Apoptotic pathway inhibition by treatment Lactic acid bacteria through survival pathway to reduce cardiac dysfunction in a rat model of diabetes

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Abstract

Cardiavascular disease (CVD) is the major cause of mortality in patients with diabetes. Many studies suggested that lactic acid bacteria exhibits diverse biological activities, including anti-oxidase, anti-inflammatory. In this study, I investigated whether G263H (*lactobacillus*) restores cardiac function in diabetic rats. The impact of diabetes mellitus (DM) on the wistar rats heart, and evaluate the role of G263H (*lactobacillus*) against DM induced cell apoptosis in wistar rats heart. Although, DM increased survival upstream proteins pIGF1R and pPI3K but can not decreased the activity of Fas-dependent and mitochondrial-dependent apoptotic pathway, were significantly increased in DM group compared to control. The evidence for which is based on increases in Fas, FADD, Caspase-8, activated Bak, Bax, Bad, Cytochrome C, Caspase-9 and activated Caspase-3. Furthermore, we found all of which are significantly attenuated by G263H treatment.

Experimental Design

Fourty Wistar rats at 5 weeks of age were randomly divided into three groups, Control, Diabetes mellitus and Diabetes mellitus treated with G263H (lactic acid bacteria) groups for 4 weeks. Cardiac function determined by echocardiography Befor sacrificed. The excised left ventricle from rats were measured by histological analysis, western blotting, and TUNEL assays. Diabetes mellitus induced cardiac abnormalities including abnormal myocardial architecture, and more cardiac TUNEL-positive apoptotic cells.

Results

Table 1. Cardiac characteristics of Control, DM group and DM with G263H

	Control	DM	DM+G263H
Number of animals	9	5	5
Body weight (BW),g	346.11 ± 50.48	269.33 ± 46.09***	299.00 ± 26.05***#
Whole heart weight (WHW),g	1.10±0.10	0.85±0.06***	0.92±0.07**
Left ventricular weight (LVW),g	0.79±0.05	0.59±0.07***	0.66±0.06**
WHW/ Tibia length ($\times 10^3$) ,g/mm	25.77±2.03	21.79±1.66**	22.40±1.79
LVW / Tibia length ($\times 10^3$) ,g/mm	18.38±1.09	15.17±1.71**	16.00±1.40
Blood sugar (mg/dL)	106.83±7.36	447.20±33.44***	334.80±16.48***###

Values are means ±SD among Wistar rats (Control), streptozotocin-induced diabetic rats (DM) and diabetic rats with G263H (DM+G263H).
**P<0.05, ** P<0.01, ***P<0.001 significant differences between Control and DM or Control and DM+G263H group.*
###P<0.05 significant differences between DM group and DM+G263H group.

Table 2. Cardiac function determined by echocardiography of Control, DM group and DM with G263H

	Control	DM	DM+G263H
EF(Teich) [%]	**78.75 ± 5.66**	**69.38 ± 4.06*** **	**74.05 ± 6.13$^{\#\#\#}$**
%FS [%]	**42.94 ± 5.54**	**34.67 ± 3.02*** **	**38.74 ± 4.79$^{\#\#}$**

Values are means ±SD among Wistar rats (Control), streptozotocin-induced diabetic rats (DM) and diabetic rats with G263H (DM+G263H).
**P<0.05, ** P<0.01, ***P<0.001 significant differences between Control and DM or Control and DM+G263H group.*
###P<0.05 significant differences between DM group and DM+G263H group.

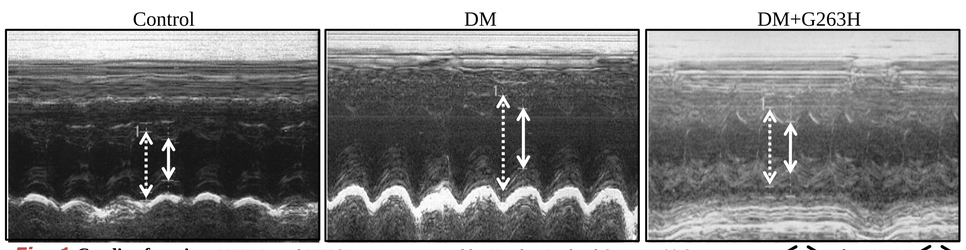

Fig. 1 **Cardiac function** LVEDD, and LVESD , were measured by Hewlett-Packard Sonos 2500 Sector scanner. ↔ is for LVEDD.↔ is for LVESD.

