Eurasian Journal of Molecular and Biochemical Sciences

https://dergi.erzurum.edu.tr/ejmbs

The Effects of Quercetin Administration on Heart Tissue and Serum Parameters in the Rats with Experimental Obesity

Cihan GÜR[1], Seçkin ÖZKANLAR[1], Semin GEDİKLİ[2], Emin ŞENGÜL[3], Volkan GELEN[4*], Adem KARA[5]

[1]*Ataturk University, Veterinary Faculty, Department of Biochemistry, 25240, Erzurum /Turkey*

[2]*Ataturk University, Veterinary Faculty, Department of Histology and Embryology, 25240, Erzurum/ Turkey*

[3]*Ataturk University, Veterinary Faculty, Department of Physiology, 25240, Erzurum/ Turkey*

[*4]*Kafkas University, Veterinary Faculty, Department of Physiology, 36100, Kars/ Turkey*

[2]*Erzurum Technical University, Faculty of Science, Department of Molecular Biology and Genetics, 25050, Erzurum/ Turkey*

Cite: *Gür C, Özkanlar S, Gedikli S, Şengül E, Gelen V, Kara A. The Effects of Quercetin Administration on Heart Tissue and Serum Parameters in the Rats with Experimental Obesity. Eurasian Mol Biochem Sci 2022;1(1): 16-21.*

Received: 16 March 2022, Accepted: 04 April 2022 *DOI. 10.54672/ejmbs.2022.316.5*

Abstract

Obesity causes structural and functional damage to various organs. Quercetin is a flavonoid and has potent antioxidant, anti-apoptotic and anti-inflammatory effects in the body cells. This study aims to investigate the therapeutic effect of Quercetin on the cardiac effect caused by obesity in rats with experimental obesity using biochemical and histological methods. In this study, 24 male Sprague Dawley rats were used. The rats were divided into three groups i.e., control, obese, and Quercetin-Obese. A high-fat diet was administrated to the obese groups for 3 months, the other groups were fed with normal pellet forage. After the formation of obesity, a 50 mg/kg dose of Quercetin was orally fed to the quercetin-obese and quercetin groups for 15 days. At the end of study, all the animals were sacrificed by taking blood under anesthesia (sevoflurane), and their heart tissues were taken. In the obtained serum samples, the levels of triglyceride (TG), cholesterol (CHOL), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), and cardiac troponin-I (cTnI) were measured by the auto-analyzer device. The heart tissues were stained with Bax and Bcl-2 antibodies as immuno-histochemical. When the results of the analysis were compared among the control and other groups, it was shown that obesity increased the levels of serum TG, CHOL, LDL-C, and cTnI whereas quercetin administration had a decreasing effect on these parameters ($P<0.05$). It was determined that the level of Serum HDL-C decreased in the obese group while quercetin administration increased the level of HDL-C ($P<0.05$). The analysis of Bax and Bcl-2 immune reactive cells showed that the apoptotic cell density increased in the heart tissues of the obese group while quercetin administration decreased the apoptotic cell density ($P<0.05$). This study shows that Quercetin may have a therapeutic effect in avoiding cardiac injuries caused by obesity.

Keywords: Obesity, Quercetin, Heart, Cholesterol, Apoptosis

* *Corresponding Author:* Volkan GELEN,
Kafkas University, Veterinary Faculty, Department of Physiology,
36100, Kars/ Turkey
E-mail: gelen_volkan@hotmail.com

Introduction

Obesity is defined as the excess of fat tissues or an increase in body mass index, which is described as a paradox that started in the 1980s and became an epidemic worldwide. Obesity can cause various complications such as heart failure, coronary heart disease, type 2 diabetes mellitus, and hypertension in humans [1,2]. One of the most significant risk factors of obesity is heart failure and is one of the biggest life-threatening health problems [3]. Previous studies showed that obesity causes heart failure due to myocardial damage, but the exact mechanism of cardiac injury is not fully understood [4]. It has been reported that the high levels of LDL-C and triglyceride values and low levels of HDL-C play an essential role in the formation of damage [5]. Troponin-I is a cardiac-specific protein used as a specific marker in measuring the extent of the injury [6]. To diagnose heart damage or to determine whether the clinical signs are due to cardiac causes, researchers have recently recommended high-sensitivity measurements of cardiac troponin-I in cardiac injury [7].

