

# Lactic acid bacteria ( LAB ) reduced cardiac dysfunction by decreasing apoptotic pathway and enhancing survival pathway activities in diabetic rats

Sheng-Chuan Lin<sup>1#</sup>、Chung-Jen Chiang<sup>1\*</sup>、Ying-Chen Lu<sup>3</sup>、Wei-Wen Kuo<sup>4</sup>、Chih-Yang Huang<sup>2\*</sup>

<sup>1</sup>Department of Medical Laboratory Science and Biotechnology, China Medical University, Taichung 404, Taiwan

<sup>2</sup>Graduate Institute of Basic Medical Science, China Medical University, Taichung 404, Taiwan

<sup>3</sup>Department of Biological Science and Technology, Chung Hwa University of Medical Technology, Tainan 717, Taiwan

<sup>4</sup>Department of Biological Science and Technology, China Medical University, Taichung 404, Taiwan

## Abstract

Cardiovascular 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 before 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

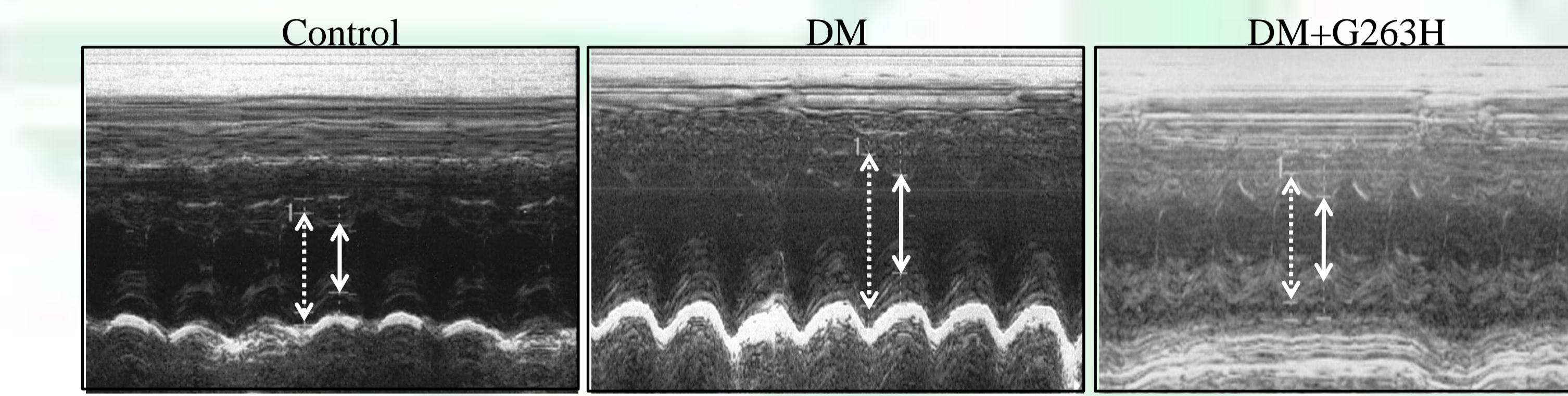
	Control	DM	DM+G263H
Number of animals	9	5	5
Body weight (BW),g	346.11 ± 50.48	269.33 ± 46.09***	299.00 ± 26.05***#
Whole heart weight (WHW),g	1.10±0.10	0.85±0.06***	0.92±0.07**
Left ventricular weight (LVW),g	0.79±0.05	0.59±0.07***	0.66±0.06**
WHW/ Tibia length (x10 <sup>3</sup> ),g/mm	25.77±2.03	21.79±1.66**	22.40±1.79
LVW / Tibia length (x10 <sup>3</sup> ),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.

