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Rosemary and hesperidin alleviate cardiac dysfunction and alter cellular signaling pathways in experimental model of diabetic cardiomyopathy

Samy A. Hussein[1], Omayma A. R. AboZaid[1], Hussein A. Ali[1], Tahya E. A. Ismael[2], Ghada F. Al lawaty[1]

[1] *Department of Biochemistry, Faculty of Veterinary Medicine, Benha University, Egypt*

[2] *Department of Nutrition & Clinical Nutrition, Faculty of Veterinary Medicine, Benha University, Egypt.*

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ABSTRACT

Alterations in the cardiac structure and function are the hallmarks of diabetic cardiomyopathy (DCM), separate coronary heart disease in diabetic patients. In a streptozotocin-induced diabetic rat model, the current research aimed to investigate the preventive effects of rosemary extract and hesperidin on DCM. Fifty male Rats were divided into five main groups. Group I: rats received no drugs. Group II: STZ (50 mg/kg b.wt.) was given intraperitoneally (i.p.) to rats just once. Group III: Rats treated with insulin at a dose of (2 U/rat/day). Group IV: rats treated with Rosemary extract at a dose of (200 mg/kg b.wt. /day). Group V: rats treated with Hesperidin at a dose of (100 mg/kg b.wt./day). The results showed a marked increase in serum total cholesterol and triacylglycerol concentrations and downregulation in Peroxisome proliferator-activated receptor-γ (PPAR-γ) and Sarcoplasmic reticulum Ca2+-ATPase (SERCA) genes expressions with upregulation in Collagen type IV (COL4) in cardiac tissue of DCM-induced group. Treatment with insulin, rosemary extract, or hesperidin to STZ-induced DCM in rats exhibited a substantial reduction in the levels of triacylglycerol and total cholesterol with an enormous downregulation of COL4 gene expression level in cardiac tissue. However, PPAR-γ and SERCA genes exhibited an enormous upregulation. In conclusion, the results indicated that rosemary extract and hesperidin have a beneficial effect in preventing lipid metabolic disorders and diabetes complications and modify several genes such as PPAR-γ, SERCA, and COL4 participating in cellular pathways which altered in DCM.

1. INTRODUCTION

Epidemiological studies showed that persons with diabetes are more likely to get heart failure, and researchers concluded that diabetes and heart failure are closely related (Drozd and Kearney, 2017). Diabetes-related cardiac abnormalities are the cause of the clinical condition known as diabetic cardiomyopathy (DCM) (Boudina and Abel, 2007). About 12 % of diabetic people are predicted to get DCM, which can cause heart failure and death (Lorenzo-Almoros *et al.*, 2017). The main characteristics of DCM are myocardial interstitial fibrosis, oxidative stress, inflammation, apoptosis, and cardiac hypertrophy (Saisho, 2014). Reactive oxygen species (ROS) and inflammation are overproduced when there is persistent hyperglycemia in diabetes, which is a major factor in DCM (Bhattacharjee *et al*., 2016).

Numerous studies revealed that the myocardium of people with DCM had a marked rise in pro-inflammatory cytokines (Atta *et al.*, 2018). Reduced left ventricular (LV) function and ROS were strongly linked with pro-inflammatory cytokine levels (Khanra *et al.*, 2015).

In the etiology of complex illnesses like diabetes and its related vascular problems, it is hypothesized that there is a crucial interaction between genes and environment. Epigenetic modifications of the genome are hypothesized to facilitate gene-environment interactions, with environmental changes frequently leading to epigenetic modifications of the genome (Bird, 2007). Numerous antioxidants have received attention as a potential strategy to reduce DCM and other diabetes problems because strong evidence implicates oxidative stress in the emergence of DCM.

The evergreen aromatic plant *Rosmarinus officinalis Linn*. (Rosemary), a member of the Lamiaceae family, is found across the Mediterranean region. It grows to a height of 1 m, has straight stems, whitish-blue blooms, and dark green leaves. One of the most widely used herbal products for flavoring and antioxidants in food preservation and cosmetics was rosemary leaf extract (Cui *et al*. 2012).

Citrus fruits including lemon, orange, lime, and grapefruit contain a lot of hesperidin, a flavanone glycoside that varies greatly depending on the species (Liu *et al.*, 2013) and it used in conventional medicines commonly, The beneficial function in cardiovascular, anti-inflammation and antioxidant properties (Mao-Qiang *et al.*, 2019). Hesperidin has multiple bioactivities which include anti-inflammatory (Wei *et al*., 2012), anti-oxidative (Selvaraj and Pugalendi, 2010), radioprotective (Petrova *et al.*, 2011) and anticancer consequences (Yumnam *et al.*, 2015).