Quercetin is a flavonoid and has potent antioxidant, anti-apoptotic and anti-inflammatory effects in the body cells [8]. Quercetin is one of the most abundant dietary flavonoids with extensive pharmacological and antioxidant properties [9]. One of the pharmacological properties of Quercetin is cardiac protection [10]. Among the essential cardiac protective properties are anti-hypertensive and anti-atherogenic effects. In addition, it prevents endothelial dysfunction and protects the myocardium from ischemia [11]. It is believed that its antioxidant property comes from its ability to chelate Fe2+ and Cu2+ ions and its scavenging feature of free radicals [12]. Besides, previous studies have revealed the protective role of inhibiting oxidative damage [13]. For this reason, this study aimed to investigate the cardiac effects of experimental obesity in rats and the role of Quercetin in the prevention of obesity-mediated cardiac damage by biochemical and histological methods.

Materials and Methods

Animal housing and procedure: This study used 24 adult male Sprague Dawley rats, 10-12 weeks old (average weight of 200-250g), obtained from Atatürk University Medical Experimental Research and Application Center. The study was approved by Atatürk University Experimental Animals Ethics Committee (Decision No: 2022/52). The rats were kept in cages at 25 ± 2 °C temperature, 60-65% humidity with 12-hour light and 12-hour dark cycles, fed *ad libitum*. The cages were cleaned daily and after one week of acclimation, rats were randomly divided into four groups Control, Obesity, Quercetin-Obesity, and Quercetin.

Obesity induction: The rats were fed a high-fat diet (35 kcal% as fat) for the experimental model of obesity for 120 days as reported in previous studies [14]. Control group animals were fed with a normal ad libitum diet (10% kcal as fat). Bodyweight gain was measured at the beginning of study and weekly reviewed for obesity assessment, along with food consumption. Rats that reached 8-20% weight gain according to the initial weight determined as the obesity criterion were accepted as obese. At the end of study, non-obese rats were excluded from the study.

Quercetin treatment: In the period following the onset of obesity (120 days later), quercetin and quercetin-obese groups were administered orally at 50 mg/kg suspended Quercetin in 1 ml corn oil for 15 days. The obese group was given 1 ml of corn oil orally for 15 days. At the end of the study, cardiac blood samples were taken from all groups of rats under deep anesthesia with sevoflurane (3%) and then were sacrificed and their heart tissues were removed for histological examination.

Biochemical analysis: To obtain sera samples, the blood samples of rats were centrifuged at 1700 g for 8

min. The serum samples were stored in the freezer at –80 °C before they were serum enzyme analyses. The serum samples were measured for the levels of triglyceride (TG), cholesterol (CHOL), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), and cardiac troponin-I (cTnI) by using the auto-analyzer (Beckman Coulter; DXI 800 USA).

Histologic analysis: Obtained heart tissues were fixed in 10% neutral formaldehyde for 72 hours and then washed with tap water and overnighted. The tissues were passed from the ascending alcohol series and cleaned with xylene. The tissues were then embedded in paraffin blocks and 5 µm thick tissue cuts were stained with Crossman modified Mallory's Triple staining. The tissue sections were examined in a trinocular light microscope (Nikon Eclipse 50i, Japan).

Immunohistochemical staining: After deparaffinized and dehydrating, the sections were left in 3% hydrogen peroxide phosphate buffer solution (PBS) at pH 7.4 for 20 minutes. Sections passed through PBS were left in sodium citrate buffer (pH 6.0) in a 600 W microwave oven to reveal antigenic receptors. Sections were incubated in Blocking Solution A (Invitrogen-Histostatin Plus Bulk Kit) for 8 min. Then Bax (1/50 dilution, Abcam) and Bcl-2 (1/50 dilution, Abcam) primary antibodies (Abcam, Ab183855) (1:500) were treated in sections for 1 hour at 37 C^{o} in the humidity chamber. After the sections were left for 30 min in biotin secondary antibody, Blocking Solution C (streptavidin peroxidase) was added and they were gone for another 30 min. Then diaminobenzidine (DAB) chromogen solution was added and hematoxylin staining was applied for contrast staining. Immunohistochemical examinations were performed under a light microscope (Nikon Eclipse 50i, Japan) and then photographs were taken. Semiquantitative analyses for all groups investigated the Bax and Bcl-2 immunoreactivity degree at the cellular level. To estimate the immune reactive cell count, staining intensity was measured using the stereological optical fractionator method, as described in detail in our previous studies [15,16].

