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ORIGINAL ARTICLE

The Effect of Intraperitoneal Injection of *Achillea millefolium* Extract on Echocardiographic Parameters and Hepatic and Renal Blood Factors in Male New Zealand White Rabbits

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Abstract

The *Achillea millefolium* has a long history of use in traditional medicine for treating various ailments, including heart conditions. This study aimed to examine the effects of hydroalcoholic extract from *Achillea millefolium* on echocardiographic parameters, as well as liver and kidney enzyme activities. Fifteen healthy male New Zealand white rabbits, weighing between 4 and 5 kg, were selected and divided into three groups. The first group received a 100 mg/kg dose of yarrow extract, the second group received 200 mg/kg, and the third group received 400 mg/kg, intraperitoneally. Echocardiography was conducted prior to extract administration and again 120 minutes' post-administration to assess 14 cardiographic indices, such as shortening fraction (FS) and ejection fraction (EF). Additionally, liver and kidney enzymes were measured to evaluate potential pathological effects, and toxicity was assessed using the MTT method on Vero cell cultures. The hydroalcoholic extract of yarrow significantly increased FS, EF, and stroke volume (SV) indices in all groups. Moreover, it significantly decreased end-systolic volume (ESV) and left ventricular dimensions in systole (LVDs) in the second and third groups ($P \leq 0.05$). The extract did not induce any detrimental effects on liver or kidney tissues and exhibited no cytotoxicity in the MTT cell culture test. Findings from this study highlight the positive inotropic effects of yarrow hydroalcoholic extract and its ability to enhance cardiac systolic function. With no observed toxicity on eukaryotic cells, these results support its potential use in treating heart diseases. However, further extensive pharmacological research is necessary to confirm its therapeutic efficacy.

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Introduction

Cardiovascular diseases are one of the leading causes of death and morbidity. Therefore, identifying new and effective methods for the prevention and treatment of these diseases is crucial (1, 2). As chemical drugs can have numerous adverse effects, developing new and safe therapeutic methods is essential (3, 4). *Achillea millefolium* is a suitable natural candidate for disease treatment due to its therapeutic potential in cardiovascular, renal, and hepatic diseases (5).

Recent advances in animal models of cardiovascular diseases have made them important tools in cardiovascular research (6). Rabbit models have been widely used in cardiac arrhythmia studies, and are more similar to human arrhythmias. Echocardiography has become a common technique in various animal models due to recent advances in imaging procedures and reliably allows non-invasive assessment of cardiovascular morphology and function (6, 7).

The liver is the main site of metabolic processes and waste elimination. It plays a critical role in regulating homeostasis and is responsible for numerous biochemical pathways (8, 9). The diagnostic utility of liver enzymes and diseases has received considerable attention. Hepatocyte damage leads to the release of various intracellular enzymes into the bloodstream, including aminotransferases such as aspartate aminotransferase (AST) and alanine aminotransferase (ALT). Other important enzymes include alkaline phosphatases. Elevated serum activity of these enzymes usually originates from the liver and bone, and their serum concentration increase during liver damage (8). Previous studies have used *Achillea millefolium* in the treatment of cardiac muscle weakness and inflammation due to its tannins and aromatic bitter compounds (10). However, few studies have investigated the effects of this plant on echocardiographic parameters and liver enzymes. Hence, the present study investigated the effects of the hydroalcoholic extract of *Achillea millefolium* leaves and flowers on the cardiovascular system, as well as its interaction with liver enzymes.

Materials and Methods

Study Design

The present study was an experimental investigation conducted on male New Zealand white rabbits from April 2024 to September 2025.

Extract Preparation

The hydroalcoholic extract used in this study was obtained from the Geya Kala Company, whose overall analysis certificate was obtained along with the extract.

In Vitro Cytotoxicity Assessment (MTT assay)

Cytotoxicity of the extract was assessed using the MTT assay on commercially available hepatic (HepG2) and renal epithelial cell lines. Cells were treated with concentrations corresponding to the in vivo doses (100, 200, and 400 mg/kg equivalent), and viability was measured after 24 h of incubation. No animals were sacrificed for this experiment. This assay provided complementary insight into the cellular-level effects to support interpretation of the acute in vivo findings (11).

Experimental Procedure

Fifteen NZW rabbits weighing 4–5 kg were randomly divided into three groups. The first group received a hydroalcoholic extract of yarrow at a dosage of 100 mg/kg, the second group received 200 mg/kg, and the third group received 400 mg/kg of the same extract. All doses were delivered intraperitoneally. The rabbits were housed under standard conditions at 22 ± 2°C and fed a standard diet. The study was approved by the Institutional Animal Ethics Committee of Islamic Azad University (IAU), Tehran, Iran.