Fig. 2 ***The protein expression levels of PI3K and Akt extracted from heart tissue were quantitated by Western blotting analysis.*** (A), (C) Total protein of cell extracts was separated by 12% SDS-PAGE, and transferred to PVDF membranes. (B), (D) The results were analyzed by one way analysis of variance (ANOVA). *P＜0.05, **P＜0.01, ***P＜0.001 vs. Control ;#P＜0.05 , ##P＜0.01, ###P＜0.001 vs. DM

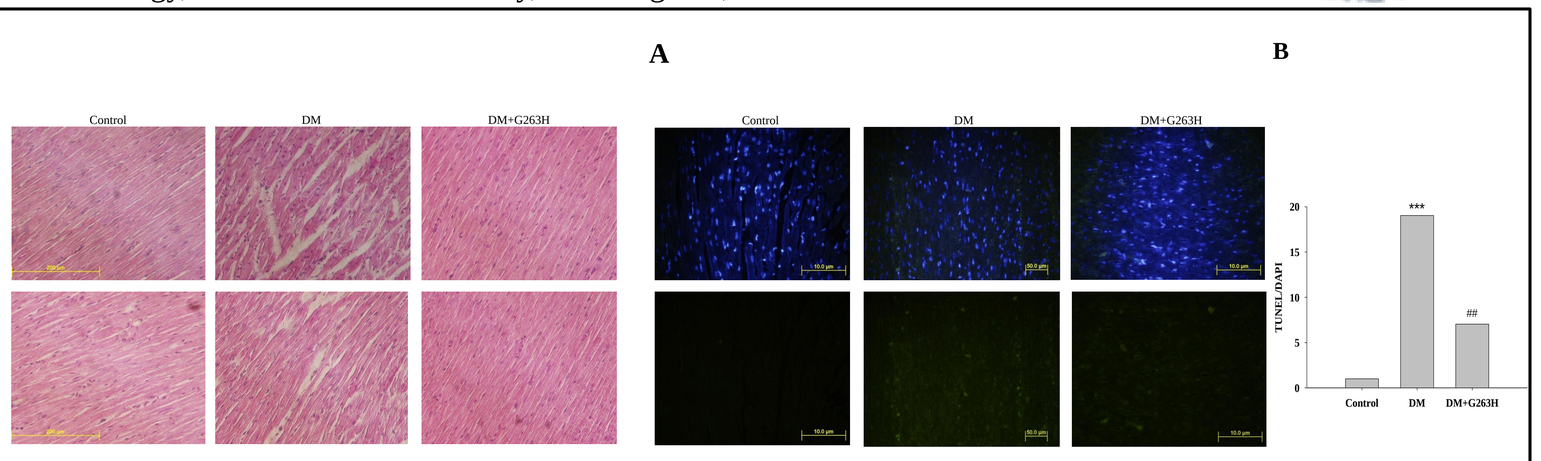

Fig. 3 **Hematoxylin-eosin staining**
Representatives of histopathological analysis in the sections of cardiac tissue stained with hematoxylin and eosin. The images of myocardial architecture were magnified and ×400.

Fig. 4 ***G263H had protective effects in diabetes- induced apoptosis.***
Influence of G263H in diabetic rat induced cardiomyoblast apoptosis by DAPI (4, 6-diamidino-2-phenylindole ; 1 g/ml) staining and TUNEL assay.

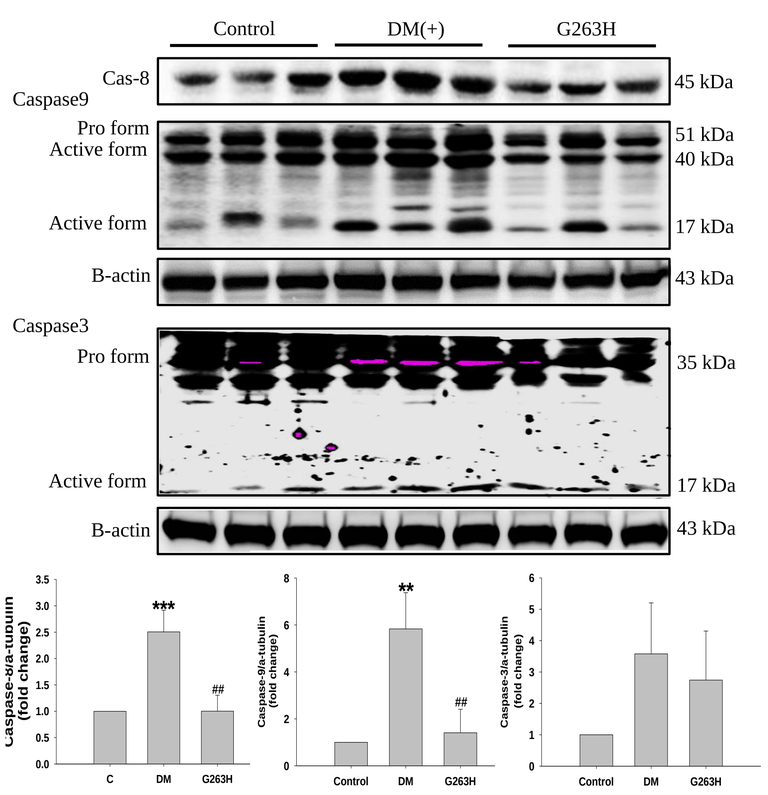

Fig. 5 ***The protein expression levels of caspaase-8, pro-caspase-9, caspase-9, pro-caspase-3 and activate-caspase-3,extracted from heart tissue were quantitated by Western blotting analysis.*** (A)Total protein of cell extracts was separated by 12% and SDS-PAGE, transferred to PVDF membranes. (B) The results were analyzed by one way analysis of variance (ANOVA). *P＜0.05, **P＜0.01, ***P＜0.001 vs. Control ;#P＜0.05 , ##P＜0.01, ###P＜0.001 vs. DM

