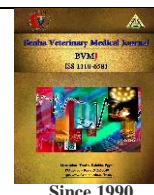




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The role of hesperidin and rosemary leaves extract on myocardium glucose transporter 4 pathway for attenuation of diabetic cardiomyopathy

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ABSTRACT

Diabetic cardiomyopathy (DCM) describes pathological changes in the myocardium in diabetes and is not directly attributable to coronary artery disease or hypertension. The cardioprotective effect of rosemary extract and hesperidin against Streptozotocin-induced diabetic cardiomyopathy in rats was evaluated. Fifty rats were divided into five groups. Group I: rats fed a normal diet. Group II: rats received a single intraperitoneal injection of streptozotocin (50 mg /kg body wt.). Group III: rats treated with insulin (2 U/rat per day). Group IV: rats administered orally rosemary extract at a dose of 200 mg/kg body weight/day. Group V: rats were given hesperidin orally at a dose of 100 mg/kg body weight/day. Blood samples and heart tissue specimens were collected after 12 weeks of the experiment for determination of some biochemical and molecular biomarkers. The obtained results showed a marked increase in blood glucose concentration, creatine kinase-MB and lactate dehydrogenase activities in addition to a significant upregulation of Histone acetyltransferase (HAT) and Histone deacetylase (HDAC) with downregulation of Glucose transporter 4 (GLUT4) gene expression in cardiac tissue of diabetic cardiomyopathy. Treatment with insulin, rosemary extract, or hesperidin exhibited a significant decrease in blood glucose level, CK-MB, and LDH activities in addition to a significant down-regulation of HAT and HDAC. However, GLUT4 translocation showed a significant upregulation. It was concluded that rosemary extract and hesperidin have potential therapeutic in DCM by attenuating GLUT4 Pathway involved in the glucose uptake by the myocardium in diabetic rats.

1. INTRODUCTION

Diabetic cardiomyopathy is characterized by an abnormal heart structure and function without further cardiac risk factors e.g., severe valvular disease, hypertension, or coronary artery disease (CAD) (Jia *et al.*, 2018). Diastolic dysfunction with retained ejection fraction is the clinical hallmark of the diabetic heart. The pathological remodeling of the heart is what results in these changes. The diabetic heart is characterized structurally by an increase in interstitial and perivascular fibrosis as well as LV hypertrophy (Tate *et al.*, 2017). Moreover, the fundamental pathophysiological mechanisms remain unknown and not restricted to abnormal extracellular matrix (Perge *et al.*, 2017). A rise in oxidative stress and inflammation, as well as mitochondrial malfunction, modifications to the metabolic profile, and variations in how much energy is produced (De Rosa *et al.*, 2018).

Diabetic cardiomyopathy (DCM) is influenced by a series of factors e.g., the activation of the renin-angiotensin-aldosterone system (RAAS), increased oxidative stress caused on by the release of reactive oxygen species (ROS), altered metabolism carried on by free-fatty acid (FFA)

oxidation, and impaired calcium homeostasis. All of these processes are connected to the main complications of diabetes, such as hyperglycemia, hypoglycemia, insulin resistance, and the chain of events that happens in response to these conditions. Early problems that have been mostly characterized in animal models include changes in myocardial structure, calcium signaling, and metabolism. These defects may occur before clinically evident heart failure (Isfort *et al.*, 2014).

With one of the greatest concentrations of antioxidant components, rosemary (*Rosmarinus officinalis* Linn) is a common culinary spice, a therapeutic plant, and a natural preservative in the food industry. (Pérez-Fons *et al.*, 2010). The primary bioactive components in rosemary leaves that are associated to the antioxidant action are the phenolic diterpenes carnosic acid and carnosol. (Bai *et al.*, 2010). A caffeic acid ester having antioxidant and anti-inflammatory properties is rosmarinic acid (Karthik *et al.*, 2011).

