# Comparative Effect of Pioglitazone and Resveratrol in Exogenous Asymmetric Dimethylarginine (ADMA) – Induced Hypertension and Cardiac dysfunction In Rats

Sawsan Abd El-Aziz Sadik[1], Nawal El- Sayed El-Gawhary[2], Mona Farag Shabana[1], Amany Nasr Ahmed Nasr [1], Mohammed M. Abdellah [3]

[1]Pharmacology Department, Faculty of Medicine, Fayoum University, Egypt
[2] Pharmacology Department, Faculty of Medicine, Cairo University, Egypt
[3] Pathology Department, Faculty of Medicine, Fayoum University, Egypt
Hamada_abdellah@yahoo.com dr.amanynasr@yahoo.com

**Abstract: Background and purpose:** This study investigates the effect of Pioglitazone and Resveratrol on Arterial blood pressure, cardiovascular dysfunction in Asymmetric dimethyl arginine induced hypertensive rats. **Results:** Systolic blood pressure was lower in pioglitazone treated and Resveratrol treated groups than in hypertensive (ADMA) group at the end of the study (mmHg, $P < 0.05$), Nitrite level in serum, Dimethyl arginine dimethyl aminohydrolase (DDAH) were reduced by pioglitazone and Resveratrol treatment. Finally There were significant pathological changes in the Pioglitazone and Resveratrol treated groups as compared to ADMA (the hypertensive) group; as fragmentation, vacuolations, edema, inflammatory infiltrate, fibrosis were found in all rats at minimal and mild degrees while contraction bands in 4-5 rats, cytolysis and hyalinization in only 1 rat. **Conclusions:**
[Sawsan Abd El-Aziz Sadik, Nawal El- Sayed El-Gawhary, Mona Farag Shabana, Amany Nasr Ahmed Nasr, ohammed M. Abdellah. **Comparative Effect of Pioglitazone and Resveratrol in Exogenous Asymmetric Dimethylarginine (ADMA) –Induced Hypertension and Cardiac dysfunction In Rats.** *J Am Sci* 2017;13(7):97-105]. ISSN 1545-1003 (print); ISSN 2375-7264 (online). http://www.jofamericanscience.org. 8. doi:10.7537/marsjas130717.08.

**Key Words**: pioglitazone; Resveratrol; Asymmetric dimethyl arginine (ADMA); hypertension.

## 1. Introduction

Hypertension is one of the major risk factors for cardiovascular diseases; it has become a major public health issue in most developed and developing countries **(Fanelli and Zatz, 2011).**

Evidence has accumulated that inhibition of NO synthesis by NOS inhibitors may be causally involved in this process. More and more attention has been paid to asymmetric dimethyl arginine (ADMA) (an endogenous nitric oxide synthase inhibitor) which contributes to endothelial dysfunction. Increasing knowledge of the role of ADMA in the pathogenesis of atherosclerosis, ADMA has become potential target for discovery of drugs decreasing its level **(Kitova, 2008).**

DDAH activity has been found in kidney, pancreas, liver, brain and aorta with immunoexpression also in neutrophils and macrophages **(Tojo et al., 1997)**. Inhibition of DDAH causes gradual vasoconstriction which is reversed by L-arginine **(MacAllister et al., 1996)**. There are two isoforms of DDAH {DDAH-1 and DDAH-2}. DDAH-1 is usually found in tissues expressing neuronal NOS while DDAH-2 is predominantly found in tissues containing the endothelial isoform of NOS **(Leiper et al., 1999)**. Increased plasma levels of glucose, oxidized LDL and homocysteine are associated with decreased levels of DDAH. Furthermore, some conventional cardiovascular risk factors may reduce DDAH activity by increasing oxidative stress **(Weis et al., 2004)**. Pharmacological inhibition of DDAH increases ADMA concentrations and reduces NO production **(Azuma et al., 1995)**.

Thiazolidinediones activate nuclear factor peroxisome proliferator-activated receptorγ (PPARγ) and affect various physiological responses, including vascular function **(Iglarz et al., 2003)**. Pioglitazone improves endothelial function by suppressing oxidative stress via increased superoxide dismutase activity and decreased NAD (P) H oxidase activity and also decreases ET-1 levels, which might be attributable to the inhibition of the transcription factor activator protein-1 (AP-1) signaling **(Matsumoto et al., 2007).**

Lifestyle modifications that effectively lower blood pressure include increased physical activity, weight loss, limited alcohol consumption, and reduced sodium intake **(Williams, 2008).** Evidence from epidemiological, in vivo, in vitro, and clinical studies indicate that plant-based diets and nutraceuticals can reduce risk of chronic diseases, particularly cancer and cardiovascular diseases **(Johnston, 2009)**. Polyphenols are classes of compounds that have received increased attention in medical research **(Espin, et al., 2007).** One of most studied polyphenols is resveratrol with tremendous health benefit potential **Smoliga et al., 2012and Yu et al., 2012).**

**Aim of Work:**

This study aimed to clarify effect of pioglitazone and resveratrol on blood pressure, heart rate, serum nitrite, cardiac gene expression of dimethylarginine dimethyl aminohydrolase (DDAH) and histopathological changes in rat cardiac tissue following ADMA-induced hypertension.

## 2. Materials and Methods

**Drugs and chemicals:**

**1-Resveratrol:** 125 mg/tablet (Docib nutritional center, UAE). Resveratrol: The tablets were crushed and dissolved in DMSO forming a suspension. It was given orally by gavage.

