The impact of Procyanidin extracted from *Crataegus azarolus* on rats with induced heart failure

Baref Z. Rashid¹, Kawa F. Dizaye²

¹Lecturer at Technical Institute, Sulaimani Polytechnic University, Sulaimaniyah, Kurdistan Region-Iraq
²College of Medicine, Hawler Medical University, Erbil, Kurdistan Region-Iraq

ARTICLE INFO

Original paper

Article history:
Received: July 01, 2022
Accepted: September 04, 2022
Published: September 30, 2022

Keywords:
Procyanidin, Spironolactone, digoxin, biomarkers, HF

ABSTRACT

This study aimed to investigate the possible use of Procyanidin extracted from *Crataegus azarolus* in the treatment of induced heart failure in rats. Thirty-six male rats were randomly assigned to three groups; the first two groups had six rats each, and the third group included four subgroups (each with six rats). The first group was regarded as the control group, while the second group (normal rats) received oral Procyanidin 30mg/kg/day for 14 days. The rest of the experimental groups were all injected intraperitoneally with 5mg/kg/day for seven days to induce heart failure. The first subgroup (IIa) served as a positive control, and the other subgroups (IIb, c, and d) received oral Procyanidin 30mg/kg/day, spironolactone 20mg/kg/day, and digoxin 7Mcg/kg/day, respectively, for 14 days. Heart failure induction in rats significantly increased levels of cardiac biomarkers, including NT-proBNP, BNP, ALP, MMP9, CPK, systolic, and diastolic blood pressure. The normal rats that received only Procyanidin experienced a significant decrease in the ALP level. Moreover, Procyanidin, accompanied by spironolactone and digoxin, significantly decreased NT-proBNP, BNP, ALP, and diastolic BP in rats with heart failure. Procyanidin extracted from *C. azarolus* significantly decreased cardiac biomarkers in rats with iso-induced HF. The final results demonstrated similar effects with both spironolactone and digoxin in induced heart failure in rats, revealing the possibility of using Procyanidin in the HF treatment.

Introduction

Heart failure (HF) as a clinical syndrome caused by various heart diseases, is a complicated disease that involves many systems and organs (1,2). Its signs and symptoms usually include breath shortness, excessive tiredness, and the leg swelling (3). Around the world, it represents the most common reason for death and readmission in patients suffering from cardiovascular diseases (4,5).

Regarding the statistics, almost more than 20 million patients suffer heart failure worldwide, and the heart failure prevalence slowly rises with age increasing (1).

Because the myocardium physiologically and structurally fails to send blood and oxygen to the peripheral tissues, or the heart works under higher filling pressures (6).

The initial pathophysiological response in HF is the activation of the neurohormonal system in response to decreased blood flow to the tissues (7). The subsequent response includes the stimulation of the renin-angiotensin system (RAS) to raise the preload pressure by increasing salt and water retention, stimulating vascular tone (to increase afterload), and regulating cardiac contractility (8).

Biomarkers in HF play an important role in pre-diagnosis, prognostic stratification, and therapeutic plan adjustment (9).

Natriuretic peptides (NPs) play a principal role as a biochemical marker of heart failure with good stability in blood and also diagnose heart failure in acute dyspnoea (10,11). They are found in the body in three different types: ANP (secreted from atria), BNP (present in the brain), and CNP (the vascular type) (12). These hormones are secreted during circulatory or hemodynamic deteriorations (13).

The increased plasma level of the brain natriuretic peptide (BNP) may indicate left ventricular failure and HF (14). The measurement of serum N-terminal NT-proBNP is regarded as a significant tool in diagnosis and/or prognosis, more than all the other natriuretic peptides (15).

Other identified cardiac biomarkers are matrix metalloproteinase MMPs and creatine phosphokinase CPK (16). The metalloproteinase enzymes such as MMP-2 and MMP-9 are the most valuable type for HF diagnosis—more useful than BNP. Medications, heart disease, kidney disease, and some other diseases may affect the level of creatine phosphokinase (CPK) (17). The serum level of CPK is increased dramatically in patients with congestive heart failure (CHF). Also, the ALP serum levels are increased considerably in patients suffering from CHF (18). Several medications are used in the treatment of HF; the primary drugs are angiotensin inhibitors, beta-blockers, diuretics and digoxin (19). Although traditional and herbal medicines play a smaller role today in developing new pharmaceuticals, they still account for 25% of all prescription drugs (20).

