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ECHOCARDIOGRAPHY FINDINGS AFTER INTRAVENOUS INJECTION OF *Achillea millefolium* (YARROW) EXTRACT IN THE DOG

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ABSTRACT

Achillea millefolium (yarrow) has been used for centuries as medicinal plant to treat different disorders in human and in traditional medicine to treat hypertension, diarrhea and shigellosis, heart failure, heart block and chest pain in thrombotic condition. There are no studies done on echocardiography in situ findings from intravenous injection *Achillea millefolium* extract in the dog. Therefore, a study was designed to evaluate echocardiography dynamics from intravenous injection of *A. millefolium* ethanolic leave extract in the male dog. This research was performed on 6 healthy male mongrel (breed) dogs in weight range of 15-30kg and age mean of 3 years. Echocardiography was performed before drug injection and then in times of 0, 60, 120 minutes after injection. Then left ventricular diameters in systole (LVDs), left ventricular diameters in diastole (LVDd), left ventricular septal thickness at end-systole (LVSs), left ventricular free wall systole (LVFWs), left ventricular free wall diastole (LVFWd), stroke volume (SV) and fractional shortening (FS) indices were measured. Mean and standard deviation was measured for each of indices in each period and were analyzed using paired t-test using SPSS as statistical software. SV, FS and EF indices before and 120 minutes after injection in 6 tested dogs showed significant difference statistically. This can be attributed to effect of alkaloids and unknown compounds available in *A. millefolium* on cardiovascular system which initially decreases blood pressure. Consequently, heart rate is increased to compensate blood pressure decreasing by activation of baroreflex and then stroke volume increases because of decreasing in afterload and increasing in preload. Antispasmoic property of compound presented in this plant decreases myocardium contraction power and in result heart fractional shortening is decreased.

Key words: *Achillea millefolium*; Dog; Echocardiography; Fractional Shortening

INTRODUCTION

Medicinal plants have been considered as reliable sources for drug preparation (Sedighi, 2013) and that has enhanced their clinical importance in modern medicine. *A. millefolium* is an old herbal remedy that has been used for different purposes in different cultural groups thus, research on its pharmacological effects has been greatly investigated (Ali *et al*., 2013; Sedighi *et al*., 2013). *A. millefolium* has showed effects on heart and nervous systems and is used in different treatments of fatigue, heart failure, kidney stone and also neurological diseases like neurasthenia, hysteria, epilepsy, hysterogenic colic (Mazandarani *et al*., 2007). This plant extracts have been shown to have anti-inflammatory, antitumor, antimicrobial, liver protective and antioxidant properties (Lin *et al*., 2002; Candan *et al*., 2003).

Huang *et al*. (2010) reported that *A. millefolium* plants have some pharmacological effects such as antispasmodic, antimicrobial, analgesic, antipyretic, choleretic, cytotoxic, and estrogenic. Moreover, phytochemical investigations of *Achillea* species have revealed that many components from this genus are highly bioactive. *Achillea* has a tradition application of dilating the peripheral arteries,

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increase the flow of blood to the surface, modifying the circulation, and thus facilitate to lower blood pressure. It is alleged that these effects are mainly attributed to the flavonoid and phenolcarbonic acid complex (Trumbeckaite *et al*., 2011). There are no studies done on echocardiography *in vivo* findings from intravenous injection *A. millefolium* extract in the dog. Therefore, this study was designed to evaluate echocardiography dynamics from intravenous injection of *A. millefolium* ethanolic leave extract in the male dog.

MATERIAL AND METHODS

All experiments were carried out under ethical guidelines of the Islamic Azad University of Shahrekord Branch, for the care and use of animals. Sonography device used in this research was dupler EX 8000 Madison model made in South Korea designed for medical and veterinary use. This device has different transducers that echocardiography can be performed by Phased Array transducer and with 2-4M-Hz frequency.

