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Research Article

# Hawthorn *(Crataegus oxyacantha)* Flavonoid Extract as an Effective Medicinal Plant Derivative to Prevent Pulmonary Hypertension and Heart Failure in Broiler Chickens

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## Abstract

The aim of this study was to investigate the effect of crateagus flavonoid extract in preventing pulmonary hypertension syndrome (PHS) in broiler chickens reared at high altitude, encountered ascites was evaluated. A 225 day-old broiler chickens (Ross-308) were randomly assigned to three treatments including different drinking levels of crateagus flavonoid extract (0, 0.1, and 0.2 mL per liter of drinking water) in a 42-day trial. Body weight gain were increased and feed conversion ratio were decreased significantly (P<0.05) when crateagus flavonoid extract was consumed by broiler chickens at levels of 0.1 and 0.2 mL per liter of drinking water in the both starting and growing stages, and throughout the trial. Over-expression of inducible nitric-oxide synthase in the heart was observed in chickens consumed different levels of crateagus flavonoid extract. Birds received crateagus flavonoid extract at levels of 0.1 and 0.2 mL had significantly (P<0.05) higher circulatory concentrations of nitric oxide but significantly (P<0.05) lower serum malondialdehyde concentration, hematocrit and heterophil to lymphocyte ratio compared to control group. Consuming crateagus flavonoid extract at levels of 0.1 and 0.2 mL reduced incidence of right ventricular hypertrophy and led to a significant decline in mortality from PHS. It was concluded that crateagus flavonoid extract is an effective medicinal plant derivative to prevent PHS and ascites in broiler chickens by lowering pulmonary blood pressure and increasing serum antioxidant capacities.

***Keywords:*** *Chicken, Crateagus flavonoid extract, Cardiac disorder, Gene expression, Ascites*

# Etlik Piliçlerde Pulmoner Hipertansiyon ve Kalp Yetmezliğini Engellemede Etkili Bir Tıbbi Bitki Türü Olarak Alıç *(Crataegus oxyacantha)* Flavanoid Ekstraktı

## Öz

Bu çalışmanın amacı, yüksek irtifada yetiştirilen broiler tavuklarında pulmoner hipertansiyon sendromunun (PHS) önlenmesinde alıç flavonoid ekstraktının etkisini araştırmaktır. 42 gün süreli çalışmada, 225 adet 1 günlük broiler tavuk (Ross-308) farklı içme suyu seviyelerindeki alıç flavanoid ekstraktlarına göre (0, 0.1 ve 0.2 mL/L içme suyu) rastgele üç uygulama grubuna ayrıldı. Başlangıç ve büyüme evrelerinde tüm çalışma süresince 0.1 ve 0.2 mL/L içme suyu oranında alıç flavanoid ekstraktı tüketen broiler tavuklarda anlamlı derecelerde vücut ağırlık kazanımı artarken yem konversiyon oranı azaldı (P<0.05). Farklı seviyelerde alıç flavanoid ekstraktı tüketen tavukların kalplerinde indüklenebilir nitrik oksit sentazın fazla ekspresyonu gözlemlendi. Kontrol grubu ile karşılaştırıldığında 0.1 ve 0.2 mL oranında alıç flavanoid ekstraktı tüketen tavuklarda anlamlı derecelerde nitrik oksitin daha yüksek dolaşım konsantrasyonuna sahip olduğu (P<0.05) ve serum malondialdehit konsantrasyonu, hematokrit ve heterofil/lenfosit oranlarının daha düşük olduğu (P<0.05) belirlendi. 0.1 ve 0.2 mL oranında alıç flavanoid ekstraktı tüketilmesi sağ ventriküler hipertrofi insidansını azalttı ve PHS'ye bağlı mortaliteyi anlamlı derecede azalmaya neden oldu. Alıç flavanoid ekstraktının, pulmoner kan basıncını düşürmek ve serum antioksidan kapasitesini artırmak suretiyle broiler tavuklarda PHS ve aşitesi önlemede etkili bir tıbbi bitki türü olduğu sonucuna varıldı.