Statistical analysis: The statistical significance among groups was analyzed by one-way ANOVA (Duncan *post hoc* test). The results were considered statistically significant when $p < 0.05$. Data are expressed as mean ± Standard deviation (SD).

Results

Biochemical results: Serum TG, CHOL, LDL-C, and cTnI levels were increased in the obesity group compared with the Control group ($p<0.05$), On the other hand, these parameters were decreased in the Obesity-Quercetin group compared with the Obesity group ($p<0.05$). While it was determined that the serum HDL-C level decreased in the obese group, quercetin administration significantly increased the HDL-C level ($P<0.05$).

Histologic analysis: Two specialists in histology blindly evaluated the possible cardiac damages. The lesions have been assessed as shown below. In the case

Table 1. Serum triglyceride (TG) cholesterol (CHOL), high-density lipoprotein (HDL-C), low-density lipoprotein (LDL-C), and Cardiac Troponin-I (cTnI) levels for all groups.

	TG	CHOL	HDL-C	LDL-C	cTnI
Control	69.24±16.32^{a}	61.80±17.39^{a}	45.33±9.28^{a}	20.17±3.64^{a}	6.28±1.75^{a}
Obesity	83.86±17.44^{b}	77.75±15.38^{b}	55.82±12.58^{b}	27.44±6.85^{b}	12.38±1.86^{b}
Quercetin-Obesity	72.54±15.53^{a}	70.33±13.32^{a}	48.10±13.8^{a}	21.75±7.42^{a}	7.72±2.62^{a}

The letters indicated the statistical differences in the column, P-value was accepted as 0.05. Data were analyzed with ANOVA (Duncan) test.