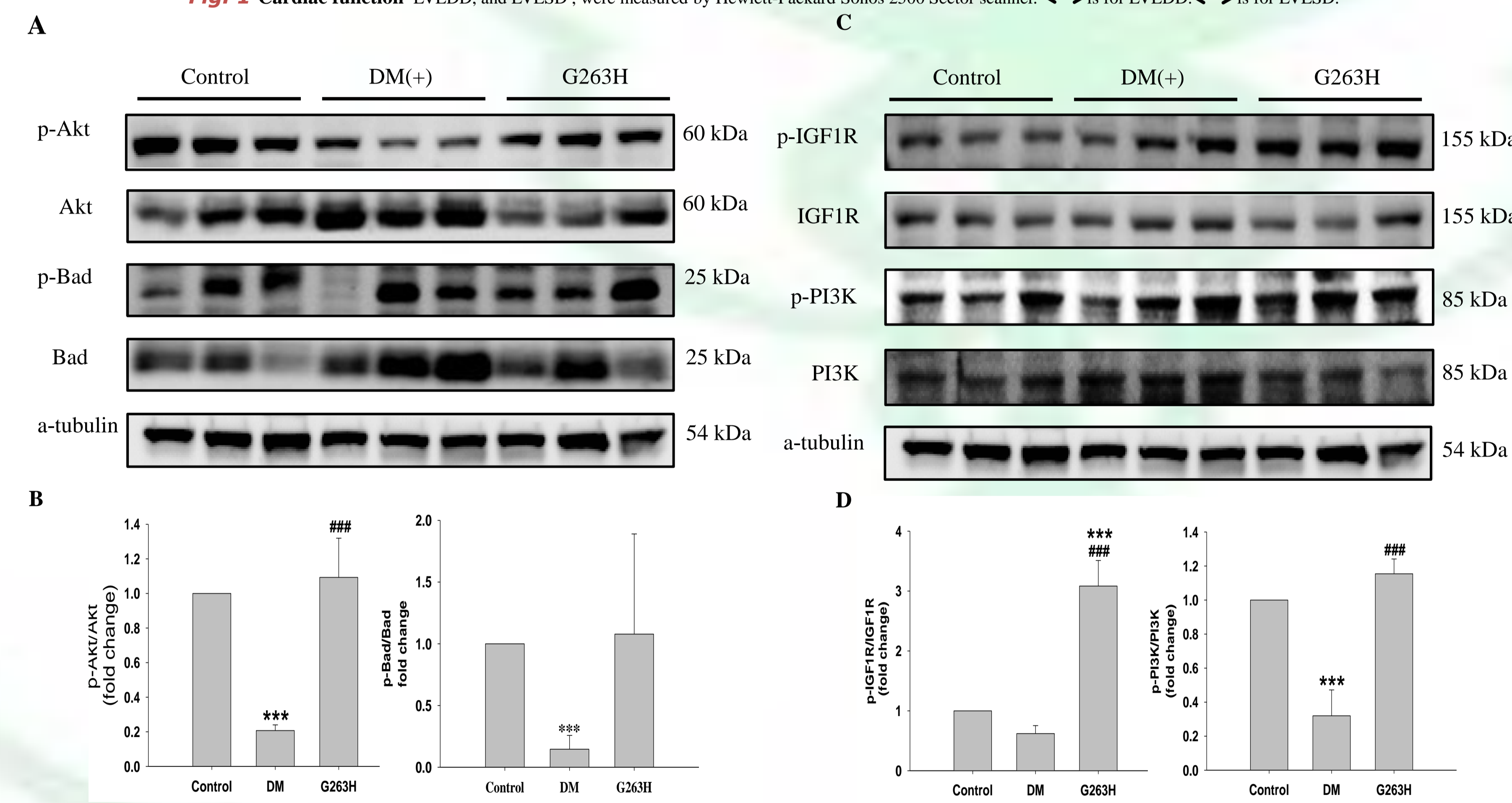
**Table 2.** Cardiac function determined by echocardiography of Control, DM group and DM with G263H

