### **The Potential Protective Effect of Probiotics on High-Fat Diet-Induced Cardiomyopathy in Male Albino Rats; the Possible Role of Peroxisome Proliferator- Activated Receptor- Gamma (**PPAR-γ)

**Abstract**:

 **Background:** Overconsumption of High Fat diet (HFD) is one of the major contributors to the current epidemic of cardiomyopathy. Probiotics provide health benefits to the host when ingested in appropriate amounts and it is one of the physiological triggers of Peroxisome Proliferator- Activated Receptor- Gamma **(**PPAR-γ) **Aim:** This study aimed to investigate the effect of probiotics on cardiomyopathy induced by HFD in rats and to explore the role of PPAR-γ in this process. **Methods**: 24 adult Wister albino male rats divided into 4 groups: group I (control group): rats received normal diet, group II(HFD group): rats received HFD for 8 weeks, group III(Probiotics group): rats received normal diet with probiotics& group IV(Probiotics + HFD group): rats received HFD with probiotics .

At the end, ejection fraction (EF), fraction shortening (FS) and left ventricular weight (LVW) were evaluated by Echocardiography and the rats were weighed. The blood samples were taken for biochemical estimation of total cholesterol (TC), triglyceride (TG), low density lipoprotein-cholesterol (LDL-C) and high density lipoprotein-cholesterol (HDL-C), and cardiac PPAR- γ protein levels **Results**: HFD caused a significant increase in body weight (BW), LVW, TC, TG, LDL-C with a significant decrease in EF and FS. Probiotics revered these effects through increased cardiac PPAR-γ . **Conclusion**: Probiotics showed a protective effect against the HFD-induced cardiomyopathy through induction of cardiac PPAR-γ.

 **Key words**: Cardiomyopathy, High fat diet, Probiotics, PPAR-γ.

**Abbreviations**: **PPAR-γ:** Peroxisome Proliferator- Activated Receptor- Gamma; **HFD:** high fat diet; **EF**: ejection fraction; **FS**: fraction shortening; **LVW**: left ventricular weight; **BW**: body weight; **TC**: total cholesterol; **TG**: triglyceride; **HDL-C:** High density lipoprotein-cholesterol; **LDL-C**: Low density lipoprotein-cholesterol.

1. **INTRODUCTION:**

Cardiomyopathy is a lifestyle-related disease and one of the largest public health issues. Risk factors for Cardiomyopathy correlate with an excessive intake of fat ***(1).***Worldwide, over 1 billion adults and 10% of children can be classified as overweight or obese. Cardiomyopathy is one of the leading cause of death globally, taking an estimated 17.9 million die each year ***(2).***

Probiotics are live microorganisms that provide health benefits to the host when ingested in appropriate amounts. A goal of probiotic use is the creation of symbiotic relationships between the human host and naturally occurring microorganisms, generating positive effects on the human host’s overall health and ability to resist illness. Probiotics are safe and widely accepted by the public. Over the past five years, they have developed into what is perceived as a natural treatment against metabolic disorders ***(3).***

PPARγ, a member of the nuclear receptor superfamily, is one of the most extensively studied ligand-inducible transcription factors. PPARγ has been identified to have the function of antimyocardial fibrosis. PPARγ has a wide spectrum of functions in regulating metabolism, attenuating inflammation, maintaining the balance of immune cells, inhibiting apoptosis and oxidative stress, and improving endothelial function. All of these biological functions will be of benefit for preventing the cardiac function from deterioration. Compounds that activate or modulate PPAR-*γ* may aid in fighting cardiomyopathy *(4).*

The current study was set to investigate the effect of probiotics on cardiomyopathy induced by HFD and to explore the role of PPAR-*γ* in this process.

Parameters chosen to assess the study included EF, FS, LVW, BW, serum TG, TC, LDL-C& HDL-C and cardiac PPAR- *γ levels*.

