

# **An Emerging Role of Beclin-1-Mediated Autophagy in the Cardioprotective Impact of Lactobacillus Acidophilus in Diabetic Hyperlipidemic Rats beside the Antioxidant and Anti-inflammatory Potency**

*Walaah H. Mohammed<sup>1</sup>, Mona K. Alawam<sup>1</sup>, Israa H. Khairat<sup>2</sup>, Sania K. Elwia<sup>3</sup>, Marwa Said El-sayed<sup>4</sup>, Mahmoud M. Hassan<sup>1</sup>, Marwa H. Muhammad<sup>1\*</sup>*

<sup>1</sup>Department of Physiology, Faculty of Medicine, Benha University, Benha 13518, Egypt

<sup>2</sup>Department of Histology, Faculty of Medicine, Benha University, Benha 13518, Egypt

<sup>3</sup>Department of Medical Biochemistry & Molecular Biology, Faculty of Medicine, Benha University, Benha 13518, Egypt

<sup>4</sup>Department of Medical Microbiology & Immunology, Faculty of Medicine, Benha University, Benha, Egypt

## **Abstract**

**Background:** Cardiac dyshomeostasis is steadily increasing in obese diabetics, with growing evidence pointing to a potential connection between diabetic cardiomyopathy (DCM) and gut microbiota alterations. The probiotic *Lactobacillus acidophilus* (Lacto-B) has garnered significant interest for its beneficial properties. This study aimed to explore the mechanisms by which Lacto-B exerts its protective effects and to assess the role of beclin-1-mediated autophagy in DCM. **Method:** Twenty-four rats were grouped into Control, Lacto-B, high-fat diet (HFD), and HFD + Lacto-B, for 8-week experimental period. Lacto-B was administrated at a dose of ( $2 \times 10^8$ ) colony-forming units/ml/d by oral gavage. Echocardiographic, biochemical, and microscopic evaluations for cardiac changes were assessed. Glycemic and lipid profiles, oxidative stress, inflammatory markers nuclear factor- $\kappa$ B, interleukin-1 $\beta$ , interleukin-10, and beclin-1 protein were evaluated. **Results:** The findings revealed that concurrent Lacto-B treatment with HFD limited weight gain, improved insulin sensitivity and dyslipidemia, preserved cardiac functions and structure evidenced by reduced serum injury biomarker, atherogenic indices, myocardial hypertrophy, and collagen deposition, along with improved ejection fraction and fractional shortening. Furthermore, there were boosts in the total antioxidant capacity and the anti-inflammatory interleukin-10 along with the reductions in the cardiac malondialdehyde, total oxidant status, nuclear factor- $\kappa$ B gene and interleukin-1 $\beta$ . Additionally, downregulation of beclin-1 autophagic protein was evident *versus* HFD group. **Conclusion:** It is noteworthy that Lacto-B reduced the HFD-induced exaggerated autophagic activity in DCM. In addition to its hypoglycemic and hypolipidemic effects, lacto-B was able to restore redox and inflammatory balance. Therefore, it could reduce the DCM risks and be a preventive strategy.

## **Keywords**

Lactobacillus acidophilus, Diabetes, Cardiomyopathy, Beclin-1, Autophagy

## Introduction

The incidence of cardiac diseases related to obesity and diabetes is greatly increasing. Diabetic cardiomyopathy (DCM) is a serious consequence and the leading cause of heart failure in diabetics. Moreover, dyslipidemia, a major risk factor for cardiovascular diseases and atherogenesis, is relatively high in diabetic people [1]. In addition, persistent hyperglycemia and the associated rise in the advanced glycation end products (AGEs) employs lethal effects on the cardiac muscle throughout the progression of DCM [2]. They promote the generation of reactive oxygen species (ROS), the secretion of pro-inflammatory cytokines, and a subsequent rise in cardiac stiffness [3].

A growing body of evidence has highlighted a relationship between diabetic cardiomyopathy (DCM) and disruptions in the intestinal microbiota, accompanied by changes in bacterial metabolites. Beyond their intestinal functions, these metabolites influence distant organs *via* intricate signaling pathways [4]. By orchestrating energy regulation, lipid metabolism, and inflammatory responses, the gut flora exerts significant influence on cardiac health and serves as a cornerstone for the gut-heart axis. This axis is a burgeoning field of research connecting gut functionality with cardiovascular pathology [5]. Since dysbiosis, alterations of intestinal microbiota, was acknowledged as a pathophysiological mechanism, it seems reasonable that microbiota modulation *via* probiotics, prebiotics, and synbiotic organisms help in restoring the eubiotic condition. *Lactobacillus acidophilus* (Lacto-B) is one of these probiotics. It is beneficial gut microbiota and is concerned with lactic acid production from carbohydrates [6]. It was proven to be therapeutically effective in many disease models.

As a physiological mechanism, autophagy promotes cellular recycling by breaking down misfolded proteins, lipids, and dysfunctional organelles. It is fundamental for supporting the health of non-dividing cells, such as neurons and cardiomyocytes [7]. It is observed in virtually all cardiovascular cell types, including cardiomyocytes, macrophages, vascular cells, and fibroblasts. Consequently, it plays a crucial role in restoring cardiac plasticity and serves as a fundamental mechanism in cardiac morphogenesis [8]. Autophagy operates in the heart during both baseline states and stress conditions like cardiomyopathies and ischemia, contributing to the development of severe heart failure [9]. This underscores its fundamental role in the diabetic heart and highlights its modulation as a potential strategy for managing DCM.

Autophagy process initiates with the formation of double-membrane vesicles, phagophores. Then, the phagophores engulf the cargo, lipid droplets, cytotoxic protein aggregates, and damaged organelles, resulting in the formation of autophagosomes that subsequently fuse with lysosomes to form autolysosomes [10]. Beclin-1 functions as a core regulator of autophagy, critically contributing

to autophagosome development. So, it is considered a critical regulator and indicator for autophagy in the cardiac muscle [9].

Although Lacto-B therapy has emerged as a potential treatment for diabetes-related complications, its precise function in the context of DCM remains undefined. Furthermore, the underlying cross-link between Lacto-B and beclin-1-mediated autophagy in the setting of DCM remains obscure, particularly with scarce and controversial available results. To address the existing gap, the research investigated the cardioprotective effects of Lacto-B in diabetic rats and explored the contribution of beclin-1-mediated autophagy in these mechanisms.

## **Materials and method:**

### **Experimental animals:**

A total of 24 adult male Wistar rats, weighing  $160 \pm 20$  grams, were used in this study. The animals were procured from the Animal Research Center at the Faculty of Veterinary Medicine, Benha University, Egypt. The rats were acclimatized for 7 days before the experiment with free access to water and food at the prevailing room temperature. All procedures were approved (RC: 2-12-2024) by the Ethical Committee, Faculty of Medicine, Benha University, Egypt.

### **Experimental diets:**

Balanced diet; (Fat 9.8%, protein 18.6% & carbohydrates 71.6%) and High-fat diet (HFD); (Fat 46.4%, protein 18.6% & carbohydrate 35%) were used. Their composition and ingredients were shown in Table 1.

**Table 1 Composition of the experimental diets**

<b>Contents (g/kg diet)</b>	<b>Balanced diet</b>	<b>High-fat diet</b>
Cornstarch	620.7	250.7
Casein ( $\geq 85\%$ protein) )	140.0	190.0
Lard	-----	320.0
Sucrose	100.0	100.0
Soybean oil	40.0	40.0
Fiber	50.0	50.0
AIN- 93 Mineral mix	35.0	35.0
AIN- 93 Vitamin mix	10.0	10.0
Choline	2.5	2.5
Antioxidant	0.008	0.008
L-Cystin	1.8	1.8

### **Preparation of Lacto-B suspension**

Lacto-B bacteria were supplied in lyophilized form. To check viability and purity, they were inoculated onto De Man–Rogosa–Sharpe (MRS) agar. One milliliter of live culture was added to 500 ml of MRS medium and incubated for 48 hours at 35°C. Following incubation, suspensions were

prepared from the liquid culture, and the bacterial colony count was determined. Following the calculation, the Lacto-B probiotic solution was finalized, including a suspension with  $10^{10}$  colony-forming units (CFU)/ml of bacteria in primary culture [11]

### **Experimental groups and procedure:**

A total of 24 male rats were grouped into four categories, each consisting of six rats, as outlined below:

**Group 1: (Control group):** For 8 weeks, rats fed balanced diet and were given by oral gavage an equivalent volume of distilled water vehicle.