Echocardiography

Cardiac function was evaluated using an echocardiographic device equipped with an 8 MHz phased array probe. Assessments were conducted at two time points: before the intraperitoneal administration of yarrow hydroalcoholic extract and 120 min post-injection. After shaving the area and cleansing it with alcohol, gel was applied to ensure optimal imaging quality. The echocardiographic device measured various cardiac parameters, including fractional shortening (FS), ejection fraction (EF), interventricular septal thickness in systole (IVSs) and diastole (IVSd), left ventricular internal diameter at end-systole (LVIDs) and end-diastole (LVIDd), left ventricular free wall thickness in systole (LVFWs) and diastole (LVFWd), stroke volume (SV), end-diastolic volume (EDV), end-systolic volume (ESV), heart rate (HR), aortic diameter (Ao), and the left atrial to aortic diameter ratio (LA/Ao). These parameters were carefully analyzed by an echocardiographer throughout the study (7).

Biochemical Parameters

Blood samples were collected before and 120 min after extract administration. Blood was collected directly from the heart, stored in tubes, and centrifuged at 3500 rpm for 10 min, and then serum samples were frozen at −20°C until analysis. Serum samples were used to measure the following parameters: aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyl transferase (GGT), total bilirubin (TB), blood urea nitrogen (BUN), creatinine (Cr), phosphorus (P), potassium (K), sodium (Na), and chloride (Cl) (12).

Data Analysis

Data analysis was performed using SPSS software with one-way ANOVA for variance analysis. The Duncan Multiple Range Test was used to identify significant differences and the differences were considered significant at $P < 0.05$.

Results

Animal Care

Throughout the housing period in specialized cages, none of the rabbits died, and no secondary illnesses were observed.

MTT Assay

The MTT assay was used to evaluate the toxicity of the hydroalcoholic extract of yarrow on eukaryotic cells at two time points, 24 and 48 h. The findings indicated that the extract reduced the viability of cancer cells in a dose-dependent manner, although its toxicity was relatively low. The IC_{50} value of the hydroalcoholic extract of yarrow was 27.6 mg/mL at the 24 h time point (Figure 1).

Echocardiography

Data analysis revealed notable increases in fractional shortening (FS), ejection fraction (EF), and stroke volume (SV) across groups one, two, and three. Additionally, groups two and three demonstrated significant reductions in left ventricular internal diameter at end-systole (LVIDs) and end-systolic volume (ESV) at 120 minutes following the administration of yarrow extract, compared to their measurements pre-injection. Importantly, the magnitude of these changes was more pronounced in group three than in group two. An example of the echocardiogram results is shown in Figure 2.

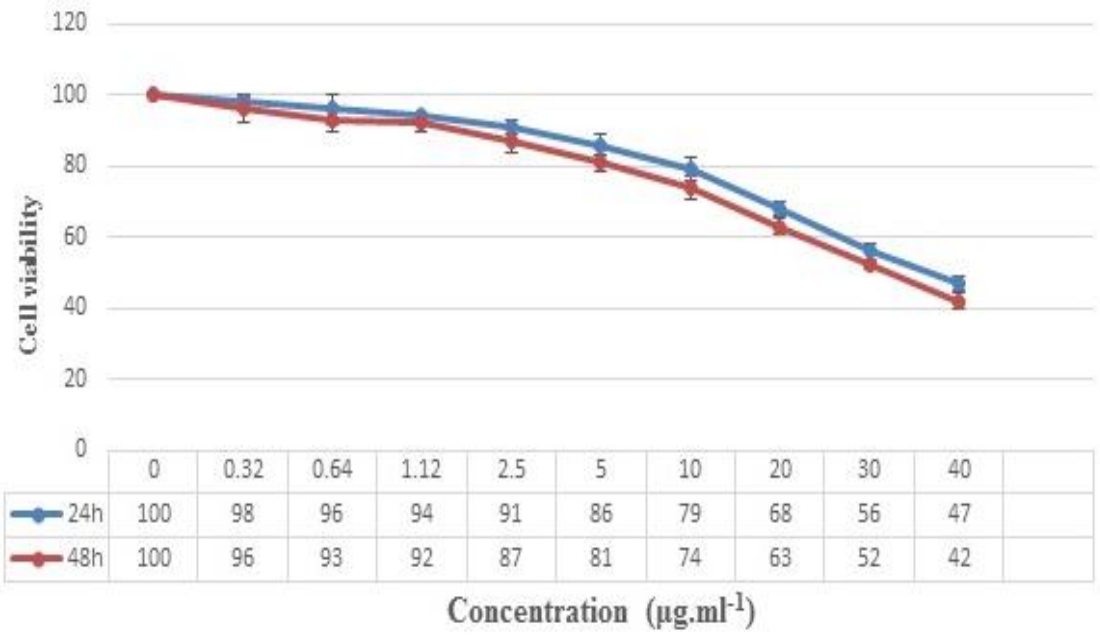

Figure 1. Viability of Vero cells following exposure to varying concentrations of the hydroalcoholic extract of yarrow.

