

Cross talk between oxidative stress, PPAR- γ and RAS system in diabetic rat heart exposed to ischemia / reperfusion injury

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ABSTRACT

Introduction: The present study aimed at clarifying the cross talk between peroxisome proliferator-activated receptor-gamma (PPAR- γ), cardiac reactive Oxygen species (ROS) and Renin-Angiotensin System (RAS). **Methods:** A total of 90 male albino rats were used. They rats were divided into: Group 1: Control group ,Group 2: Type 2 diabetic rats, Group 3: PPAR γ agonist protected type 2 diabetic rats. Group 4: Antioxidant protected type2 diabetic rats, Group 5: Metformin treated type 2 diabetic rats. Blood samples were collected for measurement of FBS and fasting insulin. Half the number of each group was sacrificed and the heart excised and perfused, from the rest of the group small piece from the heart was taken for estimation of malondialdehyde (MDA), Angiotensin 2 Receptor (AT2R) and Angiotensin Converting Enzyme 2 (ACE2) gene expression. **Results:** Treatment with pioglitazone and Vitamin E significantly lowered blood glucose, insulin levels and Homeostasis Model Assessment Insulin Resistance (HOMA IR). However, values did not return to control values. Pioglitazone and Vitamin E improved myocardial performance and percentage recovery following ischemia reperfusion. The cardioprotective effect was more pronounced in the pioglitazone group. This positively correlated with decreased MDA levels and increased AT2R and ACE2 expression in cardiac tissue. **In Conclusion,** Pioglitazone and Vitamin E in type 2 DM significantly offered cardio-protection through improving the diabetic condition and / or decreasing MDA levels.

Key words: Type 2 Diabetes mellitus, cardiac ischemia reperfusion, PPAR γ , oxidative stress, ACE2, AT2R expression.

1.INTRODUCTION

Diabetes mellitus (DM) leads to high cardiovascular morbidity and mortality as a result of functional and morphological damage in diabetic hearts.

Most of the diabetes-related cardiovascular complications can be largely attributed to overproduction of reactive oxygen species such as superoxide and hydrogen peroxide, and/or depletion of antioxidant defense mechanisms [1].

Antioxidant therapy has shown promise in prevention of development of diabetic heart complications but the results of intervention studies with classic antioxidants such as vitamin E, were shown to be elusive and unsuccessful [2]. On this matter, it has recently been suggested that antioxidant therapy with vitamin E or other antioxidants is limited to scavenging already-formed oxidants and may therefore be considered a more "symptomatic" rather than a causal treatment for vascular oxidative stress. Hyperglycemia-induced oxidative stress is accompanied by increased production of advanced glycosylation end products (AGEs), upregulation of the receptor for AGEs (RAGE) [3] and downregulation of PPAR γ system [4].

AGE binds to its cell surface receptor, RAGE, resulting in the activation of postreceptor signaling, generation of intracellular reactive oxygen species, and alteration of gene expression[5]

Reactive oxygen species have been indicated as one of the earliest and most important components of tissue injury after reperfusion of ischemic organ. Free radical production is continuous during ischemia, while during reperfusion it is primarily confined to the early stage when fresh oxygen is supplied to the ischemic region [6].

PPAR- γ system plays a critical role in oxidative stress that seems to be implicated in the development of insulin resistance and diabetic complications. Moreover, current evidence points to a cardioprotective role for PPAR- γ ligands in ischemia reperfusion animal models, but the functional role of PPAR- γ in diabetic hearts is not fully understood [7].

On the other hand, it has been recently suggested that angiotensin signaling may be regulated at multiple levels by PPAR- γ ligands, significantly reducing the expression of AT1 receptor and repressing signal transduction through this receptor to suppress oxidative stress. However, the relation between PPAR- γ , MDA levels, AT2 receptor and ACE2 expression in diabetic hearts needs to be clarified [8].

The aim of this study is to:

1. Test PPAR- γ agonist and antioxidant therapy on the development of type 2 DM in rats.
2. Test the possibility of improving the myocardial function and reducing the susceptibility to ischemia reperfusion injury through reducing MDA, one of the biomarkers of the oxidative stress.
3. Estimate the cardiac ACE2 and AT2R gene expression in relation to PPAR- γ system.
4. Compare the possible cardio-protective effects of PPAR- γ agonist, antioxidant therapy and metformin in type 2 diabetic hearts

2.MATERIALS & METHODS

2.1.Experimental Animals:

A total of 90 male albino rats were used in this study weighing 160-180 grams. These rats were treated in accordance with the guidelines approved by the Animal Use Committee of Cairo University. The animals were housed in animal house of faculty of Medicine, Cairo

University, in wire mesh cages at room temperature, with 12 hour light dark cycle. They were fed the commercial rat chew diet and had free access to water.

These rats were divided into the following groups:

Group 1: Control group

Consists of 18 normal rats.

Group 2: Type 2 diabetic rats.

Type 2 diabetic rats (n=18).

Group 3: PPAR γ agonist protected type2 diabetic rats group

Rats was treated with oral pioglitazone 20 mg/kg/day for the whole time of the experiment (n=18).

Group 4: Antioxidant protected type2 diabetic rats group

Rats was treated with oral vit E 500 mg/kg/day for the whole time of the experiment (n=18).