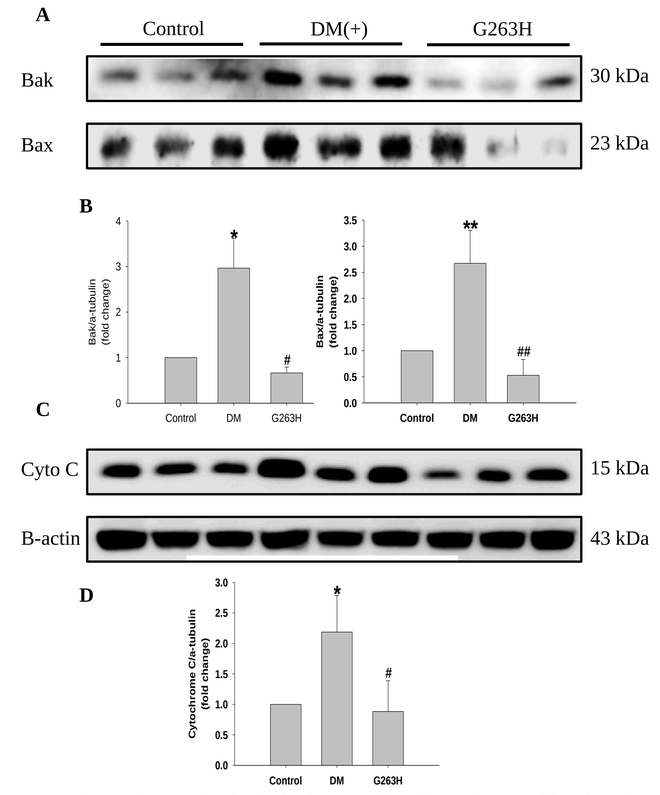

Fig. 7 ***The protein expression levels of Bak, Bax and Cytochrome C,extracted from heart tissue were quantitated by Western blotting analysis.*** (A), (C) Total protein of cell extracts was separated by 12% and SDS-PAGE, transferred to PVDF membranes. (B), (D) The results were analyzed by one way analysis of variance (ANOVA). *P＜0.05, **P＜0.01, ***P＜0.001 vs. Control ;#P＜0.05 , ##P＜0.01, ###P＜0.001 vs. DM

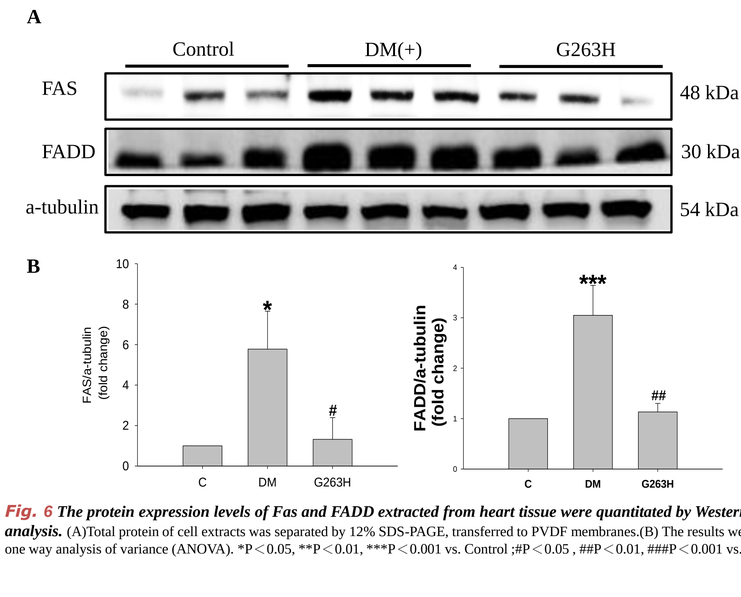

Fig. 6 ***The protein expression levels of Fas and FADD extracted from heart tissue were quantitated by Western blotting analysis.*** (A)Total protein of cell extracts was separated by 12% SDS-PAGE, transferred to PVDF membranes.(B) The results were analyzed by one way analysis of variance (ANOVA). *P＜0.05, **P＜0.01, ***P＜0.001 vs. Control ;#P＜0.05 , ##P＜0.01, ###P＜0.001 vs. DM

Conclusion

In the present investigation, dibetes mellitus induced cardiac apoptosis. Our findings demonstrate that inhibition of Fas-dependent apoptotic pathway, mitochondria-dependent apoptotic pathway and increased survival pathway by the treatment of G263H. This study suggest that G263H provides cardiovascular protection on cardiac apoptosis.