**Group 2: (Lacto-B group):** Rats fed balanced diet and were received Lacto-B at a dose of  $(2 \times 10^8)$  (CFU)/ ml /d by oral gavage, for 8 weeks [12].

**Group 3: (HFD group):** Rats fed HFD were given an equivalent volume of distilled water by oral gavage for 8 weeks [13]

**Group 4: (HFD + Lacto-B group):** Rats fed HFD and received Lacto-B  $(2 \times 10^8)$  CFU / ml /d, oral gavage, for 8 weeks.

At the end of the experimental period, the rats were weighted, Echocardiographic examination was executed. After an overnight fasting, the rats were anaesthetized using Na pentobarbital (40 mg/kg) followed by measurement of the animal length from the base of the lower incisors to the anus by a measuring tape. Blood samples were then taken by cardiac puncture for serum preparation. The hearts were then carefully dissected from the surroundings, washed, and divided into two parts; left ventricle that was kept in formaldehyde 10% for the histopathological evaluation and Masson's trichrome special staining for collagen, in addition to the immunohistochemical analysis of beclin-1 levels. The remaining cardiac tissue was stored at  $-80\text{ }^{\circ}\text{C}$  to be further analyzed.

### **Echocardiographic examination**

Two-dimensional and M-mode Echocardiography recording was performed (Samsung Madison, SONOACE-R3-Korea), after shaving of the thoracic wall. Positioned semi-left laterally with an upright inclination, the rats were anesthetized with isoflurane (2–4% inhalation), and ultrasound gel was applied to enhance the visibility of the imaging process. The following parameters were recorded: fractional shortening percentage (FS %), ejection fraction percentage (EF %), and left ventricular weight (LVW). FS % is the percentage of size reduction of the left ventricle in systole. EF % reflects the percentage of total blood volume expelled with each heartbeat and is widely utilized to assess left ventricular function [14].

### **Calculation of body mass index (BMI)**

BMI is a marker of adiposity. It was calculated from the following equation:  $\text{BMI (g/cm}^2\text{)} = \text{Final BW (g)} / (\text{length})^2$  i.e. (the measure from the base of the lower incisors to the anus)<sup>2</sup> [15]

### **Serum preparation:**

The collected blood samples were left to be clotted at room temperature and then centrifuged 3000 revolutions per minute (rpm) for 15 min. The sera were separated, and stored at  $-80^{\circ}\text{C}$  to enable the later assessment of glycemic and lipid indices, cardiac injury biomarkers, total antioxidant capacity (TAC), and total oxidant status (TOS).

### **Assessment of serum glucose profile and related indices**

According to the manufacturer's instruction, glucose enzymatic colorimetric kits (Biodiagnostics Co, Cairo, Egypt) and insulin ELISA kits (BioVendo Brno, Czech) were used. Homeostasis model assessment of insulin resistance (HOMA-IR), as a well-documented marker for insulin resistance, was calculated as follow:  $\text{HOMA-IR} = \text{fasting glucose (mg/dl)} \times \text{fasting insulin } (\mu\text{U/mL}) / 405$  [16]. Quantitative insulin-sensitivity check index (QUICKI) is taken as a measure for insulin sensitivity. It was indicated by the inverse log sum of fasting insulin ( $\mu\text{U/ml}$ ) and fasting glucose (mg/dl) [17].

### **Assessment of serum lipids profile and calculation of atherogenic indices**

Total cholesterol (TC), triglycerides (TGs), and high-density lipoprotein-cholesterol (HDL) enzymatic colorimetric assay kits (Diamond Diagnostic, Cairo, Egypt) were used. Very low-density lipoprotein-cholesterol (VLDL) =  $\text{TGs} / 5$  and low-density lipoprotein-cholesterol (LDL) =  $\text{TC} - \text{HDL} - (\text{TGs} / 5)$  [18]. Additionally, there are known lipid profiles ratios that can predict the risk of cardiovascular insults. They were calculated as follow: atherogenic index of plasma (AIP) =  $\text{Log} (\text{TGs} / \text{HDL})$ , atherosclerosis coefficient (AC) =  $(\text{TC} - \text{HDL}) / \text{HDL}$ , and cardiac risk ratio (CRR) =  $\text{TC} / \text{HDL}$  [19].

### **Assessment of serum cardiac injury biomarkers**

Lactate dehydrogenase (LDH) kinetic assay kits (Cat no: LDH117090, Biomed, Egypt) and Creatine Kinase-MB (CK-MB) Kits (catalog #E4608-100; BioVision, CA, USA) for rats were used according to the manufacturer's instructions. LDH is an enzyme in anaerobic glycolysis and a marker of cardiac injury. CK-MB is an enzyme found primarily in heart muscle cells. Their high presence in serum indicated myocardial injury.

### **Evaluation of oxidative stress-related parameters**

The cardiac lipid peroxidation as concluded from the malondialdehyde (MDA) levels were assayed using kits BML-AK171, purchased from Enzo Life Science, (NY, USA) according to the manufacturer's instructions. Total oxidant status (TOS) was assayed in the serum using colorimetric assay kits No., IKJU-002CL, purchased from Creative Diagnostics Co., NY, USA. Moreover, the

serum levels of total antioxidant capacity (TAC) were assayed using Kits No. TA2513, obtained from Biodiagnostic Co., (Gizza, Egypt). They were measured using colorimetric assay technique.

#### **Assessment of cardiac tissue interleukin-1 $\beta$ (IL-1 $\beta$ ) and AGEs:**

Cardiac tissue IL-1 $\beta$  and AGEs levels were determined using rats ELISA kits for AGEs (Kit No. LS-F39268) and IL-1 $\beta$  (Kit No. LS-F5627), obtained from LifeSpan Biosciences, Inc., USA, following sandwich ELISA protocol. To measure absorbance at 450 nm, the ChroMate 4300 microplate reader (Awareness Technologies, USA) was used.

#### **Real-time reverse transcription PCR (RT-PCR) analysis**

Quantitative estimation of the nuclear factor- $\kappa$ B (NF- $\kappa$ B) and interleukin-10 (IL-10) relative gene expressions was executed. A total RNA isolation kit (Qiagen, Hiden, Germany) was utilized for extraction of total RNA from the frozen portion of cardiac tissue according to the manufacturer's protocols. SuperScript  $\text{\textcircled{R}}$  III First-Strand Synthesis System for RT-PCR kit (Life Technologies) was used after the first step to form the first strand. Power SYBR Green PCR Master Mix (Life Technologies) was used to carry out PCR reactions. The mRNA expression of target genes was measured in relation to the housekeeping gene, Glyceraldehyde 3-phosphate dehydrogenase (GAPDH). Employing the  $2^{-\Delta\Delta\text{CT}}$  formula, the relative expression of the target and reference genes was calculated automatically based on the comparative threshold (Ct) method, facilitated by Qiagen's dedicated software (Valencia, CA, USA). The primer sequences of the assessed genes are as follows:  
**NF- $\kappa$ B:** Forward 5'-: GCTACACAGAGGCCATTGAA3' Reverse 5'-  
ATGTGCTGTCTTGTGGAGGA3', **IL-10:** 5' primer: GCAGGACTTTAAGGGTTACTTGG; 3'  
primer: GGGGAGAAATCGATGACAGC, and **GAPDH** Forwards 5'-  
:GGTGAAGTTCGGAGTCAACGGA3' and Reverse 5'-: GAGGGATCTCGCTCCTGGAAGA3'  
[20, 21]

#### **Histopathological analysis of cardiac tissue**

Excised left ventricular tissue was preserved in 10% neutral formaldehyde, dehydrated in ascending alcohol grades, clarified with xylene, embedded in paraffin, and sectioned to a thickness of 4  $\mu$ m. Hematoxylin and eosin (H&E) and Masson's Trichrome, special stain for collagen fibers detection, were applied. Cardiac fibrosis was scored as per the methodology detailed by Galati et al. [22]; No fibrosis = score 0, Fibrosis  $\leq$  30 % = score 1, 60 % > Fibrosis > 30 % = score 2, and fibrosis  $\geq$  60 % = score 3. Olympus Microscope (Olympus BX50, Tokyo, Japan) and Image J 1.47v software provided by the National Institutes of Health, USA were used for analysis by an expert pathologist. A set of five slides was prepared for each group, and five randomly chosen fields on each slide were

analyzed. Moreover, morphometric analysis of the myocardial fiber transverse diameter was assessed on Intel® Core I7® based computer using Video Test Morphology® software (Russia).