	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##

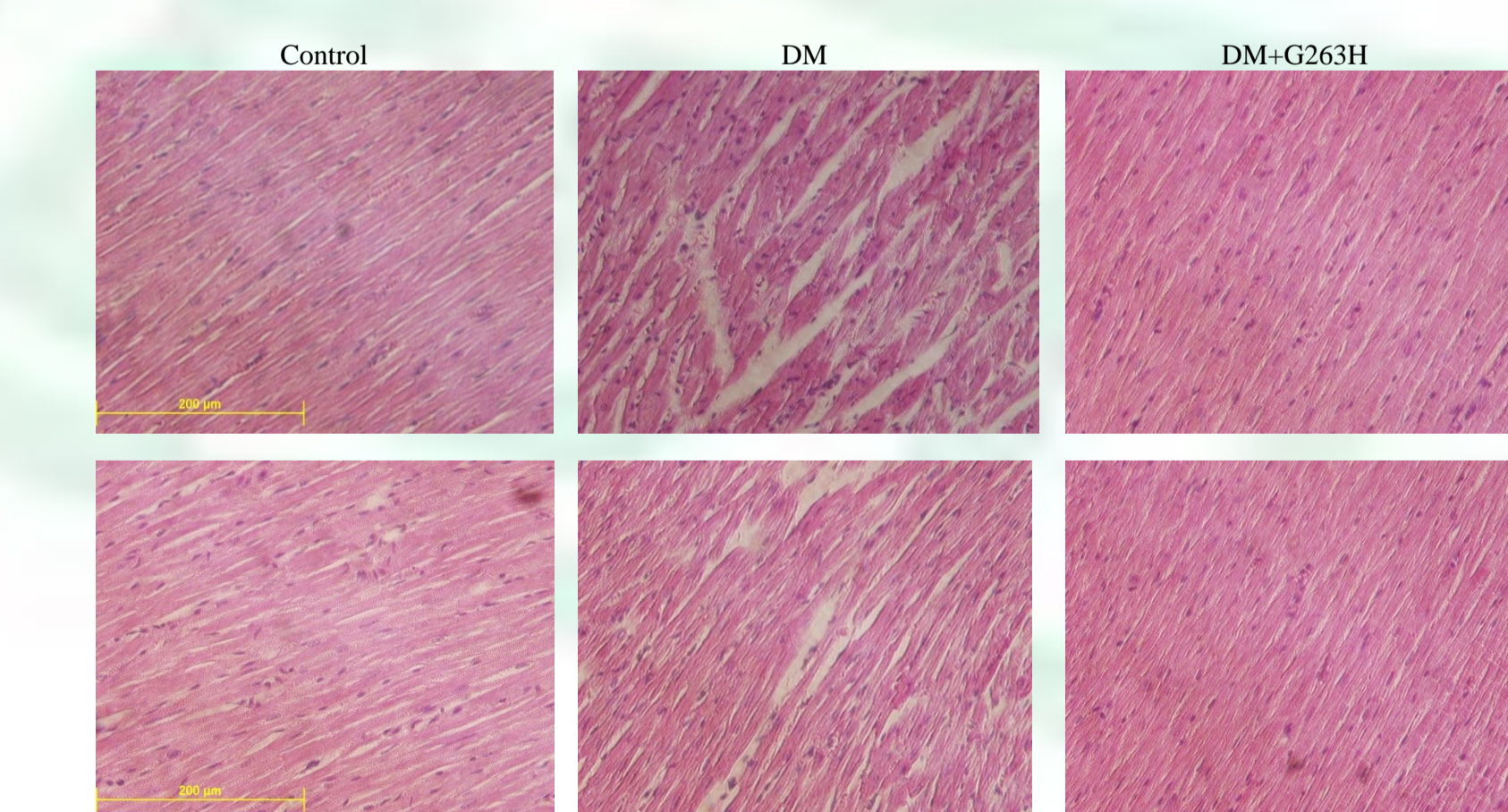
Values are means ± SD among Wistar rats (Control), streptozotocin-induced diabetic rats (DM) and diabetic rats with G263H (DM+G263H). \*P<0.05, \*\*P<0.01, \*\*\*P<0.001 significant differences between Control and DM or Control and DM+G263H group. ##P<0.05 significant differences between DM group and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