**2-Asymetric dimethyl arginine (ADMA): powder** (Sigma-Aldrich Company Limited, USA).

**3-Pioglitazone:** 30 mg/tablet. (Sigma-Aldrich Company Limited, USA) The tablets were crushed and dissolved in distilled water forming a suspension. It was given orally by gavage.

**4-DMSO:** Dimethyl sulfoxide (Gomhoria Company, Egypt ).

**5-Formalin:**"Formaldehyde sol. 38-40 %**(** Al-Nasr Pharma. Chemicals, Egypt)".

**Animals:**

24 adult male albino rats weighing 150-200 gms were used. They were housed each rat in a cage in a regulated environment with a12- hour light/dark cycle and fed with standard laboratory diet. They were allowed free access to water. All animals' procedures were performed in accordance to the Institutional Ethics Committee and in accordance with the recommendations for the proper care and use of laboratory animals.

**Methods:**

**Hypertension in Albino Rat Model:**

Induction of hypertension in albino rat model was done by administration of a symmetric dimethyl arginine (ADMA) 10mg/kg per daywas dissolved in distilled water **( Colonna et al., 2006).**

**Experimental design:**

Animals were randomly divided into the following four groupsof 6 rats each

- Control (Normal) group**:** received distilled water orally for 4 weeks.

- ADMA (Hypertensive) group: Rats in this group received (ADMA) 10mg/kg per dayfor 4 weeks.

-Pioglitazone-treated group: Pioglitazone 10mg/kg/day orally for 4 weeks **(Nakamoto et al., 2008)** and ADMA10 mg/kg per day orally for 4 weeks**.**

-Resveratrol- treated group**:** Resveratrol 5mg/kg/day orally for 4 weeks **(Osman et al., 2013)** and ADMA10 mg/kg per day orally for 4 weeks.

**Blood pressure and heart rate measurements:**

Systolic and diastolic blood pressure of animals were measured at the end of the study at 4th week by non - invasive blood pressure monitor (Ugo, Basile - Italy) in conscious rats by tail-cuff technique for which all animals were pre-trained until blood pressure was steadily recorded with minimal stress. **(Irvine et al., 1997).** Heart rate was also obtained using same method.

**Biochemical measurements:**

a. Serum nitrite level

After 4 weeks, blood samples were withdrawn from retro-orbital vein of each rat under light anesthesia by diethyl etherand collected in non-heparinized tubes. Blood was allowed to coagulate, then centrifuged. Separated serum was used for estimation of serum level of nitrite according to **Miranda et al., 2001.**

b. Quantitative real time PCR (Qpcr) for dimethyl arginine dimethyl aminohydrolase (DDAH)

Animals were sacrificed at end of 4th week by cervical dislocation **(Ajay et al., 2006).** Heart was excised and divided into two parts; one part for estimation dimethylarginine dimethyl aminohydrolase (DDAH) and other for histopathology.

Total RNA was isolated using Qiagen tissue extraction kit **(Qiagen, USA)** according to instructions of manufacturer, followed by complementary DNA synthesis. Finally Real-time qPCR using SYBR Green I AppliedBiosystem with software version 3.1 (Step One™, USA). The qPCR assay with the primer sets were optimized at the annealing temperature. All cDNA were in duplicate and including previously prepared samples (for both human VEGFR-2 and von Willbrand factor genes with 16s rRNA as an internal control for the in vitro study), (for all rat VEGFR2, VEGF, HIF-1α, CXCR4, and SDF-1 with GAPDH an internal control for the in vivo study), and non-template control (water to confirm the absence of DNA contamination in the reaction mixture).

**Histopathology of Heart**

Heart samples were fixed by immersion in 4% buffered formalin, dehydrated through a graded series of ethanol, and embedded in paraffin. Then, individual blocks of this material containing the tissue (usefully orientated) were sculpted. Each tissue was then sectioned at 4µm. The tissue was stained with hematoxylin-eosin; Masson trichrome special stain, and examined under light microscopy (Leica Queen 500).

**Pathological scoring of ADMA cardiac lesions**

Pathological scoring of the H & E stained cardiac sections was performed according to a grading system developed by **Cove-Smith et al., (2014)** and based on Billinghamand Bristow's criteria with refinement to incorporate extracellulardamage **(Billingham and Bristow, 1984)**.

**Statistical analysis:**

The Statistical Program for Social Sciences (SPSS) version16.0 was used for data management (SPSS Inc., Chicago). Mean and standard deviation (SD) were used to describe quantitative variables. Comparison of variables between groups was done using ANOVA (analysis of variance) test followed by post hoc Bonferroni test for quantitative normally distributed variables. Pearson correlation coefficient (r) was used to express correlations. P- Values less than or equal to 0.05 were considered statistically significant, while P value more than 0.05 was considered insignificant **(Dawson and Trapp, 2001).**

**3. Results:**

Effect of Pioglitazone and Resveratrol on systolic and diastolic blood pressures, heart rate, serum nitrite and DDAH cardiac gene expression in Exogenous Asymmetric Dimethylarginine (ADMA)–Induced Hypertension in rats: (Table 1).

Administration of ADMA increasedsystolic blood pressure (SBP) and diastolic blood pressure (DBP). There was significant ($P<0.05$) increase in mean SBP and DBP in ADMA group as compared to control. Pioglitazone treatment significantly lowered SBP and DBP in comparison to ADMA group.