### **Immunohistochemical evaluation of cardiac beclin-1:**

Upon deparaffinization, 4 µm sections underwent incubation with an anti-Beclin-1 antibody (ab217179, Abcam Co., Cambridge, UK) diluted at 1:150. A biotin-independent detection system is employed by the immunostainer. Mayer's haematoxylin was used as a counter-stain for the nuclei. Cytoplasmic staining appearing brown was regarded as indicative of a positive reaction. To minimize bias, the pathologist analyzing the samples was blinded to group allocation, and a semi-quantitative assessment was subsequently carried out. Staining results were determined based on the number of beclin-1-positive cells and the intensity of staining, evaluated across five randomly selected fields per slide. The assigned score quantifies the estimated percentage of cells displaying positive staining (none positive cells = 0, < 1 % positive cells = 1, positive cells 1–10 % = 2, 10–33 % positive cells = 3, positive cells 33–66% = 4, and 66–100 % positive cells = 5) [23].

### **Statistical analysis:**

The statistical processing of data was performed using SPSS for Windows (Version 20.0; SPSS Inc., Chicago, IL, USA). In order to assess the significance of differences between more than two groups, One-way analysis of variance (ANOVA) was utilized. If it showed a significant difference, and the Least Significant Difference (LSD) test was used as a post hoc test to analyze the differences within each group. Kruskal Wallis test was used for the qualitative variables. Results are given as the mean ± SD of the mean. A p-value of ≤ 0.05 was interpreted as statistically significant.

## **Results**

### **Impact of HFD and Lacto-B on rats' anthropometric parameters (Fig.1)**

Our study found that rats fed a high fat diet had a substantial rise in BW and BMI compared to control rats and those given Lacto-B ( $P < 0.05$ ). Lacto-B administration with HFD leads to a substantial decline in BW and BMI as compared to HFD group ( $P < 0.05$ ).

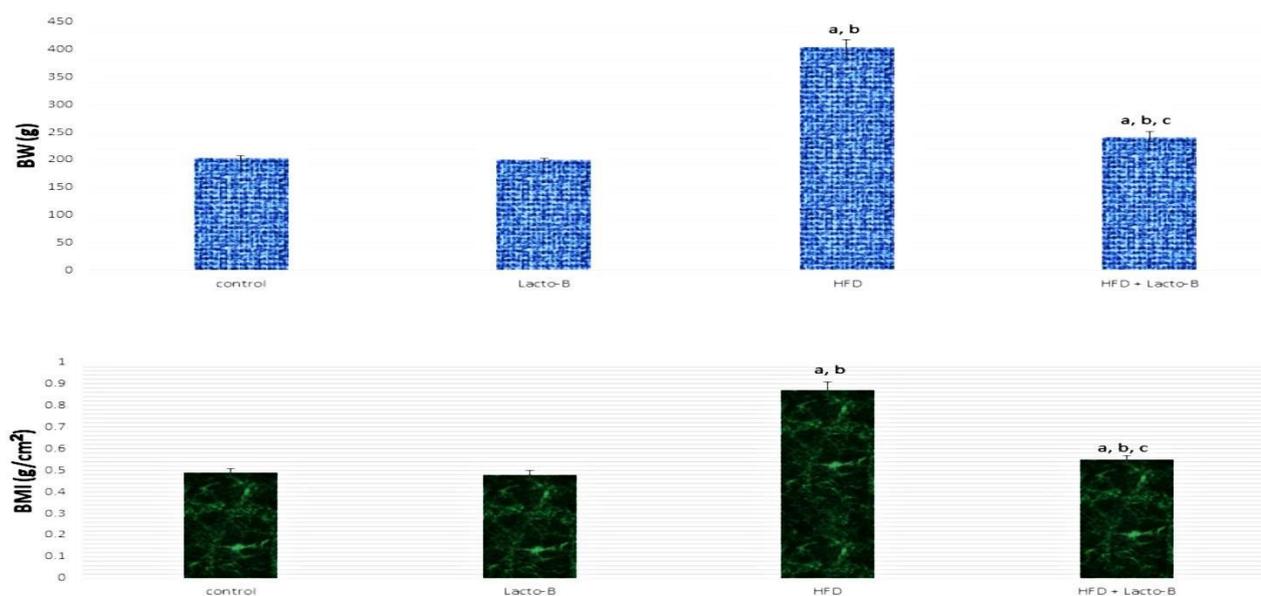
### **Impact of HFD and Lacto-B on cardiac echocardiographic and serum injury biomarkers (Table 2)**

The HFD group had significantly higher LVW ( $P < 0.05$ ) compared to the control and lacto-B groups. In contrast, the HFD group treated with Lacto-B displayed a considerable decline ( $P < 0.05$ ) in LVW compared to HFD. Furthermore, EF is much lower in the HFD group than in the control and Lacto-B groups, although Lacto-B treatment in conjunction with HFD significantly increased EF compared to the HFD group. FS was assessed in order to conduct more investigations on heart function. FS

dropped significantly in HFD group ( $P < 0.05$ ) compared to the control and Lacto-B groups. On the contrary, Lacto-B combined with HFD improved FS compared to the HFD group ( $P < 0.05$ ).

To examine how HFD affects cardiac biomarkers, we found that the HFD group had greater levels of CK-MB and LDH than the Lacto-B group and control rats ( $P < 0.05$ ). Alternatively, when assessed alongside HFD rats, lacto-B had the ability to considerably reduce this alteration in the treated (HFD + Lacto-B) rats ( $P < 0.05$ ).

**Figure 1 Rats' anthropometric parameters in the experimental groups**



Data are expressed as mean  $\pm$  standard deviation SD;  $n = 6$ , <sup>a</sup>  $P < 0.05$  significant difference compared with the Group 1; <sup>b</sup>  $P < 0.05$  significant difference compared with the Group 2; <sup>c</sup>  $P < 0.05$  significant difference compared with Group 3; Lacto-B; lactobacillus acidophilus, HFD; high-fat diet, BW: body weight, BMI; body mass index

**Table 2 Cardiac echocardiographic and serum injury biomarkers parameters**

	Group 1 Control	Group 2 Lacto-B	Group 3 HFD	Group 4 HFD + Lacto-B
LVW (g)	0.66 $\pm$ 0.03	0.67 $\pm$ 0.01	0.94 $\pm$ 0.01 <sup>a, b</sup>	0.74 $\pm$ 0.05 <sup>a, b, c</sup>
EF (%)	89.8 $\pm$ 2.8	88.8 $\pm$ 2.1	27 $\pm$ 4.6 <sup>a, b</sup>	75.3 $\pm$ 3.7 <sup>a, b, c</sup>
FS (%)	50.3 $\pm$ 2.5	48.7 $\pm$ 3.9	15.6 $\pm$ 1.13 <sup>a, b</sup>	41.8 $\pm$ 1.33 <sup>a, b, c</sup>
LDH (U/L)	1406 $\pm$ 3.88	1409 $\pm$ 5.79	2202 $\pm$ 16.8 <sup>a, b</sup>	1535 $\pm$ 33.6 <sup>a, b, c</sup>
CK-MB (ng/ml)	0.34 $\pm$ .04	0.33 $\pm$ .05	0.84 $\pm$ .07 <sup>a, b</sup>	0.40 $\pm$ .05 <sup>a, b, c</sup>

Data are expressed as mean  $\pm$  standard deviation SD;  $n = 6$ , <sup>a</sup>  $P < 0.05$  significant difference compared with the Group 1; <sup>b</sup>  $P < 0.05$  significant difference compared with the Group 2; <sup>c</sup>  $P < 0.05$  significant difference compared with Group 3; Lacto-B; lactobacillus acidophilus, HFD; high-fat diet; LVW; left ventricular weight, EF; ejection fraction, FS; fractional shortening, LDH; lactate dehydrogenase, CK-MB; creatine kinase-MB

### Impact of HFD and Lacto-B on the glycemic profile (Table 3)

Blood glucose parameters were assessed to examine the metabolic impact of HFD on rats. When compared to the control and Lacto-B groups, our study found that rats given HFD had significantly higher blood glucose and insulin hormone levels ( $P < 0.05$ ). In comparison to the HFD group, these values were considerably lower in the rats that received HFD + Lacto-B ( $P < 0.05$ ).