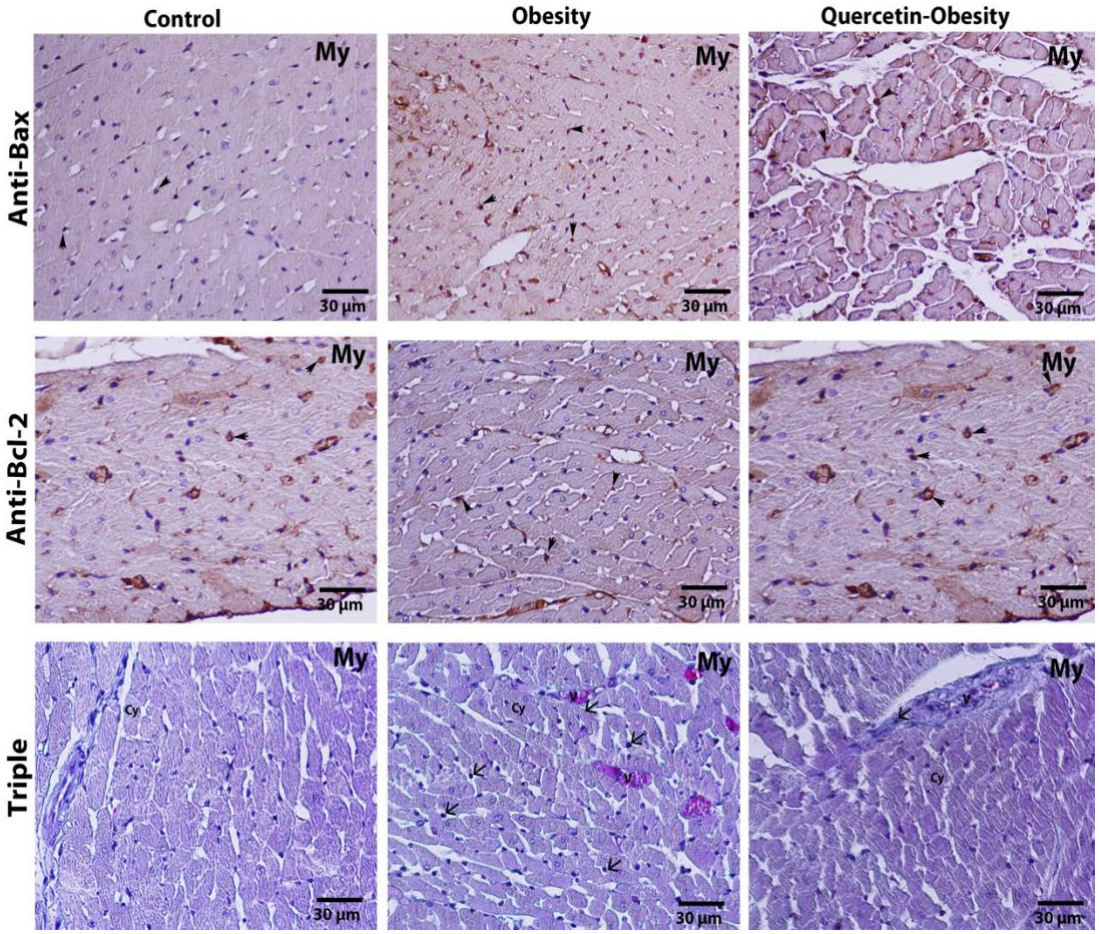

Figure 1. Illustration of anti-Bax, anti-Bcl-2, and Crossman Modified Triple staining in the heart tissues for all groups, My: myocardium, Cy: transverse area of cardiomyocyte, **arrowhead:** anti-Bax and anti-Bcl-2 immune reactive cells, **open arrows:** PNML cells

of the study of cardiomyopathy, inflammation and fibrosis have been assessed. In the examination of the myocardium and subendocardial fibrosis. On the other hand, these changes were considerably decreased in Quercetin- Obesity group (Figure 1).

Stereological Immunohistochemical analysis: In the stereological analysis of anti-Bax and anti-Bcl-2 immune reactive cells, Bax immune reactive cell density was increased in the Obesity group while decreased in the Quercetin-Obesity group ($p<0.05$). Besides, Bcl-2 cell density was reduced in the Obesity group compared with the Control group. However, it increased in the Quercetin-Obesity group ($p<0.05$). The anti-Bax and anti-Bcl-2 immune reactive cell densities and comparisons are seen in Table 2.

Table 2. Stereological evaluation of anti-Bax and anti-Bcl-2 positive cells densities in the serially obtained heart tissue sections per 1000 μm^2 area.

Groups	Bax	Bcl-2
Control	1,24±0,33[a]	3,80±0,79[a]
Obesity	3,86±0,94[b]	1,75±0,38[b]
Quercetin-Obesity	2,54±0,53[c]	2,33±0,56[c]

The letters indicated the statistical differences in the column, P-value was accepted as 0.05. Data were analyzed with ANOVA (Duncan) test.

Discussion

Quercetin is a flavonoid and its effects are currently being investigated in the prevention of many tissue or organ damages [17]. On the other hand, its anti-obese and anti-apoptotic effects have been shown in previous studies [18-21]. In the present study, a metabolic

obesity rat model was created, and Quercetin was administered at a dose of 50mg/kg which is in line with previous studies [22].

The high-fat diet used in the present study was sufficient intensity and duration to induce obesity in rats. Also, previous research suggested that rats fed with a high-fat diet for eight weeks caused obesity and impaired hypertension, inflammation, dyslipidemia, endothelial dysfunction, and cardiac fibrosis in the heart [23,24]. These changes were closely similar to the human metabolic syndrome. In the present study, Serum TG, CHOL, LDL-C, and Trop-I levels were increased and serum HDL-C level was decreased in the obesity group. But Quercetin treatment reduced TG, CHOL, LDL-C, and cTnI levels and increased the serum HDL-C level in the obese rats.

In histopathologic evaluation, eosinophilic changes, inflammatory cell infiltration, and fibrosis were observed in the obesity group heart tissues. A previous study reported the interstitial collagen deposition in the left ventricle of the obesity group [25]. However, other studies reported that did not find a histological increase in cardiac collagen in a rat model of diet-induced obesity [26] in rabbits [27]. Possible mechanisms of collagen deposition are related to inflammation and cell infiltration. Additionally, it has been reported that the higher collagen deposition is related to abnormalities in insulin metabolism [25]. Quercetin has an anti-inflammatory effect that inhibited inflammation-mediated collagen deposition and cardiac damage in obesity [28].

Increased apoptotic activity was determined in the heart tissue in the case of obesity. Bax and Bcl-2 proteins are apoptosis-related proteins that determine cell death. It has been reported that obesity-induced apoptosis is shaped by lipid accumulation in the endoplasmic reticulum in the cell [29]. In this study, the levels of the proapoptotic member of the Bcl-2 family and the anti-apoptotic member of Bax were investigated by immunohistochemical analysis. The results showed that while the Bax level increased in the obesity group, it decreased in the quercetin-obesity group. It was determined that the Bcl-2 level decreased in the obesity group and raised in the quercetin-obesity group. Similar to us, previous studies reported the cardioprotective effects of Quercetin in high-fat diet-induced obesity [28,30, 31].