Therapeutically, hesperidin has been described as an antioxidant (Ahmad *et al.*, 2012), antitumor (Coelho *et al.*, 2013), antiallergic, hypolipidemic, vasoprotective and has anti-inflammatory effects (Saiprasad *et al.*, 2013). The purpose of this study was to evaluate the cardioprotective

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effects of rosemary leaves extract and hesperidin against diabetic cardiomyopathy induced by streptozotocin through investigation of some biochemical and molecular markers and histopathological examination.

2. MATERIAL AND METHODS

2.1. Experimental Animals:

Fifty male albino rats, 5-6 weeks of age and weighing 180-200 g, were purchased from Animal Research Center, Faculty of Veterinary Medicine, Benha University. Animals were kept at room temperature (25 °C), relative humidity (50-65%) and a 12 hrs. light/dark cycle with free access to standard rodent diet and water. Rats were allowed to acclimatize at animal facility for at least 15 days before the start of the experiment. The experimental protocols were also approved by the Animal Care and Use Committee at Benha University and are in accordance with the National Institute of Health 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:

Streptozotocin (STZ) was purchased from Sigma Chemical Co. (P.O. Box. 14508, St. Louis, U.S.A.) used by intraperitoneal (i.p) injection at 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), and was subcutaneously injected (2 U/rat per day) (Shiju *et al.*, 2012).

2.2.3. Rosemary leaves extracts

2.2.3.1. Preparation of Plant Extract

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

The plant powder was mixed with ethanol (ethanol/water (70:30)) in a stoppered container, and the mixture was left to remain at room temperature for at least three days. Then the mixture was filtrated to produce liquid extract. A rotary evaporator was used to concentrate the extract at 50 °C and low 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).

2.2.3.2. Hesperidin:

Hesperidin (95%) was purchased from Al-dawlya Company. Hesperidin (HSP) was dissolved in saline (50 mg/ml) and given orally at a dose of 100 mg/kg per day (Pires Das Neves *et al.*, 2004).

2.4. Induction of diabetic cardiomyopathy:

Diabetes was induced in rats by a single i.p. injection of streptozotocin at a dose of 50 mg/kg dissolved in citrate buffer. After a week of receiving STZ, rats were allowed to fast for 12 hours before blood was drawn to check their blood glucose levels. Rats in the diabetic group were classified as diabetic and enrolled in subsequent studies when their blood glucose levels exceeded 250 mg/dl (Ramanathan *et al.*, 1999).

2.5. Experimental design:

Six weeks after DCM induction, therapeutic treatment with insulin, rosemary extracts and hesperidin were delivered and

carried out for six weeks. Rats were classified into five groups as following:

- Group I: Control Normal group: 7 rats were given a standard diet and no medication.
- Group II: Diabetic cardiomyopathy non-treated group: STZ (50 mg/kg body weight) was administered intraperitoneal ly to 13 rats.
- Group III: STZ + Insulin treated group: 10 rats were given a single intraperitoneal (i.p.) injection of STZ (50 mg/kg BW), followed by daily subcutaneous injections of long-acting insulin (2 U/rat) for 6 weeks.
- Group IV: STZ + Rosemary Extract treated group: 10 rats were given a single intraperitoneal (i.p.) injection of STZ (50 mg/kg bw) and were given Rosemary extract orally at a dose of (200 mg/kg bw) once daily for 6 weeks.
- Group V: STZ + Hesperidin treated group: 10 rats were injected i.P. with STZ (50 mg/kg body weight) followed by administration of hesperidin (100 mg/kg bw) orally once daily for 6 weeks.

2.6. Sampling:

2.6.1. Serum samples:

Serum was separated by centrifugation at 2500 rpm for 15 minutes after being collected by ocular vein puncture in screw-capped tubes. Serum was separated using an automatic pipette, received in a dry, sterile samples tube, and then stored in a deep freezer at -20°C and used for determination of glucose, Creatine kinase-MB and Lactate dehydrogenase

2.6.2. Tissue specimens:

At the end of experiment (12 weeks), after blood collection rats were sacrificed by decapitation according to Animal Ethics Committees and abdomen was opened, then hearts were collected.