***Anahtar sözcükler:*** *Tavuk, Alıç flavanoid ekstraktı, Kardiyak bozukluk, Gen ekspresyonu, Ascites*

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# INTRODUCTION

Rapid growth in modern broiler chickens has disposed these birds to pulmonary hypertension syndrome (PHS) due to the imbalance between oxygen-demanding muscles and oxygen-supplying organs such as heart and lungs [1]. Intensive genetic selection through the past decades in broiler chickens for rapid growth has reduced the heart and lungs ratio against body muscles mass, whereas increased sensitivity of broiler chickens to PHS will be increase if they are raised at high altitudes with limited atmospheric oxygen supply [2,3]. Succeed vasoconstriction of arterioles will be respond to hypoxia and broiler chickens develops pulmonary hypertension with subsequent right ventricular failure (RVF) that finally leads to ascites and pulmonary vascular remodeling which results from pulmonary hypertension [4,5]. Research has demonstrated the impact of different factors on the development of PHS [6-8]. It is of particularly important to know the effects of herbal medicine in prevention and control of PHS in broiler chickens due to negative effects of PHS on the world broiler chickens industry.

*Crataegus oxyacantha* (common hawthorn) is an endemic member of the *Rosaceae* family that grows in Europe, Africa, and Asia, where is commonly found as a shrub or small tree 5-10 m tall [9]. Scientific evidence has demonstrated that hawthorn fruit, leaves, and flowers possesses potent antioxidant and free radical scavenging activities, due to the presence of different bioactive compounds, such as epicatechin, hyperoside, and chlorogenic acid [9]. These compounds are reported to have many pharmacological effects, including neuroprotective, hepatoprotective, cardioprotective, and nephroprotective [9,10]. Furthermore, hawthorn fruit possesses tonic effects on the heart and could reduce cardiovascular occurrence [11].

In broiler chickens potential of free radicals in creation of PHS has been addressed [12]. Antioxidants play a vital role in protecting cells against reactive oxygen species (ROS) by reducing chemical radicals and disrupting the process of lipid peroxidation [13]. Low quantities of antioxidants in the body of birds with PHS could therefore lead to an inability to control lipid peroxidation [12]. Cawthon et al.[14] observed lower levels of primary antioxidants, and α-tocopherol, and glutathione (GSH) in the mitochondria in the liver of birds with PHS. Dietary supplementation of vitamin E [15], or as an implant [12], and vitamin C in the diet [15,16] have been used to improve body antioxidant status and to prevent ascites.

Ahmadipour et al.[1] showed that body weight gain and feed to gain responses improved when *Kelussia odoratissima Mozzaf* (KOM) was included in broiler diets at 0.05 and 0.75% in the growing stage and throughout the trial. Over-expression of inducible nitric oxide (iNOS) synthase in the heart, higher circulatory concentrations of NO, but lower serum MDA concentration, hematocrit and heterophil to lymphocyte ratio were observed in chickens fed KOM compared to the birds fed the control diet. Feeding KOM prevented from right ventricular hypertrophy and led to a significant decline in mortality from PHS ($P<0.05$).

Based on the report of Tekeli [17] the use of 10 and 20 g/kg of rosehip in the rations under cold stress conditions in broiler chickens significantly reduced T3 hormone, Na, cholesterol, RBC, HCT and HGB compared to the control group ($P<0.05$).

There is no information about the antioxidant effect of crateagus flavonoid extract on the antioxidant status, PHS and ascites incidence in broiler chickens. According to the facts that some compounds in *crataegus oxyacantha* have strong antioxidant potential and some of its compounds have lowering blood pressure effects, the objectives of the present study were to examine the effects of different drinking levels of crateagus flavonoid extract in preventing pulmonary PHS of broiler chickens. To the best of our knowledge, there has been no report on the effect of *crataegus oxyacantha* on pulmonary hypertension in birds.

# MATERIAL and METHODS

### *Experimental Facility and Hypoxic Condition*

The experiment was conducted in the experimental facility of Shahrekord University, Shahrekord, Iran. The study was ethically approved by the Ethical Review Committee of College of Public Health and Medical Sciences of Shahrekord University, Shahrekord, Iran. Management of the chickens in the experimental setting followed the guidelines for animal handling, care and use as pre-scribed by the Ethical Review Committee at Shahrekord University.