SBP of resveratrol group was significantly lower than ADMA group. DBP of resveratrol treated group was significantly decreased as compared with ADMA group. Comparing pioglitazone to resveratrol group, SBP was significantly lower. DBP in Pioglitazone treated group was significantly decreased compared to Resveratrol treated group.

Heart rate insignificantly decreased in ADMA compared with control group. There was an insignificant increase in heart rate in pioglitazone treated group as compared with ADMA.

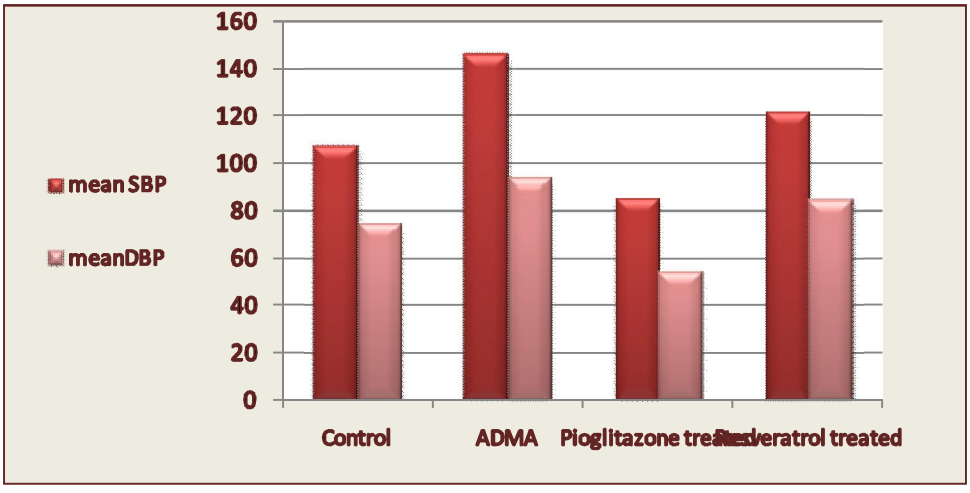
Administration of ADMA produced a significant decrease in serum nitrite in ADMA as compared to control group. Meanwhile, both pioglitazone and resveratrol treatment significantly increased serum nitrite as compared to ADMA-group**.**

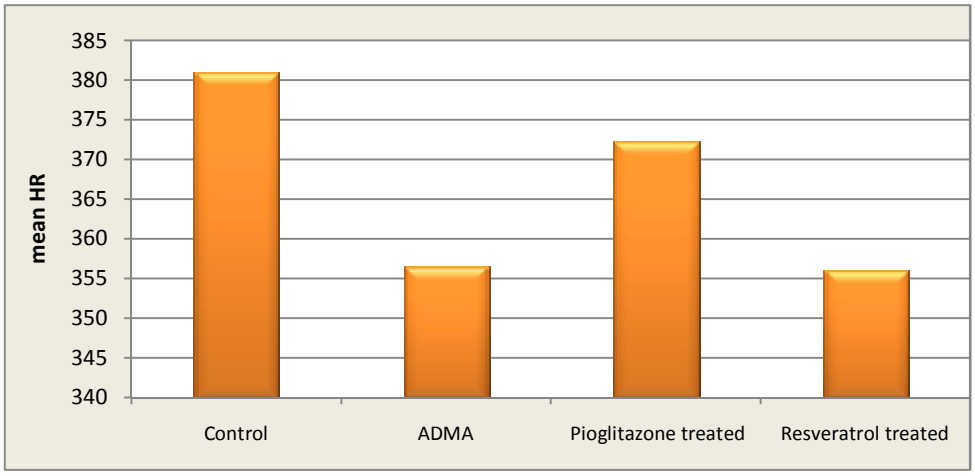
Effect of Pioglitazone and Resveratrol on histopatholgical features of heart in Exogenous Asymmetric Dimethylarginine (ADMA)–Induced Hypertension in rats (Table 2 ).

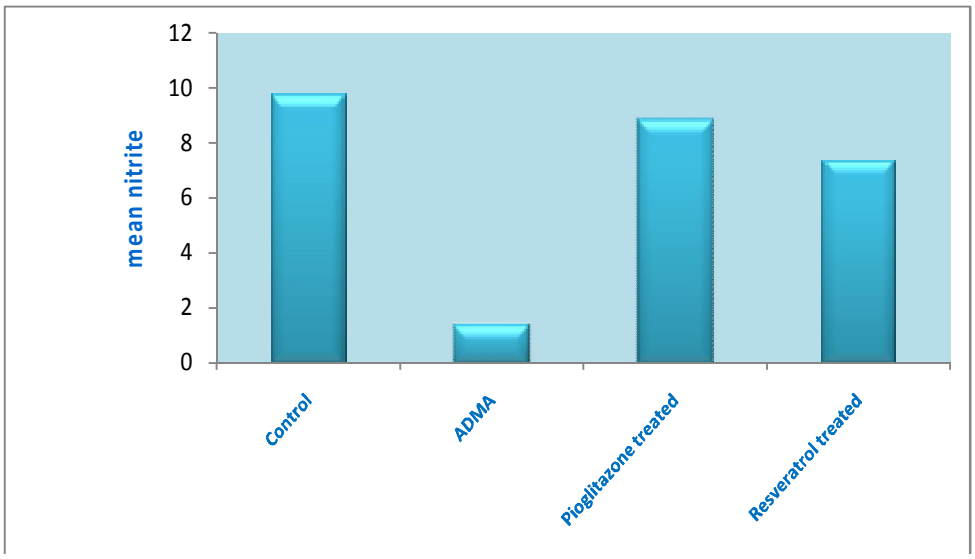
**Table (1): Blood pressure, heart rate, serum nitrite level and DDAH gene expression in Experimental Groups**