**2. MATERIAL AND METHODS:**

**2.1. Animals:**

It is a prospective experimental study. This work was achieved using 24 adult male albino rats. They were obtained from the animal research center in Faculty of Veterinary Medicine, Benha University. The experiment lasted 8 weeks from September 2020 to November 2020 in physiology department, Faculty of medicine, Benha University. All procedures are approved by ethical committee of Benha faculty of medicine. The rats were fed a standard diet, with free access to food and water. They were placed at suitable room temperature. These conditions were continued for 10 days before the experiment for acclimatization. The study period lasted for 8 weeks.

**2.2. Composition of the diet used:**

**Table (I):** Balanced diet and High fat Diet (HFD) Composition(***5)****.*

|  |  |  |
| --- | --- | --- |
| **Ingredients%** | **Balanced diet** | **HFD** |
| **Soy beans** | 18.6% | 18.6% |
| **Yellow corn** | 71.6% | 35% |
| **Fat** | 9.8% | 46.4% |

**2.3. Drugs and chemicals used:**

***Probiotics Acidophilus tablets****:* purchased from (Puritans pride, Ronkonkoma, USA lot no: 11779) provided as white tablets, Each tablet contains 100 million active lactobacillus bacteria. ***Total Cholesterol (TC) estimation kits:*** purchased from Santa Coloma,Spain; lot no:456A. ***HDL-cholesterol(HDL-C) estimation kits:*** purchased from Weisbaden-Germany; lot no:H130DA. ***Triglyceride(TG) estimation kits:*** purchased from Santa Coloma,Spain; lot no:605. ***PPAR-γ*** ***ELISA kits:*** purchased from Abbexa LLC, Houston, TX, USA ; lot no:abx155996

**2.4. Experimental design:**

Male rats were divided into 4 groups, each containing 6 rats:

**Group 1: (Control group):** Rats received balanced diet had 9.8% fat, 18.6% protein & 71.6 carbohydrate components for 8 weeks ***(5)****.* **Group 2: (HFD):** Rats received HFD contained 46.4% fat, 18.6% protein & 35% carbohydrate components for 8 weeks ***(5)****.***Group 3**: **(Probiotics):** Rats received probiotics, one tablet dissolved in 10 ml distilled water/day, by oral gavage at a dose of (4.48x107 colony forming unit (CFU)/ml) for 8 weeks ***(3)****.* **Group 4: (Probiotics+HFD):** Rats received HFD and the same previous dose of probiotics by oral gavage (4.48x107 CFU/ml) for 8 weeks ***(3)****.*

 After the end of the experiment, cardiac functions and LVW were evaluated by Echocardiography and after overnight fasting, the rats were weighed. Then, the rats were anaesthetized with Na pentobarbital (40 mg/kg). the blood samples were taken for biochemical estimation of lipid profiles.

**2.5. Echocardiography:**

Echocardiographywas performed in different groups at the end of the experimental period (8weeks). Two –dimensional and M-mode recording of short axis view was performed using a 8-10MHz liner transducer (maximum depth of 3 cm) attached to an ultra-sonographic machine (Samsung Madison, SONOACE-R3-Korea). The rats were anesthetized using 2– 4% isoflurane by inhalation according to their weight. Then, rats were placed in the proper posture (semi-left lateral position with upright tilt) after the thoracic walls were shaved clean. Ultrasound gel was placed on the thorax to optimize visibility. The following measurements were recorded: fractional shortening (FS%), ejection fraction (%EF) and left ventricular weight (LVW) ***(6).***

 **2.6. Estimation of serum TC, TG, LDL-C& HDL-C:**

 ***2.6.1. Serum preparation:***

 3 ml of each sample was left until clotting. Serum was separated by centrifugation at 3000 revolution per minute (rpm) for 15 min. After centrifugation, serum was pipetted out. Approximately 1.5 ml of serum was obtained from each blood sample, transferred to serum tubes (Eppendorf tubes) which were labeled and stored at -20°C in dark containers in deep freezer.

 ***2.6.2 Estimation of lipid profiles:***

* **TC, TGs and HDL-C** were measured using a colorimetric reflectance spectrophotometric method ***(7).***
* **LDL-C:** Calculated by this equation:

 ***Total Cholesterol – {HDL + (Triglycerides/5)}*** ***(8)***

**2.7. Tissue sampling:**

The heart was immediately washed with normal saline and stored at -80° C for biochemical estimations of Cardiac PPAR-*γ* using ELISA method.