Birds were reared at altitude of 2.100 m above sea level under hypoxic conditions known as hypobaric hypoxia faced with ascites. Hypoxic condition was defined as reduced partial pressure of oxygen that occurs at high altitude as the altitude increases up to 1.800 m [1]. The partial pressure of oxygen falls down 7 mmHg for each 1.000 m altitude approximately. This is equal to a reduction of approximately 2.5% of the air oxygen for each 1.000 m altitude [18]. Therefore, compared to sea level with partial pressure of oxygen equal to 21%, the partial pressure of oxygen in the experimental facility of Shahrekord University was calculated to be 15.75%. At this altitude, hypobaric hypoxia will be associated with a high degree of PHS occurrence and could be leads to ascites [5].

### *Birds and Management*

A total of 225 day-old mixed broiler chickens (Ross 308) from a parent stock of age 42 weeks were randomized across 15 floor pens with 2 square meter area (15 birds per pen). All chicks were allocated to pens so that all pens had equal average body weights (46.8±1.2 g). Each pen was equipped with a bell drinker and a feed trough. The temperature of the experimental house was set at about 32°C during week 1, then at a rate of 3°C reduced through week 2 to week 4, and finally fixed at 22°C until the end of trail. All chicks had free access to feed and water and provided with 23 h light and 1 h dark throughout the trial.

### Treatments

A mash diet based on corn and soybean meal were formulated for the starting (1-3 weeks of age, AME:CP=139) and growing (3-6 weeks of age, AME:CP=160) stages according to NRC (1994) recommendations for all treatments *(Table1)*. Experimental treatments were prepared by adding 0.0, 0.1 and 0.2 mL of crateagus flavonoid extract (HE 00152, Crateagus-Drop 6260) per liter of drinking water (pH=7.05; TDS=2.000 ppm) of broiler chickens. So each liter of drinking water contained 0.25 and 0.50 mg of total flavonoids compounds. In this way, birds in groups of 0.1 and 0.2 received 0.05 to 0.10 mg of total flavonoids compounds daily. Generally, flavonoid extract of c*rataegus oxyacantha* containing biologically active flavonoid compounds (polyphenols) like anthocyanidins and proanthocyanidins (also known as bioflavones or procyanidins). Each mL of oral crataegus-Drop 6260 contained 2.5 mg of total flavonoids compounds in form of hyperoside (21.4% polyphenols and 19.7% procyanidins), produced by Iran-Darouk Pharmacy Co, under production code of 3067-88-02. Determination of total phenolic compounds in crataegus-Drop 6260 was done through colorimetric method according to the standard extraction procedure by mentioned company [19].

### Measurements

Mass body weight of birds in each pen was obtained at 21 and 42 days of age. Body weight gain and feed intake were calculated for 1-21 day, 21-42 day, and 1-42 day periods. Feed conversion ratio (FCR) data corrected for mortality weights, was also calculated for all of periods. At 42 days of age, 10 birds per treatment were selected for blood collection and processing. The selected birds had body weights within approximately 5% of the average pen body weight. Blood samples (3 mL) were collected from the brachial vein and centrifuged at 2500 g for 10 min to obtain sera. Serum samples were used for the determination of NO and MDA. Serum NO was measured according to the method described by Chapman and Wideman [20]. Serum MDA concentration as biomarker of oxidative stress was assayed by the method of Nair and Turner [21].

For measuring hematocrit, samples of blood were collected in micro-hematocrit tubes. An aliquot of blood was also obtained on glass slides to prepare the blood smear for the determination of differential leukocyte count. Thereafter the May-Grunwald and Giemsa staining, 100 leukocytes, including granular (heterophils) and non-granular (lymphocytes) were enumerated and the heterophil to lymphocyte ratio (H:L) was calculated. All chemical reagents were obtained from Sigma-Aldrich Co. (Sigma-Aldrich Co., St. Louis, MO, USA). After the blood collection, the birds were killed by decapitation. Data obtained at processing time were included live body weight, hot carcass weight, breast weight, and thigh weight. The hearts were also removed and the ventricles were dissected and weighed to calculate the right-to-total ventricular weight ratio (RV: TV ratio). The RV: TV is indicative of pulmonary hypertension [7]. In addition, total mortality and mortality from PHS was