QUICKI and HOMA-IR are the respective indices employed for the assessment of insulin sensitivity and insulin resistance. In comparison to the Lacto-B group and control rats, HFD-induced insulin resistance was confirmed by a substantial drop in QUICKI and a significant rise in HOMA-IR ( $P < 0.05$ ). Alternatively, Lacto-B supplementation significantly restored these variances in HOMA-IR and QUICKI levels as compared to the HFD group ( $P < 0.05$ ).

Furthermore, AGEs were significantly higher in the HFD group than in the control and Lacto-B groups. Alternatively, the HFD + Lacto-B group's AGEs were notably lower than those of the HFD group ( $p < 0.05$ ).

**Table 3 Glucose homeostasis-related parameters**

	Group 1 Control	Group 2 Lacto-B	Group 3 HFD	Group 4 HFD + Lacto-B
Glucose (mg/dl)	89.17 ± 3.7	88.17 ± 5.6	202.33 ± 2.3 <sup>a, b</sup>	130.5 ± 3.6 <sup>a, b, c</sup>
Insulin (μU/mL)	7.12 ± 0.42	7.07 ± 0.32	18.9 ± 0.59 <sup>a, b</sup>	9.07 ± 0.40 <sup>a, b, c</sup>
HOMA-IR	1.57 ± 0.08	1.54 ± 0.14	9.44 ± 0.35 <sup>a, b</sup>	2.92 ± 0.14 <sup>a, b, c</sup>
QUICKI	0.504 ± 0.003	0.506 ± 0.007	0.427 ± 0.00 <sup>a, b</sup>	0.466 ± 0.002 <sup>a, b, c</sup>
AGEs (μg/mg protein)	6.1 ± 0.6	6.5 ± 0.52	19.3 ± 1.6 <sup>a, b</sup>	7.9 ± 0.4 <sup>a, b, c</sup>

Data are expressed as mean ± standard deviation SD;  $n = 6$ , <sup>a</sup>  $P < 0.05$  significant difference compared with the Group 1; <sup>b</sup>  $P < 0.05$  significant difference compared with the Group 2; <sup>c</sup>  $P < 0.05$  significant difference compared with Group 3; Lacto-B; lactobacillus acidophilus, HFD; high-fat diet; HOMA-IR, homeostasis model assessment of insulin resistance, QUICKI; Quantitative insulin-sensitivity check index, AGEs; advanced glycation end products

### Impact of HFD and Lacto-B on lipid profile and the atherogenic indices (Table 4)

Notably, compared to both control and Lacto-B, HFD rats showed considerably lower HDL and significantly higher TGs, TC, LDL, and VLDL ( $P < 0.05$ ). Nevertheless, in comparison to HFD group, lacto-B delivery yielded a notable decrease in TGs, TC, LDL, and VLDL while also significantly increasing HDL ( $P < 0.05$ ). Unsurprisingly, rats on HFD exhibited markedly higher atherogenic indices CRR, AIP, and AC than their counterparts in both the control and Lacto-B groups. Supplementing with Lacto-B significantly decreased the levels of AIP, AC, and CRR in the HFD + Lacto-B group compared to the HFD group.

**Table 4 Lipid profile and the atherogenic indices**

	Group 1 Control	Group 2 Lacto-B	Group 3 HFD	Group 4 HFD + Lacto-B
TC (mg/dl)	130.6 ± 4.1	128.67 ± 6.1	270.8 ± 19.3 <sup>a, b</sup>	162.5 ± 9.3 <sup>a, b, c</sup>
LDL (mg/dl)	34.23 ± 1.6	37.37 ± 7.56	177.9 ± 13.7 <sup>a, b</sup>	68 ± 14.6 <sup>a, b, c</sup>
HDL (mg/dl)	77.8 ± 3.4	74.3 ± 1.7	35.7 ± 3.7 <sup>a, b</sup>	60.6 ± 3.6 <sup>a, b, c</sup>
VLDL (mg/dl)	18.27 ± 1.45	20.13 ± 2.44	45.43 ± 5.41 <sup>a, b</sup>	25.17 ± 3.8 <sup>a, b, c</sup>
TGs (mg/dl)	93.0 ± 4.8	102.3 ± 11.1	234.2 ± 26.5 <sup>a, b</sup>	130.2 ± 14.4 <sup>a, b, c</sup>
AIP	0.076 ± .04	0.137 ± 0.05	0.817 ± 0.07 <sup>a, b</sup>	0.331 ± 0.03 <sup>a, b, c</sup>
AC	0.680 ± 0.04	0.731 ± 0.1	6.653 ± 1.01 <sup>a, b</sup>	1.68 ± 0.3 <sup>a, b, c</sup>
CRR	1.680 ± 0.04	1.731 ± 0.1	7.654 ± 1.0 <sup>a, b</sup>	2.68 ± 0.3 <sup>a, b, c</sup>

Data are expressed as mean ± standard deviation SD;  $n = 6$ , <sup>a</sup>  $P < 0.05$  significant difference compared with the Group 1; <sup>b</sup>  $P < 0.05$  significant difference compared with the Group 2; <sup>c</sup>  $P < 0.05$  significant difference compared with Group 3; Lacto-B; lactobacillus acidophilus, HFD; high-fat diet; TC; total cholesterol, HDL; High-density lipoprotein, LDL; Low-density lipoprotein, VLDL; Very low-density lipoprotein, TG; triglyceride, AIP; atherogenic index of plasma, AC; atherosclerosis coefficient, CRR; cardiac risk ratio.

#### Impact of HFD and Lacto-B on the oxidative stress biomarkers (Table 5)

Compared to the Lacto-B and control groups, HFD caused oxidative stress, as evidenced by a substantial decline in serum TAC ( $P < 0.05$ ) and a considerable increase in cardiac MDA and serum TOS levels. Nevertheless, as compared to the HFD group, Lacto-B treatment significantly increased serum TAC while simultaneously causing substantial decreases in cardiac MDA and serum TOS ( $P < 0.05$ ).

**Table 5 Oxidative homeostasis-related parameters**

	Group 1 Control	Group 2 Lacto-B	Group 3 HFD	Group 4 HFD + Lacto-B
Cardiac MDA (nmol/g)	32.33 ± 2.6	27.0 ± 2.09	82.83 ± 4.26 <sup>a, b</sup>	37.0 ± 2.37 <sup>a, b, c</sup>
Serum TOS (nmol/ml)	7.12 ± 0.45	6.48 ± 0.23	13.8 ± 0.49 <sup>a, b</sup>	9.17 ± 0.66 <sup>a, b, c</sup>
Serum TAC (nmol/ml)	7.83 ± 0.82	9.92 ± 0.79	1.96 ± 0.28 <sup>a, b</sup>	5.15 ± 0.67 <sup>a, b, c</sup>

Data are expressed as mean ± standard deviation SD;  $n = 6$ , <sup>a</sup>  $P < 0.05$  significant difference compared with the Group 1; <sup>b</sup>  $P < 0.05$  significant difference compared with the Group 2; <sup>c</sup>  $P < 0.05$  significant difference compared with Group 3; Lacto-B; lactobacillus acidophilus, HFD; high-fat diet; MDA; malondialdehyde, TOS; total oxidant status, TAC; total antioxidant capacity.