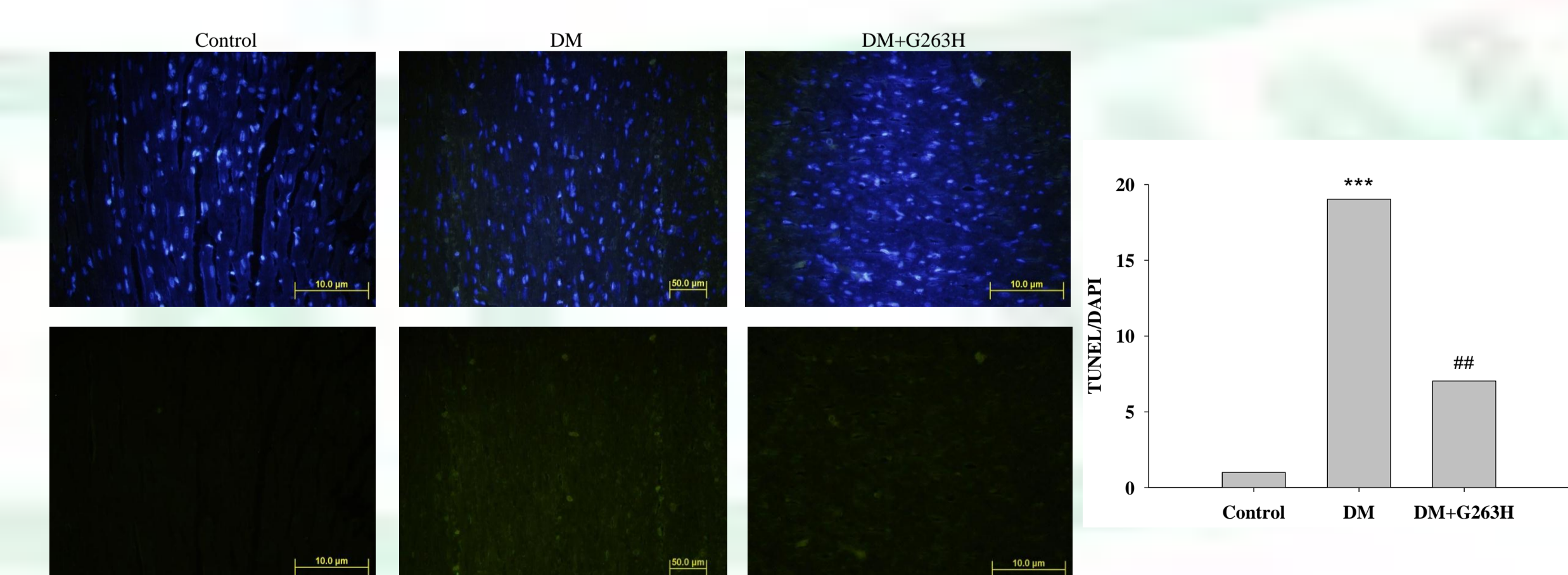
**Fig. 1** Cardiac function LVEDD, and LVESD, were measured by Hewlett-Packard Sonos 2500 Sector scanner. ←→ is for LVEDD, ←→ is for LVESD.