***Table1.*** *Composition of the basal diet for broiler chickens during starter and grower stages*

| Item (% Unless Noted) | Starter (1-21 Days) | Grower (22-42 Days) |
|---|---|---|
| Corn | 47.4 | 55.8 |
| Soybean meal (44% CP) | 37.3 | 33.5 |
| Fish meal (60% CP) | 3.6 | 1.1 |
| Wheat bran | 0.5 | 1.3 |
| Soy oil | 7.5 | 4.7 |
| Dicalcium phosphate | 1.3 | 1.2 |
| Oyster shell | 1.45 | 1.5 |
| Salt | 0.35 | 0.3 |
| DL-Methionine | 0.1 | 0.1 |
| L-Lysine | - | - |
| Mineral supplement[a] | 0.25 | 0.25 |
| Vitamin supplement[b] | 0.25 | 0.25 |
| **Calculated composition** | | |
| AME (kcal/kg) | 3200 | 3200 |
| CP | 23 | 20 |
| AME:CP | 139 | 160 |
| Met | 0.52 | 0.41 |
| Met+Cys | 0.86 | 0.74 |
| Lys | 1.3 | 1.06 |
| Thr | 1 | 0.91 |
| Arg | 1.46 | 1.29 |
| Ca | 1 | 0.91 |
| Available P | 0.45 | 0.35 |
| Na | 0.18 | 0.15 |
| Cl | 0.27 | 0.29 |
| K | 0.91 | 0.92 |
| Na + K – Cl (mEq/kg) | 237 | 238 |

[a] *Provided the following per kg of diet: vit. A (trans retinyl acetate), 3600 IU; vit. $D_3$ (cholecalciferol), 800 IU; vit. E (dl-α-tocopheryl acetate), 7.2 mg; vit. $K_3$, 1.6 mg; thiamine, 0.72 mg; riboflavin, 3.3 mg; niacin, 0.4 mg; pyridoxin, 1.2 mg; cobalamine, 0.6 mg; folicacid, 0.5 mg; choline chloride, 200 mg.*
[b] *Provided the following per kg of diet: Mn (from $MnSO_4.H_2O$), 40 mg; Zn (from ZnO), 40 mg; Fe (from $FeSO_4.7H_2O$), 20 mg; Cu (from $CuSO_4.5H_2O$), 4 mg; I [from $Ca(IO_3)_2.H_2O$], 0.64 mg; Se (from sodium selenite),0.08 mg*

checked daily throughout the trial and whenever the RV: TV was greater than 0.25 are considered as pulmonary hypertension [22].

### PCR Analysis

At the end of trail (42 days of age), 10 chickens from the control group and the groups received different levels of crateagus flavonoid extract were randomly selected, weighed and killed by decapitation. The hearts were harvested and the right ventricles were dissected and immediately frozen in liquid nitrogen and stored at -70°C for subsequent RNA analysis. Specific primers of SOD1, iNOS and β-actin were designed with Primer-Blast (NCBI). Details of the primers are listed in *Table 2*.

Polymerase chain reactions (PCRs) were carried out in a realtime PCR cycler (Rotor Gene Q6000, Qiagen, USA) in three replicates for each sample of ventricles. The quantitative polymerase chain reaction (qPCR) methodology was followed as explained with slight modification [23]. One microliter cDNA (complementary DNA) was added to the 10 μL of SYBR® Premix Ex Taq II Mix and 1 μL of each specific primer in a total volume of 20 μL. The thermal profile was 95°C for 30 s, 40 cycles of 94°C for 40 s, 64°C for 35 s and 72°C for 30 s. At the end of each phase, the measurement of fluorescence was done and used for quantitative objectives. Gene expression data were normalized to β-actin. Data were analyzed using LinReg PCR software version 2012.0 (Amsterdam, Netherland), to give the threshold cycle number and reaction efficiency [24]. Relative transcript levels and fold changes in transcript abundance were calculated using efficiency adjusted Paffl methodology [25].