2.6.2.1. Samples for molecular analysis:

The heart tissues were collected from all animals groups, put in Eppendorf tubes and were immediately kept in liquid nitrogen and stored at - 80°C till RNA extraction for determination of the following gene expression: Histone acetyltransferase (HAT) and Histone deacetylase (HDAC) in addition to Glucose transporter4 (GULT4).

2.6.2.2. Samples for histopathological examination:

Histopathological analysis of hearts' tissue specimens treated in neutral buffered formalin solution at 10% according to Bancroft and Gamble (2008). After proper fixation, the samples were dehydrated in ascending grades of ethyl alcohol, then cleared in xylol, embedded in paraffin, and finely blocking occurred. These samples were sectioned at 5 µm in thickness and stained with hematoxylin and eosin for microscopical examination.

2.7. Analysis:

2.7.1. Biochemical analysis:

Serum glucose, creatine kinase-MB and lactate dehydrogenase (LDH) were enzymatically determined according to Tietz (1995), Urdal and Lanndaa (1979) and Dito (1979), respectively.

2.7.2. Molecular analysis:

The mRNA expression contents of HAT, HDAC, and GULT4 were determined by real-time quantitative polymerase chain reaction (real- time qPCR) analysis (Table 1) in the hearts of rats. B-actin was used as a loading control.

Table 1 Forward and reverse primers sequence used in qPCR:

Gene	Forward primer (5'-----3')	Reverse primer (5'-----3')
<i>GLUT4</i>	AGTTGGAAGAGAGCGTCCACTGT	GCTGCAGCACCACTGCAATAATCA
<i>HAT1</i>	CGACTGCTGGTGACTGACAT	TGCTCGTGCTGCACACTTAT
<i>HDAC1</i>	GTGGCCCTGGACACAGAGAT	GCTTGAATCTGGTCCAAAGT
<i>B-actin</i>	AAGTCCCTCACCTCCCAAAG	AAGCAATGCTGTCACCTTCCC

Total RNA was isolated from liver using the High Pure RNA Isolation Kit (iNtRON Biotechnology, easy-REDTM Total RNA Extraction Kit) according to the manufacturers' instructions. From each sample, cDNA was reverse transcribed using a RevertAidTM First Strand cDNA Synthesis Kit (Thermo Scientific, Fermentas, #EP0451, USA). Then, real-time quantitative PCR amplification carried out on Faststart Universal SYBR Green Master (Roche, GER). Target gene was normalized with β-actin by used the 2-ΔΔCt method (Livak and Schmittgen, 2001).

2.8. Statistical analysis:

All the data were expressed as means ±SEM using SPSS software (Version 13.0, 2009). 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). When p<0.05, values were determined statistically significant (Steel et al., 1997).

3. RESULTS

When compared to the normal group, a significant rise in blood glucose levels, CK-MB, and LDH activities were seen in STZ-induced DCM. Treatment of STZ-induced DCM with insulin, rosemary extracts, or hesperidin resulted in a considerable reduction in blood glucose levels, CK-MB, and LDH activities compared to the untreated group, with insulin group showing the greatest reduction (Table 2 and Figs. 1, 2, 3).

When compared to the normal group, STZ-induced diabetic cardiomyopathy markedly upregulated the expression of HAT1 and HDAC1 genes in cardiac tissue. On the other side, GULT4 gene expression was markedly downregulated. As compared to the STZ-induced diabetic cardiomyopathy group, rats treated with insulin, rosemary extracts, or hesperidin for STZ-induced DCM showed a considerable downregulation of HAT and HDAC. With highest downregulation in (G3). However, GULT4 gene showed a

significant upregulation as shown in (Table 3 and Figs. 4, 5, 6).

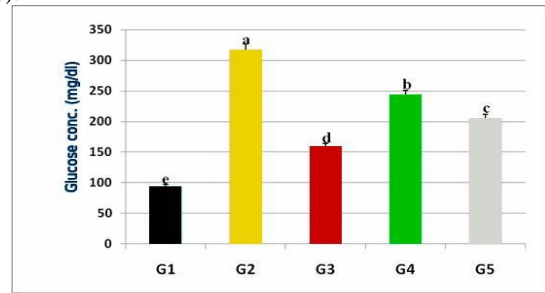


Fig. (1): Effect of insulin, rosemary extract or hesperidin treatment on blood glucose concentration in experimental model of diabetic cardiomyopathy in rats.