| Group ( n= 6 ) / Parameter | Control | ADMA | Pioglitazone treated | Resveratrol-treated | P-value ANOVA |
|---|---|---|---|---|---|
| **SBP ( mmHg )** | **107.5±0.55** | **145.67±14.5$^{a}$** | **85±11.03$^{b}$** | **121.5 ±7.03$^{c}$** | |
| **DBP ( mmHg )** | **74.5 ±2.25** | **94 ±11.87$^{a}$** | **54.5 ±2.28$^{b}$** | **84.17 ±6.62$^{c}$** | |
| **Heart rate ( beats/min)** | **381 ±3.79** | **356.5±34.89** | **372.17±18.4** | **356 ±26.66** | |
| **Serum nitrite level ( mmol/l)** | **9.77±0.41** | **1.42±0.31$^{a}$** | **8.82±1.65$^{bc}$** | **7.35±1.67$^{bc}$** | |
| **DDAH gene expression** | **1.03±0.02** | **0.203±0.03$^{a}$** | **0.807±0.23$^{bc}$** | **0.85±0.07$^{bc}$** | |

Data are presented as mean±SD. Numbers carrying same superscript letters are not statistically significant.
ANOVA Analysis of Variance.
P≤ 0.05 is considered significant.




mean HR
385
380
375
370
365
360
355
350
345
340
Control
ADMA
Pioglitazone treated
Resveratrol treated


mean nitrite
12
10
8
6
4
2
0
Control
ADMA
Pioglitazone treated
Resveratrol treated

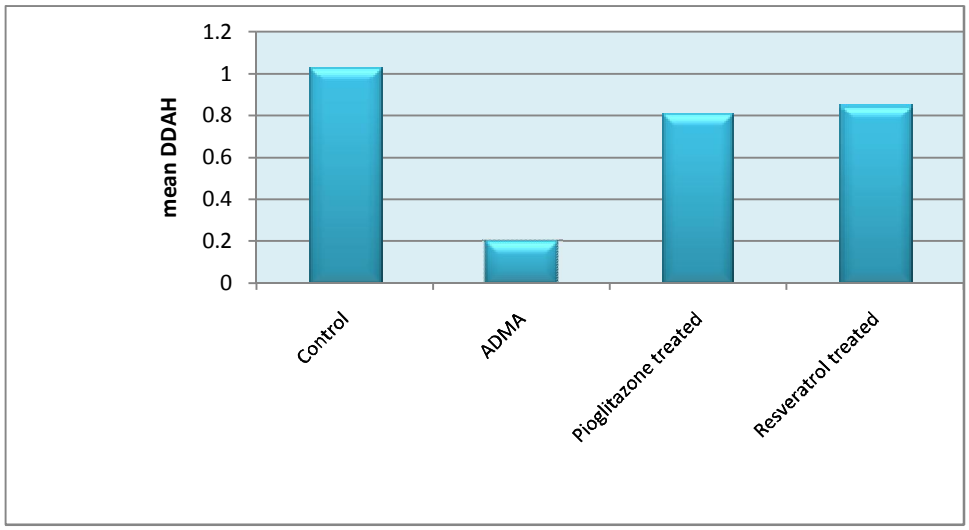
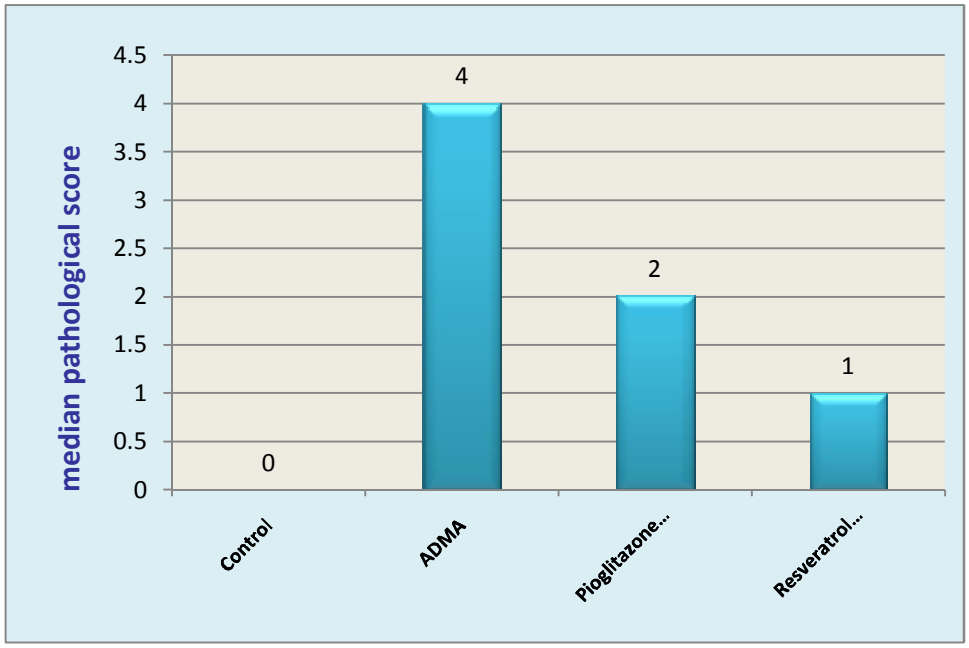
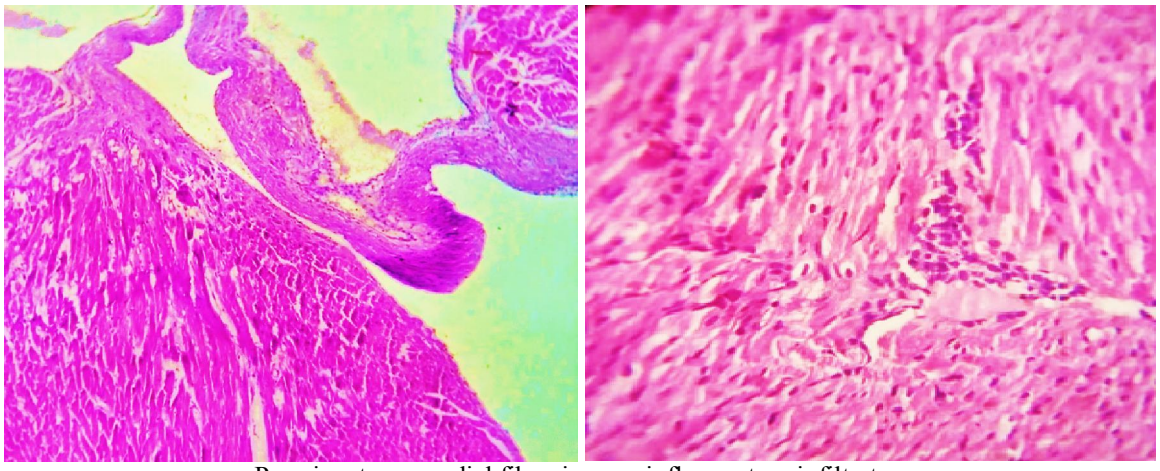