Animals and experimental design

Six healthy male mongrel (mixed breed) dogs in weight range of 15-30kg and age mean of 3 years were selected. Vital signs of animals were carefully studied and recorded before starting work and function of their heart and lung were examined by auscultation, and examination was implemented with complete blood count (CBC) and obtaining electrocardiogram (ECG). Initially, heart rate of each dog was recorded before starting echocardiography. In order to study heart function by echo-cardiography, fur on the right side between 3rd-6th intercostal spaces at thorax were completely clipped off and the skin was washed with alcohol to remove surface fats. The animal was transferred to radiology and sonography section after decreasing environmental stresses and providing a calm and dark environment. Normal echocardiography was performed without using chemical and physical restrain and while dog was standing. Two-dimensional method (lighting mode) echo-cardiography pictures were obtained from right side approach in longitudinal axis. After identifying and evaluating cardiac structures, by rotating transducer to *90* degrees in site, pictures in vertical axis were obtained and studied indices were measured and recorded by movement mode method. LVDd, LVDs, LVSs, LVSd, SV, EF, FS, LVfwd, LVfws and HR were the indices measured by the movement mode method. The data obtained before injection with *A. millefolium* was used as the before treatment data. After measuring and recording above indices and recording natural heart rate, 20mg/kg (the rate at which it has been used in other researches) of *A. millefolium* ethanolic leave extract was injected into the animal through *cephalic* vein and heart rate measuring and recording was repeated. Echocardiography was repeated in 0, 60 and 120 minutes times after injection and all intended factors were evaluated and measured. Mean and standard deviation was measured for each of indices in each period and were analyzed using paired t-test using SPSS as statistical software.

Preparation method of *A. millefolium* ethanolic extract

Fifty grams of powdered plant was added to 700 ml of 50% ethanol (350 ml distilled water and 350 ml ethanol) and Soxhlet apparatus was used to prepare hydroethanol extract. The solvent was filtered under reduced pressure. The plant ingredient concentration in the final extract was adjusted to 0.1 g/ml by adding distilled water to the dried extract. The extract was prepared each week and stored in refrigerator (Boskabady *et al*., 2006).

RESULTS

Generally, significant changes were observed in all measured parameters 120 minutes after injection of *A. millefolium* ($P<0.05$). Ejection fraction, fractional shortenings, interventricular septal thickness at end-diastole, left ventricular posterior posterior wall thickness at end-diastole and left ventricular posterior wall thickness at end-systole all increased significantly after injection of *A. millefolium* (Fig. 1 to 4). However, heart rate, stroke volumes, left ventricular internal diameter at end-diastole and left ventricular internal diameter at end-systole showed significant decreasing after the injection of *A. millefolium* (Table 1).

DISCUSSION

The experiment showed significant effects of intravenous injection of *A. millefolium* ethanolic leave extraction echocardiography findings in the male dogs. Achillea contains various bioactive compounds mentioned by Bocevska and Sovov, (2007) achilleine, apigenin, luteolin, azulene, camphor, coumarin, inulin, menthol, quercetin, rutin, succinic, salicylic and caffeic acids. Khoori *et al.* (1999) showed that methanolic extract of this plant could decrease relative activity of atrio-ventricular node.

Effect of apigenin available in *Achillea* causes aortic-endothelium dependent relaxation (Ko *et al*., 1991). Therefore, it is possible that observed anti-contraction effects in this research resulted from apigenin flavonoides existing in *Achillea* extracts. *Achillea* had antispasmodic effect on smooth muscles (Khoori *et al*., 1999). Moreover, there are reports that some chemical compositions which are found in *Achillea* such as apigenin, luteolin and lignans showed vasorelaxant effects (Schussler *et al*., 1995; Woodman *et al*., 2004; Ko *et al*., 2005; Oh *et al*., 2008).

Quercetin is one of the flavonoides which has antihypertensive effect (Qian *et al*., 2010). Consequently, antihypertensive effect of *Achillea* which was also confirmed in this study might partly be due to this negative inotropic and chronotropic effects. *Achillea* showed reduction in pulse pressure indicates reduction of arterial compliance due to arterial relaxation. In many studies, *Achillea* extracts demonstrated the suppression of smooth muscle spasms and decreasing of vascular pressure (Baser, 2008; Peixoto-Neves *et al*., 2010), luteolin (Jiang *et al*., 2005), apigenin (Jin *et al*., 2009) and 1, 8-cineole (Lahlou *et al*., 2002; Nascimento *et al*., 2009).

Effect of increasing ejection fractions observed in this study showed that the extracts initiated vigorous and effective cardiac contractions. This can reduce symptoms of heart failure or cardiomyopathy due to increased percentage of blood ejected from the left ventricle systole in relation to the total end-diastolic volume. Lignans have negative inotropic effect but louteolin has positive inotropic effect (Boskabady and Jandaghi, 2003). Luteolin has vasorelexant effect by inhibition of sarcolemmal Ca^{2+} channels release from intracellular Ca^{2+} stores and activation of K^{+} channels (Peixoto-Neves *et al*., 2010).

