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Akt/FOXO3a/SIRT1 Mediated Cardioprotection by n-Tyrosol against Ischemic Stress in Rat In vivo model of Myocardial Infarction: Switching Gears towards Survival and Longevity

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Abstract

Moderate consumption of wine has been associated with decreased risk of cardiovascular events. Recently we have shown that white wine is equally cardioprotective like red wine. However, unlike resveratrol (polyphenol in red wine), the white wine component, n-Tyrosol/2-(4-hydroxyphenyl) ethanol has not been explored for its cardioprotective effect and mechanism of action. Therefore, the present study was designed to evaluate the effect of tyrosol treatment (5mg/kg/day for 30 days) on myocardial ischemic stress in rat *in vivo* model of Myocardial Infarction (MI) and to identify key molecular targets involved in this mechanism. MI was induced by Left Anterior Descending (LAD) coronary artery ligation. We have observed reduced infarct size (% area at risk, 32.42 vs 48.03) and cardiomyocyte apoptosis (171 vs 256 counts /100HPF) along with improvement in the myocardial functional parameters such as LVIDs (5.89 vs 6.58 mm), % Ejection Fraction (51.91 vs 45.09 %) and % Fractional Shortening (28.46 vs 23.52 %) as assessed by echocardiography in the tyrosol treated animals when compared to the non-treated controls. We have also observed significant, increase in the phosphorylation and activation of Akt (1.4 fold) and eNOS (3 fold), phosphorylation and inhibition of FOXO3a (2.6 fold) pro-apoptotic activity and increase in the expression of nuclear longevity protein SIRT1 (3.2 fold) in the tyrosol treated MI group as compared to the non-treated MI control. We have demonstrated for the first time that tyrosol induces myocardial protection against ischemia induced stress thereby prompting the development of a new drug to combat IHD, while also revealing potential therapeutic molecular targets such as FOXO3a and SIRT1 that can be modulated to precondition the heart to overcome an ischemic stress.

Keywords

Tyrosol; Akt; FOXO3a; SIRT1; eNOS; Myocardial Infarction

Introduction

Over the past few years there have been significant advances in the therapy of Ischemic Heart Diseases (IHD). However there is still a major unmet need for better drugs/therapies for many IHD. Basic scientific research is being directed towards isolating and studying the mechanism of action of the active principles from naturally occurring compounds that are known to be cardioprotective. Mounting scientific evidence suggests that regular but moderate consumption

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of wine is associated with decreased risk of cardiovascular events (1). The cardioprotective effect of this natural ataractic agent has been related to the high content of biophenols (2). In the recent past we have reported that red wine polyphenol, resveratrol, confers protection against the myocardial injury caused by ischemia-reperfusion (IR), hypercholesterolemia and diabetes (3-5). Bromelain (a proteolytic enzyme obtained from the stem of pineapple) mediated cardioprotection against ischemia reperfusion injury through the phosphorylation of FOXO3a has been demonstrated (6). Moreover, we have recently reported for the first time that white wine pretreatment renders cardioprotection against myocardial ischemia reperfusion injury via Akt/FOXO pathway (7).

The FOXO proteins are known to be crucial regulators of a variety of cellular processes such as apoptosis, cell cycle progression and oxidative stress resistance (8). The mammalian system has four FOXO family members, namely, FOXO1, FOXO3a, FOXO4 and FOXO6, which are known to be regulated by the Akt/PKB signaling pathway (9,10). The FOXO transcription factors are known to induce the expression of pro-apoptotic proteins such as Bim and Fas-L while downregulating the expression of pro-survival proteins such as Bcl-X_L (11). Among the FOXO proteins, FOXO3a has been studied for its ability in mediating stress response by acting as an important sensor for cellular stresses (11-13). The transcriptional activity of FOXO3a is modulated not only by Akt but also by SIRT1 (14,15). The mammalian sirtuin proteins (SIRT1 through 7) share a common catalytic domain with Sir2 (silent information regulator 2), an NAD⁺ dependent deacetylase (16) that controls longevity in lower eukaryotes (an increase in Sir2 tends to increase the life expectancy of the cells) (17), of which the nuclear SIRT1 has the highest sequence similarity with Sir2 (18).