mean DDAH
1.2
1
0.8
0.6
0.4
0.2
0
Control
ADMA
Pioglitazone treated
Resveratrol treated

**Table (2):** Effect of Pioglitazone and Resveratrol on Median histopathological changes in (ADMA) induced hypertension in rats

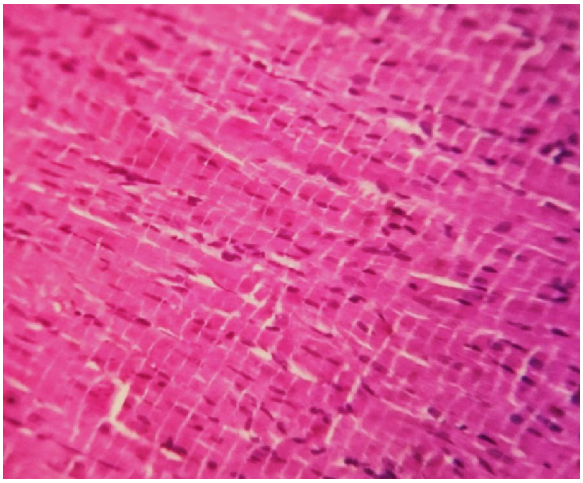
| Group | Median histopathological changesscore |
|---|---|
| **Control** | **0** |
| **ADMA** | **4[a]** |
| **Pioglitazone treated** | **2[b]** |
| **Resveratrol treated** | **1[c]** |

Data are presented as median pathological change. Numbers carrying same superscripy letters are not statistically significant. 0 = no histopathology changes. 1 = Mild changes 2= Moderate changes 3= Severe changes

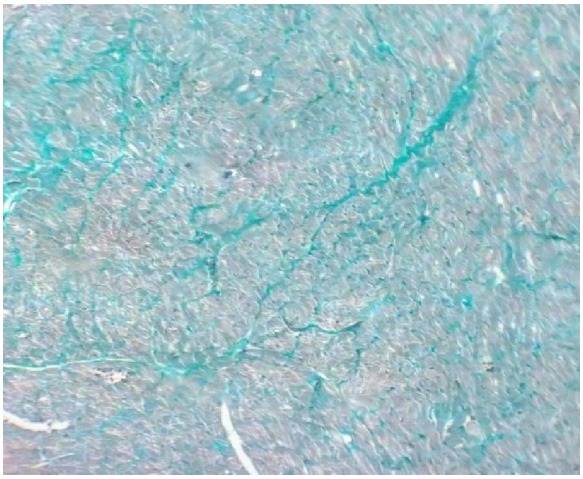


Prominent myocardial fibrosis inflammatory infiltrate



Contraction bands



Fibrosis demonstrated by (Masson trichrome) special stain.

Administration of ADMA increased significantly the histopathological changes in the ADMA as compared to normal group. The changes were in the form of fragmentation, contraction bands, vacuolations, edema, inflammatory infiltrate, fibrosis, cytolysis and hyalinazation in all the rats at various degrees. There were significant histopathological changes in the Pioglitazone treated groupas compared to ADMA group; as fragmentation, vacuolations, edema, inflammatory infiltrate, fibrosis were found in all rats at minimal and mild degrees, while contraction bands in five rats, cytolysis and hyalinization in only one rat. Significant pathological changes in resveratrol treated groupas compared to ADMA group; as fragmentation, vacuolations, edema, inflammatory infiltrate, fibrosis were found in all rats at minimal and mild degrees while contraction bands in four rats, cytolysis in only one rat and no hyalinization. In pioglitazone and resveratrol treated group there were significant differences in histopathological. Resveratrol group showed contraction bands in four rats, cytolysis in only one rat and no hyalinization at all, while in pioglitazone group contraction bands were in five rats with cytolysis and hyalinization in only one rat.