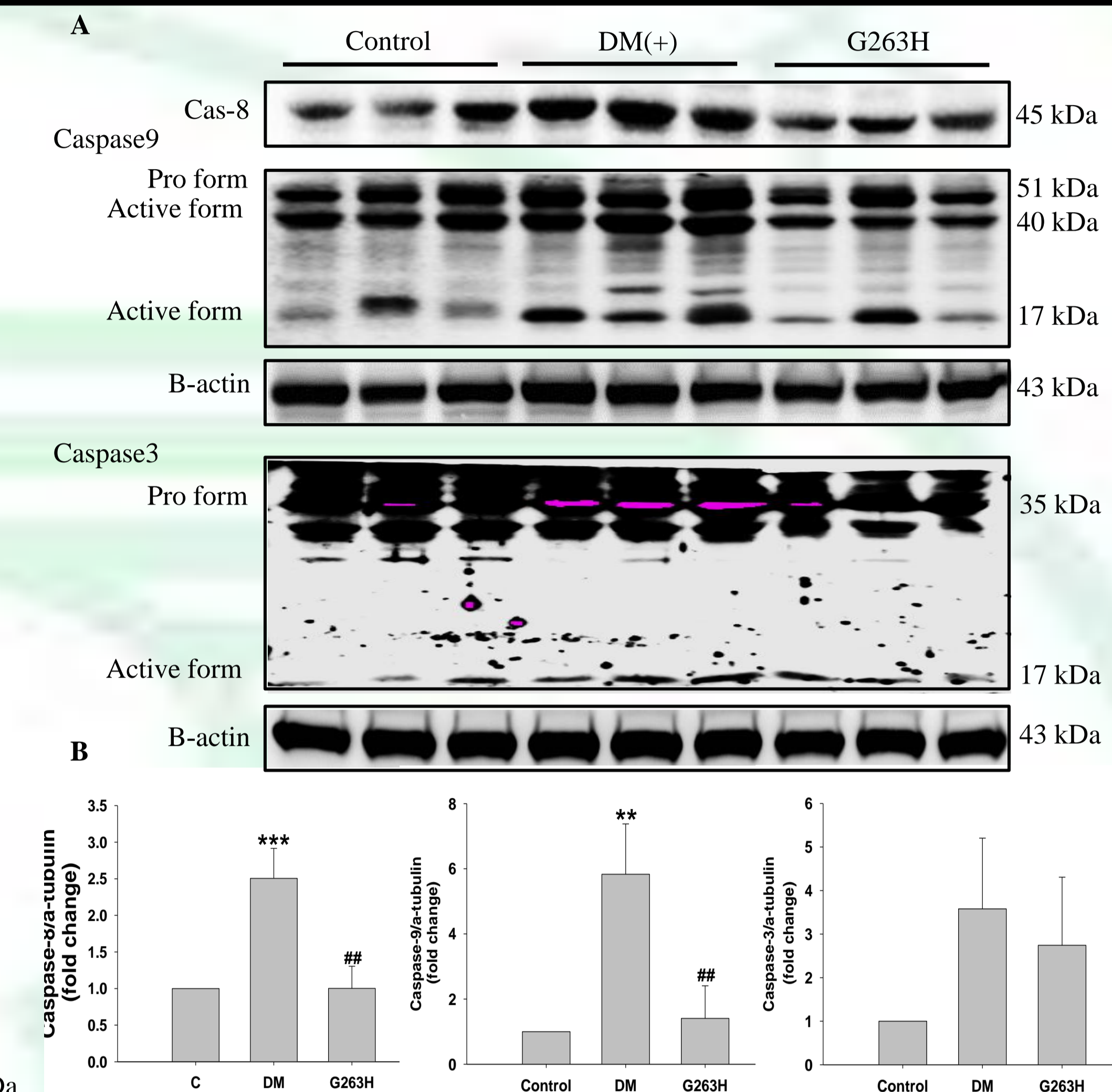
**Fig. 2** The protein expression levels of PI3K and Akt extracted from heart tissue were quantitated by Western blotting analysis. (A), (C) Total protein of cell extracts was separated by 12% SDS-PAGE, and transferred to PVDF membranes. (B), (D) The results were analyzed by one way analysis of variance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