White wine is known to be rich in active components including shikimic acid, caffeic acid, n-tyrosol and hydroxy-tyrosol (7). n-Tyrosol/2-(4-hydroxyphenyl) ethanol, a major component of olive oil, is a monophenolic active compound that has been shown to protect Caco-2 cells against oxidized LDL induced oxidative cellular damage and exposure of these cells to n-tyrosol have shown to prevent cell retraction caused by oxidized LDL (19). Vivancos M et al recently reported that n-tyrosol treatment reduced oxidized LDL stimulated oxidative stress in RAW 264.7 macrophages (20). Plotnikov et al, have shown that intra-gastric n-tyrosol treatment in male Wistar rats exhibited pronounced reduction in platelet aggregation and blood viscosity in these rats (21). It was also shown that intravenous administration of n-tyrosol 10 min prior to coronary occlusion in *in vivo* acute myocardial ischemia model of Wistar rats, significantly reduced the arrhythmic activity that occurs during myocardial ischemia and reperfusion (22). Hydroxy-tyrosol treatment of HepG2 cells, improved the antioxidant defense system of these cells while increasing cellular integrity and stress resistance capabilities (23). Hydroxy-tyrosol have also been shown to protect the aorta against oxidative stress mediated impairment of nitric oxide (NO) and vessel relaxation (24). Moreover, increased myocardial apoptosis and infarct size has been reported in eNOS knock out mice demonstrating the role of eNOS in cardioprotection (25). Review of recent literature has demonstrated the protective effects of tyrosol however; very little data exists on the molecular mechanisms involved in the protective effects of tyrosol. Therefore in the present study we aimed at investigating whether and how n-tyrosol pretreatment could confer cardioprotection against an ischemic insult caused by permanent LAD ligation in *in vivo* model of rat myocardial infarction.

Materials and Methods

Animal maintenance and treatment

All animals used in this study received humane care in compliance with the principles of the laboratory animal care formulated by the National Society for Medical Research and with the *Guide for the Care and Use of Laboratory Animals* prepared by the National Academy of Sciences and published by the National Institutes of Health (Publication No. 85-23, Revised

1996). The experimental protocol was approved by the Institutional Animal Care Committee of the University of Connecticut Health Center (Farmington, CT).

Experimental design

Male Sprague-Dawley rats weighing 275 - 300 g were used for the study. The rats were randomized into four groups (n=24 in each group): 1) Control Sham (CS); 2) Tyrosol Sham (TS); 3) Control MI (CMI); and 4) Tyrosol MI (TMI). The experimental rats were gavaged with n-Tyrosol/2-(4-hydroxyphenyl) ethanol (Sigma Aldrich, St-Louis, MO, USA) at a dosage of 5mg/kg/rat/day for 30 days. Myocardial infarction was induced by permanent Left Anterior Descending (LAD) coronary artery ligation. The myocardial infarct size was measured 24 hr after MI, while the protein expression profile for the phosphorylated proteins (p-Akt, p-eNOS and p-FOXO3a) and the longevity protein SIRT1 was observed in the left ventricular tissue 8 hr and 4 days after MI, respectively. The cardiac functions and the extent of cardiac fibrosis were measured after 45 days of MI.

Surgical procedure

The experimental model has been described previously (3,26). Briefly, under anesthesia with ketamine (100 mg/kg i.p.) and xylazine (10 mg/kg i.p.) and artificial ventilation, the heart was exposed via left lateral thoracotomy followed by pericardiectomy. The LAD was ligated permanently with 6-0 polypropylene suture. Sham operated rats underwent thoracotomy and pericardiectomy followed by passing the suture under the LAD without ligation. After positive end-diastolic pressure was applied to fully inflate the lung, the chest was closed with 4-0 polypropylene suture. Cefazolin (25 mg/kg i.p.) was administered as a preoperative antibiotic cover. After surgery analgesic buprenorphine (0.1 mg/kg s.c.) was given and the animals were weaned from the respirator, and were then placed on a heating pad for recovery.

Assessment of infarct size

Twenty-four hours after MI, infarct size was measured in eight horizontal sections between the point of ligation and the apex. The area at risk (AAR) was recognized as the area not perfused with 50% Unisperse Blue (Ciba-Geigy, Glen Ellyn, IL), whereas the noninfarcted and infarcted areas were demarcated after incubation with 1% triphenyltetrazolium chloride (Sigma Aldrich, St-Louis, MO, USA). With the use of Scion image software (Scion Corporation), the volumes of both infarcted myocardium and area at risk were calculated. Infarct size was reported as a percentage of the area at risk (3,26).