### Statistical Analysis

Results were analyzed by GLM procedure using SAS (2007) software in a completely randomized design. Data were subjected to a nested design when there was sampling effect within pens. The statistical model used for growth performance data was $Y_{ij} = \mu + T_i + e_{ij}$. For other traits, the model was $Y_{ijk} = \mu + T_i + e_{ij} + \varepsilon_{ijk}$. In these models, $Y_{ij}$ and $Y_{ijk}$ are observations; μ is the general mean; Ti is the effect of treatment i; $e_{ij}$ is random error; and $\varepsilon_{ijk}$ is subsampling error. Means were separated by Duncan's multiple range test.

## RESULTS

Effects of different drinking levels of crateagus flavonoid extract on broiler chickens growth performance and the rate of mortality are shown in *Table 3*. Body weight gain and FCR improved when drinking crateagus flavonoid extract was used by broiler chickens at levels of 0.1 and 0.2 mL throughout the trial (P<0.05). However, no significant effect was observed among treatments in terms of feed intake in 1-21 days of age. Significant decline in mortality percentage of birds was observed through different stages of trail in the groups received 0.1 and 0.2 mL of crataegus flavonoid extract compared to the control group (P<0.05).

*Table 4* indicates blood and serum variables of broiler chickens received different levels of drinking crateagus flavonoid extract. Broiler chickens received drinking crateagus flavonoid extract at levels of 0.1 and 0.2 mL had higher concentrations of NO, but lower concentrations of MDA than that of control group (P<0.05). Both levels of drinking crateagus flavonoid extract caused a reduction in heterophil to lymphocyte ratio and hematocrit when compared to the control (P<0.05).

The expression of SOD1, iNOS, and ET-1 genes in the heart of broiler chickens affected by different levels of drinking crateagus flavonoid extract *(Table 5)*. Superoxide dismutase-1 was highly over-expressed in broiler chickens consumed drinking crateagus flavonoid extract at both levels of 0.1 and 0.2 mL. Inducible nitricoxide synthase was also highly over-expressed in the right ventricle of birds consumed drinking crateagus flavonoid extract at levels of 0.1 and 0.2 mL. On the other hand, crateagus flavonoid extract significantly suppressed the expression of ET-1.

*Table 6* depicts the carcass characteristics of broiler chickens consumed different levels of drinking crateagus flavonoid extract at 42 days of age. Carcass yield was higher in broiler chickens consumed levels of 0.1 and 0.2 drinking crateagus flavonoid extract compared to control group, but breast and thigh yields were not affected by different levels of drinking crateagus flavonoid extract. However, inclusion of crateagus flavonoid extract in drinking water of broiler chickens reduced the proportions of liver, heart and abdominal fat when compared to the

***Table 2.*** *Details of the primers used for quantitative real time PCR analysis of chicken mRNAs*

| Target | Primers | PCR Product (bp) | Accession No |
|---|---|---|---|
| β-Actin | 5′-AGCGAACGCCCCCAAAGTTCT-3′<br>5′-AGCTGGGCTGTTGCCTTCACA-3′ | 13 | NM_205518.1 |
| SOD1 | 5′-CACTGCATCATTGGCCGTACCA-3′<br>5′-GCTTGCACACGGAAGAGCAAGT-3′ | 223 | NM_205064.1 |
| iNOS | 5′-AGGCCAAACATCCTGGAGGTC-3′<br>5′-TCATAGAGACGCTGCTGCCAG-3′ | 371 | U46504 |
| ET-1 | 5′-GGACGAGGAGTGCGTGTATT-3′<br>5′-GCT CCAGCAAGCATCTCTG-3′ | 141 | XM418943 |

*SOD1: superoxide dismutase 1; iNOS: inducible nitric oxide synthase; ET-1: endothelin 1; bp: base pair*