After injection of *A. millefolium,* fractional shortenings increased to greater than 28% and this can substantiate that the treatment increased myocardial contractility. A decrease in systemic blood pressure or a decrease in myocardial stiffness increase fractional shortenings. The *A. millefolium* treatment has showed the same effect as catecholamine release. Some authors reported on cardiovascular effect of *Achillea* like electro-cardiogram and cardiac enzymes (Rahchamani *et al*., 2008), hypotensive (Farrokh *et al*., 2005). Asgary *et al*. (2000) demonstrated the antihypertensive and antihyperlipidemia effects of *Achillea* in a clinical trial but its cardiac effect was not shown.

It may be conclude that the *A. millefolium* extract has chemical compositions with fast action and short duration negative inotropic effect and also compositions with late action and positive inotropic effect. The same as in the previous study *A. millefolium* showed a positive inotropic effect after 2 hours of intravenous injection in sheep (Rahchamani *et al*., 2008).

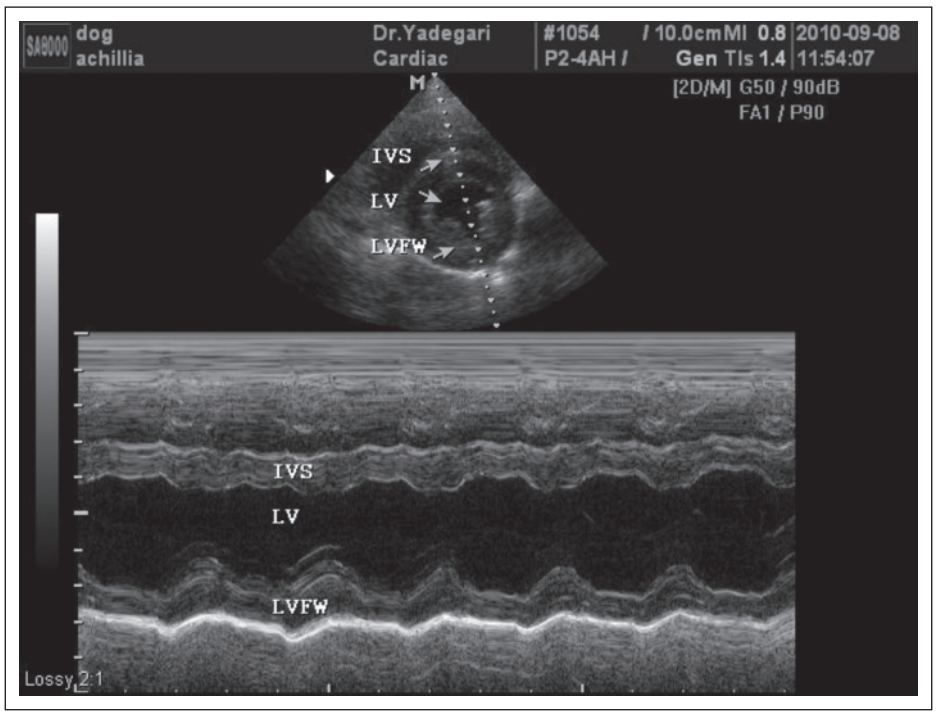

Fig. 1. Left ventricular diameters in systole (LVDs) and left ventricular diameters in diastole (LVDd) indices were measured.