In this research, we look into the harmful complications of Streptozotocin-induced diabetes mellitus and intracellular pathways activated in the heart. Moreover, the molecular and hypolipidemic consequences of two natural agents

* Correspondence to: ghada.fathy.la@gmail.com

(rosemary extract and Hesperidin) in experimentally induced diabetic cardiomyopathy in rats with molecular and biological characteristics are assessed in blood and cardiac tissue. In this study, we explore the harmful complications of STZ-induced DCM and intracellular signaling pathways activated and involved in pathophysiology of diabetic cardiomyopathy (DCM). Also, the ameliorating role of two natural agents (rosemary extract and Hesperidin) towards molecular alterations of cardiac tissue and lipid metabolic abnormalities has been checked.

2. MATERIAL AND METHODS

2.1. Experimental animals:

In this investigation, 50 white male albino rats' weight 180-200 g aged 5-6 weeks were used. Throughout the course of the experiment, rats were kept in separate metallic cages under constant environmental and dietary circumstances. The rats were given a consistent ration of food, and unlimited access to fresh, clean drinking water was provided. Prior to the start of the trial, all rats underwent a minimum 15-day acclimation period. The Benha University Animal Care and Use Committee authorized the experimental protocols, which adhere to the National Institutes of Health's guide for the care and use of laboratory animals (Approval no. BUFVTM 02-8-21).

2.2. Chemicals and antioxidant agents:

2.2.1. Streptozotocin:

We obtained streptozotocin (STZ) from (Sigma Chemical Co. P.O. Box. 14508, St. Lowis, U.S.A.) uses a single intraperitoneal (i.p.) injection of 50 mg/kg body weight of freshly dissolved, citrate buffered medication for induction of hyperglycemia (Ramanathan *et al.,* 1999).

2.2.2. Insulin:

Long-acting insulin was purchased from (Lantus Solostar, Sanofi-Aventis, Germany). It is subcutaneously injected (2 U/rat per day) (Shiju *et al.,* 2012).

2.2.3. Rosemary extract preparation

About 250 g of the dried rosemary leaves were milled into a fine powder after being dried in the shade to prevent the chemical contents from decomposing.

The plant powder was placed in a stoppered container with ethanol (ethanol/water (70:30)) and allowed to stand at room temperature for at least 3 days. After this period, the mixture was filtrated to obtained liquid extract. Then, the extract was concentrated using a rotary evaporator at 50 °C under reduced pressure. This process was repeated at least 3 times. Finally, the extract was weighted and stored at -20 °C till usage. Each rat was orally administered with 0.5 ml of Rosemary extract daily (Abdul- Rahim and Taha, 2011).

2.2.4. Hesperidin:

Hesperidin (95%) was purchased from Al-dawlya Company, dissolved in saline, and given orally (50 mg/ ml) at a dose of 100 mg/kg per day, using intra-gastric tubes after 6 weeks of STZ induction (Pires Das Neves *et al.,* 2004).

2.3. Induction of diabetic cardiomyopathy:

Rats were given free access to water after an 18-hour fast. To lower the animals' blood glucose levels to a point where circulating glucose does not out-compete streptozotocin for GluT-2 binding and transport -cells, fasting is necessary prior to streptozotocin injection. Streptozotocin (STZ), freshly dissolved in citrate buffer, PH 4.5, was administered as a single intraperitoneal (i.p.) injection dosage to male rats to experimentally develop diabetes. After six weeks of starting diabetes, experimental diabetic cardiomyopathy develops. Similar doses of merely the vehicle (citrate buffer) were given to control rats. Following a week of treatment with STZ, rats were fasted for 12 hours, and blood samples were taken from the orbital venous sinus to measure glucose levels. Diabetic rats were defined as those in a group with blood glucose levels greater than 250 mg/dl and were involved in additional research (Ramanathan *et al.,* 1999). To prevent any possible Streptozotocin-induced hypoglycemia, the animals were given access to glucose solution (5%) w/v overnight (STZ).