Cardiomyocyte Apoptosis

The formaldehyde-fixed left ventricle was embedded in paraffin and were cut into transverse sections (4 μ m thick) and deparaffinized with a graded series of histoclear and ethanol solutions. Immunohistochemical detection of apoptotic cells was carried out using TUNEL reaction using In Situ Cell Death Detection Kit, Fluorescein as per the kit protocol (Roche Diagnostics, Mannheim, Germany). In brief, the TUNEL reaction preferentially labels DNA strand breaks generated during apoptosis which can be identified by labeling free 3'-OH termini with modified nucleotides in an enzymatic reaction catalysed by Terminal deoxynucleotidyl transferase (TdT). Fluorescein labels incorporated in nucleotide polymers are detected by fluorescence microscopy. The sections were washed thrice in PBS, blocked with 10% normal goat serum in 1% BSA in PBS and incubated with mouse monoclonal anti- α -sarcomeric actin (Sigma, St Louis, MO, USA) followed by staining with TRITC-conjugated rabbit anti-mouse IgG (1:200 dilution, Sigma, St Louis, MO, USA). After incubation, the sections were rinsed thrice in PBS and mounted with Vectashield mounting medium (Vector, Burlingame, CA). The sections were observed and images were captured using a confocal laser Zeiss LSM 410

microscope. For the quantitative purpose, the number of TUNEL-positive cardiomyocytes was counted on 100 high power fields (HPF) (6).

Isolation of nuclear and cytosolic protein fractions

The myocardial tissue for analysis was harvested from the risk zone or border zone of the left ventricle adjacent to the infarct (avoiding any infarcted tissue). The protein was isolated according to the kit protocol of CellLytic NuCLEAR Extraction kit from Sigma (St. Louis, MO, USA). In brief, 100 mg of tissue were homogenized in 1 ml of buffer containing 100 mM HEPES (pH 7.9) with 15 mM MgCl₂, 100 mM KCl, and 0.1 M DTT solution and centrifuged at 10,000 g for 20 min. The supernatant containing the cytosolic fraction was transferred to a fresh tube. The pellet was resuspended in 150 µl of extraction buffer containing 1.5 µl of 0.1 M DTT and 1.5 µl protease inhibitor cocktail (104mM AEBSF, 80µM Aprotinin, 4mM Bestatin, 1.4mM E-64, 2mM Leupeptin, and 1.5mM Pepstatin A). The solution was allowed to stand on ice for 30 min by shaking at brief intervals followed by centrifugation at 20,000 g for 5 min. The supernatant was transferred to a clean chilled tube and contained the nuclear protein fraction. The cytosolic and nuclear total protein concentrations were determined using a bicinchoninic acid protein assay kit (Pierce, Rockville, IL, USA). (6,7)

Western Blot analysis for phosphorylated Akt, FOXO3a, eNOS and nuclear SIRT1

To quantify the p-Akt, p-FOXO3a, p-eNOS (8 hrs sample/ cytosolic fraction) and SIRT1 (4days sample/Nuclear fraction) standard SDS/PAGE Western blot technique was performed (3,5-7,27). The proteins were run on polyacrylamide electrophoresis gels (SDS-PAGE) typically using 7% for p-eNOS and SIRT1; 10% for p-Akt and p-FOXO3a. The antibodies for p-eNOS (the phosphorylation site is specific to serine-1177, 1:500), eNOS (1:500), p-Akt (the phosphorylation site is specific to serine-473, 1:500), Akt (1:500), p-FOXO3a (the phosphorylation site is specific to threonine-32, 1:500), FOXO3a (1:500) were purchased from Cell Signaling Technology, Danvers, MA, USA and for SIRT1 (1:250) from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Primary antibody binding was visualized by HRP-conjugated secondary antibodies and enhanced chemiluminescence (ECL).

Assessment of Cardiac Fibrosis (Masson's Trichrome staining)