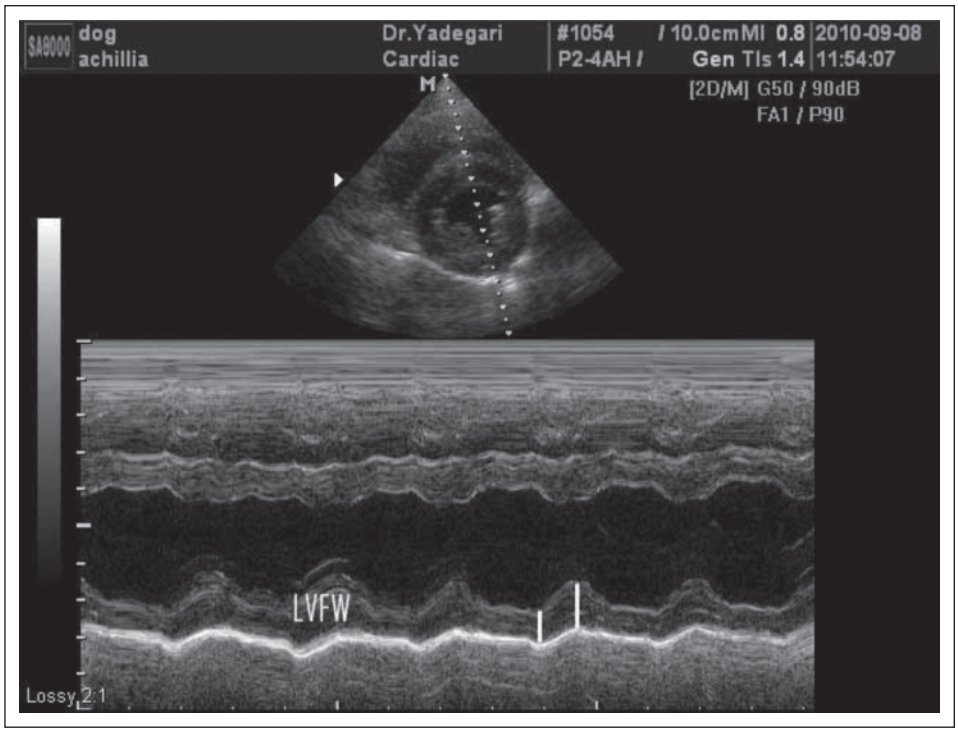

Fig. 2. Left ventricular free wall systole (LVFWs) and left ventricular free wall diastole (LVFWd) indices were measured.

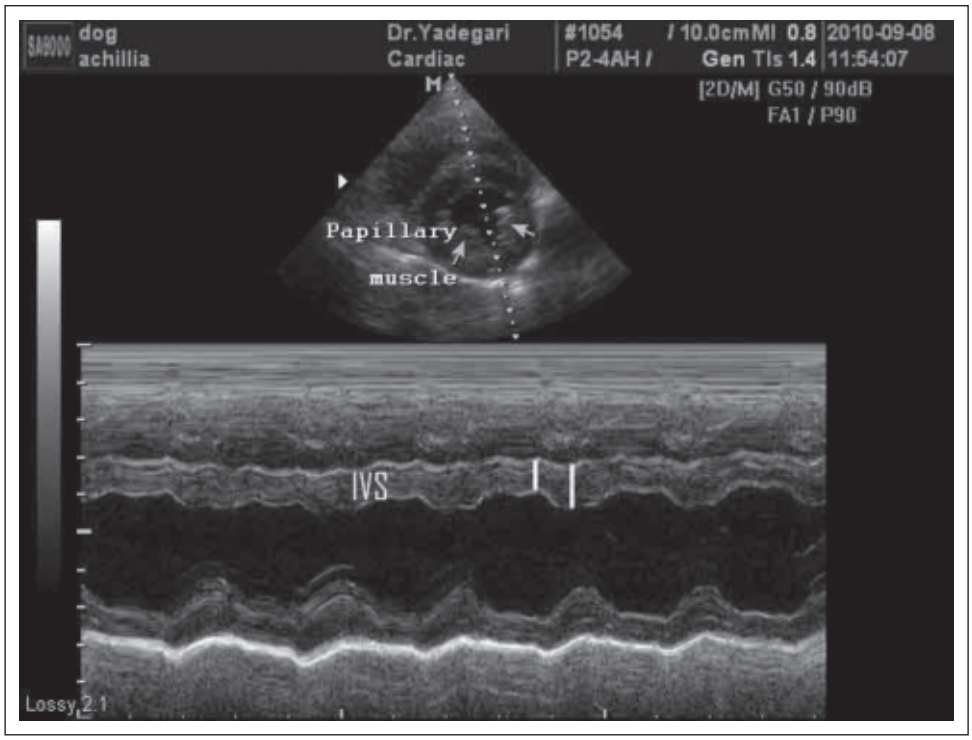

Fig. 3. Interventricular septal thickness at end-diastole and inter ventricular septal thickness at end-systole indices were measured.