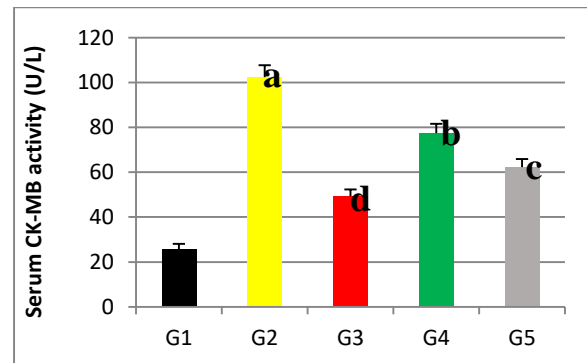


Fig. (2): Effect of insulin, rosemary extract or hesperidin treatment on CK-MB activity in experimental model of diabetic cardiomyopathy in rats.

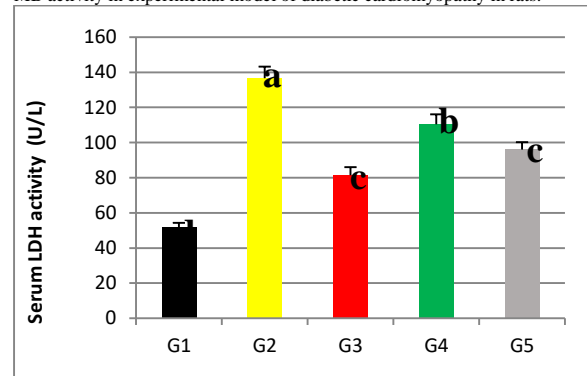


Fig. (3): Effect of insulin, rosemary extract or hesperidin treatment on serum LDH activity in experimental model of diabetic cardiomyopathy in rats.

Table 2 Effect of insulin, rosemary extract or hesperidin treatment on blood glucose concentration, CK-MB and LDH activities in control and different treated groups:

Animal groups	Glucose (mg/dl)	CK_MB (U/L)	LDH (U/L)
Control Normal (G1)	95.33 ± 4.03 ^e	25.66 ± 2.35 ^e	51.72 ± 2.63 ^d
DCM (G2)	321.33 ± 9.19 ^a	102.46 ± 5.25 ^a	136.72 ± 6.57 ^a
DCM + Insulin (G3)	163.33 ± 5.19 ^d	49.28 ± 3.09 ^d	81.44 ± 4.51 ^c
DCM + Rosemary Extract (G4)	247.4 ± 7.32 ^b	77.46 ± 4.15 ^b	110.49 ± 5.62 ^b
DCM + Hesperidin (G5)	209.6 ± 6.68 ^c	62.38 ± 3.54 ^c	96.04 ± 4.26 ^c

Data are presented as (Mean ± SEM). SEM= Standard error of mean. Mean values with different superscript letters in the same column are significantly different at (P≤0.05).

Table 3 Effect of insulin, rosemary extract or hesperidin treatment on cardiac tissue HAT1, HDAC1 and GLUT4 gene expression in control and different treated groups:

Animal groups	HAT1 Fold change \pm SEM	HDAC1 Fold change \pm SEM	GLUT4 Fold change \pm SEM
Control Normal (G1)	1.00 \pm 0.07 ^d	1.00 \pm 0.05 ^d	1.00 \pm 0.05 ^a
DCM (G2)	4.92 \pm 0.22 ^a	5.94 \pm 0.28 ^a	0.13 \pm 0.01 ^e
DCM + Insulin (G3)	1.08 \pm 0.08 ^d	2.17 \pm 0.11 ^c	0.79 \pm 0.04 ^b
DCM + Rosemary Extract (G4)	2.87 \pm 0.12 ^b	3.34 \pm 0.13 ^b	0.38 \pm 0.02 ^d
DCM + Hesperidin (G5)	1.58 \pm 0.09 ^c	3.07 \pm 0.12 ^b	0.55 \pm 0.03 ^c