 ***2.7.1 Assessment of tissue PPAR-γ:***

This ELISA kit used Sandwich-ELISA as the method. The micro ELISA plate provided in this kit has been pre-coated with an antibody specific to Rat PPAR-*γ*. Samples were added to the appropriate micro ELISA plate wells and bound by the specific antibody. Then a biotinylated detection antibody specific for Rat PPAR-*γ* and HRP conjugate was added to each micro plate well successively and incubated. Free components are washed away. The substrate solution was added to each well. Only those wells that contain Rat PPAR-*γ*, biotinylated detection antibody and HRP conjugate appeared blue in color. The enzyme-substrate reaction was terminated by the addition of a sulphuric acid solution and the color turns yellow. The optical density (OD) was measured spectrophotometrically at a wavelength of 450 nm +/- 2 nm. The OD value was proportional to the concentration of Rat PPAR-*γ* calculated the concentration of Rat PPAR-*γ* in the samples by comparing the OD of the samples to the standard curve

 **2.8. Statistical analysis:**

The collected data were summarized in terms of mean ± Standard Deviation (SD). Comparisons between the different study groups were carried out using the one-way analysis of variance (ANOVA; F value) followed by post hoc tests using the LSD method using the Statistical Package for Social Science (SPSS) program, version 20. P-value < 0.05 was considered statistically significant.

**3. RESULTS:**

 ***3.1.Comparison between the study groups regarding*** ***EF (%) & FS(%)(Chart 1):***

 We can observe that, when rats received HFD contained 46.1% fat, 18.6% protein & 35% carbohydrate components for 8 weeks **inHFD** **group** resulted in a significant decrease (P < 0.05) in EF when compared with their corresponding in the **Control group** .Additionally, administration of probiotic alone to rats in the **Probiotics group** at a dose of (4.48x107 colony forming unit (CFU)/ml) for 8 weeksorally resulted a non-significant change (P > 0.05) in EFwhen compared with their corresponding in the **Control group** .

Furthermore, combined administration of probiotic and HFD in the **Probiotics +HFD** group resulted in a significant increase (P < 0.05) in EF when compared with their corresponding **in** **HFD** **group.** Also, there was a significant decrease (P < 0.05) in their levels when compared with their corresponding in the **Control group** and the **Probiotics group**.

Regarding FS, the **HFD group** showed a significant decrease (P < 0.05) in FS when compared with their corresponding in the **Control group.** Additionally, **Probiotics group** showed a significant increase (P < 0.05) in FS when compared with their corresponding in the **Control group** and **HFD group** **.**

Interestingly, combined administration of probiotic and HFD in the **Probiotics +HFD** group resulted in a significant increase (P < 0.05) in FS when compared with their corresponding **in** **HFD** **group** .Also, there was a significant decrease (P < 0.05) in their levels when compared with their corresponding in the **Probiotics group**

**Chart (1):** Mean & SD of EF (%) & FS (%) in the studied groups:

Data are represented as mean ± standard deviation (SD). P < 0.05 is significant tested by one-way analysis of variance (ANOVA) and post hoc multiple comparison LSD method.

**a** P < 0.05 *vs*. control group **b** P < 0.05*vs* HFD group

 **c** P < 0.05 *vs.* probiotics group

**HFD:** high fat diet; **EF**: ejection fraction; **FS**: fraction shortening

***3.2.Comparison between the study groups regarding LVW(gm)&BW(gm)(Chart 2A, B):***

We can observe that, rats in the **HFD group** showed a significant increase (P < 0.05) in LVW & BW when compared with their corresponding in the **Control group** .

Additionally, administration of probiotic alone to rats in the **Probiotics group** resulted in a non-significant change (P > 0.05) in LVW & BW when compared with their corresponding in the **Control group** .

Furthermore, combined administration of probiotic and HFD in the **Probiotics +HFD** group resulted in a significant decrease (P < 0.05) in LVW & BW when compared with their corresponding **in** **HFD** **group**. Also, there was a significant increase (P <0.05) in their levels when compared with their corresponding in the **Control group** and the **Probiotics group** .