2.4. Experimental design:

After six weeks of diabetic cardiomyopathy induction all rats were randomly placed in individual cages and classified into five main groups as following:

- Group I: Control Normal group: Consisted of 7 rats, rats fed with ordinary diet only without any treatment during the entire experimental period of 12 weeks.
- Group II: Diabetic cardiomyopathy non treated group: Consisted of 13 rats received a single intraperitoneal (i.p) injected dose of STZ (50 mg /kg body wt.) freshly dissolved in citrate buffer, PH 4.5.
- Group III: STZ + Insulin treated group: Consisted of 10 rats received a single intraperitoneal (i.p) injected dose of STZ (50 mg /kg body wt.) and treated with daily subcutaneous injections of long-acting insulin 2 U/rat per day for 6 weeks after induction of diabetic cardiomyopathy.
- Group IV: STZ + R.E. treated group: Consisted of 10 rats received a single intraperitoneal (i.p) injected dose of STZ (50 mg /kg body wt.) and treated with Rosemary extract orally once per day at a dose of (200 mg/kg body weight/day) for 6 weeks after induction of diabetic cardiomyopathy.
- Group V: STZ + Hesperidin treated group: Consisted of 10 rats received a single intraperitoneal (i.p) injected dose of STZ (50 mg /kg body wt.) and treated with Hesperidin orally once per day at a dose of (100 mg/kg body weight/day) for 6 weeks after induction of diabetic cardiomyopathy.

2.5. Sampling:

2.5.1. Blood samples:

Blood samples were collected from all rat groups at the end of experiment after overnight fasting. Blood was centrifugation at 2500 rpm for 15 minutes for serum separation. Then, collected by an automatic pipette, in a dry, sterile Eppendorf, and stored at -20°C for estimation of total cholesterol and triacylglycerols

2.5.2. Tissue specimens (For molecular analysis):

Rats were slaughtered by decapitation and the abdomen was then opened. Heart tissues were collected, placed in Eppendorf tubes, and immediately kept in liquid nitrogen and stored at- 80°C until RNA extraction for the determination of the following gene expression: Peroxisome proliferator-activated receptor-γ (PPAR-γ) and Sarcoplasmic reticulum Ca2+-ATPase (SERCA) in addition to Collagen type IV (COL4).

2.6. Analysis:

2.6.1. Biochemical analysis:

Serum total cholesterol and triacylglycerols were determined enzymatically utilizing the approach outlined by (Ellefson and Caraway, 1976) and Stein, (1987), respectively.

2.6.2. Molecular analysis:

Real-time quantitative polymerase chain reaction analysis (real-time qPCR) was used to identify the mRNA expression levels of PPAR-γ, SERCA2, and COL4 (Table 1) in rat hearts. The load was managed with -actin. Following the manufacturer's instructions, total RNA was extracted from the heart using a High Kit for extraction of pure RNA (Thermo Scientific, Fermentas, #K0731) RNA Extraction kit. The RevertAid TM First Strand cDNA synthesis kit (#EP0451, Thermo Scientific, Fermentas, USA) was used to reverse-transcribe the material for each cDNA. Then, using the Faststart Universal SYBR Green Master, real-time quantitative PCR amplification was carried out (Roche, GER). Using the 2-Ct technique, the target gene was normalized with β-actin (Livak and Schmittgen, 2001).

Table 1 Forward and reverse primers sequence for primers used in qPCR:

Gene	Forward primer (′5 ------ ′3)	Reverse primer (′5 ------ ′3)
PPAR-γ	GCCAAGAACATCCCCAACTTC	GCAAAGATGGCCTCATGCA
SERCA2	CAGTTCATCCGCTACCTCATCTCC	CGCAGTGGCAGGCAGACC
COL4a3	CCCAAAGGCATCAGGGGAAT	ATCCTGGTAAACCAGCCAGC
B-actin	AAGTCCCTCACCCTCCCAAAAG	AAGCAATGCTGTCACCTTCCC

2.7. Statistical analysis:

The means and SEM were used to express all the data. One-way analysis of variance (ANOVA) was used to assess the statistical significance, and Duncan's multiple range test was used to get individual comparisons (DMRT). SPSS, version 18.0 software was used. When $p<0.05$, values were deemed statistically significant (**Steel *et al*., 1997).**

3. RESULTS

3.1. Effect of treatment with insulin, rosemary extract, or hesperidin on total cholesterol (TC) and triacylglycerols (TAG) concentrations of rats with STZ-induced diabetic cardiomyopathy are depicted in table (2) and figures (1, 2): When compared to the healthy control group, a substantial rise in TC and TG concentrations was seen in STZ-induced diabetic cardiomyopathy. Injection of insulin (G3), administration of rosemary extracts (G4), or hesperidin (G5) to STZ-induced diabetic cardiomyopathy showed a noticeable decline in TC and TG concentration. With the highest decrease in (G4) followed by (G3) and finally (G5).