## 4. Discussion

Exogenous ADMAin present study produced significant elevation in systolic and diastolic blood pressure but insignificant decrease in heart rate. ADMA significantly reduced serum nitrite and cardiac dimethylarginine dimethyl aminohydrolase (DDAH) gene expression. Histopathological changes in cardiac tissue were fragmentation, contraction bands, vacuolations, edema, inflammatory infiltrate, fibrosis, cytolysis and hyalinization. These findings are consistent with **De Gennaro Colonna et al., (2007)**. **Mihout et al., (2011)** On the contrary to the present study, there was no reduction of DDAH expression **Achan et al., (2003**) clinical trial showed that ADMA increased mean blood pressure, reduced heart rate and cardiac output.

ADMA is eliminated principally by metabolism to L-citrulline by the enzyme dimethyl arginine dimethyl aminohydrolase (DDAH) with a small contribution from renal excretion. Two isoforms of DDAH have been identified, DDAH1 and DDAH2. Overexpression of either DDAH1 or DDAH2 in transgenic mice led to decreased plasma ADMA levels, increased NO bioavailability, and decreased blood pressure **Hasegawa et al., 2007. Hu et al., 2009** demonstrated that DDAH1 was highly expressed in heart and vascular endothelium, and that endothelial DDAH1 plays an important role in regulating blood pressure.

In present study, pioglitazone significantly reduced systolic and diastolic blood pressure but insignificant increase in heart rate. It significantly elevated serum nitrite and DDAH cardiac gene expression. Fragmentation, vacuolations, edema, inflammatory infiltrate and fibrosis were minimal. Contraction bands were seen in five rats while cytolysis and hyalinization in one rat. **Suzuki et al., (2013)** in a prospective trial of patients with hypertension and type 2 diabetes mellitusobserved that co-administration of candesartan with pioglitazone had beneficial effects with regard to hypertension, inflammation, and adipose tissue metabolism. In **Nakamoto et al., (2008)** work, pioglitazone significantly decreased blood pressure in rats and contributed to left ventricularhypertrophy regression. Besides their insulin-sensitizing action, thiazolidinediones exhibit direct vascular protection by inhibiting tissue inflammation, modulating the growth

and proliferation of vascular smooth muscle cells, and improving endothelial cell function. These mechanisms confer the antihypertensive action of thiazolidinedione drugs **(Benkirane et al., 2006)**.

Resveratrol significantly decreased systolic and diastolic blood pressure with no change in heart rate. Resveratrol produced significant elevation in serum nitrite and cardiac DDAHgene expression. It improved histopathological features of cardiac tissue as fragmentation, vacuolations, edema, inflammatory infiltrate. fibrosis were found in all rats at minimal and mild degrees, while contraction bands were present in four rats, cytolysis in only one rat and no hyalinization. Resveratrol is a natural polyphenol present in various plants, including grapes and peanuts. It is a potent antioxidant and modulates lipid and lipoprotein metabolism. It has anti-inflammatory properties by inhibiting cyclooxygenase-2 expression **(Seidman et al., 2013)**. Inanimal models of cardiovascular disease, resveratrol has been shown to protect heart from ischemia-reperfusion injury, reduce blood pressure and cardiac hypertrophy in hypertensive animals and has shown to slow progression of atherosclerosis **(Li et al., 2012)**. The exact mechanism of cardioprotective effect of resveratrol could be due to inhibition of the rapid increase in ROS accumulation in cardiac cell mitochondria **(Danz et al., 2009)** by increasing superoxide dismutase activity **(Tatlidede et al., 2009)**. However, antioxidant therapies have failed to produce satisfactory results in clinical trials **(Gianni et al., 2008). Osman et al., (2013)** found resveratrol to have potent cardioprotective effect while doxorubicin showed cardiac myocytes with cytoplasmic vacuolization or fragmentation. The resveratrol inhibited doxorubicin-induced increase in ROS in cardiac mitochondria.

**Cheng et al., (2014)** examined whether resveratrol decreases the generation of reactive oxygen species (ROS), the major findings were that activation of adenosine monophosphate kinase (AMPK) decreases blood pressure and production of ROS. AMPK is an important regulator of NADPH oxidase activity. **Dong et al., (2014)** demonstrated reversed left ventricular hypertrophy by resveratrol and modulation of activation and expression of calcium handling proteins. **Han et al., (2015)** found that DOCA salt-induced hypertension was prevented by resveratrol. Resveratrol reduced blood ADMA level in hypertensive and normotensive animals. This maybe an important mechanism for its beneficial effect on heart. **Qin et al., 2012, Thandapilly et al., 2013**and **Chan et al., 2011**findings were consistent with presenthistopathological findings. As in resveratrol group, fibrosis (demonstrated also by massontrichrome special stain) was minimal. A double-blind, placebo controlled trial, involved 40 post-infarction Caucasian patients who received 10 mg resveratrol capsule daily for 3 months showed improved ventricular diastolic function and lowered LDL cholesterol level **Magyar et al.,2012**.