One of the most important plants for cardiovascular...
disorders is hawthorn (Crataegus spp.). Hawthorn extract has been used to treat mild to moderate heart failure (HF) and as an adjunct therapy in patients suffering from HF (New York Heart Association classes I – III (21). The therapeutic effects of Crataegus spp., specifically in HF, are due to the presence of flavonoids such as procyanidins, a class of polyphenolic compounds that improve pulse rate, coronary blood flow, and left ventricular filling pressure. Several studies indicate that proanthocyanidins (Procyanidin) help to protect the myocardium and improve blood circulation by dilating the blood vessels (22). Spironolactone, as a non-selective aldosterone receptor antagonist, has the ability to improve survival in patients with symptomatic chronic HF and acute myocardial infarction (AMI) was used in this study. While the exact molecular mechanisms of spironolactone are not completely clarified (23). Moreover, digoxin, as of several medications used to treat HF symptoms, was applied in this research (24).

The present study was undertaken to identify the therapeutic effect of Procyanidin extracted from Crataegus spp. on HF and compare it with other drugs by assessing the levels of serum cardiac biomarkers (BNP, NT-proBNP, MMP9, and CPK), alkaline phosphatase, and hemodynamic assays (BP and HR).

Materials and Methods

Animals

In this study, 36 male Wistar albino rats with average weights between 210–250 g (mean ± SD 233.36 ± 14.51 g) were prepared for the experiments. They were provided by the rat house department of the College of Medicine/ Hawler Medical University (Erbil, Iraq). The rats were kept in individual, well-organized plastic cages, with water and standard regular rat chow available 24 hours a day for each cage. Further, their room was set to 12/12 hours of light-dark cycles and 23° C to 25° C.

Plant extraction

Procyanidin was extracted from the fruit of the hawthorn plant (Crataegus azarolus) obtained from Piramgroon Mountain (Sulaymaniyah, Iraq) at the beginning of autumn. The fruit was dried and ground to a fine powder. Then, the sample was withdrawn directly from the heart chamber by a cardiac puncture technique; whole blood was collected from each animal for estimating serum biomarkers such as serum NT-proBNP, BNP, CPK, MMP 9, and ALP. For measuring blood pressure (systolic, diastolic, and mean) and heart rate, a tail-cuff blood pressure measurement and CODA device were used (25).

Materials

The following materials were used in the present study: serum N-terminal pro-B-type natriuretic peptide (NT-proBNP), BNP, alkaline phosphatase, matrix metalloproteinase 9 (MMP9), creatine phosphokinase (CPK), ELISA rat kits, a CODA noninvasive blood pressure monitoring device, isoprenaline HCl powder, digoxin and spironolactone powder purchased from a qualified and authorized pharmacy, and procyanidin extract from Crataegus azarolus fruit.

Study design

Thirty-six male rats were randomly assigned to three groups. The first two groups had six rats each, and the third group included four subgroups (each with n = 6). The groups were distinguished as follows:

- Group I: a control group with no drug treatment;
- Group II: a normal rats group, receiving oral Procyanidin 30mg/kg/day for 14 days;
- Group III: included 24 rats, all injected with isoprenaline 5mg/kg/day for seven days to induce heart failure, being then subdivided into four groups as follows:
  - Group IIIa: received no treatment,
  - Group IIIb: received oral Procyanidin 30mg/kg/day for 14 days,
  - Group IIIc: treated with oral spironolactone 20mg/kg/day for 14 days,
  - Group IIId: treated with oral digoxin 7Mg/kg/day for 14 days.

All the oral medications were administered through oral gavage after mixing them with a convenient solvent (water).

To separate the serum, the blood samples were collected once the animal was injected with intraperitoneal Ketamine 125 mg/kg and Xylazine 10 mg/kg to be anesthetized. Then, the sample was withdrawn directly from the heart chamber by a cardiac puncture technique; whole blood was collected from each animal for estimating serum biomarkers such as serum NT-proBNP, BNP, CPK, MMP9, and ALP. For measuring blood pressure (systolic, diastolic, and mean) and heart rate, a tail-cuff blood pressure measurement and CODA device were used (26).