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

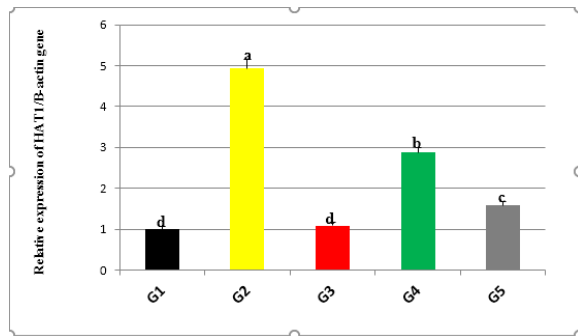


Fig. (4): Effect of insulin, rosemary extract or hesperidin treatment on HAT1 gene expression in experimental model of diabetic cardiomyopathy in rats.

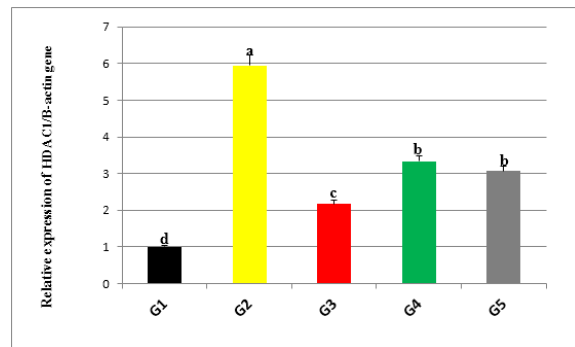


Fig. (5): Effect of insulin, rosemary extract or hesperidin treatment on HDAC1 gene expression in experimental model of diabetic cardiomyopathy in rats.

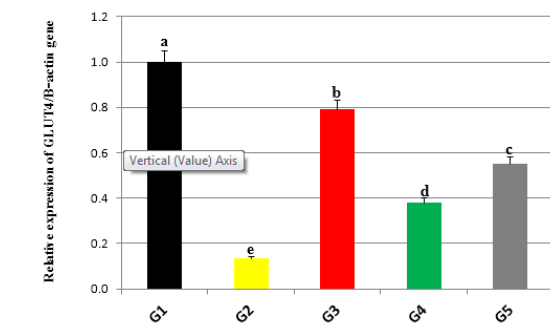


Fig. (6): Effect of insulin, rosemary extract or hesperidin treatment on GLUT4 gene expression in experimental model of diabetic cardiomyopathy in rats.

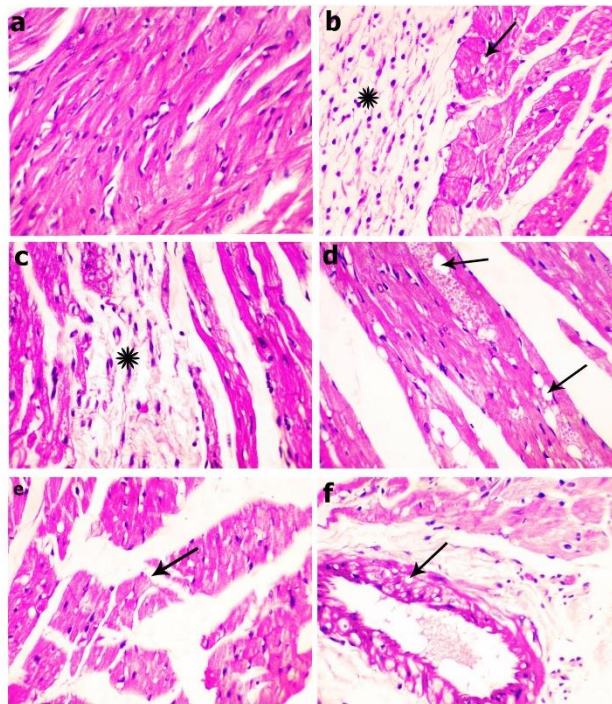
Histopathological findings:

Compared to the normal histological structure of cardiac tissue (Graph 1a) obtained from the negative control group,

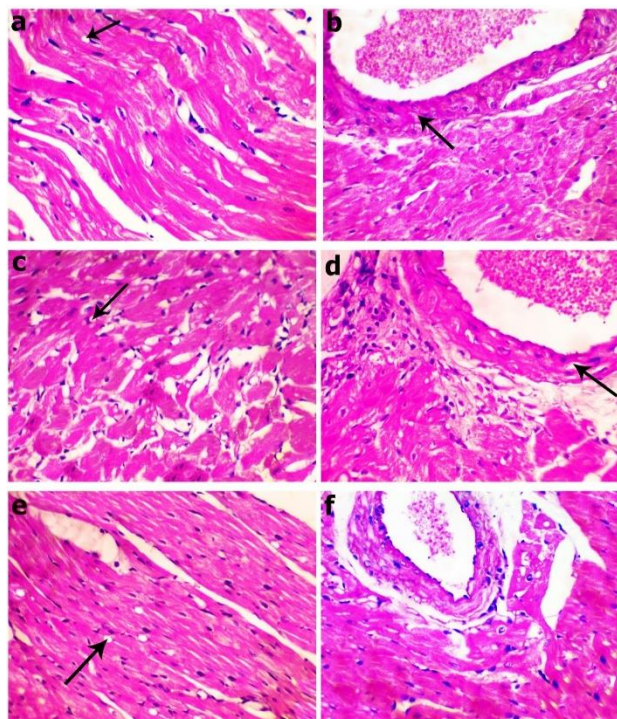
pericarditis is characterized by an increase in the thickness of pericardium with mononuclear leukocytic cellular infiltrations was detected in diabetic rats, in association with congestion of myocardial blood vessels and marked degeneration of myocytes characterized by discrete clear sarcoplasmic vacuoles of variable size, with the presence of flattened nucleus at the periphery (Graph 1b). Additionally, cardiomalacia (Graph 1c) in association with the presence of intravascular clear fat vacuoles (Graph 1d) and tear of muscle bundles (Graph 1e) were the main pathological change detected in diabetic rats of group 2. Moreover, degenerative change in the wall of myocardial blood vessels that were represented in vacuolation in the tunica media was demonstrated (Graph 1f).

The microscopical examination of the heart, of rats treated with insulin for 6 weeks revealed a marked reduction in the pathological alterations induced by streptozotocin (STZ) in the cardiac tissue post-treated with insulin. Histopathological examination of the cardiac tissue obtained from rats in this group displayed normal histological structure of the cardiac muscle in most examined cases as only mild congestion of the myocardial blood vessels with mild degeneration of the cardiac muscle in the form of small clear vacuoles in the myocardial sarcoplasm (Graph 8a) was demonstrated in one case, in association with mild degeneration of the tunica intima with vacuolation of sarcoplasm of tunica media of the examined blood vessels was demonstrated in some examined rats (Graph 8b).

However, the pathological alterations induced by streptozotocin (STZ) in the cardiac tissue post-treated with rosemary extract were moderately reduced, as mild, focal areas of degeneration in the cardiac muscle in the form of vacuolation in their sarcoplasm were also detected (Graph 8c). Additionally, degeneration of the examined blood vessels represented by vacuolation in its tunica media was noticed with marked degeneration of myocytes characterized by discrete clear sarcoplasmic vacuoles of variable size, flattened, and displaced the nucleus to the periphery (Graph 8d). Meanwhile, improvement in the cardiac tissue was detected in the diabetic rats treated with hesperidin, as the cardiac muscle showed normal histological structure in most examined cases only mild cytoplasmic vacuolation (Graph 8e) was demonstrated in two cases. Also, the cardiac blood vessels showed mild degeneration with marked degeneration of myocytes characterized by discrete clear sarcoplasmic vacuoles of variable size, flattened, and displaced the nucleus to the periphery (Figure 8f).