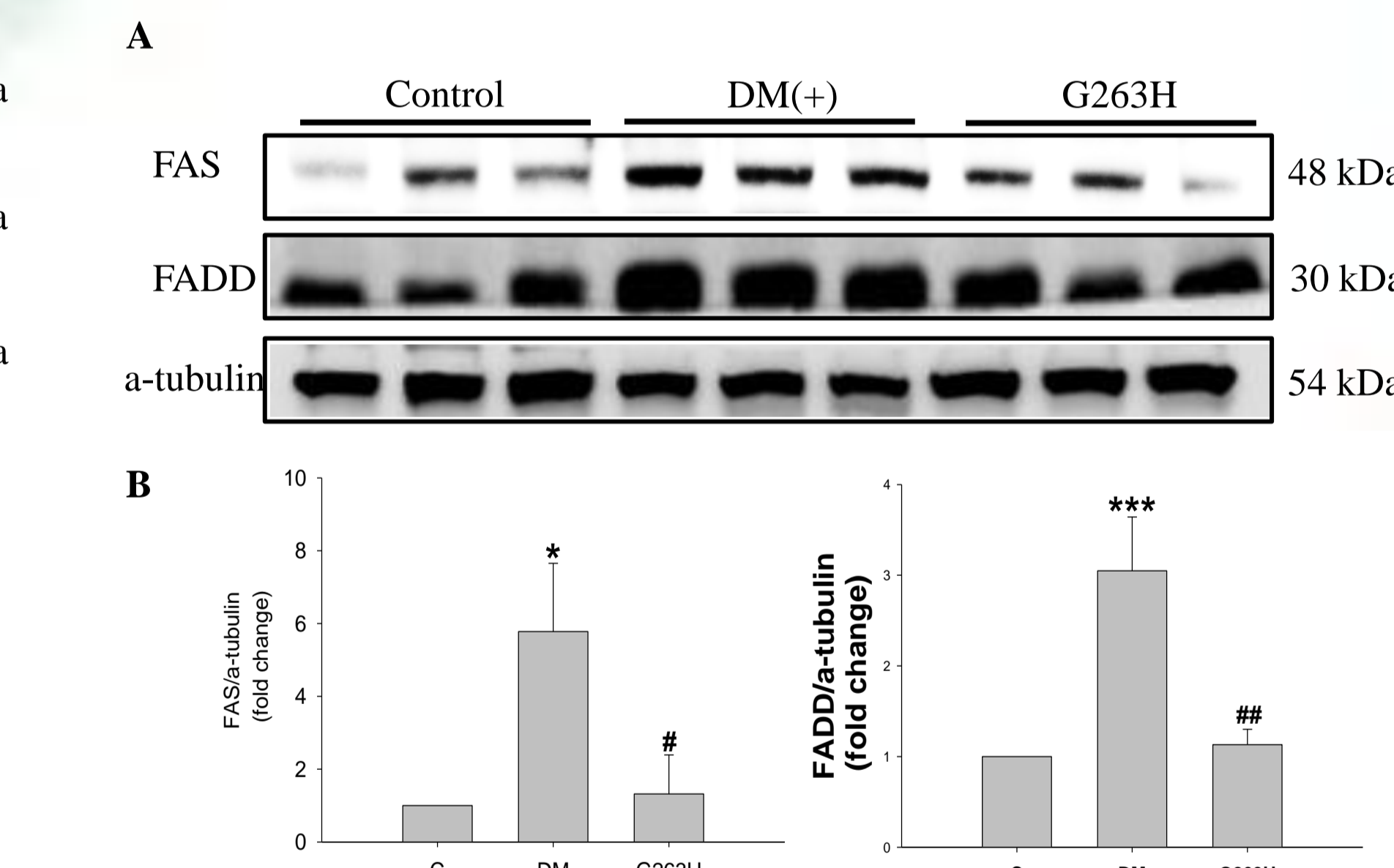
**Fig. 3** Hematoxylin-eosin staining. Representatives of histopathological analysis in the sections of cardiac tissue stained with hematoxylin and eosin. The images of myocardial architecture were magnified and ×400.