Table 2 Effect of insulin, rosemary extract or hesperidin treatment on total cholesterol and triacylglycerols concentrations of STZ-induced diabetic cardiomyopathy in rats:

Animal groups	Total Cholesterol (mg/dl)	Triacylglycerols (mg/dl)
Control Normal (G1)	98.17 ± 3.67^{c}	40.49 ± 2.15^{d}
DCM (G2)	162.09 ± 6.11^{a}	105.37 ± 4.45^{a}
DCM + Insulin (G3)	125.63 ± 5.02^{b}	76.33 ± 3.34^{b}
DCM + R.E. (G4)	114.00 ± 4.39^{b}	62.67 ± 2.85^{c}
DCM + HES (G5)	130.04 ± 5.23^{b}	82.55 ± 3.70^{b}

Data are presented as (Mean ± S.E). S.E = Standard error. Mean values with different superscript letters in the same column are significantly different at ($P \leq 0.05$).

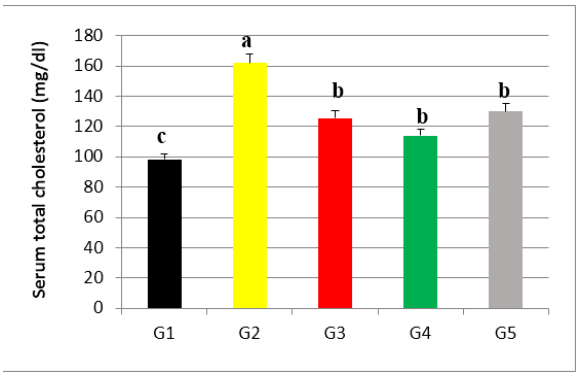

Fig. (1): Effect of insulin, rosemary extract or hesperidin treatment on total cholesterol concentration in experimental model of diabetic cardiomyopathy in rats.

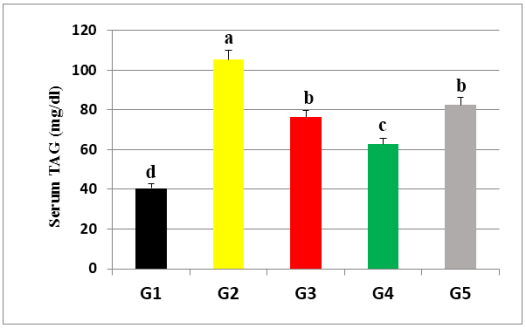

Fig. (2): Effect of insulin, rosemary extract or hesperidin treatment on triacylglycerols concentration in experimental model of diabetic cardiomyopathy in rats.

3.2. Effect of treatment with insulin, rosemary extract, or hesperidin on Peroxisome proliferator-activated receptor-γ (PPAR-γ), Sarcoplasmic reticulum Ca2+-ATPase (SERCA), and Collagen type IV (COL4) gene expression of cardiac tissue in STZ-induced DCM in rats are presented in table (3) and figures (3, 4, 5): A significant downregulation of PPAR-γ and SERCA gene expression were noticed in STZ-induced DCM in comparison to the normal control group. However, a significant COL4 gene expression level was significant upregulation. Treatment with insulin (G3), rosemary extracts (G4), or hesperidin (G5) to STZ-induced DCM in rats exhibited a significant upregulation of PPAR-γ and SERCA gene expression as compared with the STZ-induced DCM group. With the highest upregulation in (G3) followed by (G5) and finally (G4). However, COL4 gene expression showed a significant downregulation.

Table 3 Effect of insulin, rosemary extract or hesperidin treatment on cardiac tissue PPAR-γ, SERCA and COL4 gene expression of STZ-induced diabetic cardiomyopathy in rats:

Animal groups	PPAR-γ	SERCA	COL4
Control Normal (G1)	1.00 ± 0.04^{a}	1.00 ± 0.04^{a}	1.00 ± 0.06^{e}
DCM (G2)	0.22 ± 0.01^{e}	0.15 ± 0.01^{e}	11.63 ± 0.42^{a}
DCM + Insulin (G3)	0.86 ± 0.03^{b}	0.83 ± 0.04^{b}	1.85 ± 0.08^{d}
DCM + R.E. (G4)	0.46 ± 0.02^{d}	0.32 ± 0.02^{d}	8.40 ± 0.33^{b}
DCM + HES (G5)	0.65 ± 0.02^{c}	0.51 ± 0.03^{c}	3.76 ± 0.16^{c}

Data are presented as (Fold chain Mean ± SEM). SEM = Standard error mean. Mean values with different superscript letters in the same column are significantly different at ($P \leq 0.05$).