Statistical analysis

The study data and measurements were analyzed using SPSS (Version 26) computer program. The data were recorded as the mean ± standard error means (M ± SEM). An ANOVA (analysis of variance) test was performed to estimate the differences in all control and treated rat parameters. The Tukey test and student t-test were used to compare the groups. All the changes were regarded as significant when p < 0.05.

Results

Effects of Procyanidin, spironolactone, and digoxin treatments on different biomarker measures on rats with HF

NT-proBNP significantly increased during heart failure in Group IIId (12.75 ± 0.46 ng/ml) compared to the control group, Group I (11.12 ± 0.28 ng/ml). Compared to the control group, serum NT-proBNP decreased significantly after 14 days of treatment with Procyanidin (IIId) (11.34 ± 0.18 ng/ml), spironolactone (IIIdc) (11.36 ± 0.18 ng/ml) and digoxin (IIId) (11.4 ± 0.21 ng/ml) (Table 1). However, when Procyanidin was administrated to normal rats (Group II) for 14 days, serum NT-proBNP (11.81 ± 0.37 ng/ml) was not changed significantly (p > 0.05) compared to the control group (Table 2).

The level of BNP was elevated significantly (1.46 ± 0.16 ng/ml) during heart failure compared with the control group (0.70 ± 0.02 ng/ml). The BNP levels were decreased significantly after treatment with Procyanidin (0.84 ± 0.02 ng/ml), spironolactone (0.99 ± 0.04 ng/ml), and digoxin (1.06 ± 0.08 ng/ml), as recorded in Table 1. BNP did not change significantly in normal rats after receiving Procyanidin compared with the control group, as observed in Table 2.
The Impact of Procyanidin on rats with induced heart failure, 2022, 68(9): 179-185

Table 1. Effects of Procyanidin, spironolactone, and digoxin treatments on different biomarker measures in rats with HF.

<table>
<thead>
<tr>
<th>DATA</th>
<th>Control GROUP I</th>
<th>Heart failure GROUP IIIa</th>
<th>Procyanidin 30mg GROUP IIIb</th>
<th>Spironolactone GROUP IIIc</th>
<th>Digoxin GROUP IIId</th>
</tr>
</thead>
<tbody>
<tr>
<td>NT-Pro BNP ng/ml</td>
<td>11.12 ± 0.28</td>
<td>12.75 ± 0.46</td>
<td>11.34 ± 0.18</td>
<td>11.36 ± 0.18</td>
<td>11.40 ± 0.21</td>
</tr>
<tr>
<td></td>
<td>a</td>
<td>b</td>
<td>a</td>
<td>a</td>
<td>a</td>
</tr>
<tr>
<td>BNP ng/ml</td>
<td>0.70 ± 0.02</td>
<td>1.46 ± 0.16</td>
<td>0.84 ± 0.02</td>
<td>0.99 ± 0.04</td>
<td>1.06 ± 0.08</td>
</tr>
<tr>
<td></td>
<td>a</td>
<td>c</td>
<td>ab</td>
<td>ab</td>
<td>b</td>
</tr>
<tr>
<td>ALP ng/ml</td>
<td>135.16 ± 7.58</td>
<td>177.33 ± 11.56</td>
<td>103.20 ± 2.85</td>
<td>130.17 ± 5.33</td>
<td>145 ± 5.13</td>
</tr>
<tr>
<td></td>
<td>b</td>
<td>c</td>
<td>a</td>
<td>ab</td>
<td>b</td>
</tr>
<tr>
<td>MMP9 ng/ml</td>
<td>254 ± 19.22</td>
<td>473.83 ± 47.88</td>
<td>453.60 ± 15.44</td>
<td>442.33 ± 11.78</td>
<td>429.33 ± 6.68</td>
</tr>
<tr>
<td></td>
<td>a</td>
<td>b</td>
<td>b</td>
<td>b</td>
<td>b</td>
</tr>
<tr>
<td>CPK ng/ml</td>
<td>93.83 ± 1.88</td>
<td>120.50 ± 7.03</td>
<td>105.60 ± 1.60</td>
<td>106.16 ± 3.17</td>
<td>110.66 ± 6.05</td>
</tr>
<tr>
<td></td>
<td>a</td>
<td>b</td>
<td>ab</td>
<td>ab</td>
<td>ab</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM. Different letters indicate significant differences. (p-value < 0.05).