**References**


1. Achan V, Broadhead M, Malaki M, Whitley G, Leiper J, MacAllister R, Vallance P (2003). Asymmetric Dimethylarginine Causes Hypertension and Cardiac Dysfunction in Humans and Is Actively Metabolized by Dimethylarginine Dimethylaminohydrolase. Arterioscler Thromb Vasc Biol. 23:1455-1459.
2. Ajay M, Achike F.I, Mustafa A.M and Mustafa M.R (2006). Effect of quercetin on altered vascular reactivity in aortas isolated from STZ-induced diabetic rats. Diabetes Res. Clin.Pract; 73; 1–7.
3. Azuma H, Sato J, Hamasaki H, Sugimoto A, Isotani E, Obayashi S (1995). Accumulation of endogenous inhibitors for nitric oxide synthesis and decreased content of L-arginine in regenerated endothelial cells. Br J Pharmacol 115: 1001-4.
4. Benkirane K, Viel EC, Amiri F, Schiffrin EL (2006): Peroxisome proliferator−activated receptor γ regulates angiotensin II− stimulated phosphatidylinositol 3-kinase and mitogen-activated protein kinase in blood vessels in vivo. Hypertension 47: 102–108.
5. Billingham M., and Bristow M. R. (1984). Evaluation of anthracycline cardiotoxicity: Predictive ability and functional correlation of endomyocardial biopsy. Cancer Treat. Symptoms. 3,71–76.
6. Chan V, Fenning A, Iyer A, Hoey A, Brown L (2011). Resveratrol improves cardiovascular function in DOCA-salt hypertensive rats. Curr Pharm Biotechnol 12:429–436.
7. Cheng P.W, Ho W.Y, Su Y.T, Lu P.J, Chen B.Z, Cheng H.W, et al (2014). Resveratrol decreases fructose-induced oxidative stress, mediated by NADPH oxidase via an AMPK-dependent mechanism British Journal of Pharmacology 171: 2739–2750.
8. Colonna V D. G, Bonomo S, Ferrario P, Bianchi M, Berti M, Guazzi M, Manfredi B (2006). Asymmetric dimethylarginine (ADMA) induces vascular endothelium impairment and aggravates post-ischemic ventricular dysfunction in rats. Eur J J Pharmacol: 17258196 Cit: 3.
9. Cove-Smith L, Woodhouse N, Hargreaves A, Kirk J, Smith S, Price S.A, Galvin M et al., (2014). An Integrated Characterization of Serological, Pathological, and Functional Events

in Doxorubicin Induced Cardiotoxicity. Toxicological Science 140(1), 3–15.
10. Danz ED, Skramsted J, Henry N, Bennett JA, Keller RS (2009). Resveratrol prevents doxorubicin cardiotoxicity through mitochondrial stabilization and the Sirt1 pathway. Freeradic. Biol Med 46(12):1589–1597.
11. Dawson B and Trapp RG (2001). Basic and clinical biostatistics; Mcgraw-Hill Inc. Third edition.
12. De Gennaro Colonna V, Bonomo S, Ferrario P, Bianchi M, Berti M, Guazzi M, et al (2007). Asymmetric dimethylarginine (ADMA) induces vascular endothelium impairment and aggravates post-ischemic ventricular dysfunction in rats.Eur J Pharmacol. 557(2-3):178-85.
13. Dong Q, Wu Z, Li X, Yan J, Zhao L, Yang C, Lu J, Deng J and Chen M (2014). Resveratrol ameliorates cardiac dysfunction induced by pressure overload in rats via structural protection and modulation of Ca2+ cycling proteins. Journal of Translational Medicine 12:323.
14. Espin, J. C., Garcia-Conesa, M. T., & Tomas-Barberan, F. A. (2007). Nutraceuticals: facts and fiction. Phytochemistry 68 (22-24), 2986-3008.
15. Fanelli and Zatz R (2011). "Linking oxidative stress, the renin-angiotensin system, and hypertension," Hypertension 57: 373–374.
16. Gianni L, Herman E.H, Lipshultz S.E, Minotti G, Sarvazyan N, Sawyer DB (2008). Anthracyclinecardiotoxicity: from bench to bedside. J Clin Oncol 26: 3777–3784.
17. Han S, Uludag M.O, Usanmaz S.E, Ayaloglu-Butun F, Akcali K.C, Demirel-Yilmaz E (2015). Resveratrol affects histone 3 lysine 27 methylation of vessels and blood biomarkers in DOCA salt-induced hypertension Mol Biol Rep 42:35–42.
18. Hasegawa K, Wakino S, Tatematsu S, Yoshioka K, Homma K, Sugano N, Kimoto M, Hayashi K, Itoh H (2007). Role of Asymmetric Dimethylarginine in Vascular Injury in Transgenic Mice Overexpressing Dimethylarginie Dimethylaminohydrolase 2. Circ Res101:e2–10.
19. Hu X, Xu X, Zhu G, Atzler D, Kimoto M, Chen J, Schwedhelm E, Luneburg N, Boger RH, Zhang P et al (2009). Vascular endothelial-specific dimethylarginine dimethylaminohydrolase-1-deficient mice reveal that vascular endothelium plays an important role in removing asymmetric dimethylarginine. Circulation 120: 2222 – 2229.
20. Iglarz M, Touyz RM, Amiri F, Lavoie MF, Diep QN, Schiffrin EL (2003). Effect of peroxisome proliferator-activated receptor-alpha and -gamma activators on vascular remodeling in endothelin-dependent hypertension. Arterioscler Thromb Vasc Biol 23:45–51.
21. Irvine R. White J, Chan R (1997). The influence of restraint on blood pressure in the rat. J Pharmacol Toxicol Methods 38:157-162.
22. Johnston, C. (2009). Functional Foods as Modifiers of Cardiovascular Disease. Am J Lifestyle Med 3: 39S-43S.
23. Kitova L.V (2008). Effect of Fenofibrate On The level of Asymmetric Dimethyl Arginine In Type 2 Diabetes Mellitus. Journal of IMAB - Annual Proceeding (Scientific Papers) book 1.
24. Leiper JM, Santa Maria J, Chubb A, et al (1999). Identification of two human dimethylarginine dimethylaminohydrolases with distinct tissue distributions and homology with microbial arginine deiminases. Biochem J 1999; 343 Pt 1: 209-14.
25. Li, H., Xia, N., & Forstermann, U. (2012). Cardiovascular effects and molecular targets of resveratrol. Nitric Oxide, 26(2), 102-110.
26. MacAllister RJ, Parry H, Kimoto M, et al (1996). Regulation of nitric oxide synthesis by dimethylarginine dimethylaminohydrolase. Br J Pharmacol 119: 1533-40.
27. Magyar, K., Halmosi, R., Palfi, A., Feher, G., Czopf, L., Fulop, A., et al. (2012). Cardioprotection by resveratrol: A human clinical trial in patients with stable coronary artery disease. Clin Hemorheol Microcirc 50(3):179-87.
28. Matsumoto T, Noguchi E, Kobayashi T, Kamata K (2007). Mechanisms underlying the chronic pioglitazone treatment-induced improvement in the impaired endothelium-dependent relaxation seen in aortas from diabetic rats. Free Radic Biol Med 42:993–1007.
29. Mihout F, Shweke N, Big N, Jouanneau C, Dussaule J. C, Ronco P Chatziantoniou C and Boffa J.J (2011). Asymmetric dimethylarginine (ADMA) induces chronic kidney disease through a mechanism involving collagen and TGF-β1 synthesis. J Pathol 223: 37–45.
30. Miranda K.M, Espey M.G, Wink D.A (2001). A rapid, simple spectrophotometric method for simultaneous detection of nitrate and nitrite, Nitric Oxide 5; 62–71.
31. Nakamoto M, Ohya Y, Shinzato T, Mano R, Yamazato M, Sakima A, and Takishita S (2008). Pioglitazone, a Thiazolidinedione Derivative, Attenuates Left Ventricular Hypertrophy and Fibrosis in Salt-Sensitive Hypertension Hypertens Res 31: 353–361.
32. Osman A.M, Al-Harthi S. E, Al Arabi O.A, et al (2013).Chemosensetizing and cardioprotective