**Chart (2A):** Mean & SD of LVW (gm) in the studied groups:

**Chart (2B):** Mean & SD of BW (gm) in the studied groups:

Data are represented as mean ± standard deviation (SD). P < 0.05 is significant tested by one-way analysis of variance (ANOVA) and post hoc multiple comparison LSD method.

**a** P < 0.05 *vs*. control group **b** P < 0.05*vs* HFD group

 **c** P < 0.05 *vs.* probiotics group **HFD:** high fat diet; **LVW**: left ventricular weight; **BW**: body weight

***3.3. Comparison between the study groups regarding serum TG, TC, HDL-C &LDL-C (mg/dl)(Chart 3A, B):***

We can observe that, when rats received HFD contained 46.1% fat, 18.6% protein & 35% carbohydrate components for 8 weeks **inHFD** **group** resulted in a significant increase (P < 0.05) in serum TC , TG& LDL-C when compared with their corresponding in the **Control group** .

Additionally, administration of probiotic alone to rats in the **Probiotics group** at a dose of (4.48x107 colony forming unit (CFU)/ml) for 8 weeksorally resulted a non-significant change (P > 0.05) in serum TC , TG & LDL-C when compared with their corresponding in the **Control group**.

Interestingly, combined administration of probiotic and HFD in the **Probiotics +HFD** group resulted in a significant decrease (P < 0.05) in serum TC, TG & LDL-C when compared with their corresponding **in** **HFD** **group.**  Also, there was a significant increase (P < 0.05) in their levels when compared with their corresponding in the **Control group** and the **Probiotics group**.

Regarding serum HDL-C, the **HFD group** showed a significant decrease (P < 0.05) in serum HDL-C when compared with their corresponding in the **Control group.**

Additionally, **Probiotics group** showed a non-significant change (P > 0.05) in serum HDL-C when compared with their corresponding in the **Control group.**

Interestingly, combined administration of probiotic and HFD in the **Probiotics +HFD** group resulted in a significant increase (P < 0.05) in serum HDL-C when compared with their corresponding **in** **HFD** **group**. But, there was a significant decrease (P < 0.05) in their levels when compared with their corresponding in the **Control group** and the **Probiotics group**.

**Chart (3A):** Mean & SD of serum TC, TG &LDL-C (mg/dl) in the studied groups:

**Chart (3B):** Mean & SD of serum HDL-C (mg/dl) in the studied groups:

Data are represented as mean ± standard deviation (SD). P < 0.05 is significant tested by one-way analysis of variance (ANOVA) and post hoc multiple comparison LSD method.

**a** P < 0.05 *vs*. control group **b** P < 0.05*vs* HFD group

 **c** P < 0.05 *vs.* probiotics group

**HFD:** high fat diet; **TC**: total cholesterol; **TG**: triglyceride

***3.4. Comparison between the study groups regarding cardiac* PPAR-*γ (Chart 4):***

Regarding cardiac PPAR-*γ,*the **HFD group** showed a significant decrease (P < 0.05) in cardiac PPAR-*γ* when compared with their corresponding in the **Control group.**

Additionally, **Probiotics group** showed a non-significant change (P > 0.05) in cardiac PPAR-*γ* when compared with their corresponding in the **Control group.**

Interestingly, by combined administration of probiotic and HFD in the **Probiotics +HFD** group resulted in a significant increase (P < 0.05) in cardiac PPAR-*γ* when compared with their corresponding **in** **HFD** **group**. But, there was a significant decrease (P < 0.05) when compared with their corresponding in the **Control group** and the **Probiotics group.**

**Chart (4):** Mean & SD of cardiac PPAR-*γ* (ng/mg) in the studied groups:

Data are represented as mean ± standard deviation (SD). P < 0.05 is significant tested by one-way analysis of variance (ANOVA) and post hoc multiple comparison LSD method.

