Hyperglycemia is an important risk factor for cardiovascular diseases. High glucose-induced generation of reactive oxygen species (ROS) can lead to diabetic cardiomyopathy. In our previous study, we showed that NADPH oxidase-derived ROS-induced apoptosis is mediated via the JNK-dependent activation of NF-κB in cardiomyocytes exposed to high glucose (HG). In this study, we investigated the mechanisms governing the anti-apoptotic effect of diallyl trisulfide (DATS) on HG-exposed cardiac cells both in vitro and in vivo. H9c2 cells were incubated with media containing 5.5 or 33 mM of glucose for 36hr in the presence or absence of DATS. We found that DATS treatment led to a dose-dependent decrease in ROS levels as well as protein levels of p22, gp91, phosphorylated JNK, and phosphorylated c-Jun. In addition, DATS inhibited the HG-induced activation of caspase 3 as well as the nuclear translocation of NF-κB. Similar results were observed in HG-exposed neonatal primary cardiomyocytes and streptozotocin-treated diabetic rats. Echocardiographic data showed that DATS administration led to a marked increase in fractional shortening and cardiac output. Therefore, DATS appears to suppress high glucose-induced cardiomyocyte apoptosis by inhibiting NADPH oxidase-derived ROS and its downstream JNK/NF-κB signaling.