To determine the effect n-Tyrosol treatment on cardiac fibrosis, the collagenous fibrotic area of the heart was stained by Masson's Trichrome staining protocol. The rats were sacrificed 45 days after the surgical procedure; hearts were removed and fixed overnight in 4% paraformaldehyde. After fixation the paraffin embedded sections (of 4 µm thick) were made and the extent of fibrosis was analysed using Masson's trichrome staining (28). In brief, the paraffin sections were deparaffinized in histoclear and rehydrated using sequential passage through 100, 95, 80 and 70% ethanol for 6 min each followed by washing in distilled water 3 times. The slides were then stained with Weigert's iron hematoxylin for 10 min and placed under tap water for 10 min. The sections were again washed in distilled water and then stained with Biebrich scarlet-acid fuchsin solution for 15 min, in phosphomolybdic-phosphotungstic acid solution for 15 min and aniline blue solution and stained for 10 min. The sections were rinsed briefly in distilled water and were treated with 1% acetic acid solution for 5 min. After a final wash in distilled water the sections were dehydrated through sequential gradient of 70-100% alcohol followed by histoclear wash and then mounted using Permount. The heart tissue sections were digitally imaged in high pixel resolution on an Epson Scanner and enlarged images were captured using a phase contrast microscope with a high resolution digital camera (Olympus).

Echocardiography

After 45 days of MI, rats were sedated using isoflurane (3%, inhaled). When adequately sedated, the rat was secured with tape in the supine position in a custom-built platform designed to maintain the rat's natural body shape after fixation. The hair on the chest wall was removed with a chemical hair remover. Ultrasound gel was spread over the precordial region, and ultrasound biomicroscopy (UBM) (Vevo 770, Visual-Sonics Inc., Toronto, ON, Canada) with a 25-MHz transducer was used to visualize the left ventricle. The left ventricle was analyzed in apical, parasternal long axis, and parasternal short axis views for left ventricular (LV) systolic function, LV cavity diameter, wall thickness, diastolic function, LV end-systolic and end-diastolic volume determination. 2D directed M-mode images of the LV short axis were taken just below the level of the papillary muscles for analyzing ventricular wall thickness and chamber diameter. All left ventricular parameters were measured according to the modified American Society of Echocardiography–recommended guidelines. Ejection fraction (EF) and fractional shortening (FS) were assessed for left ventricular systolic function. All measurements represent the mean of at least 3 consecutive cardiac cycles. Throughout the procedure ECG, respiratory rate, and heart rate were monitored as previously described (6, 27,29).

Statistical analysis

The values are expressed as means $\pm$ SEM. The ANOVA test was first carried out followed by Bonferroni correction to test for any differences between the mean values of all groups. The results were considered significant if $p < 0.05$.

Results

Effect of n-Tyrosol on infarct size and cardiomyocyte apoptosis

Quantitative analysis indicated that tyrosol treatment significantly reduced myocardial infarct size 24 h after MI compared with non treated group. Tyrosol treatment reduced the infarct size approximately to 32% as compared to 48% in CMI group (Figure 1A). Significant decrease in the cardiomyocyte apoptosis was also observed in the TMI as compared to CMI (171 vs 256 counts / 100HPF) (Figure 1B).

Effect of n-Tyrosol on phosphorylation of Akt, eNOS and FOXO3a

The phosphorylation status of Akt, eNOS and FOXO3a proteins was observed after 8hr of LAD occlusion. n-Tyrosol treatment has shown significant increase in the p-Akt (Figure 2A) both in TS and TMI groups (4 and 1.4 fold) as compared to corresponding CS and CMI groups. Similarly, n-tyrosol treatment has shown significant increase in the p-eNOS level (Figure 2B) both in TS and TMI groups (3.5 and 3 fold) as compared to corresponding CS and CMI groups. The p-FOXO3a levels (Figure 2D) in TS and TMI were also found to be significantly increased (1.8 and 2.6 fold) as compared to corresponding controls. No significant difference was observed in the non-phosphorylated protein levels of these proteins.

Effect of n-Tyrosol on SIRT1 expression

The levels of nuclear longevity protein SIRT1 was examined in the groups 4 days after MI. There was a significant decrease in the SIRT1 expression in the CMI group as compared to the CS group. However, n-tyrosol treatment has shown significant increase in the levels of nuclear SIRT1 (Figure 2C) in the TS (1.7 fold) and TMI (3.2 fold) as compared to the respective controls CS and CMI. Nuclear histone H3 was used as the loading control.

Effect of n-Tyrosol on cardiac fibrosis

The n-Tyrosol treated MI group demonstrated significantly reduced collagen staining and fibrosis compared with the respective non-treated control. Figure 3D shows a smaller infarct with less fibrosis in the n-Tyrosol treated TMI heart when compared with larger infarct with fibrosis and ventricular dilatation in a CMI heart (Figure 3C). Figure 3E-F shows an enlarged image of the infarct and peri-infarct regions of CMI and TMI groups respectively. A significant increase in regions of viable cardiac muscle was observed in the infarct and peri-infarct regions of the n-Tyrosol treated MI group as compared with the non-treated control (Figure 3E-F). Figure 3A and 3B represents the non-treated and n-Tyrosol treated Sham respectively with no significant fibrosis.