### Impact of HFD and Lacto-B on the inflammatory status (Table 6)

Cardiac NF-kB mRNA relative expression and IL-1 $\beta$  were considerably higher in rats on HFD, while IL-10 gene expression was substantially reduced in rats on an HFD compared to the control and Lacto-B groups ( $P < 0.05$ ). Additionally, Lacto-B treatment in HFD+ Lacto-B rats reduces HFD-induced inflammation, as evidenced by a substantial increase in IL-10 expression ( $P < 0.05$ ) and a marked decrease in cardiac IL-1 $\beta$  and NF-kB expression as compared to the HFD group.

**Table 6 Inflammatory-related parameters in the experimental groups**

	Group 1 Control	Group 2 Lacto-B	Group 3 HFD	Group 4 HFD + Lacto-B
Cardiac IL-1 $\beta$ (pg/mg protein)	46.3 $\pm$ 1.08	45.0 $\pm$ 1.52	103.8 $\pm$ 5.7 <sup>a, b</sup>	63.0 $\pm$ 5.17 <sup>a, b, c</sup>
NF-kB mRNA relative expression	3.91 $\pm$ 0.58	3.73 $\pm$ 0.51	8.83 $\pm$ 1.21 <sup>a, b</sup>	5.33 $\pm$ 0.41 <sup>a, b, c</sup>
IL-10 mRNA relative expression	5.16 $\pm$ 0.61	5.42 $\pm$ 0.49	1.16 $\pm$ 0.61 <sup>a, b</sup>	4.00 $\pm$ 1.18 <sup>a, b, c</sup>

Data are expressed as mean  $\pm$  standard deviation SD;  $n = 6$ , <sup>a</sup>  $P < 0.05$  significant difference compared with the Group 1; <sup>b</sup>  $P < 0.05$  significant difference compared with the Group 2; <sup>c</sup>  $P < 0.05$  significant difference compared with Group 3; Lacto-B; lactobacillus acidophilus, HFD; high-fat diet; NF-kB; nuclear factor-kappa B, IL-1 $\beta$ ; interleukines-1beta, IL-10; interleukines-10.

### Impact of HFD and Lacto-B on cardiac histopathological changes and fibrosis (Fig. 2 & 3)

The normal control and Lacto-B groups displayed regular branching cardiomyocytes with center rounded nuclei, according to the histological analysis of the myocardium (Fig 2a, 2b respectively). Cardiomyocyte breakdown and hyalinization, interstitial gap widening, inflammatory cell infiltration, and extravasated red blood cells were also observed in HFD rats (Fig. 2c). On the other hand, HFD +Lacto-B group showed largely preserved cardiac cells with little widening of interstitial spaces and extravasated RBCs (Fig. 2d). Additionally, there was a substantial increase in myocardial fiber transverse diameter in HFD group compared to control and Lacto-B groups and significant reduction in HDF + Lacto-B group relative to HFD group ( $p < 0.05$ ) (Fig. 2e).

Masson's trichrome staining was applied to myocardial sections from various groups to assess collagen deposition. Few collagen fibers were seen between cardiomyocytes in the control and Lacto-B groups (Fig. 3a, 3b). Large, blue bundles of collagen fibers between cardiomyocytes and noticeable fibrosis were seen in the HFD group (Fig. 3c). On the other hand, a considerable collagen fiber between cardiomyocytes was detected in the HDF + Lacto-B group (Fig. 3d). Furthermore, the cardiac fibrosis score of the HFD rats was statistically noticeably higher than that of the Lacto-B and normal control groups ( $P < 0.05$ ). The HFD + Lacto-B group's cardiac fibrosis score was markedly lower than HFD group's ( $P < 0.05$ ) (Fig. 3e).

### **Impact of HFD and Lacto-B on myocardial Beclin-1 protein expression (Fig. 4)**

Immunohistochemical analysis of myocardial Beclin-1 protein expression showed negative expression of Beclin-1 in cardiac muscle cells in control and Lacto-B groups (Fig. 4a, 4b respectively). On the other hand, there was an intense Beclin-1 expression in cardiac muscle cells in HFD group (Fig. 4c). While HFD + Lacto-B group showed moderate Beclin-1 expression in cardiac muscle cells (Fig. 4d). In keeping with these findings, there was a considerable rise in digital morphometric scoring of Beclin-1 immunohistochemical expression in HFD group *vs.* controls and Lacto-B groups ( $P < 0.05$ ), while there was a significant decline observed in HFD *vs.* HFD + Lacto-B group ( $P < 0.05$ ) (Fig. 4e).

### **Figure 2 Photomicrographs of rats' myocardium stained with Hematoxylin and Eosin (H&E)**

Photomicrographs of sections in cardiac muscle cells of rats stained with Hematoxyline and Eosin (**a-d**). (**a, b**) represent control and Lacto-B group respectively: showing regular branching cardiomyocytes (CM) with central rounded nuclei (N) (**c**) HFD group: showing disruption (D) and hyalinization (H) of Cardiomyocytes, Widening of interstitial spaces (S) with inflammatory cells infiltrate (stars) and extravasated RBCs (black arrows) (**d**) HDF + Lacto-B group: showing largely preserved cardiac cells with little widening of interstitial spaces (S) and extravasated RBCs (black arrows). (Magnification power, 400x) (**e**) Chart showing changes in the myocardial fiber transverse diameter in the experimental groups. Data are expressed as mean  $\pm$  standard deviation SD;  $n = 6$ , <sup>a</sup>  $P < 0.05$  significant difference compared with the Group 1; <sup>b</sup>  $P < 0.05$  significant difference compared with the Group 2; <sup>c</sup>  $P < 0.05$  significant difference compared with Group 3; Lacto-B; lactobacillus acidophilus, HFD; high-fat diet.

### Figure 3 Photomicrographs of rats' myocardium stained with Masson's trichrome special stain

Photomicrographs of sections in cardiac muscle cells of rats stained with Masson's Trichrome stain **(a-d)** **(a, b)** represent control and Lacto-B group respectively: showing few collagen fibers between cardiomyocytes **(c)** HFD group: showing marked fibrosis and large, blue bundles of collagen fibers between cardiomyocytes (stars) **(d)** HDF + Lacto-B group: showing moderate collagen fibers between cardiomyocytes (stars) (Magnification power,400x). **(e)** Chart showing cardiac fibrosis score in the experimental groups.

Data are expressed as mean  $\pm$  standard deviation SD;  $n = 6$ , <sup>a</sup>  $P < 0.05$  significant difference compared with the Group 1; <sup>b</sup>  $P < 0.05$  significant difference compared with the Group 2; <sup>c</sup>  $P < 0.05$  significant difference compared with Group 3; Lacto-B; lactobacillus acidophilus, HFD; high-fat diet.

**Figure 4 Photomicrographs of rats' myocardial Beclin-1 protein immunohistochemical expressions**

Photomicrographs of sections in cardiac muscle cells of rats stained with anti-Beclin-1 antibody (**a-d**). (**a, b**) represent control and Lacto-B group respectively showing : negative expression of Beclin-1 in cardiac muscle cells. (**c**) HFD group: showing strong Beclin-1 expression in cardiac muscle cells (black arrows) (**d**) HFD+Lacto-B group: showing moderate Beclin-1 expression in cardiac muscle cells (black arrows). (Magnification power, 400x). (**e**) Chart showing Beclin-1 expression levels in the experimental groups compared to control and Lacto-B groups and significant decrease in HDF + Lacto-B group compared to HDF group. Data are expressed as mean  $\pm$  standard deviation SD;  $n = 6$ , <sup>a</sup>  $P < 0.05$  significant difference compared with the Group 1; <sup>b</sup>  $P < 0.05$  significant difference compared with the Group 2; <sup>c</sup>  $P < 0.05$  significant difference compared with Group 3; Lacto-B; lactobacillus acidophilus, HFD; high-fat diet.