Table 2. Effect of Procyanidin 30mg/kg/day administration for 14 days to normal rats on different biomarker measures.

<table>
<thead>
<tr>
<th>DATA</th>
<th>Control Means ± SE GROUP I</th>
<th>Procyanidin 30mg normal rats GROUP II</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>NT-ProBNP ng/ml</td>
<td>11.12 ± 0.28</td>
<td>11.81 ± 0.37**</td>
<td>0.170</td>
</tr>
<tr>
<td>BNP ng/ml</td>
<td>0.70 ± 0.02</td>
<td>0.80 ± 0.04**</td>
<td>0.065</td>
</tr>
<tr>
<td>ALP ng/ml</td>
<td>135.17 ± 7.58</td>
<td>106.67 ± 6.11*</td>
<td>0.015</td>
</tr>
<tr>
<td>MMP9 ng/ml</td>
<td>254 ± 19.22</td>
<td>267 ± 43.09**</td>
<td>0.789</td>
</tr>
<tr>
<td>CPK ng/ml</td>
<td>93.83 ± 1.88</td>
<td>91 ± 2.90**</td>
<td>0.440</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM. * p < 0.05 when compared with Group I. **p > 0.05 when compared with Group I.

Table 3. Effects of Procyanidin, spironolactone, and digoxin treatments on different hemodynamic measures in rats with HF.

<table>
<thead>
<tr>
<th>DATA</th>
<th>Control</th>
<th>Heart Failure</th>
<th>Procyanidin 30 mg</th>
<th>Spironolactone</th>
<th>Digoxin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Systolic mmHg</td>
<td>110.66 ± 5.40</td>
<td>134.24 ± 6.64</td>
<td>122.66 ± 1.85</td>
<td>113.43 ± 5.32</td>
<td>118.46 ± 6.61</td>
</tr>
<tr>
<td></td>
<td>a</td>
<td>b</td>
<td>ab</td>
<td>ab</td>
<td>ab</td>
</tr>
<tr>
<td>DSP mmHg</td>
<td>86.60 ± 2.59</td>
<td>116.41 ± 8.43</td>
<td>83.46 ± 5.49</td>
<td>81.76 ± 4.26</td>
<td>91.70 ± 4.26</td>
</tr>
<tr>
<td></td>
<td>a</td>
<td>b</td>
<td>a</td>
<td>a</td>
<td>a</td>
</tr>
<tr>
<td>MBP mm Hg</td>
<td>95.56 ± 5.96</td>
<td>92.06 ± 5.83</td>
<td>93.08 ± 4.91</td>
<td>85 ± 4.81</td>
<td>90.76 ± 4.27</td>
</tr>
<tr>
<td></td>
<td>a</td>
<td>a</td>
<td>a</td>
<td>a</td>
<td>a</td>
</tr>
<tr>
<td>HR pulse/minute</td>
<td>409.55 ± 14.91</td>
<td>417.16 ± 27.91</td>
<td>438.16 ± 15.39</td>
<td>424 ± 16.40</td>
<td>437.16 ± 12.71</td>
</tr>
<tr>
<td></td>
<td>a</td>
<td>a</td>
<td>a</td>
<td>a</td>
<td>a</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM. Different letters indicate significant differences. (p-value < 0.05).

Alkaline phosphatase was significantly elevated during HF (177.33 ± 11.56 ng/ml) compared with the control group (135.16 ± 7.58ng/ml). The levels of ALP decreased significantly compared to the control group after 14 days of treatment with Procyanidin (103.2 ± 2.85ng/ml), spironolactone (130.17 ± 5.33 ng/ml), and digoxin (145 ± 5.13 ng/ml), as presented in Table 1. When Procyanidin was administered to normal rats for 14 days, the ALP level decreased significantly (106.67 ± 6.11ng/ml) compared to the control group (93.83 ± 1.88 ng/ml); however, during treatment with Procyanidin, spironolactone, and digoxin, the enzyme levels decreased insignificantly (Table 1).