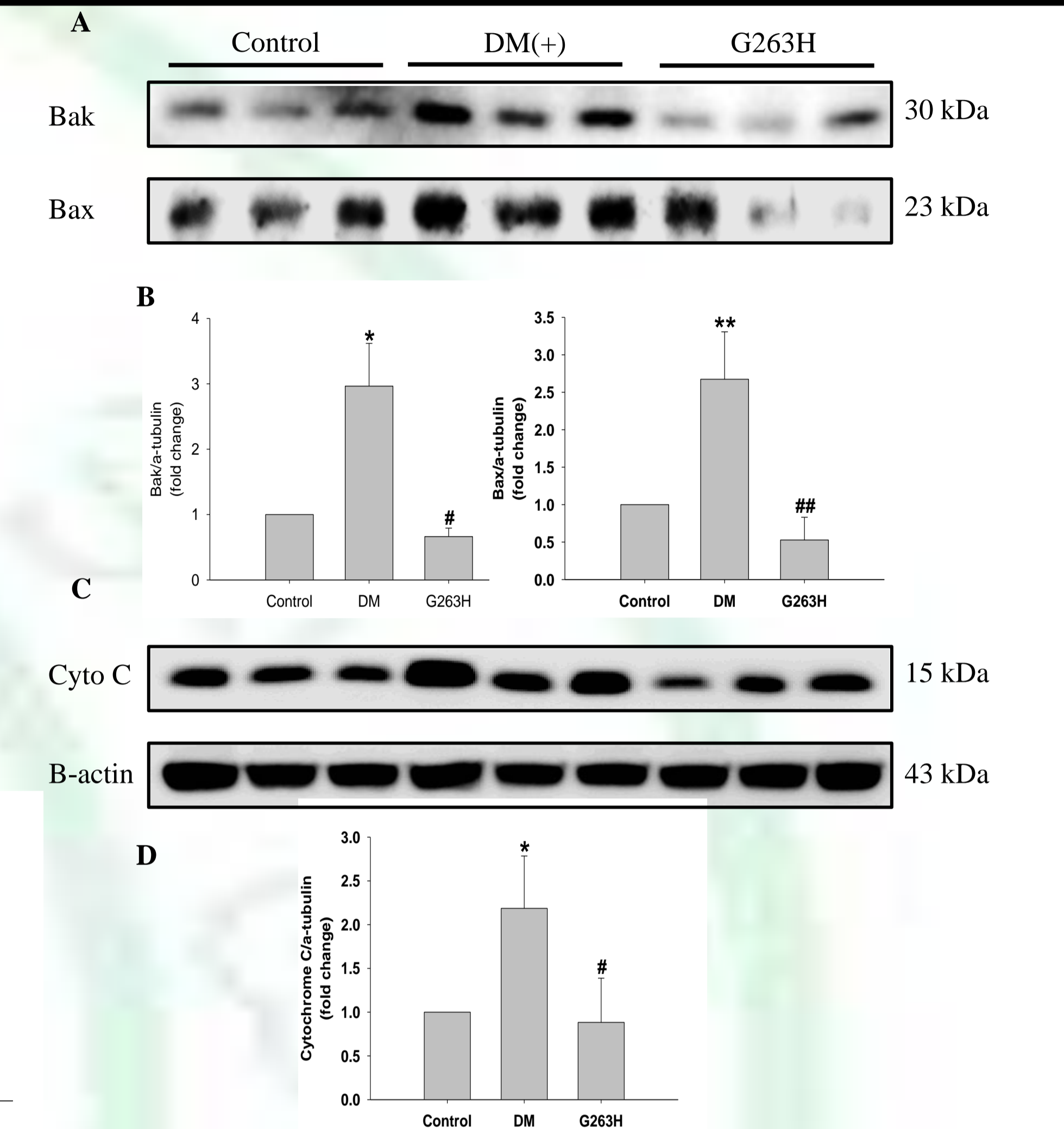
**Fig. 4** G263H had protective effects in diabetes-induced apoptosis. Influence of G263H in diabetic rat induced cardiomyoblast apoptosis by DAPI (4, 6-diamidino-2-phenylindole; 1 g/ml) staining and TUNEL assay.



**Fig. 5** The protein expression levels of caspase-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. 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



**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

## Conclusion

In the present investigation, diabetes 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.