**a** P < 0.05 *vs*. control group **b** P < 0.05*vs* HFD group **c** P < 0.05 *vs.* probiotics group  **HFD:** high fat diet

**4. DISCUSSION:**

We have found that HFD feeding for 8 weeks contained 46.1% fat, 18.6% protein & 35% carbohydrate components resulted in obesity and dyslipidemia in the form of a significant increase (p < 0.05) in BW, TG, TC& LDL-C and a significant decrease (p < 0.05) in HDL-C in HFD group (group II) when compared with control group (group I). These results were in agreement with ***(9)*** showed that excess body weight is associated with higher plasma triglyceride, LDL cholesterol, fasting glucose levels, and lower HDL cholesterol content and a higher prevalence of dyslipidemia. Early phases of abdominal obesity are characterized by coronary endothelial dysfunction, vascular oxidative stress, and lipid profile abnormalities.

According to ***(5),*** HFD induced obesity and inflammation by increasing pro-inflammatory cytokines (TNF-α) & decreased levels of IL-10 in cardiac tissue resulting in cardiac fibrosis and decreased EF.

Interestingly, combined administration of probiotic at a dose of (4.48x107 colony forming unit (CFU)/ml) for 8 weeksorally and HFD in the **Probiotics +HFD** group (group IV) showed improvement in the cardiac dysfunction parameters and prevented dyslipidemia with a significant decrease (p < 0.05) in BW, TG, TC& LDL-C and a significant increase(p < 0.05) in HDL-C, EF &FS when compared with HFD group (group II). These results were in agreement with ***(10)***

The mechanisms by which probiotics prevented dyslipidemia and cardiac dysfunction include **Direct mechanism** through the homeostatic effect of probiotics on gut microbiota which can be explained by ***(11)*** who suggested that probiotics have hypocholesterolemic effectsthrough numerous mechanisms such as binding of cholesterol to cellular surface, interference with the formation of micelle for intestinal absorption, and bile acids deconjugation through the secretion of bile salt hydrolase. Hypocholesterolemic effects exhibited by probiotics is mostly claimed due to bile salt hydrolase activity and it can be detected in all lactobacilli. The major role of bile salt hydrolase is deconjugation of bile acid, which makes the bile salt less soluble and be excreted out as free bile acid via feces. This will reduce the cholesterol in serum and increase the de novo bile acids synthesis to replace the lost bile acid. Besides that, cholesterol could be removed in greater amount in the presence of bile as it act as a surfactant and allows cholesterol to attach on to bacterial cell membrane.

Another **indirect mechanism** by which probiotics prevent dyslipidemia and cardiac dysfunction through its effect on PPAR-γ pathway.

Concerning the role of PPAR-*γ* in cardiomyopathy, we found that HFD feeding for 8 weeks resulted in a significant decrease (p < 0.05) in cardiac PPAR-*γ* in HFD group (group II) when compared with control group (group I). These results were in agreement with ***(12)*** who reported that HFD feeding for 8 weeks resulted in a significant decrease in cardiac PPAR-*γ.*

On studying the protective effect of probiotics on HFD induced cardiomyopathy, probiotics showed a significant increase (P < 0.05) in PPARγ in the **Probiotics +HFD** group (group IV) when compared with HFD group (group II). These results were in agreement with ***(13)*** who showed that probiotics cause increase the expression of mediators involved in PPARγ signaling and attenuated the development of signs and symptoms of cardiomyopathy by reversing cardiac down-regulation of PPAR-γ induced by HFD

These findings provide novel support for the role of probiotics as cardioprotective dietary elements. This study may have important implications for effective nutritional intervention toward the prevention of cardiac disease ***(14).***

**CONCLUSION:**

Consumption of HFD caused obesity and dyslipidemia in the form of increase in BW, TC, TG & LDL-C and decrease in HDL-C. in addition to cardiac dysfunction in the form of decreased EF& FS with suppression of PPAR-*γ* in cardiac tissue.

However, administration of probiotics in adequate amounts in combination with HFD prevented the effect of HFD induced cardiomyopathy and reversed the measured parameters through induction of PPAR-*γ.*

**CONFLICT OF INTEREST:**

There is no conflict of interest.

