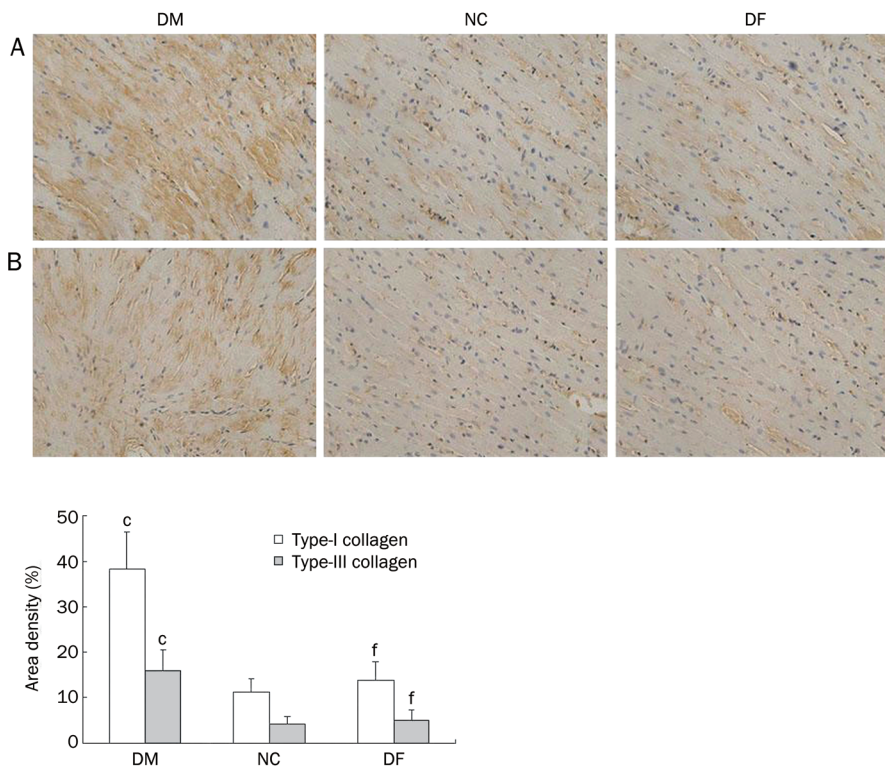


**Figure 1.** (A) Cardiac matrix deposition in Masson-stained sections of diabetic group (DM), control group (NC), and diabetic rats treated with fasudil group (DF). Heart tissues from the DM group show focal regions of fibrosis (blue) in the interstitium (collagen area  $10.15\pm0.79\%$ ,  $P<0.01$  vs NC:  $2.42\pm0.33\%$  and DF:  $2.20\pm0.31\%$ ). Magnification,  $\times 400$ . The number of rats in each group was six. (B) Representative electron micrograph obtained from the DM, NC, and DF. In diabetic rat hearts, “packages” of collagen fibrils (arrow) can also be seen between myocardiocytes (magnification,  $\times 6000$ ). The control heart shows regular myofibrillar organization with evident Z lines (magnification,  $\times 5000$ ). The ultrastructural appearance of diabetic rats treated with fasudil is similar to that of control rats (magnification,  $\times 6000$ ).

reduced the deposition of collagen. To identify collagen features in the cardiac fibrotic areas, SABC immunohistochemistry staining for type-I and type-III collagen was undertaken. An increase in deposition of type-I and type-III collagen was shown in untreated diabetic rats compared with that seen in control rats ( $38.4\pm8.2\%$  and  $15.9\pm4.6\%$  vs  $11.2\pm2.9\%$  and  $4.3\pm1.5\%$ , respectively,  $P<0.01$ ). This increase was completely inhibited by treatment with fasudil ( $13.7\pm4.1\%$  and  $5.1\pm2.3\%$ , respectively,  $P<0.01$  vs DM) (Figure 2A and 2B).

The results of TEM observations showed the ultrastructure of the myocardium (Figure 1B). Untreated diabetic rats showed dense packages of collagen fibrils between cardiomyocytes, and large areas of cytoplasm had lost their regular myofibrillar organization. Control rats showed fibrils with Z lines regularly organized in the cardiomyocytes. The cardiac morphology of fasudil-treated diabetic rats was similar to that of control rats: regularly organized cytoplasm with numerous myofibrils arranged in parallel.



**Figure 2.** Immunostaining for type-I collagen (A) and type-III collagen (B) in the hearts of diabetic group (DM), control group (NC), and diabetic rats treated with fasudil group (DF). A brown color denotes positive staining. Diabetes is associated with increased immunostaining of type-I and type-III collagen in interstitial areas, whereas there is minimal immunostaining in control animals. Treatment of diabetic rats with fasudil was associated with a reduction in immunostaining of type-I and type-III collagen. Magnification,  $\times 400$ . The number of rats in each group was six.  $^cP<0.01$  vs NC;  $^fP<0.01$  vs DM.









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