Matrix metalloproteinase 9 enzyme increased significantly with heart failure (473.83 ± 47.88 ng/ml) compared to the control group (254 ± 19.22 ng/ml). After treatment with Procyanidin, spironolactone, and digoxin, the levels of MMP9 also decreased; however, the changes were not significant compared to rats with heart failure (Table 1). Procyanidin received by normal rats for 14 days did not affect the enzyme level significantly (267 ± 43.09 ng/ml) compared with the control group (Table 2).

Creatinine phosphokinase enzyme level increased significantly by heart failure (120.50 ± 7.03 ng/ml) compared to the control group (93.83 ± 1.88 ng/ml); however, during treatment with Procyanidin, spironolactone, and digoxin, the enzyme levels decreased insignificantly (Table 1). When Procyanidin alone was administered to normal rats, the decrease in the enzyme level was not significant (91 ± 2.90 ng/ml) compared to the control group (93.83 ± 1.88 ng/ml), (p-value < 0.05) (Table 2).

Effects of Procyanidin, spironolactone, and digoxin treatments on different hemodynamic measures (systolic, diastolic, mean blood pressure, and heart rate) in rats with HF

Systolic blood pressure increased significantly with heart failure (Group IIIa) (134.24 ± 6.64 mm Hg) compared to the control group (110.66 ± 5.40 mm Hg). After 14 days of receiving Procyanidin (Group IIIb), the SBP recorded 122.66 ± 1.85 mm Hg; with spironolactone, Group IIIc recorded 113.43 ± 5.32 mm Hg, and with digoxin, Group IIIId recorded 118.46 ± 6.61 mm Hg. Overall, the SBP decreased in these three groups but not significantly compared to the control group (Table 3). Moreover, the
Procyanidin administration for 14 days in Group II (normal rats) resulted in a non-significant change, as recorded in Table 4.

Diastolic blood pressure increased significantly with heart failure (116.41 ± 8.43 mm Hg) compared to the control group (86.60 ± 2.59 mm Hg). After 14 days of the Procyanidin administration, the DBP recorded 83.46 ± 5.49 mm Hg, spironolactone recorded 81.76 ± 4.26 mm Hg, and digoxin recorded 91.70 ± 4.26 mm Hg, indicating that the DBP decreased significantly in all the three groups compared to the control group (Table 3). However, when normal rats received Procyanidin for 14 days, the DBP did not change significantly (Table 4).

Mean blood pressure results showed no significant change either during heart failure or after receiving different treatments. Moreover, the measurements remained close to each other (Table 3). The MBPs remained static when Procyanidin was given for 14 days to normal rats, with no significant difference before and after administration (Table 4).

Heart rate was not affected significantly either during HF or while receiving Procyanidin, spironolactone, and digoxin compared with the control group (Table 3). Moreover, the heart rate was not affected significantly in normal rats after receiving Procyanidin for 14 days (Table 4).

### Discussion

Heart failure is among the most aggressive cardiovascular diseases. Its frequency and morbidity have increased every year, with a relatively weak prognosis (27). In the present study, when isoproterenol was injected in a daily dose of 5mg/kg intraperitoneally, HF was induced after one week in rats. Isoproterenol induces HF by non-selectively stimulating β1, (mostly located in the heart) and β2 receptors, causing positive chronotropic and inotropic effects. Positive chronotropic and inotropic effects may cause increased cardiac load (prior to HF), possibly accompanied by decreased coronary blood flow. Production of reactive oxygen species by ISO may cause myocardial injury (28). Sympathetic over-activation by ISO may cause myocardial injury (27). Sympathetic over-activation by ISO may cause myocardial injury (27).

Matrix metalloproteinase (MMP) enzymes are another biomarker. They use zinc as their main substrate. This study found a significant elevation in the MMP9 level during heart failure compared with the control group. These enzymes degrade extracellular proteins; therefore, they can be involved in tissue development and remodeling, both of which are observed in HF (34). This group of enzymes acts as a strong biomarker because of their diversity, being classified into three main types (collagenase, gelatinase, and stromelysin). The gelatinase type, which contains MMP9, is more dependable for determining the remodeling process caused by HF. The rise in the level of MMPs might be related to the increase in the level of BNP during HF induction; it is believed that BNP can inhibit collagen production and elevate the expression of MMPs (35).