**REFERENCES:**

1. ***Takata, T., Sakasai-Sakai, A., Ueda, T., & Takeuchi, M.*** (2019). Intracellular toxic advanced glycation end-products in cardiomyocytes may cause cardiovascular disease. *Scientific reports*, *9*(1), 1-9
2. ‏ ***Benatti, F. B., and Pedersen, B. K***. (2015). Exercise as an anti-inflammatory therapy for rheumatic diseases-myokine regulation. *Nat. Rev. Rheumatol*. 11, 86–97.
3. ***Lai, C. H., Tsai, C. C., Kuo, W. W., Ho, T. J., Day, C. H., Pai, P. Y., et al.*** (2016). 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.‏
4. ***Hernandez-Quiles, M., Broekema, M. F., & Kalkhoven, E.*** (2021). PPARgamma in metabolism, immunity, and cancer: Unified and diverse mechanisms of action. Frontiers in Endocrinology, 12.‏
5. ***Kesherwani, V., Chavali, V., Hackfort, B. T., Tyagi, S. C., & Mishra, P. K.*** (2015). Exercise ameliorates high fat diet induced cardiac dysfunction by increasing interleukin 10. *Frontiers in physiology*, *6*, 124.‏
6. ***Shoukry, H. S., Ammar, H. I., Rashed, L. A., Zikri, M. B., Shamaa, A. A., Abou Elfadl, S. G., et al.*** (2017). Prophylactic supplementation of resveratrol is more effective than its therapeutic use against doxorubicin induced cardiotoxicity. *PLoS One*, *12*(7), e0181535.‏
7. ***Karaşahin, T., Aksoy, N. H., Haydardedeoglu, A. E., Dursun, Ş., Bulut, G., Çamkerten, G. et al.***  (2019). Serum cholesterol levels in Hair goats of Aksaray Region. Indian Journal of Animal Research, 53(1), 63-66.‏
8. ***Martin, S. S., Blaha, M. J., Elshazly, M. B., Brinton, E. A., Toth, P. P., McEvoy, J. W., et al.***(2013). Friedewald-estimated versus directly measured low-density lipoprotein cholesterol and treatment implications. *Journal of the American College of Cardiology*, *62*(8), 732-739.‏
9. ***Qin, X. D., Qian, Z., Vaughn, M. G., Trevathan, E., Emo, B., Paul, G., et al.*** (2015). Gender-specific differences of interaction between obesity and air pollution on stroke and cardiovascular diseases in Chinese adults from a high pollution range area: A large population based cross sectional study. *Science of the Total Environment*, *529*, 243-248.‏
10. ***Tunapong, W., Apaijai, N., Yasom, S., Tanajak, P., Wanchai, K., Chunchai, T., et al.***(2018). Chronic treatment with prebiotics, probiotics and synbiotics attenuated cardiac dysfunction by improving cardiac mitochondrial dysfunction in male obese insulin-resistant rats. European journal of nutrition, 57(6), 2091-2104.‏
11. ***Kim, S. J., Park, S. H., Sin, H. S., Jang, S. H., Lee, S. W., Kim, S. Y., et al.*** (2017). Hypocholesterolemic effects of probiotic mixture on diet-induced hypercholesterolemic rats. Nutrients, 9(3), 293.
12. ***Ma, Z. G., Yuan, Y. P., Zhang, X., Xu, S. C., Wang, S. S., & Tang, Q. Z. (***2017). Piperine attenuates pathological cardiac fibrosis via PPAR-γ/AKT pathways. *EBioMedicine*, *18*, 179-187.‏
13. ***Imam, M. U., Ismail, M., Ithnin, H., Tubesha, Z., & Omar, A. R.*** (2013). Effects of germinated brown rice and its bioactive compounds on the expression of the peroxisome proliferator-activated receptor gamma gene. Nutrients, 5(2), 468-477.‏
14. ***Moludi, J., Alizadeh, M., Davari, M., Golmohammadi, A., & Maleki, V.*** (2019). The efficacy and safety of probiotics intervention in attenuating cardiac remodeling following myocardial infraction: Literature review and study protocol for a randomized, double-blinded, placebo controlled trial. Contemporary clinical trials communications, 15, 100364.‏