**Table 3.** *Effects of drinking crataegus flavonoid extract on broiler's growth performance and mortality percentage*

| Parameter | Age | Drinking Levels of *Crataegus* Flavonoid Extract | | | SEM |
|---|---|---|---|---|---|
| | | Control (0 mL) | 0.1 (mL) | 0.2 (mL) | |
| Weight gain (g/bird) | 1-21 days of age | 664.13[b] | 713.00[a] | 703.49[a] | 16.84 |
| | 22-42 days of age | 1334.09[b] | 1473.64[a] | 1534.79[a] | 41.64 |
| | 1-42 days of age | 1998.22[b] | 2186.64[a] | 2238.18[a] | 40.47 |
| Feed intake (g/bird) | 1-21 days of age | 1051.75 | 1031.25 | 1012.93 | 26.42 |
| | 22-42 days of age | 2860.96[b] | 2945.33[a] | 2898.24[ab] | 33.61 |
| | 1-42 days of age | 3887.71[b] | 4001.58[a] | 3961.17[ab] | 41.48 |
| Feed conversion ratio | 1-21 days of age | 1.58[a] | 1.45[b] | 1.44[b] | 0.02 |
| | 22-42 days of age | 2.14[a] | 2.00[b] | 1.89[c] | 0.02 |
| | 1-42 days of age | 1.95[a] | 1.83[b] | 1.77[c] | 0.03 |
| Mortality percentage (%) | 1-21 days of age | 8.25[a] | 6.09[b] | 5.98[b] | 0.38 |
| | 22-42 days of age | 23.27[a] | 16.43[b] | 16.17[b] | 0.66 |
| | 1-42 days of age | 31.52[a] | 22.52[b] | 22.15[c] | 0.91 |

**Table 4.** *Effect of drinking crataegus flavonoid extract on serum and blood variables in broiler chickens measured at 42 days of age*

| Parameter | Drinking Levels of *Crataegus* Flavonoid Extract | | | SEM |
|---|---|---|---|---|
| | Control (0 mL) | 0.1 (mL) | 0.2 (mL) | |
| Plasma nitric oxide (µmol/L) | 5.32[c] | 6.71[b] | 8.06[a] | 0.36 |
| Malondialdehyde (µmol/L) | 2.09[a] | 1.06[b] | 0.84[c] | 0.11 |
| Heterophil to lymphocyte (%) | 1.03[a] | 0.70[b] | 0.61[b] | 0.16 |
| Hematocrit (%) | 39.75[a] | 36.13[b] | 32.50[c] | 1.65 |

*Superscripts in the same row with different letters are statistically different (P<0.05)*
*Each mean represents values from 10 replicates*

**Table 5.** *Effect of drinking crataegus flavonoid extract on expression of SOD1, iNOS, and ET-1 genes in the right ventricle of broiler chickens measured at 42 days of age*

| Item | Control (T1) | 0.1 (T2) | 0.2 (T3) | T2/T1 Ratio | T3/T1 Ratio | SEM |
|---|---|---|---|---|---|---|
| SOD1 | 0.0001[c] | 0.006[b] | 0.021[a] | 60 | 210 | 0.008 |
| iNOS | 0.001[c] | 0.024[b] | 0.603[a] | 24 | 603 | 0.016 |
| ET-1 | 0.047[a] | 0.001[b] | 0.001[b] | 0.021 | 0.021 | 0.024 |

*Superscripts in the same column with different letters are statistically different (P<0.05). SOD1: superoxide dismutase1; iNOS: inducible nitricoxide; ET-1:endothelin1; CAT: Catalase. Number of observation=20*

**Table 6.** *Effect of drinking crataegus flavonoid extract on carcass characteristics of broiler chickens at 42 days of age (as % of carcass weight)*

| Item (%) | Drinking Levels of *Crataegus* Flavonoid Extract | | | SEM |
|---|---|---|---|---|
| | Control (0 mL) | 0.1 (mL) | 0.2 (mL) | |
| Carcass yield | 67.68[b] | 70.78[a] | 70.66[a] | 1.39 |
| Breast yield | 35.27 | 35.05 | 36.35 | 0.87 |
| Thigh yield | 30.21 | 30.54 | 30.22 | 0.45 |
| Abdominal fat | 1.39[a] | 1.14[b] | 1.05[b] | 0.10 |
| Liver | 2.88[a] | 2.52[b] | 2.26[c] | 0.07 |
| Heart | 0.83[a] | 0.71[ab] | 0.63[b] | 0.06 |
| RV:TV (ratio) | 0.32[a] | 0.25[b] | 0.22[b] | 0.02 |