In conclusion, the present data provide scientific evidence that Quercetin may provide protection against cardiac damage and may reduce lipid accumulation and inflammation in the heart tissue. Quercetin contained multiple bioactive compounds which might act synergistically to produce protective effects.

Declaration of Interest: No potential conflict of interest relevant to this article was reported.

Authors' Contributions: CG, SÖ, SG, EŞ, VG and AK contributed to the study conception and design. CG, VG, AK contribution to laboratory work. Writing the article (SÖ, EŞ, SG and AK). All authors read and approved the final manuscript. CG: Cihan Gür, SÖ:Seçkin Özkanlar, SG: Semin Gedikli, EŞ: Emin Şengül, VG: Volkan Gelen, AK: Adem Kara.

ORCID

Cihan GÜR 0000-0001-6775-7858
Seçkin ÖZKANLAR 0000-0001-7717-797X
Semin GEDİKLİ 0000-0001-8238-7226
Emin ŞENGÜL 0000-0003-1566-1816
Volkan GELEN 0000-0002-5091-1262
Adem KARA 0000-0002-5766-6116

References

1- Lavie CJ, Alpert MA, Arena R, Mehra MR, Milani RV, Ventura HO. Impact of obesity and the obesity paradox on prevalence and prognosis in heart failure. JACC: Heart Failure 2013;1:93-102.
2- Poirier P, Giles TD, Bray GA, Hong Y, Stern JS, Pi-Sunyer FX, et al. Obesity and cardiovascular disease: pathophysiology, evaluation, and effect of weight loss: an update of the 1997 American Heart Association Scientific Statement on Obesity and Heart Disease from the Obesity Committee of the Council on Nutrition, Physical Activity, and Metabolism. Circulation 2006;113:898-918.
3- Kenchaiah S, Evans JC, Levy D, Wilson PW, Benjamin EJ, Larson MG, et al. Obesity and the risk of heart failure. NEJM 2002;347:305-313.
4- Ndumele CE, Coresh J, Lazo M, Hoogeveen RC, Blumenthal RS, Folsom AR, et al. Obesity, subclinical myocardial injury, and incident heart failure. JACC: Heart Failure 2014;2:600-607.