## Discussion

The growing burden of obesity-driven metabolic diseases, particularly cardiomyopathy, has become a major health concern globally, drawing substantial attention in both developed and developing countries. As a result, the study of their origins, prevention, and treatments has become a global research priority. The present investigation sought to elucidate the potential cardioprotective effects of Lacto-B on myocardial dysfunction and the accompanying histopathological alterations in a diabetic hyperlipidemic rat model.

The results presented revealed that in relation to the control rats, the hyperlipidemic diet ingestions augmented BW and BMI as obesity indices. Moreover, dyslipidemia was documented by the rise in TGs, TC, LDL and VLDL along with the decline in HDL. Unsurprisingly, the atherogenic indices CRR, AIP, and AC were also aggravated. These data refer to the increased risk for cardiovascular diseases and were in agreement with Zuo et al [24]. Regarding the glycemic profile findings; hyperglycemia, hyperinsulinemia along with increased insulin resistance marker HOMA-IR and reduced insulin sensitivity QUICKI marker were documented in the HFD-fed group *versus* the control one. Also, there was an increase in the AGEs levels that irreversibly accumulated in the heart.

Glucotoxicity is considered the primary cause of the initiation of DCM. To sustain systolic and diastolic function, glycolysis provides only about one-third of the energy cardiomyocytes need under normal physiological conditions. Under high glucose conditions a decrease in the myocardial glucose transporter-4 and -1 occurs, contributing to metabolic disturbances in glucose metabolism and diminished glucose uptake [25]. Glucotoxicity further promotes the accumulation of AGEs in myocardial cells, facilitating cross-linking with extracellular matrix proteins. This interaction accelerates collagen deposition and glycation, subsequently impairing its degradation, reducing cardiac muscle compliance, and contributing to myocardial stiffening [26].

Both hyperinsulinism and cardiac insulin resistance play a critical role in limiting glucose utilization [27]. As a consequence of impaired glucose utilization, myocardial energy metabolism is largely sustained by the oxidation of non-esterified fatty acids. This metabolic shift fosters lipid deposition and the accumulation of lipid intermediates, which in turn disrupt calcium handling and contractility. These alterations contribute to myocardial dysfunction, necrosis of cardiomyocytes, and fibrosis, ultimately leading to structural and functional cardiac abnormalities that culminate in DCM [26].

Left ventricular hypertrophy accompanied by myocardial fibrosis represents a hallmark pathophysiological feature of DCM [28]. This was clearly demonstrated in our current rat model. A

rise in serum cardiac injury biomarkers, LDH and CK-MB, was evident. Moreover, the echocardiographic findings referred to increased LVW and a decline in EF and FS markers of cardiac dysfunction in the HFD group when compared with the control group. In addition, an increase in myocardial fiber transverse diameter, widening of interstitial spaces with inflammatory cells infiltrate, and large bundles of collagen fibers between cardiomyocytes were confirmed by histopathological examination and Masson's trichrome staining of myocardium. These results were in agreement with Tong et al. [29], and proving the validity of this model to study the effect of Lacto-B on the diabetic heart changes.

HFD induces gut dysbiosis with increased gut permeability. The augmented bacterial metabolites induce metabolic endotoxemia with increased fatty acids production, and subsequently, TGs lipogenesis, bad cholesterol formation, and fat deposition within the myocardium [30]. These findings underscore the functional role of gut microbiota in the etiology of cardiomyopathy, identifying it as a potential target for pharmacological therapy. Thus, maintenance of microbiota balance seemed reasonable in management of obesity-related disorders and capable to constitute an innovative preventive approach in DCM.

Diet is a key factor in influencing the gut-cardiac axis, with its effectiveness arising from its ability to modify the intestinal microbiota's composition and function. Probiotic-rich diets help maintain a balanced and diverse gut microbiota, promoting gastrointestinal health and positively impacting cardiac health [31].

Lacto-B treatment besides the HFD contributed to the improvement of atherogenic indices and cardiovascular risk factors that was in agreement with those of [12, 32, 33] findings. The hypocholesterolemic effect may be ascribed to multiple mechanisms, including the deconjugation of bile acids by bile salt hydrolase, the incorporation of cholesterol into the cell walls of Lacto-B, and the production of short-chain fatty acids, which can suppress hepatic cholesterol biosynthesis.

A growing body of literature supports the notion that chronic hyperglycemia, AGEs accumulation, and hyperlipidemia associating HFD ingestion, promote the excessive release of ROS and reactive nitrogen species from the malfunctioning mitochondria that can damage vascular endothelial cells. Increased cardiac MDA levels and serum TOS simultaneously with depleted TAC led to oxidative stress and deleterious cardiac proteins, lipids, and DNA oxidative damage. Moreover, simultaneous Lacto-B administration with HFD exerted potent antioxidant potency and prevented such oxidative injury as previously reported by Hoffmann et al. [34]. Thus, Lacto-B can be introduced as natural antioxidants strains.

Nuclear factor- $\kappa$ B (NF- $\kappa$ B) serves as a critical transcription factor that regulates the expression of genes associated with pro-inflammatory, pro-fibrotic, and hypertrophic processes. The generated AGEs and the induced ROS release, activate NF- $\kappa$ B, and then initiate the transcription of the pro-inflammatory factors, causing vasculopathy and tissue injury [35]. Our data revealed NF- $\kappa$ B overexpression and IL-1 $\beta$  elevated in the cardiac tissue of HFD-fed rats *versus* the control that was in agreement with the results of Farrg et al. [3]. IL-1 $\beta$ , due to its excessive production by pancreatic  $\beta$ -cells under hyperglycemic conditions, is one of the most elevated circulating pro-inflammatory agents in diabetic models [36]. In the same context, rats fed HFD showed a reduction in the anti-inflammatory IL-10 levels. Similar results were previously reported by Kesherman et al. [37] that proved obesity-associated inflammatory response.

Upon stimulation by probiotic lactobacilli, macrophages and dendritic cells secrete cytokines that play a key role in modulating immune responses. The balance between the anti-inflammatory IL-10 and the pro-inflammatory IL-1 $\beta$  production stimulated by a probiotic is essential for identifying the direction of the immune response [38]. Our results demonstrated that concurrent Lacto-B administration besides the HFD significantly decreased cardiac IL-1 $\beta$  and augmented IL-10 levels. This is congruent with the evidence presented by Solfaïne et al. [39] which underscored the capability of Lacto-B to effectively reducing IL-1 $\beta$ . Thus, Lacto-B promotes the anti-inflammatory status and limiting the development and progression of DCM.

Dysregulated autophagy is increasingly recognized as a major contributor to the molecular mechanisms underlying DCM [40]. Untreated rats receiving an HFD showed a considerable increase in cardiac beclin-1 protein expression, as established by the present investigation. This was in line with a previous observation that has shown dysregulation of autophagy is an important factor in the development of DCM [40]. Moreover, Che et al [41] have reported beclin-1 rise in HFD group versus the control with imbalance of other autophagy-related proteins. A marked increase in autophagosome formation and lipid droplet was observed upon electron microscopy examination. Mei et al. [42] likewise reported that HFD interferes with autophagosome maturation by impairing their fusion with lysosomes, thereby inhibiting autophagosome degradation and disrupting autophagic flux. This dysfunction has been linked to mitochondrial impairment, heightened apoptosis, dysregulated intracellular Ca<sup>2+</sup> levels, and compromised cardiac function. A growing body of evidence supports the notion that NF- $\kappa$ B and autophagy engage in a complex regulatory interaction in both pathological and physiological processes, explaining this observation. It has been established that NF- $\kappa$ B contributes to autophagy activation by directly regulating beclin-1 expression [43 - 45].

Based on previously cited evidence, autophagy is commonly considered cardioprotective in different physiological and pathological conditions; however, its effects are highly context-dependent and may contribute to cell death and cardiac dysfunction, as seen in ischemia/reperfusion, which enhances autophagy in the mouse heart [46] and DCM [40]. Accordingly, beclin-1-mediated autophagy upregulation in obesity, driven by inflammatory stimuli or metabolic and endocrine alterations, appears to function as a protective mechanism for clearing protein aggregates, lipid droplets, and impaired organelles. Nevertheless, when excessively activated, it may exert harmful effects.