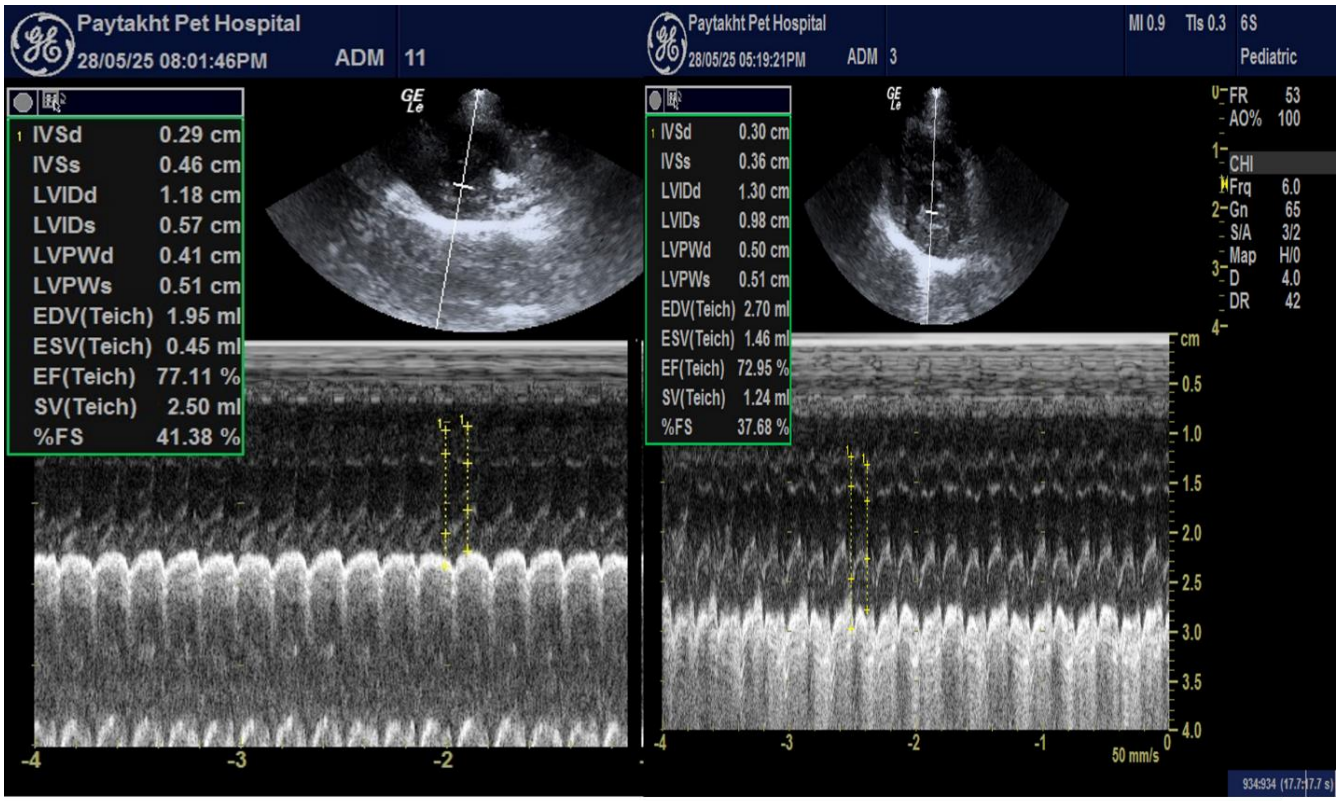

Figure 2. Representative echocardiographic images from group 2, illustrating the condition pre-injection of yarrow hydroalcoholic extract (left) and 120 min post-injection (right).

Biochemical Parameters

The effects of yarrow hydroalcoholic extract on liver and kidney enzyme function in male rabbits at 120 min after intraperitoneal administration are shown in Table 1. No parameter in any of the groups showed a significant difference between pre-injection and post-injection values, although a slight and non-significant increase or decrease was observed in most parameters.