5- Rubins HB, Robins SJ, Collins D, Fye CL, Anderson JW, Elam MB, et al. Gemfibrozil for the secondary prevention of coronary heart disease in men with low levels of high-density lipoprotein cholesterol. N Engl J Med 1999;341:410-418.
6- Boccara G, Pouzeratte Y, Troncin R, Bonardet A, Boularan AM, Colson P, et al. The risk of cardiac injury during laparoscopic fundoplication: cardiac troponin I and ECG study Note. Acta Anaesthesiol Scand 2000;44:398-402.
7- Adams JE, Sicard GA, Allen BT, Bridwell KH, Lenke LG, Davila-Roman VG, et al. Diagnosis of perioperative myocardial infarction with measurement of cardiac troponin I. N Engl J Med 1994;330:670-674.
8- Sözüer EC, Tarladaçalişir YT. Quercetin Ameliorates the Streptozotocin-Induced Diabetic Renal Injury by Inhibiting Apoptosis. Trak Univ J Nat Sci 2021;22:131-138.
9- Panda S, Kar A. Combined effects of vincristine and Quercetin in reducing isoproterenol-induced cardiac necrosis in rats. Cardiovascular Tox 2015;15:291-299.
10- Perez-Vizcaino F, Duarte J. Flavonols and cardiovascular disease. Mol Aspects Med 2010;31:478-494.
11- Panda S, Kar A. Combined effects of vincristine and Quercetin in reducing isoproterenol-induced cardiac necrosis in rats. Cardiovascular tox 2015;15:291-299.
12- Sestili P, Guidarelli A, Dachà M, Cantoni O. Quercetin prevents DNA single-strand breakage and cytotoxicity caused by tert-butyl hydroperoxide: free radical scavenging versus iron-chelating mechanism. Free Radic Biol Med 1998;25:196-200.
13- Lin CF, Kuo YT, Chen TY, Chien CT. Quercetin-Rich Guava (Psidium guajava) juice in combination with trehalose reduces autophagy, apoptosis, and pyroptosis formation in the kidney and pancreas of type ıı diabetic rats. Molecule 2016;21:334.
14- Gelen V, Şengül E, Gedikli S, Gür C, Özkanlar S. Therapeutic effect of Quercetin on renal function and tissue damage in the obesity-induced rats. Biomed Pharmacother 2017;100: 524-528.
15- Arabacı T, Kermen E, Özkanlar S, Köse O, Kara A. Kızıldağ A, et al. Therapeutic effects of melatonin on alveolar bone resorption after experimental periodontitis in rats: A biochemical and immunohistochemical study. J Periodontol 2015;86:874–881.
16- Akman S, Canakci V, Kara A, Tozoglu U, Arabaci T, Dagsuyu IM. Therapeutic effects of alpha-lipoic acid and vitamin C on alveolar bone resorption after experimental periodontitis in rats: A biochemical, histochemical, and stereologic study. J Periodontol 2013;84:666–674.
17- Rivera L, Morón R, Sánchez M, Zarzuelo A, Galisteo M. Quercetin ameliorates metabolic syndrome and improves the inflammatory status in obese Zucker rats. Obesity 2008;16:2081-2087.
18- Erboga M, Aktas C, Erboga ZF, Donmez YB, Gurel A. Quercetin ameliorates methotrexate-induced renal damage, apoptosis, and oxidative stress in rats. Renal failure 2015;37:1492-1497.
19- Sengul E, Gelen V, Gedikli S. Cardioprotective activities of quercetin and rutin in Sprague Dawley rats treated with 5-fluorouracil. JAPS 2021;31:423-431.
20- Gelen V, Şengül E, Çınar DA. The effects of rutin and quercetin on ECG parameters in 5-FU-induced cardiotoxicity rat model. WJARR 2021;9:253-257.
21- Sengul E, Gelen V, Gedikli S, Ozkanlar S, Gur C, Çelebi F. The protective effect of quercetin on cyclophosphamide-induced lung toxicity in mice. Biomed Pharmacother 2017;92:303-7.
22- Levin BE, Triscari H, Sullivan AC. Altered sympathetic activity during development of diet-induced obesity in rat. Am J Physiol Regul Integr Comp Physiol 1983; *244*: R347-R355.
23- Panchal SK, Poudyal H, Arumugam TV, Brown L. Rutin attenuates metabolic changes, nonalcoholic steatohepatitis, and cardiovascular remodeling in high-carbohydrate, high-fat diet-fed rats. The J Nut 2011;141:1062-1069.
24- Poudyal H, Campbell F, Brown L. Olive leaf extract attenuates cardiac, hepatic, and metabolic changes in high carbohydrate–, high fat–fed rats. The J Nut 2010;140:946-953.
25- Leopoldo AS, Sugizaki MM, Lima-Leopoldo AP, do Nascimento AF, Luvizotto RDAM, de Campos DHS, et al. Cardiac remodeling in a rat model of diet-induced obesity. Can J Cardiol 2010;26:423-429.
26- Carroll JF, Zenebe WJ, Strange TB. Cardiovascular function in a rat model of diet-induced obesity. Hypertension 2006;48:65-72.
27- Carroll JF, Tyagi SC. Extracellular matrix remodeling in the heart of the homocysteinemic obese rabbit. Am J Hypertens 2005;18:692-698.
28- Sato S, Mukai Y. Modulation of Chronic Inflammation by Quercetin: The Beneficial Effects on Obesity. J Inflamm Res 2020;13:421–431.
29- Ozcan U, Cao Q, Yilmaz E, Lee AH. Iwakoshi NN, Ozdelen E, et al. Endoplasmic reticulum stress links obesity, insulin action, and type 2 diabetes. Science 2004;306:457-461.
30- Ahn J, Lee H, Kim S, Park J, Ha T. The anti-obesity effect of Quercetin is mediated by the AMPK and MAPK signaling pathways. Biochem Biophys Res Commun 2008;373:545-549.
31- Gedikli S, Ozkanlar S, Gur C, Sengul E and Gelen V. Preventive effects of quercetin on liver damages in high-fat diet-induced obesity. *J Histol Histopathol* 2017; 4:7.