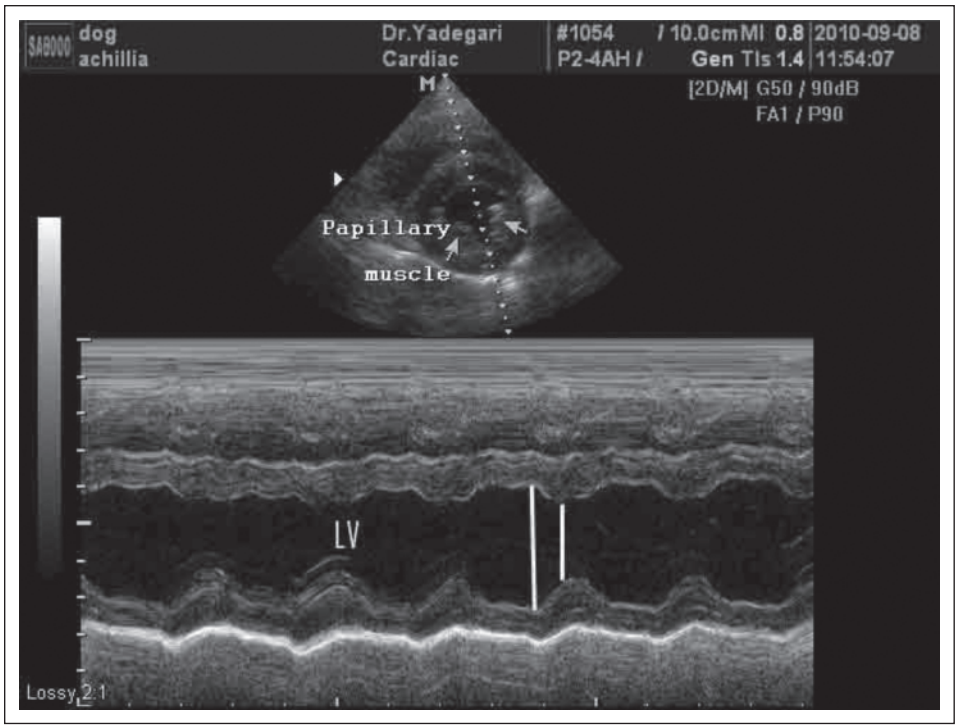

Fig. 4. Left ventricular septal thickness at systole and diastole indices were measured.

Table 1. Echocardiography indices measured by the movement mode method

Parameter	Before treatment	Treatment		
		0 min	60 min	120 min
EF (%)[1]	57.21±3.25[a]	55.02±4.08[ab]	66.89±5.66[ab]	70.07±3.02[b]
FS (%)[2]	32.05±1.67[a]	28.70±1.65[ab]	36.36±2.64[ab]	38.65±2.07[b]
HR (beat/minute)[3]	65.33±2.84[a]	55.33±3.02[b]	64±3.08[ab]	62.67±2.46[ab]
SV (ml)[4]	28.68±5.06[a]	16.62±6.7[ab]	23.67±6.15[ab]	26.49±8.03[ab]
IVSd (cm)[5]	0.96±0.16[a]	0.99±0.36[ab]	0.97±0.04[ab]	0.97±0.12[ab]
IVSs (cm)[6]	1.33±0.28[a]	1.39±0.33[ab]	1.26±0.20[ab]	1.29±0.21[ab]
LVFWd (cm)[7]	0.88±0.33[a]	0.88±0.24[ab]	0.92±0.14[ab]	0.87±0.16[ab]
LVFWs (cm)[8]	1.19±0.23[a]	1.18±0.28[ab]	1.22±0.18[ab]	1.22±0.21[ab]
LVIDd (cm)[9]	3.19±0.54[a]	2.73±0.49[ab]	2.99±0.48[ab]	3.11±0.45[ab]
LVIDs (cm)[10]	2.05±0.39[a]	1.95±0.36[ab]	1.92±0.43[ab]	1.88±0.27[ab]

[a,b] Numbers with different superscripts in the same column differ significantly $p<0.05$

1- Ejection Fraction. 2- Fractional Shortenings. 3- Heart Rate. 4- Stroke Volume. 5- Interventricular septal thickness at end-diastole. 6- Inter ventricular septal thickness at end-systole. 7- Left ventricular posterior wall thickness at end-diastole. 8- Left ventricular posterior wall thickness at end-systole. 9- Left ventricular internal diameter at end-diastole. 10- Left ventricular internal diameter at end-systole.

CONCLUSIONS

The used cardiodynamic parameters provide valuable data and assisted to detect incremental effects of treatment and permits detection of subtle changes. Calculations used in the current study basically dependent on measurement of the left ventricular outflow blood system.

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