Discussion

Chemical medications are commonly used to treat various illnesses, including cardiovascular diseases. However, these drugs can cause numerous adverse effects, some of which may persist throughout the patient's lifetime (13). Due to these concerns, natural and herbal remedies have gained increasing attention from researchers in recent years. Studies have shown that, in certain cases, herbal medicines provide more effective therapeutic outcomes than chemical drugs while also presenting significantly fewer adverse effects (4).

Yarrow demonstrates notable therapeutic benefits for cardiovascular health. Studies have shown that compounds found in this plant, including synthol, luteolin, and carvacrol, can help lower blood pressure and enhance blood circulation. Harandizadeh et al. (2012) reported that yarrow extract exhibits vasodilatory effects in rats and contributes to improved cardiac performance (14). Additionally, its potent antioxidant properties help protect the heart from oxidative damage. Yarrow extract is also considered a promising antiarrhythmic candidate for treating supraventricular tachyarrhythmia (10, 15).

The present study investigated acute in vivo cardiovascular and biochemical responses to the hydroalcoholic extract of *Achillea millefolium* in rabbits using a within-subject repeated-measures design, in which each animal served as its own baseline control. This approach minimizes inter-individual variability and allows precise detection of treatment-related changes. Although the absence of an independent control group may theoretically limit the ability to fully exclude confounding factors such as handling or injection-related stress, using each animal as its own control provides robust internal reference points, particularly for acute short-term responses. Three doses of the extract—100, 200, and 400 mg/kg—were administered via intraperitoneal injection. Results demonstrated notable improvements in key cardiac indices, including FS, EF, SV, LVIDs, and ESV. Specifically, the extract caused a significant increase in FS, EF, and SV while reducing LVIDs and ESV parameters.

Table 1. Echocardiographic results for the three groups of rabbits before injection and 120 min after intraperitoneal injection of yarrow hydroalcoholic extract. The asterisk (*) indicates a significant difference between the post-administration and pre-injection time points.

Echocardiographic Parameter						
Value	**Group 1**		**Group 2**		**Group 3**	
	Before Treatment	**120 min after Treatment**	**Before Treatment**	**120 min after Treatment**	**Before Treatment**	**120 min after Treatment**
FS (%)	37.71	40.77*	36.81	43.26*	32.81	48.9*
EF (%)	71.66	75.73*	70.41	77.08*	70.76	83.5*
LVIDs (cm)	0.88	0.78	0.92	0.62*	0.98	0.61*
ESV (mL)	1.23	1.12	1.32	0.77*	1.39	0.71*
SV (mL)	2.82	3.12*	2.78	3.28*	2.72	3.57*
Biochemical Parameters						
ALP (IU/L)	514 ± 10	530 ± 8	622 ± 9	631 ± 12	598 ± 11	602 ± 5
ALT (IU/L)	52 ± 5	55 ± 7	57 ± 5	61±5	64 ± 7	67 ± 4
AST (IU/L)	41 ± 6	45 ± 6	39 ± 7	42 ± 3	45 ± 3	47 ± 3
GGT(IU/L)	12 ± 3	14 ± 3	14 ± 4	16 ± 2	14 ± 2	15 ± 3
Cr (mg/dL)	1.2 ± 0.3	1.4 ± 0.4	1.3 ± 0.3	1.4 ± 0.3	1.4 ± 0.2	1.9 ± 0.5
BUN (mg/dL)	21 ± 2	24 ± 3	20 ± 3	18 ± 3	26 ± 3	21 ± 4
TB (mg/dL)	2.2 ± 0.4	2.8 ± 0.2	2.4 ± 0.3	2.3 ± 0.6	2.2 ± 0.5	2.7 ± 0.7
P (mg/dL)	7.2 ± 0.5	8.2 ± 0.4	7.1 ± 0.6	9 ± 2	8.2 ± 0.4	8.7 ± 0.6
Na (mEq/L)	135 ± 10	142 ± 10	128 ± 8	145 ± 8	151 ± 8	157 ± 9
Cl (mEq/L)	101 ± 6	106 ± 5	104 ± 8	102 ± 4	123 ± 7	113 ± 3
K (mEq/L)	5.2 ± 0.5	5.5 ± 0.4	5.3 ± 0.4	4.9 ± 1.5	6.5 ± 0.6	6.2 ± 0.4

