. Cardioprotective effects of fucoidan against hypoxia-induced apoptosis in H9c2 cardiomyoblast cells. Pharm. Biol., 53: 1352-1357.
29. Wang, J., S.Y. Ji, S.Z. Liu, R. Jing and W.J. Lou, 2015. Cardioprotective effect of breviscapine: Inhibition of apoptosis in H9c2 cardiomyocytes via the PI3K/Akt/eNOS pathway following simulated ischemia/reperfusion injury. Pharmazie, 70: 593-597.
30. Vejdani-Jahromi, M., J. Freedman, Y. Joong-Kim, J. Osborn, G. Trahey and P. Wolf, 2016. ARFI ultrasound elastography measures isovolumic relaxation time constant for assessment of diastolic function. Circulation, Vol. 134.
31. Correale, M., A. Totaro, A. Ferraretti, T. Passero and F. de Rosa *et al*., 2014. Peak myocardial acceleration during isovolumic relaxation time predicts the occurrence of rehospitalization in chronic heart failure: Data from the Daunia heart failure registry. Echocardiography, 31: 434-440.
32. Brand, M., M. Christ, J. Roeing, S. Amirie and M. Grett *et al*., 2016. Poster session 2 The imaging examination and quality assessment. Eur. H. J.-Cardiovasc. Imag., 17: ii88-ii94.
33. Olson, J.M., B.A. Samad and M. Alam, 2016. Myocardial performance index determined by tissue doppler imaging in patients with systolic heart failure predicts poor long-term prognosis: An observational cohort study. J. Cardiac Failure, 22: 611-617.

34. Naresh, N.K., Y. Yang, J.W. Holmes, M. Salerno and F.H. Epstein, 2016. High temporal-resolution phase-contrast MRI demonstrates impaired left-ventricular diastolic relaxation in mice fed a high-fat high-sucrose diet. J. Cardiovasc. Magnetic Resonance, Vol. 18. 10.1186/1532-429X-18-S1-P50.
35. Pedersen, F., I. Raymond, L.H. Madsen, J. Mehlsen, D. Atar and P. Hildebrandt, 2004. Echocardiographic indices of left ventricular diastolic dysfunction in 647 individuals with preserved left ventricular systolic function. Eur. J. Heart Failure, 6: 439-447.
36. Teshima, K., K. Asano, K. Iwanaga, H. Koie and M. Uechi *et al*., 2006. Evaluation of right ventricular Tei index (index of myocardial performance) in healthy dogs and dogs with tricuspid regurgitation. J. Vet. Med. Sci., 68: 1307-1313.
37. Sousa, M.G., R. Carareto, A.B. De-Nardi, F.L. Brito, N. Nunes and A.A. Camacho, 2007. Effects of isoflurane on Tei-index of myocardial performance in healthy dogs. Can. Vet. J., 48: 277-282.
38. Baumwart, R.D., K.M. Meurs and J.D. Bonagura, 2005. Tei index of myocardial performance applied to the right ventricle in normal dogs. J. Vet. Internal Med., 19: 828-832.
39. Vogel, M., M.R. Schmidt, S.B. Kristiansen, M. Cheung, P.A. White, K. Sorensen and A.N. Redington, 2002. Validation of myocardial acceleration during isovolumic contraction as a novel noninvasive index of right ventricular contractility: Comparison with ventricular pressure-volume relations in an animal model. Circulation, 105: 1693-1699.