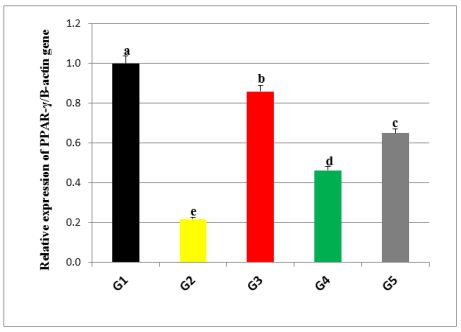

Fig. (3): Effect of insulin, rosemary extract or hesperidin treatment on PPAR-γ gene expression in experimental model of diabetic cardiomyopathy in rats.

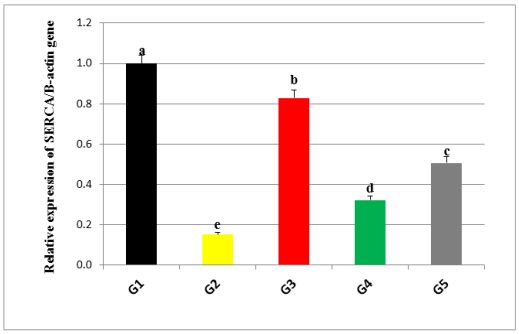

Fig. (4): Effect of insulin, rosemary extract or hesperidin treatment on SERCA gene expression in experimental model of diabetic cardiomyopathy in rats.

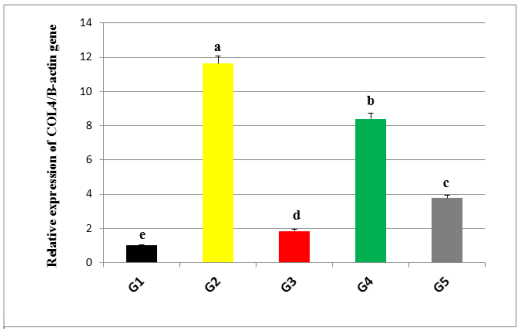

Fig. (5): Effect of insulin, rosemary extract or hesperidin treatment on COL4 gene expression in experimental model of diabetic cardiomyopathy in rats.

4. DISCUSSION

Without any other heart conditions, cardiomyopathy is a separate consequence of diabetes mellitus (Huynh *et al.*, 2014). The pathophysiology of DCM is likely influenced by oxidative stress, inflammation, and apoptosis that are brought on by hyperglycemia and hyperlipidemia (Falcao and Leite, 2012); (Suzuki *et al.*, 2015). In this investigation, STZ-induced diabetic cardiomyopathy was found to significantly raise TC and TG concentrations, as shown in Table (1) and images (1, 2). Similar to this, it appears that the changed lipid profile in the serum of STZ-induced diabetic rats is a key element in the emergence of atherosclerosis, a condition associated with diabetes (Chattopadhyay and Bandyopadhyay, 2005). Increased levels in serum TG and TC in diabetes are inconsistent with observations of Maiti *et al*. (2005) and by others (Yadav *et al.*, 2008). A substantial reduction in TC and TG concentrations was seen in rats with STZ-induced diabetic cardiomyopathy that were treated with insulin, rosemary extracts, and hesperidin. These outcomes have been supported by Iweala and Oludare (2011) who claimed that taking a rosemary leaf extract by mouth significantly reduced blood levels of TG, LDLC, and total cholesterol while increasing HDL-C. Due to its gradual metabolic influence over the systems involved in fat removal from the body, rosemary leaf extract has the potential to be hypolipidemic. The antioxidant properties of Rosemary's ingredients, such as rosmarinic acid, which altered the rate of fatty acid oxidation in the liver and decreased the rate of triglycerides production in rats, may be the cause of this drop. Xinhui *et al*. (2011) detected Hesperidin caused a drop in the range of total cholesterol, TG, and LDL cholesterol as well as a rise in the levels of HDL cholesterol in the serum of rats fed a high-cholesterol diet.