Effect of n-Tyrosol on myocardial functions by echocardiography

There were no significant differences at baseline between treated and non treated groups in the echocardiographic measurements either in cardiac structure or function. Cardiac function measured after 45 days of MI showed increased left ventricular functions in n-tyrosol treated group as compared with that in control animals. Compared with the sham group (CS and TS), decreased LV function and increased LV dilatation was observed in both CMI and TMI groups after LAD ligation. The Ejection Fraction (in %, 51.91 vs 45.09, Figure 4C) and Fractional Shortening (in %, 28.46 vs 23.52, Figure 4D) of the left ventricle were significantly increased in the TMI compared to the CMI group. As seen in Figure 4A, representative M-mode images demonstrated increased systolic and diastolic LV chamber dimensions after MI. The left ventricular chamber was dilated in the CMI compared to TMI as assessed by measuring LVIDs (in mm, 5.89 vs 6.58, Figure 4B). There was a compensatory increase in the posterior (LVPW) and lateral wall systolic thickness in tyrosol treated group as compared to the non treated group (data not shown).

Discussion

In the present study we have documented for the first time that white wine component n-tyrosol/ 2-(4-hydroxyphenyl) ethanol renders cardioprotection against the ischemic stress induced by myocardial infarction in a rat *in vivo* LAD occlusion (MI) model. n-Tyrosol pretreatment significantly reduced the myocardial infarct size and the extent of cardiomyocyte apoptosis followed by decreased ventricular remodeling as evidenced by the significant reduction in the collagenous fibrotic tissue and improvement in the left ventricular myocardial functions in conjunction with significant increase in the levels of phosphorylated forms of Akt, eNOS and FOXO3a along with increased expression of nuclear SIRT1 in the n-tyrosol administered groups as compared to untreated controls.

Serine 473 (Ser⁴⁷³) phosphorylated active Akt is known to play a critical role in promoting cell survival by phosphorylation of its downstream target molecules such as the FOXO proteins, a subclass of forkhead group of transcription factors (8,9). Akt activation not only increased cell survivability in *in vivo* model but also improved regional and overall myocardial function (30). Phosphorylation of FOXO3a by Akt in the cytoplasm has been shown to sequester FOXO3a in the cytoplasm and enhance its degradation (11), while the same event of phosphorylation when occurs in the nucleus significantly reduces the DNA binding activity of FOXO3a and creates 14-3-3 protein docking sites on it thereby facilitating its nuclear export and subsequent entry into the proteasomal degradation pathway in the cytosol (31). Thus phosphorylation of FOXO3a by Akt has been shown to have effect on the transcriptional regulation of FOXO3a dependent genes thereby inhibiting Bim and Fas-L dependent apoptosis. In the present study the increase in the phosphorylation of Akt and FOXO3a thus corresponds to the reduced myocardial infarct size, lesser collagenous fibrotic tissue, increased islands of

viable cardiac muscle as a result of increased cell survivability in the n-Tyrosol treated MI groups as compared to non-treated control MI group.

SIRT1 has been shown to regulate stress response and survival of cells by modulating the FOXO3a transcription factors (32). Red wine polyphenol, resveratrol, has also shown to increase SIRT1 activity in favor of cell survival (33). SIRT1 is known to localize in the nucleus irrespective of the cellular stress status while cytoplasmic FOXO3a relocates to the nucleus in response to various stress stimuli (14). SIRT1 has been shown to interact with phosphorylated FOXO3a, and deacetylates it in the nucleus (34). This interaction increased FOXO3a's capability to induce cell cycle arrest, resist stress and induce DNA repair mechanisms, while inhibiting FOXO3a's ability to induce apoptosis (34-36). Thus the FOXO3a dependent responses towards stress may be modulated away from cell death and towards stress resistance by the nuclear SIRT1. In the present study, we have documented the effect of n-tyrosol treatment on the levels of nuclear SIRT1. We have observed significant increase in the expression of nuclear SIRT1 in the tyrosol treated groups as compared to respective non-treated controls.