Graph 1: -H&E-stained histological sections of heart at 12 weeks from normal un-treated rats (a), diabetic rats (b-f), showing (a) normal cardiac muscles (x400), (b), pericarditis (asterisk) with clear vacuoles in the myocardium (arrow, x400), (c) : cardiomyopathy (arrow, x400), (d) intra-vascular clear lipid vacuoles (arrow, x400), (e) tear of the cardiac muscles (arrow, x400), (f) clear degeneration in the wall of cardiac blood vessel (arrow, x400).



Graph 2: -H&E-stained histological sections of heart at 12 weeks from diabetic rats treated with insulin (a-b), diabetic rats treated with rosemary extract (c-d), diabetic rats treated with hesperidin (e-f), showing (a) mild vacuolation of the cardiac muscle (arrow x400), (b), mild degeneration in the wall of cardiac blood vessels (arrow, x400), (c) clear small vacuoles in the sarcoplasm (arrow, x400), (d) degeneration in the wall of cardiac blood vessel with marked degeneration of myocytes characterized by discrete clear sarcoplasmic vacuoles of variable size, flattened and displaced the nucleus to the periphery (arrow, x400), (e) mild vacuolation of the cardiac muscle (arrow, x400), (f) mild degeneration in the wall of cardiac blood vessel with marked degeneration of myocytes characterized by discrete clear sarcoplasmic vacuoles of variable size, flattened and displaced the nucleus to the periphery (x400).

4. DISCUSSION

Diabetes doubles or triples the chance of developing heart failure and even hastens the decline of heart function in people with hypertension, ischemic heart disease, and atrial fibrillation. Apoptosis and inflammatory responses in cardiac tissues are important factors in the occurrence and progression of DCM (Lebeche, 2015).

The obtained results revealed that, when compared to the normal control group, the diabetic group of rats showed a substantial increase in blood glucose. These outcomes are remarkably similar to those mentioned by Mestry *et al.* (2017) who recorded that, STZ administration resulted in a significant increase in serum glucose level in the diabetic group compared with normal control group throughout 8 weeks after induction. Due to its ability to induce specific necrosis of the pancreatic β cells, STZ has been an agent of

choice to induce experimental DM resulting in degranulation and loss of capacity to secrete insulin.

When compared to the DCM non-treated group, treatment with rosemary extract considerably reduced the serum glucose concentration. This outcome was consistent with Bakirel *et al.* (2008) who linked the anti-diabetic properties of numerous *Labiatae* species, including rosemary, to their essential oils, which contain mono sesquiterpenes, phenolic compounds, and flavonoids with hypoglycemic properties. Rosemary leaf extract's ability to lower blood sugar levels may be due to its ability to stimulate insulin production or to improve insulin action (Alnahdi, 2012). Also, our results were in agreement with the results of Akiyama *et al.*, (2010) who showed that hesperidin can reduce blood sugar in type 1 diabetic rats. These results confirmed the anti-hyperglycemic activity of rosemary extract and hesperidin. In the current study, serum cardiac biomarkers such as CK-MB and LDH demonstrated a significant rise in the DCM untreated group. These results agreed with Suanarunsawat *et al.* (2016) who discovered that diabetes affected the liver, renal, and cardiac functioning of diabetic rats by raising serum levels of AST, ALT, creatinine, blood urea nitrogen, LDH, and CK-MB. Likewise, Feng *et al.* (2008) who suggested that peak rise in LDH is proportional to the extent of injury to the myocardial tissue.

Treatment with rosemary extract significantly decreased CK-MB, and LDH activities when compared with DCM non-treated group. This result came in agreement with Alnahdi, (2012) who stated that the dramatically reduced AST, CK, and LDH activities at 200 mg/kg body weight "scientifically suggest" that the rosemary extract may be able to lessen the risk factors for myocardial infarction. When compared to the DCM non-treated group, treatment with hesperidin dramatically reduced the serum levels of CK-MB and LDH in STZ-induced DCM rats. These findings indicated that hesperidin might lessen myocardial Ischemia/Reperfusion (I/R) damage (Li *et al.*, 2016).