Ejection fraction (EF), which measures the heart's overall pumping efficiency, exhibited a marked increase across all treatment groups ($P < 0.05$). This enhancement can be attributed to the positive inotropic effects of yarrow extract, which is consistent with previous findings regarding the beneficial influence of flavonoid and antioxidant compounds in yarrow on myocardial contractility (16). Additionally, the FS index showed substantial improvement in all experimental groups, further corroborating enhanced left ventricular systolic function (17). Stroke volume (SV), reflecting the volume of blood ejected by the heart with each contraction, also increased significantly in response to the extract. The collective rise in EF, FS, and SV indicates enhanced cardiac performance and tissue perfusion. Moreover, reductions in LVIDs and ESV parameters observed in groups treated with 200 and 400 mg/kg doses indicate improved systolic function and more effective left ventricular emptying. These findings suggest that higher doses of the hydroalcoholic extract optimize overall cardiac function, highlighting its potential as a supportive agent in cardiovascular health management.

Yadegari et al. (2015) reported that the hydroalcoholic extract of yarrow significantly enhanced EF, FS, and SV indices in six healthy male dogs. These improvements were attributed to the alkaloid compounds in yarrow, which are known for their blood pressure-lowering effects (18). Additionally, findings from other studies have attributed reductions in LVIDs and ESV observed on echocardiography to the antispasmodic properties of yarrow extract. These compounds reduce myocardial contractility, which subsequently leads to lower LVIDs and ESV values (18, 19). Furthermore, Rahchamani et al. (2008) found that intravenous administration of 20 mg/kg of yarrow extract over a two-hour period resulted in decreased end-systolic volume (ESV) (19). In the current study, a similar reduction in ESV was observed with yarrow doses of 200 and 400 mg/kg after 120 minutes, which is consistent with previous findings. The 120 min observation period was chosen to capture immediate cardiovascular and biochemical changes. This duration is appropriate for evaluating acute physiological responses, as many hemodynamic and functional changes manifest within minutes to hours.

Nevertheless, it does not allow assessment of delayed or cumulative effects, and future studies with longer observation periods are recommended.

This study measured enzymes and biochemical parameters to evaluate the pathological effects of yarrow extract on liver and kidney function. Findings revealed no significant changes in these parameters before and after extract administering, suggesting the doses used did not cause tissue damage in rabbits and supported its potential medicinal use. While medicinal plants are widely recommended for treating cardiovascular diseases, the potential presence of toxic compounds can limit their application in both human and veterinary medicine. To address this, the toxic effects of yarrow extract were assessed on Vero cells, and the results indicated minimal toxicity, further supporting its therapeutic potential. Nevertheless, additional comprehensive research is necessary to investigate potential adverse effects, such as allergic reactions, and to develop appropriate measures if such issues arise.

Limitations include the small sample size, absence of an independent control group, and short 120 min observation period. Future studies should consider longer-term monitoring, inclusion of independent control groups, and larger sample sizes to fully evaluate delayed or cumulative effects and the long-term safety profile of the extract.

Conclusion

The main focus of this study was the acute in vivo cardiovascular and biochemical responses to the hydroalcoholic extract of *Achillea millefolium* in rabbits. Complementary in vitro evaluation using the MTT assay on HepG2 and renal epithelial cell lines provided additional insight into cellular-level cytotoxicity. These in vitro results help contextualize the in vivo observations and support the safety assessment; however, they are supplementary and cannot replace comprehensive in vivo toxicological evaluation. The observed changes in cardiac parameters likely reflect acute hemodynamic or functional responses, whereas the absence of significant biochemical changes within 120 min is consistent with the short observation period, as markers of hepatic and renal function often require longer to show measurable alterations. Investigation of the effects of yarrow extract on echocardiographic indices in rabbits revealed that doses of 200 and 400 mg/kg can significantly enhance cardiac function. Furthermore, the absence of liver and kidney damage in the rabbits, coupled with the low toxicity observed in Vero cells, underscores the extract's potential as a therapeutic option for cardiovascular diseases. However, extensive pharmacological studies are still required to fully evaluate its efficacy in this area.

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Authors' Contributions

Roya Rezaeiyan: Analysis and interpretation of data, Drafting of the manuscript. **Mohammad Nasrollahzadeh Masouleh:** Study concept and design. **Varya Touhidi:** Study concept and design. **Parviz Tajik:** Statistical analysis. **Ali Moradganjeh:** Study concept and design.

Data Availability

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

Ethical Approval

The study was approved by the Institutional Animal Ethics Committee of Islamic Azad University, Science and Research Branch, Tehran, Iran (Ethics Code: IR.IAU.SRB.REC.1404.275). All applicable international, national, and institutional guidelines for the care and use of animals were followed.

Conflict of Interest

The authors declare that they have no conflict of interest.

Consent for Publication

Not applicable.

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