We have also observed a significant increase in the serine 1177 (Ser¹¹⁷⁷) phosphorylation of eNOS in the tyrosol treated group as compared to the non-treated controls. Reports have documented that active Akt phosphorylates and activates eNOS thereby reducing myocardial infarct size in a rat model of ischemia reperfusion injury (37,38). It was shown that eNOS inhibition and reduction of NO levels compromised endothelial dependent vasodilation and ventricular function in eNOS deficient mice while transgenic expression of eNOS have been shown to attenuate congestive heart failure and myocardial ischemia reperfusion injury in mice (39-41). Moreover FOXO3a has been known to be a transcriptional repressor of eNOS (42).

Therefore, the tyrosol induced phosphorylation of Akt, subsequent phosphorylation of FOXO3a and eNOS, increase in the expression of SIRT1 should have played a critical role in increasing the cell survivability bringing about significant reduction of myocardial infarct size due to reduced apoptosis (Figure 1A and B), lesser collagenous fibrotic tissue (Figure 3D) and increased regions of viable cardiac muscle (Figure 3F) and improvement in the left ventricular functional parameters (Figure 4A-4D) in the n-tyrosol treated MI group as compared to the non-treated controls. The inhibition of FOXO3a by phosphorylation also might explain the increased levels of p-eNOS, since FOXO3a is considered to be a transcriptional repressor of eNOS.

In conclusion, we have demonstrated for the first time that white wine component tyrosol induces myocardial protection against ischemia induced stress by significantly reducing the myocardial infarct size and improving the left ventricular myocardial functions might be through the activation of Akt, eNOS and SIRT1, which in turn may co-ordinate in shifting the FOXO3a dependent stress response away from inducing programmed cell death response but towards stress resistance and longevity. The present study is of significant clinical importance since it has elucidated a possible mechanism for the cardioprotective effect of tyrosol, prompting the development of a new drug to combat IHD, while also revealing potential therapeutic molecular targets such as FOXO3a and SIRT1 that can be modulated to precondition the heart to overcome an ischemic stress.

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Figure 1.

Effect of n-Tyrosol on A) Infarct Size - Graphical representation of infarct size in % area at risk between the control MI and n-Tyrosol treated MI groups 24 hours after LAD ligation. Values are mean $\pm$ SEM (n = 6). B) Cardiomyocyte apoptosis - Graphical representation of cardiomyocyte apoptosis between the control MI and n-Tyrosol treated MI groups 24 hours after LAD ligation. * $p < 0.05$ represents TMI compared with CMI group. CMI represents Control MI group and TMI represents n-Tyrosol treated MI group.

Figure 2.

Representative Western blots showing the protein expression of p-Akt (A), p-eNOS (B), SIRT1 (C) and p-FOXO3a (D). Akt, eNOS, Histone-H3 and FOXO3a were used as the respective loading controls. Graphs represent the quantitative comparison between the groups. * $p < 0.05$ represents TS compared with CS group, † $p < 0.05$ represents TMI compared with CMI group. CS represents Control Sham group, TS represents n-Tyrosol treated Sham group, CMI represents Control MI group and TMI represents n-Tyrosol treated MI group.

Figure 3.

Representative images showing effect n-Tyrosol treatment on collagenous fibrotic area of the heart 45 days after surgical procedure (Masson's Trichrome Staining). Images A and B represents the CS and TS groups respectively with no significant fibrosis while C and D represents the collagenous fibrotic staining in blue in the CMI and TMI groups respectively. Images E and F shows enlarged images of regions of the infarct and peri-infarct area of CMI and TMI groups respectively showing viable cardiac tissue in the TMI group (the area that has been focused and zoomed in to obtain the images E and F are outlined in boxes on images C and D respectively). CS represents Control Sham group, TS represents n-Tyrosol treated Sham group, CMI represents Control MI group and TMI represents n-Tyrosol treated MI group.

Figure 4.

Effect of n-Tyrosol on cardiac functions by echocardiography. (A) Representative M-mode images of CS, TS, CMI and TMI 45 days after the surgical procedure. Graph represents the LVIDs in mm (B), % EF (C) and % FS (D) in CS, TS, CMI and TMI 45 days after the surgical procedure. Values are mean $\pm$ SEM (n = 6). *p<0.05 represents TMI compared with CMI group. CS represents Control Sham group, TS represents n-Tyrosol treated Sham group, CMI represent Control Myocardial Infarction group and TMI represents n-Tyrosol treated Myocardial Infarction group, LVIDs = Left Ventricular Internal Diameter in systole, EF = Ejection Fraction and FS = Fractional Shortening