A significant upregulation of cardiac tissue HAT and HDAC gene expression were observed in DCM untreated. Similarly, Miao *et al.*, (2004) reported that chronic hyperglycemia causes increased acetylation of various histone lysine residues, a general epigenetic marker associated with increased gene transcription. They showed that monocytes exposed to high glucose concentrations and blood monocytes from diabetic patients specifically increased histone lysine acetylation of promoter regions of cyclooxygenase-2 (COX-2) and tumor necrosis factor α (TNF- α) genes, with a corresponding increase in their gene expression, patients indicating relevance of histone acetylation in diabetes (Williams., *et al.*, 2014). This result demonstrated that class I HDACs have the potential to be targeted for a variety of organ fibrosis treatments, as it also occurs in bone marrow-derived fibroblasts (Zhang, *et al.*, 2018). In our study, the upregulation of HAT and HDAC gene expression in STZ-induced DCM in rats was significantly downregulated on treatment with insulin, rosemary extract and hesperidin. These outcomes were consistent with Wolfram, (2007) who mentioned that dietary polyphenols' antioxidant and/or anti-inflammatory properties have all been proven to influence chromatin remodeling or nuclear factor kappa B (NF-B) activation through modifying HDAC activity, which in turn affects the expression of inflammatory genes.

The obtained results showed a significant downregulation of GLUT4 gene in STZ-induced diabetic cardiomyopathy. These outcomes supported Tremblay, *et al.*, (2001) who discovered that insulin triggers the translocation of GLUT4 from an intracellular deposit site to the membrane,

increasing glucose absorption in skeletal muscles. As a defense against excessive glucose uptake during hyperglycemia and insulin resistance, glucose causes a reduction in plasma membrane levels of GLUT4 (Marette *et al.*, 1999).

Treatment with rosemary extract exhibited a significant upregulation of GLUT4 translocation when compared with DCM non treated group. These results agree with Runtuwene *et al.*, (2016) who showed that rosmarinic acid raised the amount of GLUT4 that was expressed in skeletal muscle, and this action stopped the progression of insulin resistance. An increase in GLUT4 expression in skeletal muscle improves insulin sensitivity (Richter and Hargreaves, 2013). A significant upregulation of GLUT4 translocation was also observed in treatment with hesperidin. Similarly, Agrawal *et al.*, (2014) reported that, hesperidin upregulates GLUT 4 translocation and the Peroxisome Proliferator-Activated Receptor Gamma (PPAR γ), which together help reduce blood sugar levels. In STZ-induced diabetic rats, hesperidin administration resulted in a decrease in glucose 6 phosphatase (G6Pase) activities, which reduces glucose exports from the cells by a glucose transporter membrane protein (Akiyama *et al.*, 2010). In the current research, the downregulation of GLUT 4 translocation in STZ-induced DCM in rats was significantly upregulated on treatment with insulin, rosemary extract and hesperidin.

Biochemical observations were in keeping with the morphological changes in the heart. Supplementation with rosemary leaves extract or hesperidin were moderately reduced the pathological alterations induced by streptozotocin (STZ) in the cardiac tissue and restored the cardiac cytoarchitecture nearly similar to that of normal rats. However, perivascular fibrosis was detected but no myocardial fibrosis was noticed this could be attributed to the occurrence of fibrosis required long duration than in the current experiment. As most fibrosis conditions were demonstrated in patients, aged between 58 and 73 years, with a clinical and laboratory diagnosis of dilated cardiomyopathy (Radu, *et al.*, 2012).

5. CONCLUSION

The current study concluded that hesperidin and rosemary extracts have therapeutic potential in the treatment of DCM by inhibiting the GLUT4 Pathway, which is involved in the myocardium's uptake of glucose in diabetic rats. Additionally, it was shown that the transcriptional repressor HDAC controls and activates the expression of GLUT4 in heart muscle.

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