In STZ-induced diabetic cardiomyopathy, compared to the group of healthy controls, the expression of the PPAR-γ gene was significantly downregulated, according to the data. However, treatment of STZ-induced diabetic cardiomyopathy in rats with insulin, rosemary extracts, and hesperidin resulted in a considerable elevation of PPAR-γ level in comparison to the STZ-induced diabetic cardiomyopathy group. These findings were consistent with those of Rau *et al*. (2006) who claimed that aqueous ethanol extract of rosemary was successful in activating PPAR-γ in 80% of cases utilizing a reporter gene experiment. Since both the phenolic diterpene chemicals carnosol and CA were identified as the active ingredients, it is possible that they are a part of the mechanism behind the anti-hyperglycemic properties of rosemary, which are similar to those of the glucose-lowering medication glitazone (Rau *et al*. 2006). Based on dichloromethane methanol's anti-oxidant and relaxing effects on type 2 diabetes mellitus and obesity issues, Tu *et al*. (2013) outlined the mechanism of the rosemary extract's metabolic control in HepG2 cells. By activating signaling pathways like AMP-activated protein kinase (AMPK), which induces glycolysis, and PPAR-γ. The rosemary extracts also upregulated sirtuin-1, which boosts fatty acid oxidation, Peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α) (which activates PPAR- γ), and low-density lipoprotein cholesterol (LDL-C) receptor, which is in charge of endocytosis of LDL-C from circulation to liver hepatocytes (Tu *et al.* 2013). Also, Agrawal *et al.*, (2014) discovered that Hesperidin stimulates GLUT 4 translocation and PPAR to successfully lower blood glucose levels.

In STZ-induced DCM, a substantial downregulation of SERCA expression was seen. Similar to this, repeated tests have demonstrated that type 1 diabetes caused by streptozotocin (STZ) has a down-regulated SERCA2a mRNA expression, protein level, and activity (Zhong *et al.*, 2001). The uptake of Ca2+ into the sarcoplasmic reticulum is a major mediator of the heart's diastolic relaxation. Numerous investigations have demonstrated an association between poor relaxation and reduced contractility in diabetic cardiomyopathy (Belke and Dillmann, 2004). The primary factor influencing how quickly the heart relaxes is a decreased capacity to sequester calcium into the SR, which has been linked to these changes. In the current work, treatment of STZ-induced diabetic cardiomyopathy in rats with insulin, rosemary extracts, and hesperidin resulted in a substantial elevation of SERCA expression. These outcomes were consistent with (Golfman *et al.*, 1999) and (Zhong *et al.*, 2001) who observed that "Insulin therapy can restore the decrease in SERCA2a activity in STZ-treated rats, suggesting a direct stimulatory impact of insulin on SERCA2a." Other studies showing an upregulation of SERCA1 in skeletal muscle after stimulation with insulin provide additional evidence for this notion (Dibb *et al.*, 2007). The current in vitro experiments demonstrating a direct influence of insulin on SERCA2a transcription in isolated cardiac myocytes further support these observations, which suggest a probable relationship between insulin and expression of SR calcium ATPase. Further evidence for insulin's critical role in heart health comes from Kim *et al.*, (2008) who showed that both with and without the transplantation of smooth muscle cells, insulin protects heart function in streptozotocin-induced diabetic heart failure.

In STZ-induced DCM, COL4 level was found to be significantly upregulated. In contrast to the STZ-induced DCM group, however, treatment with insulin, rosemary extracts, and hesperidin showed a significant downregulation of COL4 level. Similarly, Haneda *et al.* (1991) have demonstrated that glucose increases the synthesis of type IV collagen in glomerular mesangial cells from cultured rats. Patients with higher blood glucose levels have seen higher serum type IV collagen concentrations (Matsumoto *et al.*, 1991). Increased matrix protein deposition, including collagen, and subsequent increased expression of the type I and type IV collagen genes are characteristics of DCM.

5. CONCLUSION

By reducing metabolic irregularities and cellular pathways that are disrupted in DCM, the results showed that rosemary extract and hesperidin may have potential therapeutic benefits for treating DCM and other cardiovascular diseases.