Noteworthy, this study demonstrated, for the first time to the best of our knowledge, that Lacto-B treatment decreased HFD-induced rise of beclin-1 levels in the setting of DCM. This was owing to the hypolipidemic, hypoglycemic, potent anti-inflammatory and antioxidant activities that significantly reduce the deleterious load on the myocardium and need for autophagic machinery with subsequent reduction of beclin-1. In support of our data, similar finding was reported by Yang et al. [47] in placentas of undergoing spontaneous delivery during normal pregnancy. On the contrary, Lai et al [48] reported beclin-1 mediated autophagy pathway in high fat diet group was increased after probiotic supplementation. This controversy could be attributed to use of multi-strain probiotic powder, or the discrepancy in the environmental situations. Finally, evaluation of other proteins in the autophagic flux has come as a limitation in our work.

## **Conclusion**

While much progress has been achieved in decoding the pathophysiological mechanisms underlying DCM we identified, for the first time, beclin-1 upgrading as a possible pathway. Concurrent Lacto-B administration with HFD down-regulated HFD-induced autophagy and restored its balance. Moreover, lacto-B probiotics exerted a beneficial impact by preventing body weight gain and improving atherogenic indices, improving the lipid and glycemic profiles. It can maintain the redox and the inflammatory homeostasis. Hence, it may be considered a potential food supplement for mitigating the risk of DCM.

**Competing interest and disclosure statement:** No potential conflicts of interest.

**Compliance with ethical standards:** The study was executed by following the rules and guidelines of the Institutional Animal Ethics Committee, Benha University, Egypt (RC- No. 2-12-2024).

**Author contributions:** All authors contributed equally to the study design, collection of samples, data analysis, and manuscript writing.

**Acknowledgments:** The authors sincerely acknowledge the support provided by the Departments of Physiology, Histology, Biochemistry, and Microbiology, Faculty of Medicine, Benha University, Egypt, for their generous support in this study.

## References

1. Ghosh, N., Chacko, L., Bhattacharya, H., Vallamkondu, J., Nag, S., Dey, A., ... & Dewanjee, S. Exploring the complex relationship between diabetes and cardiovascular complications: understanding diabetic cardiomyopathy and promising therapies. *Biomedicines*, 11(4), 1126, 2023
2. Huo, J. L., Feng, Q., Pan, S., Fu, W. J., Liu, Z., & Liu, Z. Diabetic cardiomyopathy: Early diagnostic biomarkers, pathogenetic mechanisms, and therapeutic interventions. *Cell death discovery*, 9(1), 256, 2023
3. Farrag, E. A., Hammad, M. O., Safwat, S. M., Hamed, S., & Hellal, D. Artemisinin attenuates type 2 diabetic cardiomyopathy in rats through modulation of AGE-RAGE/HMGB-1 signaling pathway. *Scientific Reports*, 13(1), 11043, 2023
4. Khalaf, E. M., Hassan, H. M., El-Baz, A. M., Shata, A., Khodir, A. E., Yousef, M. E., ... & Eissa, H. A novel therapeutic combination of dapagliflozin, Lactobacillus and crocin attenuates diabetic cardiomyopathy in rats: Role of oxidative stress, gut microbiota, and PPAR $\gamma$  activation. *European Journal of Pharmacology*, 931, 175172, 2022
5. Akshay, A., Gasim, R., Ali, T. E., Kumar, Y. S., & Hassan, A. Unlocking the Gut-Cardiac Axis: A Paradigm Shift in Cardiovascular Health. *Cureus*, 15(12), e51039, 2023
6. María Remes Troche, J., Coss Adame, E., Ángel Valdovinos Díaz, M., Gómez Escudero, O., Eugenia Icaza Chávez, M., Antonio Chávez-Barrera, J.,... & Solana Ortiz, R.. Lactobacillus acidophilus LB: a useful pharmabiotic for the treatment of digestive disorders. *Therapeutic advances in gastroenterology*, 13, 1756284820971201, 2020
7. Gómez-Virgilio, L., Silva-Lucero, M. D. C., Flores-Morelos, D. S., Gallardo-Nieto, J., Lopez-Toledo, G., Abarca-Fernandez, A. M., ... & Cardenas-Aguayo, M. D. C. Autophagy: a key regulator of homeostasis and disease: an overview of molecular mechanisms and modulators. *Cells*, 11(15), 2262, 2022
8. Li, J., Xie, Y., Zheng, S., He, H., Wang, Z., Li, X., ... & Sun, Y. Targeting autophagy in diabetic cardiomyopathy: From molecular mechanisms to pharmacotherapy. *Biomedicine & Pharmacotherapy*, 175, 116790, 2024
9. Maejima, Y., Isobe, M., & Sadoshima, J. Regulation of autophagy by Beclin 1 in the heart. *Journal of molecular and cellular cardiology*, 95, 19-25, (2016).
10. Dewanjee, S., Vallamkondu, J., Kalra, R. S., John, A., Reddy, P. H., & Kandimalla, R. Autophagy in the diabetic heart: a potential pharmacotherapeutic target in diabetic cardiomyopathy. *Ageing research reviews*, 68, 101338, 2021
11. Çiftci, G., Çiftci, A., Onuk, B., Çenesiz, M., Savaşan, S., & Çenesiz, S., Investigation of the effects of atorvastatin and Lactobacillus acidophilus on some hormones and oxidative stress in experimental hypercholesterolemia, *Prostaglandins & Other Lipid Mediators*, 165, 106716, 2023
12. Kar, P., Çiftci, G., & Çiftci, A. Potential benefit of Lactobacillus acidophilus supplementation to rats fed with a high-fat diet on serum lipid profile, kidney amyloid protein and tumor necrosis factor-alpha level. *Prostaglandins & Other Lipid Mediators*, 175, 106911, 2024
13. Guo, X. X., Wang, Y., Wang, K., Ji, B. P., & Zhou, F. Stability of a type 2 diabetes rat model induced by high-fat diet feeding with low-dose streptozotocin injection. *Journal of Zhejiang University. Science. B*, 19(7), 559–569, 2018