As another biomarker, creatinine phosphokinase enzyme or creatine kinase (CK) also increased significantly during HF compared with the control group. CKP increases because it mediates the reaction between creatine and adenosine triphosphate (ATP) to hydrolyze and produce phosphocreatine plus adenosine diphosphate (ADP). Heart disease is one of the major causes that may affect the heart rate (prior to HF), possibly accompanied by decreased coronary blood flow. Production of reactive oxygen species by ISO may cause myocardial injury (28).

### Tables

#### Table 4. Effect of Procyanidin 30mg/kg/day administration for 14 days to normal rats on different hemodynamic measurements.

<table>
<thead>
<tr>
<th>DATA</th>
<th>Control Means± SE</th>
<th>Procyanidin 30mg normal rats</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Systolic mm Hg</td>
<td>108.50 ± 5.05</td>
<td>115.12 ± 7.62*</td>
<td>0.53</td>
</tr>
<tr>
<td>DSP mm Hg</td>
<td>78 ± 5.36</td>
<td>81.58 ± 4.59*</td>
<td>0.68</td>
</tr>
<tr>
<td>MAP mm Hg</td>
<td>85.91 ± 5.34</td>
<td>91.76 ± 6.10*</td>
<td>0.53</td>
</tr>
<tr>
<td>HR pulse/minute</td>
<td>392 ± 13.76</td>
<td>418.03 ± 14.52*</td>
<td>0.14</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM. *p > 0.05 when compared with Group I.
blood pressure parameters and heart rate. Measurement of the above variables is crucial in monitoring heart failure. Any decrease in BP in patients suffering from ventricular failure may decrease excess remodeling and the ratio of HF. It is believed that decreasing 10 mm Hg of SBP reduces the incidence of HF by 12%. For monitoring HR, the pulse rate should be maintained near 60 bpm; however, for the BP, no targeted limit is determined (38).

The SBP and DBP were both elevated significantly during HF compared with the control group. This increase can be explained by the increased aldosterone, angiotensin II, sympathetic tone, and wall stress, as previously mentioned.

After inducing heart failure in the rats, to compare the effect of Procyanidin in treating HF, spironolactone and digoxin were used in the present study. After two weeks of administering the drugs with the Procyanidin, all the biomarkers and hemodynamic measurements were recorded, all of which decreased.

The Procyanidin used in this study was extracted from *Crataegus azarolus*, which is thought to have antioxidant activity (39); this explains the reason behind the decreasing levels of MMP9 and CPK after using Procyanidin. Procyanidin's vasodilator activity can improve blood flow in the brachial artery, which may be related to its antioxidant activity (40).

BNP, NT-ProBNP, and ALP levels were decreased because vasodilation decreased preload and post-load and improved the portal blood flow, in turn decreasing the wall stress on the heart and affecting BNP, NT-ProBNP, and ALP. The SBP, DBP, and MBP also decreased; this can largely be explained through recent studies that have defined the role of Procyanidin with its powerful antihypertensive effect (41).

Spironolactone was one of the two drug treatments used in our study for 14 days. The aldosterone antagonist was used because the angiotensin release in HF elevates aldosterone levels, which may cause more sodium retention and increase sympathetic output, baroreceptor dysfunction, and myocardial and vascular fibrosis (42). By inhibiting aldosterone, spironolactone increases sodium/water excretion and potassium retention, which may explain the decrease in the levels of BNP, NT-ProBNP, ALP, SBP, DBP, and MBP observed in the current study. Moreover, through the inhibition of mineralocorticoid receptors, they mediate pleiotropic effects that lead to ventricular and vascular remodeling, which is found with decreased levels of MMP9 and CPK after treatment (43).

The other drug treatment used was digoxin. The use of digitalis in HF may decrease symptoms and the frequency of hospitalization (but not the mortality rate) (44) by the inhibition of Na-K-ATPase, which increases extracellular potassium and the concentration of intracellular Na and Ca. Thus, digoxin causes a positive inotropic and a negative chronotropic and dromotropic effect. It has a positive effect on parasympathetic tone; the negative effect on sympathetic tone may be enough to explain the decrease in all the biomarker and hemodynamic levels recorded in the present study (45).