*Superscripts in the same row with different letters are statistically different (P<0.05)*
*Each mean represents values from 10 replicates. RV:TV right ventricle to total ventricle weight ratio*

**Table 7.** *Effect of drinking crataegus flavonoid extract on total mortality and mortality from PHS in broiler chickens reared up to 42 days of age*

| Item (%) | Drinking Levels of *Crataegus* Flavonoid Extract | | | SEM |
|---|---|---|---|---|
| | Control (0 mL) | 0.1 (mL) | 0.2 (mL) | |
| Total mortality | 31.52[a] | 25.52[b] | 22.15[b] | 2.91 |
| PHS mortality | 28.72[a] | 22.32[b] | 18.41[b] | 2.88 |

*Superscripts in the same row with different letters are statistically different (P<0.05)*

control. In addition, using of drinking crateagus flavonoid extract decreased the RV:TV ratio (P<0.05).

In *Table 7* compared the total mortality with mortality from PHS in broiler chickens consumed different levels of drinking crateagus flavonoid extract up to 42 days of age. Consuming different levels of drinking crateagus flavonoid extract at both levels of 0.1 and 0.2 mL caused a reduction in PHS mortality (P<0.05).

## DISCUSSION

In this study increase of body weight gain and decrease in FCR was observed in broiler chickens after consuming different levels of drinking crateagus flavonoid extract which can be attributed to positive effects of this compound. As a rule, the RV:TV ratio is an index of pulmonary hypertension in chickens so that the RV:TV values greater than 0.25 regards as pulmonary hypertension [1,6]. In the control group the mean value of RV: TV was greater than 0.25, implicated to further number of birds in this group which suffered from pulmonary hypertension. Increased growth performance of birds in the groups received drinking crateagus flavonoid extract can also be attributed to the polyphenols (flavonoids) and oligomeric proanthocyanidins (OPCs) compounds in c*rataegus oxyacantha*. The plant polyphenols including flavonoids and non-flavonoids exhibit a broad spectrum of beneficial biological properties such as growth-promoting, anti-oxidative, sedative, antibacterial and anti- viral actions [26]. Increased serum concentration of NO as a result of consuming different levels of crateagus flavonoid extract to broiler chickens is due to the over- expression of iNOS gene in broiler's heart. It has been demonstrated that iNOS gene is normally expressed in the heart of broiler chickens and contributed in normal NO production in myocardio-cytes. NO is an important regulator of cardiac function by involvement in the control of myocardial energetics, myocardial regeneration, hyper- trophic remodeling and improvement of ventricular diastolic distensibility [1,27]. It has been suggested that impaired NO synthesis and local reduction of iNOS gene expression in the heart ventricles are involved in the pathophysiology of cardiac failure in broiler chickens with pulmonary hypertension [28]. On the other hand, consuming different levels of crateagus flavonoid extract especially at level of 0.2 mL per liter in drinking water of broiler chickens caused significant reductions in circulatory level of MDA. MDA is an indicator of lipid oxidation in the body and it is an index of oxidative stress. It is clear that chickens are very susceptible to oxidative stress because of their higher metabolic rate [29]. Increased metabolic rate resulted in higher production of ROS [1,12]. Moreover, birds have a body temperature about 3°C higher than in mammals, which expands the production of ROS [30,31].

As well as, chicken's blood glucose concentration as a potent oxidative factor is at least twice as high as that of mammals [32], so antioxidant potency is crucial to broiler chickens against oxidative agents [33]. Some of compounds in crateagus flavonoid extract such as flavonoids, particularly OPCs contribute to the productive roles against oxidative stress and lipid peroxidation (reduced MDA concentration) along with increase the activities of the antioxidant enzymes glutathione peroxidase, super-oxide dismutase and catalase, which counteract the oxidative stress [9,34]. Additionally, some organic acids such as chlorogenic acid and ferulic acid has ferric reducing ability and by involvement in $Fe^{2+}/H_2O_2$ systems exerts antioxidant activity [34-36]. Following mechanisms through using flavonoids can also prevent oxidative stress including: direct scavenging of ROS, activation of anti-oxidant enzymes, metal chelating activity, reduction of α-tocopheryl radicals, inhibition of oxidases, and increase in uric acid level [3,26]. Flavonoids have great amount of vitamin P (citrin bioflavonoid) which can synergistically act with vitamin C which counteract the oxidative products [37,38]. Significant reductions observed in the H:L ratio and hematocrit in birds consumed different levels of crateagus flavonoid extract are in accordance with decreased oxidative stress. The H:L ratio is an index to describe stress in the chicken [39]. Therefore, consuming crateagus flavonoid extract suppresses ROS production and deduces the oxidative stress of birds, which led to increase the growth performance and reducing the MDA level and H:L ratio.