5. REFERENCES

1. Abdul-Rahim, A. and Taha, A., 2011. Effects of Rosemary (Rosmarinus officinalis) on lipid profile of diabetic rats. Indian Journal of Experimental Biology, 37. 124-130
2. Agrawal Y.O.; Sharma P.K.; Shrivastava B.; Ojha S.; Upadhya H.M.; Arya D.S. and Goyal S.N., 2014. Hesperidin produces cardioprotective activity via PPAR-gamma pathway in ischemic heart disease model in diabetic rats. PLoS ONE.; 9. e111212
3. Atta M.S.; A.H. El-Far; F.A. Farrag; M.M. Abdel-Daim; S.K. Al Jaouni and S.A. Mousa, 2018. Thymoquinone attenuates cardiomyopathy in streptozotocin-treated diabetic rats. Oxid Med Cell Longev. 2018. 7845681.
4. Belke D.D. and Dillmann W.H., 2004. Altered cardiac calcium handling in diabetes. Curr Hypertens Rep, 6.424–429.
5. Bhattacharjee N.; S. Barma; N. Konwar; S. Dewanjee and P. Manna, 2016. "Mechanistic insight of diabetic nephropathy and its pharmacotherapeutic targets. an update," European Journal of Pharmacology, 791, 8–24.
6. Bird A., 2007. Perceptions of epigenetics. Nature;447. 396–8.
7. Boudina S. and E. D. Abel, 2007. "Diabetic cardiomyopathy revisited," Circulation, 115, 25, 3213–3223.
8. Chattopadhyay RR and Bandyopadhyay M., 2005. Effect of Azadirachta Indica on serum lipid profile changes in normal and Streptozotocin induced diabetic rats, Afr. J. Biomed. Res.8. 101-104.
9. Cui L. *et al.,* 2012. Abietane diterpenoids of Rosmarinus officinalis and their diacylglycerol acyltransferase-inhibitory activity. Food Chem 132.1775–1780
10. Dibb K.M.; Graham H.K.; Venetucci L.A.; Eisner D.A. and Trafford A.W., 2007. Analysis of cellular calcium fluxes in cardiac muscle to understand calcium homeostasis in the heart. Cell Calcium, 42.503–512.
11. Drozd M. and M. T. Kearney, 2017. "Diabetes mellitus and heart failure. A deadly duo," Journal of Thoracic Disease; 9(1). 16–18.
12. Ellefson, R.D. and Caraway, W.T., 1976. fundamentals of clinical chemistry.Ed Tietz NW; p474-542.
13. Falcao-Pires I. and A. F. Leite-Moreira, 2012. Diabetic cardiomyopathy. understanding the molecular and cellular basis to progress in diagnosis and treatment. Heart Failure Reviews; 17(3). 325–344.
14. Golfman L.; Dixon I.M.; Takeda N.; Chapman D. and Dhalla N.S., 1999. Differential changes in cardiac myofibrillar and sarcoplasmatic reticular gene expression in alloxan-induced diabetes. Mol Cell Biochem, 200.15–25.
15. Haneda M.; Kikkawa R.; Horide N.; Togawa M.; Koya D. *et al.,* 1991. Glucose-induced overproduction of type IV collagen in cultured glomerular mesangial cells. J Diabet Complications; 5.199-200.
16. Huynh K.; B. C. Bernardo; J. R. McMullen and R. H. Ritchie, 2014. "Diabetic cardiomyopathy. mechanisms and new treatment strategies targeting antioxidant signaling pathways," Pharmacology & Therapeutics, 142, 3, 375–415.
17. Iweala, E.J. and Oludare, F.D., 2011. Hypoglycemic effect, biochemical and histological changes of Spondias mombin linn, and Painari polyandra benth. Seeds Ethanolic extracts in alloxan induced diabetic rats. J Pharma Toxic., 6(2). 101-112.
18. Khanra, R.; S. Dewanjee; T.K. Dua; R. Sahu; M. Gangopadhyay; V. De Feo; M. Zia-Ul- Haq and Abroma augusta L., 2015. (Malvaceae) leaf extract attenuates diabetes induced nephropathy and cardiomyopathy via inhibition of oxidative stress and inflammatory response. J. Transl. Med. 13.6.
19. Kim B.O.; Verma S.; Weisel R.D.; Fazel S.; Jia Z.Q.; Mizuno T. and Li R.K., 2008. Preservation of heart functions in diabetic rats by the combined effects of muscle cell implantation and insulin therapy. Eur J Heart Fail, 10.14–21.
20. Liu, E.H.; Zhao, P. and Duan L., 2013. Simultaneous determination of six bioactive flavonoids in Citri Reticulatae Pericarpium by rapid resolution liquid chromatography coupled with triple quadrupole electrospray tandem mass spectrometry, Food Chemistry, 141, 4, 3977–3983.
21. Livak, K.J. and Schmittgen, T.D., 2001. Analysis of relative gene expression data using real-time quantitative PCR and the 2− CT Method. Methods 25(4). 402-408.