Overall, the decrease of the MMP9 level observed while treating the rats with Procyanidin, spironolactone, and digoxin was non-significant, which may be explained as the effect of several factors that control the MMP9 level such as specific MMP inhibitors defined as tissue inhibitors of matrix metalloproteinases (TIMPs). The imbalance between the MMPs and TIMP, possibly caused by pathological conditions, may lead to an irregularity of the MMP9 level and, especially, the TIMP4 level, which is specific to cardiovascular tissues (46).

The decrease in the CPK level after treatment by Procyanidin, spironolactone, and digoxin, also was not significant compared to the HF group or even the control group. Myocardial necrosis and apoptosis are major initiators of CHF (47). Apoptosis (i.e., programmed cell death), which degrades DNA and is regarded as a strong cause of cell death in HF, requires a significant amount of ATP (48); this may explain the slow decline in the serum level of CPK in the current study.

The ALP level in rats treated with Procyanidin, spironolactone, and digoxin during HF decreased significantly, and the ratio of the decrease compared to the level of other biomarkers was high, which may be explained partly by the improved liver perfusion and partly by the decreased congestion and its complications.

The 30mg/kg/day administration of Procyanidin alone for 14 days did not change any of the hemodynamic and biomarkers in normal rats (Group II) statistically significantly. The negligible effect of the extract on normal rats may be in agreement with Smeriglio et al. (49), who found that the biological action of Procyanidin is sophisticated and most probably appears after continuous intake of these phytochemicals over a long time. In addition, changes in gene expression and their effect on the molecular level may explain the complex reactions in vivo.

HR was not changed statistically significantly during all the stages of HF and treatment in all the groups. The results of this study are not consistent with the study of Dan Liu (50), who evaluated the effect of Procyanidins as useful after myocardial ischemia-reperfusion in rats.

In ancient medicine, drugs were obtained from plants. Herbal chemicals determine their therapeutic effect according to their action in the human body. Therefore, medicinal plants are classified into certain groups according to their radius of action. A medicinal plant does not always have a specific effect, and the spectrum of its effects may increase or decrease. This means that one plant may be effective in treating several diseases; on the contrary, a mixture of several plants is often prepared to multiply their effect to strengthen their therapeutic effect (51-57).

Procyanidin extracted from *Crataegus azarolus* can significantly change several biomarkers and hemodynamic measurements in rats with HF. The effect of Procyanidin compared to spironolactone and digoxin regarding both serum biomarker levels and blood pressure data is close in value. This study reveals the possible uses of Procyanidin for rats suffering from HF.

Acknowledgments
The authors appreciate Firdaus N. Ahmed and Nazar M. Shareef for their grateful help and involvement practically in the research.

Funding statement
The authors received no financial support for conducting this research.

Interest conflict
The authors declare that there are no conflicts of interest.
regarding the publication of this paper.

**Authors’ contribution**

KD: Visualization, data analysis, typewritten review, modification, and supervision; BZR: Literature review, typewritten, data collection, and performance; This article was reviewed and checked critically by the authors for significant rationalistic content to approve the final version of the manuscript.

**Data availability**

Data recorded and/or analyzed statistically for this research are preserved by the corresponding author and can be revealed on formal request.

**Ethical approval**

The present study was approved by the ethics committee in the College of Medicine/Hawler Medical University and recorded with paper code number 11, meeting code 4. We committed to the "Guide for the care and use of laboratory animals" arranged by the National Academy of Science and published by the National Institute of Health.

**Author Details**

1. College of Medicine, Hawler Medical University, Royal city C-05, Erbil, Iraq
2. Technical Institute of Sulaimani, Sulaimani Polytechnic University, Dania city B-14, Sulaimani, Iraq

**References**

Baref Z. Rashid and Kawa F. Dizaye / The Impact of Procyanidin on rats with induced heart failure, 2022, 68(9): 179-185


Ambrosy AP, Bhatt AS, Stebbins AL, Wruuck LM, Fudim M, Greene SJ, et al. Prevalent digoxin use and subsequent risk of death or hospitalization in ambulatory heart failure patients with a reduced ejection fraction—Findings from the Heart Failure: A Controlled Trial Investigating Outcomes of Exercise Trai-