Also in this study similar to previous experiment [1], abdominal fat deposition and liver percentage was significantly reduced in chickens consumed different levels of crateagus flavonoid extract. crateagus flavonoid extract has lipolytic effect [40,41]. Lipolytic effect of crateagus flavonoid extract is attributed to flavonoids and OPCs as well as phenolic compounds [40,42]. Lipid-lowering effects of flavonoids have been well documented [43]. Reduced liver percentage compare to live body weight in chickens consumed different levels of crateagus flavonoid extract is in line with decreased lipogenesis as appeared in reduced abdominal fat. Liver in the chicken is the primary site of lipogenesis [44] and declined liver weight reflects lower lipogenesis due to the consuming of crateagus flavonoid extract in broiler chickens diets. The proportion of heart percentage to live body weight and RV:TV ratio have been reduced by consuming of crateagus flavonoid extract to birds. These observations confirm ability of *crataegus oxyacantha* flavonoid extract to prevent heart hypertrophy and particularly right ventricular hypertrophy. It is evident that birds of the control group (RV:TV more than 0.25) are in pre-ascitic condition and this situation has been improved when birds consumed crateagus flavonoid extract at levels of 0.1 and 0.2 mL per liter of drinking water. Although other research findings in this regard point to this fact that the RV:TV more than 0.27 can be considered as ascetic condition [43]. In this regard, a significant decline in total mortality and mortality from PHS was observed in the groups received consumed different levels of crateagus flavonoid extract when compared to the control group.

*Crataegus oxyacantha* flavonoid extract significantly promoted over expression of SOD in the heart of chickens. Research has shown that over expression of SOD reduces hypertension, increases availability of NO and endothelium-dependent relaxation in different models of hypertension [1,45]. This finding explains significant reduction in the incidence of PHS in birds consumed crateagus flavonoid extract at levels of 0.1 and 0.2 mL per liter of drinking water. According to previous reports, the vascular remodeling in lung vessel beds contributes to mortality of broiler chickens with PHS [5,7]. Flavonoid content of crateagus extract prevents the proliferation of vascular smooth muscle cells and inhibits thickening of the intima and narrowing of the vessels, as well as exerts considerable collagen stabilizing effect [46,47]. Thus, flavonoids and OPCs contribute to be the effective factor in preventing cardiovascular diseases [11,47]. Flavonoids and OPCs has also vaso-relaxant potential and considering the fact that flavonoids and OPCs is the main constitute of c*rataegus oxyacantha*, this medicinal plant could effectively prevent PHS in broiler chickens. Furthermore, flavonoids as the main constitute of *crataegus oxyacantha* have endothelium-independent vaso-dilating effects and by possessing lowering blood pressure potential [33,48] further improved cardio-pulmonary function and helped to prevent PHS [11,46]. It is worth noting that the vasodilatory effect of flavonoid compounds may be intensified by over production of NO synthesis [1,48]. Significant decrease in the expression of ET-1 by consuming of crateagus flavonoid extract further suggests the potential of this plant extract in preventing pulmonary hypertension.

In conclusion, consuming different levels of crateagus flavonoid extract could significantly prevent PHS and cardiac disorders in broiler chickens reared at high altitudes encountered to ascites. Beneficial effects of crateagus flavonoid extract are attributed to antioxidant actions that mediated through flavonoids and OPCs bioactive compounds. Therefore, crateagus flavonoid extract is an effective medicinal plant derivative to prevent pulmonary hypertension in broiler chickens under the terms of ascites and reared at high altitude.

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## Authors Conflict

All the authors have not conflict.

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