22. Lorenzo-Almoros A.; J. Tunon; M. Orejas; M. Cortés; J. Egido; and Ó. Lorenzo, 2017. Diagnostic approaches for diabetic cardiomyopathy. Cardiovascular Diabetology; 16(1). 28.
23. Maiti R; Das UK and Ghosh D., 2005. Attenuation of hyperglycemia in STZ induced diabetic rats by aqueous extract of seed of Tamarindus Indica, Biol. Pharm. Bull. 28. 1172-1176.
24. Mao-Qiang, M; Bin, Y. and Peter, M.E. 2019. Benefits of Hesperidin for Cutaneous Functions Evidence-Based Complementary and Alternative Medicine.Volume, 19 pages.
25. Matsumoto E.; Matsumoto G.; Bessho H.; Kikuoka H. and Nanjo K., 1991. Serum concentrations of intact type IV collagen in diabetics. J Diabet Complications; 5.189-90.
26. Petrova A.; Davids LM.; Rautenbach F. and Marnewick JL., 2011. Photoprotection by honeybush extracts, hesperidin and mangiferin against UVB-induced skin damage in SKH-1 mice. Journal of Photochemistry and Photobiology B. Biology; 103.126-139.
27. Pires Das Neves RN; Carvalho F; Carvalho M; *et al.*, 2004. Protective activity of hesperidin and lipoic acid against sodium arsenite acute toxicity in mice. Toxicol Pathol 32.527–35.
28. Ramanathan, M.; Jaiswal, A.K. and Bhattacharya, S.K., 1999. Superoxide dismutase, catalase jand glutahiones peroxidase activities in the brain of streptozotocin induced diabetic rats. Indian. J.Exp.Biol.; 37. 182-183
29. Rau O.; Wurglics M.; Paulke A. *et al.,* 2006. Carnosic acid and carnosol, phenolic diterpene compounds of the labiate herbs rosemary and sage, are activators of the human peroxisome proliferator-activated receptor gamma. Planta Med 72. 881–887.
30. Saisho, Y., 2014. Glycemic variability and oxidative stress. a link between diabetes and cardiovascular disease?. International Journal of Molecular Sciences, 15(10). 18381–18406.
31. Selvaraj P. and Pugalendi KV., 2010. Hesperidin, a flavanone glycoside, on lipid peroxidation and antioxidant status in experimental myocardial ischemic rats. Redox Rep; 15. 217-223.
32. Shiju Thomas Michael, Rajesh Nachiappa Ganesh and Pragasam Viswanathan., 2012. Effect of long acting insulin supplementation on diabetic nephropathy in Wistar rats. Indian J of Exp Biology; 50, 867-874.
33. Steel R; Torrie J and Dickey D., 1997. Principles and procedures of Statistics. A Biometrical Approach, 3rd ed, McGraw-Hill, New York, NY.
34. Stein, E.A., 1987. lipids, lipoproteins, and apolipoproteins. In NW Tietz, ed. Fundamentals of clinical chemistry, 3rd ed. Philadelphia. WB Saunders; 448-481.
35. Suzuki H.; Y. Kayama; M. Sakamoto; *et al.,* 2015. "Arachidonate 12/15-lipoxygenase-induced inflammation and oxidative stress are involved in the development of diabetic cardiomyopathy," Diabetes, 64, 2, 618–630.
36. Tu Z.; Moss-Pierce T.; Ford P. and Jiang TA., 2013. Rosemary (*Rosmarinus officinalis L.)* extract regulates glucose and lipid metabolism by activating AMPK and PPAR pathways in HepG2 cells. J Agric Food Chem 61.2803–2810.
37. Wei D.; Ci X.; Chu X.; Wei M.; Hua S. and Deng X., 2012. Hesperidin suppresses ovalbumin-induced airway inflammation in a mouse allergic asthma model. Inflammation; 35.114-121.
38. Xinhui Wang; Junichi Hasegawa; Yoshiyuki Kitamura; Zhongzhi Wang; Akiko Matsuda; Waka Shinoda; Norimasa Miura and Koji Kimura, 2011. Effects of Hesperidin on the Progression of Hypercholesterolemia and Fatty Liver Induced by High-Cholesterol Diet in Rats. J Pharmacol Sci 117, 129 – 138
39. Yadav JP; Saini S; Kalia AN. and Dangi AS., 2008. Hypoglycemic and hypolipidemic activity of Ethanolic extract of Salvadora oleoides in normal and alloxan-induced diabetic rats, Ind. J. Pharmacol. 40. 23-27.
40. Yumnam S.; Hong GE.; Raha S.; Venkatarame Gowda Saralamma V.; Lee HJ.; Lee WS.; Kim EH. and Kim GS., 2015. Mitochondrial Dysfunction and Ca Overload Contributes to

Hesperidin Induced Paraptosis in Hepatoblastoma Cells, HepG2. J Cell Physiol; 231(6).1261-1268.

41. Zhong Y.; Ahmed S.; Grupp I.L. and Matlib M.A., 2001. Altered SR protein expression associated with contractile dysfunction in diabetic rat heart. Am J Physiol Heart Circ Physiol, 281(3).H1137–47.