14. Shoukry, H. S., Ammar, H. I., Rashed, L. A., Zikri, M. B., Shamaa, A. A., Abou Elfadl, S. G.,... Prophylactic supplementation of resveratrol is more effective than its therapeutic use against doxorubicin induced cardiotoxicity. *PLoS One*, 12(7), e0181535, 2017
15. Muhammad, M. H., Elsharkawy, S. M., Abd Elhameed, A., Khairat, E., Ahmed, N., & Ibrahim, R. M. Exercise and/or Liposomal Curcumin Modulates Sarcolipin/Sarcoplasmic reticulum Ca<sup>2+</sup>-ATPase pump to Counteract Fibrillin/Asprosin/OLFR734 Pathway in Obese Diabetic Rats. *Bulletin of Egyptian Society for Physiological Sciences*, 51-76, 2024
16. Matthews, D. R., Hosker, J. P., Rudenski, A. S., Naylor, B. A., Treacher, D. F., & Turner, R. C. Homeostasis model assessment: insulin resistance and  $\beta$ -cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia*, 28(7), 412-419, 1985
17. Cacho, J., Sevillano, J., de Castro, J., Herrera, E., & Ramos, M. D. P. Validation of simple indexes to assess insulin sensitivity during pregnancy in Wistar and Sprague-Dawley rats. *American journal of physiology-endocrinology and metabolism*, 295(5), E1269-E1276, 2008
18. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without the use of the preparative ultracentrifuge. *Clin Chem*; 18: 499–502, 1972
19. Muhammad, M. H., Hussien, N. I., & Elwia, S. K. Vitamin D Replacement Mitigates Menopause-Associated Dyslipidaemia and Atherogenic Indices in Ovariectomized Rats; A Biochemical Study. *Experimental and Clinical Endocrinology & Diabetes*, 128(03), 144-151, 2020
20. Carniglia, L., Durand, D., Caruso, C. and Lasaga, M. Effect of NDP- $\alpha$ -MSH on PPAR- $\gamma$  and  $-\beta$  Expression and Anti-Inflammatory Cytokine Release in Rat Astrocytes and Microglia. *PLOS ONE* 8(2): e57313, 2013
21. O'Bryan, M. K., Gerdprasert, O., Nikolic-Paterson, D. J., Meinhardt, A., Muir, J. A., Foulds, L. M., ... & Hedger, M. P. Cytokine profiles in the testes of rats treated with lipopolysaccharide reveal localized suppression of inflammatory responses. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology*, 288(6), R1744-R1755, 2005
22. Galati, G., Leone, O., Pasquale, F., Olivotto, I., Biagini, E., Grigioni, F., ... & Rapezzi, C. Histological and histometric characterization of myocardial fibrosis in end-stage hypertrophic cardiomyopathy: a clinical-pathological study of 30 explanted hearts. *Circulation: Heart Failure*, 9(9), e003090.1177–21184, 2016
23. Barca, I., Mignogna, C., Novembre, D., Ferragina, F., & Cristofaro, M. G. Immunohistochemical analysis of the beclin-1 expression predicts the progression of oral squamous cell carcinoma. *International Journal of Environmental Research and Public Health*, 18(21), 11125, 2021
24. Zuo, A., Zhao, X., Li, T., Li, J., Lei, S., Chen, J., & Guo, Y.. CTRP9 knockout exaggerates lipotoxicity in cardiac myocytes and high- fat diet- induced cardiac hypertrophy through inhibiting the LKB1/AMPK pathway. *Journal of Cellular and Molecular Medicine*, 24(4), 2635-2647, 2020
25. Fazakerley DJ, Lawrence SP, Lizunov VA, Cushman SW, Holman GD. A common trafficking route for GLUT4 in cardiomyocytes in response to insulin, contraction and energy-status signalling. *J Cell Sci*.;122: 727–34, 2009.
26. Bugger H, Abel ED. Molecular mechanisms of diabetic cardiomyopathy. *Diabetologia*; 57:660–71, 2014

27. Jia G, Whaley-Connell A, Sowers JR. Diabetic cardiomyopathy: a hyperglycaemia- and insulin-resistance-induced heart disease. *Diabetologia*;61:21–28, 2018
28. Mohan, M., Dihoum, A., Mordi, I. R., Choy, A. M., Rena, G., & Lang, C. Left ventricular hypertrophy in diabetic cardiomyopathy: a target for intervention. *Frontiers in Cardiovascular Medicine*, 8, 746382., 2021
29. Tong, M., Saito, T., Zhai, P., Oka, S. I., Mizushima, W., Nakamura, M., ... & Sadoshima, J. Mitophagy is essential for maintaining cardiac function during high fat diet-induced diabetic cardiomyopathy. *Circulation research*, 124(9), 1360-1371, 2019
30. Al-Muzafar, H. M., & Amin, K. A. Probiotic mixture improves fatty liver disease by virtue of its action on lipid profiles, leptin, and inflammatory biomarkers. *BMC complementary and alternative medicine*, 17(1), 1-9, 2017
31. Horn, J., Mayer, D. E., Chen, S., & Mayer, E. A. Role of diet and its effects on the gut microbiome in the pathophysiology of mental disorders. *Translational psychiatry*, 12(1), 164, 2022
32. Ejtahed, H. S., Mohtadi-Nia, J., Homayouni-Rad, A., Niafar, M., Asghari-Jafarabadi, M., Mofid, V., & Akbarian-Moghari, A. Effect of probiotic yogurt containing *Lactobacillus acidophilus* and *Bifidobacterium lactis* on lipid profile in individuals with type 2 diabetes mellitus. *Journal of dairy science*, 94(7), 3288-3294, 2011
33. Ali, S. M., Salem, F. E., Aboulwafa, M. M., & Shawky, R. M. Hypolipidemic activity of lactic acid bacteria: Adjunct therapy for potential probiotics. *Plos one*, 17(6), e0269953, 2022
34. Hoffmann, A., Kleniewska, P., & Pawliczak, R. Antioxidative activity of probiotics. *Archives of medical science: AMS*, 17(3), 792, 2019
35. Lorenzo, O., Picatoste, B., Ares-Carrasco, S., Ramírez, E., Egido, J., & Tuñón, J. Potential role of nuclear factor  $\kappa$ B in diabetic cardiomyopathy. *Mediators of inflammation*, (1), 652097, 2011
36. Alfadul, H., Sabico, S. & Al-Daghri, N. M. The role of interleukin-1 $\beta$  in type 2 diabetes mellitus: A systematic review and metaanalysis. *Front. Endocrinol.* 13, 901616, 2022
37. Kesherwani, V., Chavali, V., Hackfort, B. T., Tyagi, S. C., & Mishra, P. K. Exercise ameliorates high fat diet induced cardiac dysfunction by increasing interleukin 10. *Frontiers in physiology*, 6, 124, 2015
38. Kaji, R., Kiyoshima-Shibata, J., Tsujibe, S., Nanno, M., & Shida, K. Probiotic induction of interleukin-10 and interleukin-12 production by macrophages is modulated by co-stimulation with microbial components. *Journal of dairy science*, 101(4), 2838-2841, 2018
39. Solfaine, R., Fikri, F., Maslamama, S. T., Purnama, M. T. E., & Hamid, I. Exploring the protective efficacy of *Lactobacillus acidophilus* against interleukin-1 $\beta$  (IL-1 $\beta$ ) expression in mice induced with canine coronavirus vaccine. *Adv. Anim. Vet. Sci*, 12(4), 673-678, 2024
40. Zhou, Y., Zheng, Z., Wu, S., & Zhu, J. Ubiquitin-conjugating enzyme E2 for regulating autophagy in diabetic cardiomyopathy: A mini-review. *Journal of Diabetes*, 16(3), e13511, 2024
41. Che, Y., Wang, Z. P., Yuan, Y., Zhang, N., Jin, Y. G., Wan, C. X., & Tang, Q. Z. Role of autophagy in a model of obesity: A long-term high fat diet induces cardiac dysfunction. *Molecular medicine reports*, 18(3), 3251-3261, 2018
42. Mei, Y., Thompson, M. D., Cohen, R. A., & Tong, X. Autophagy and oxidative stress in cardiovascular diseases. *Biochimica et Biophysica Acta (BBA)Molecular Basis of Disease*, 1852(2), 243-251, 2015

43. Salminen, A., Hyttinen, J. M., Kauppinen, A., & Kaarniranta, K. Context-Dependent Regulation of Autophagy by IKK-NF- $\kappa$ B Signaling: Impact on the Aging Process. *International journal of cell biology*,(1), 849541, 2012
44. Trocoli, A., & Djavaheiri- Mergny, M. The complex interplay between autophagy and NF- $\kappa$ B signaling pathways in cancer cells. *American journal of cancer research*, 1(5), 629, 2011
45. Nivon, M., Richet, E., Codogno, P., Arrigo, A. P., & Kretz-Remy, C. Autophagy activation by NF $\kappa$ B is essential for cell survival after heat shock. *Autophagy*, 5(6), 766-783, 2009
46. Sciarretta, S., Hariharan, N., Monden, Y., Zablocki, D., & Sadoshima, J. Is autophagy in response to ischemia and reperfusion protective or detrimental for the heart?. *Pediatric cardiology*, 32, 275-281, 2011
47. Yang, P., Li, Z., Tye, K. D., Chen, Y., Lu, T., He, Z., ... & Xiao, X. Effects of an orally supplemented probiotic on the autophagy protein LC3 and Beclin1 in placentas undergoing spontaneous delivery during normal pregnancy. *BMC Pregnancy and Childbirth*, 20, 1-8, 2020
48. Lai, C. H., Tsai, C. C., Kuo, W. W., Ho, T. J., Day, C. H., Pai, P. Y., ... & Huang, C. Y. Multi-strain probiotics inhibit cardiac myopathies and autophagy to prevent heart injury in high-fat diet-fed rats. *International journal of medical sciences*, 13(4), 277, 2016