effects of resveratrol in doxorubicin- treated animals Cancer Cell International 13:52.
33. Qin F, Siwik DA, Luptak I, Hou X, Wang L, Higuchi A, Weisbrod RM, Ouchi N, Tu VH, Calamaras TD, Miller EJ, Verbeuren TJ, Walsh K, Cohen RA, Colucci WS: (2012). The polyphenols resveratrol and S17834 prevent the structural and functional sequelae of diet-induced metabolic heart disease in mice. Circulation 125:1757–1764.
34. Seidman MD, Tang W, Bai VU, Ahmad N, Jiang H, Media J, et al (2013). Resveratrol decreases noise-induced cyclooxygenase-2 expression in the rat cochlea. Otolaryngol Head Neck Surg 148: 827-33.
35. Smoliga, J. M., Baur, J. A., & Hausenblas, H. A. (2012). Resveratrol and health—a comprehensive review of human clinical trials. Mol Nutr Food Res, 55(8), 1129-1141.
36. Suzuki H, Sakamoto M, Hayashi T, Iuchi H, Ohashi K, Isaka T, Sakamoto N, Kayama Y, Tojo K, Yoshimura M and Utsunomiya K (2013). Effects of co-administration of candesartan with pioglitazone on inflammatory parameters in hypertensive patients with type 2 diabetes mellitus: a preliminary report. Cardiovascular Diabetology 12:71.
37. Tatlidede E, Sehirli O, Velioğlu-Oğünc A, Cetinel S, Yeğen BC, Yarat A, Süleymanoğlu S, Sener G (2009). Resveratrol treatment protects against doxorubicin-induced cardiotoxicity by alleviating oxidative damage. Free Radic Res 43:195–205.
38. Thandapilly SJ, Louis XL, Behbahani J, Movahed A, Yu L, Fandrich R, Zhang S, Kardami E, Anderson HD, Netticadan T (2013). Reduced hemodynamic load aids low-dose resveratrol in reversing cardiovascular defects in hypertensive rats. Hypertens Res 36:866–872.
39. Tojo A, Welch WJ, Bremer V et al (1997). Colocalizationof demethylating enzymes and NOS and functional effects of methylarginines in rat kidney. Kidney Int 52: 1593-601.
40. Weis M, Kledal TN, Lin KY, et al (2004). Cytomegalovirus infection impairs the nitric oxide synthase pathway: role of asymmetric dimethylarginine in transplant arteriosclerosis. Circulation 109: 500-5.
41. Williams, B. (2008). The year in hypertension. J Am Coll Cardiol, 51(18), 1803-1817.
42. Yu, W., Fu, Y. C., & Wang, W. (2012). Cellular and molecular effects of resveratrol in health and disease. J Cell Biochem, 113(3): 752-759.

7/9/2017