Group 5: Metformin treated type2 diabetic rats group

Treatment was initiated at 350 mg/kg/ day and was gradually increased to a dose of 650 mg/kg/ day for the whole time of the experiment (n=18).

Half the number of each group was sacrificed and the heart excised and perfused according to the Langendorff technique, from the rest of the group small piece from the heart was taken for estimation of AT2R and ACE2 expression and malondialdehyde (MDA).

2.2.Experimental protocol:

2.2.1.Induction of Type 2 Diabetes:

Beginning on day 0, rats were fed either normal rodent chow (12% calories as fat)(group 1) or high fat diet (60% calories as fat)(group 2) for two weeks. On day 14, rats on the high fat diet (HFD) were injected with a single low dose of streptozotocin (STZ, 35 mg/kg i.p., in 0.01M citrate buffer pH 4.3) to induce type 2 diabetes [9]. Consumption of high fat diet leads to insulin resistance and is considered to be a major predisposing factor for type 2 diabetes [10]. Subsequent to STZ treatment, rats had free access to food and water and were continued on their respective diets for the duration of study.

2.2.2.Characterization of Type 2 Diabetes Model

On day 20, in the HFD + STZ group 2, type 2 diabetes was confirmed by measuring fasting plasma glucose and insulin levels. Blood sampled from the retro orbital plexus under diethyl ether anesthesia was used to measure plasma glucose and insulin concentration.

2.2.3.Heart Perfusion:

Was performed according to the Langendorff technique with Krebs-Heinseleit medium at a hydrostatic pressure of 55 cm H₂O and bubbled with a mixture of 95% O₂ and 5% CO₂ [11].

100 **2.2.4. Measurements of myocardial function**

101 Mechanical performances of the left ventricle of the heart was determined by the systolic
102 pressure, the diastolic pressure, the heart rate and the peak rate of maximum left
103 ventricular pressure rise (dp/dt) which is considered as a good index of contractility.
104 These mechanical performances were monitored during pre-ischemic; ischemic and
105 post-ischemic reperfusion phases by a balloon inserted into the left ventricle and
106 connected to a polygraph apparatus (San-ei instrument LTD Nee, Tokyo, Japan). The
107 developed pressure was calculated (systolic pressure – Diastolic pressure).

108 **2.2.5. Morphological Measurements:**

109 At the end of the perfusion, hearts were removed from the perfusion apparatus, excess
110 water was absorbed on tissue paper, and the heart mass was weighed.

111 **2.2.6. Measurement of fasting blood insulin**

112 Insulin concentrations were measured in previously frozen and thawed serum
113 samples by enzyme immunoassay using the Rat Insulin ELISA kits.

114 **2.2.7. Measurement of fasting blood glucose**

115 The blood glucose was assayed by the method adopted by **Trinder P (1969)** [12].
116 The test materials for this method were supplied as kits by “Diamond Diagnostics”.

117 **2.2.8. HOMA-IR**

118 Calculated as the product of fasting insulin (microunits/ml) and fasting glucose
119 (mMol/L) divided by 22.5. Lower index indicates greater insulin sensitivity [13].

120 Fasting insulin (μIU/ml) × fasting glucose (mmol/L)

121 HOMA- IR = -----

122 22.5

123 **2.2.9. Semi-Quantitation of AT2R and ACE2 gene expression by reverse**
124 **transcriptase polymerase chain reaction (RT-PCR):**

125 About 30 mg of heart tissue was homogenized in RNA lysis buffer containing β-
126 mercaptoethanol then the tissue homogenate was centrifuged at 10000 rpm for 20 min,
127 then the clear lysate was subjected to the following

128 **A) RNA extraction:**

- 129 1. The clear lysate solution was transferred to microcentrifuge tube by pipetting.
- 130 2. 200 μl 95% ethanol was added to the cleared lysate, mixed by pipetting 3-4 times,
131 this mixture was transferred to spin column assembly, centrifuged at 12000-14000
132 rpm for 1 min.
- 133 3. The spin basket was taken from the spin column assembly, the fluid was discarded
134 in the collection tube, and the spin basket was put into the collection tube.
- 135 4. 600 μl of RNA wash solution was added to the spin column assembly, and then
136 centrifuged at 14000 rpm for 1 min.

- 137 5. The collection tube was empty and placed in the rack, Dnase mix was prepared by
138 mixing 40 ml yellow core buffer, 5 ml 0.09 Mol mncl_2 and 5 ml of Dnase solution
139 was kept on ice for 1 min then about 50 ml of freshly prepared DNase solution was
140 added directly to the membrane inside the spin basket, and incubated for 20 min at
141 20°C, after the incubation, 200 ml of DNase stop solution was added to the spin
142 basket and centrifuged at 14000 rpm for 2 min.
- 143 6. 600 ml RNA wash solution was added and centrifuged at 14000 rpm for 1 min.
- 144 7. The spin basket was transferred from the collection tube to the elution tube, and 100
145 ml nuclease free water was added to the membrane, centrifuged at 12000 rpm for 1
146 min, the spin basket was removed and discarded; the elution tube containing the
147 purified RNA capped and stored at -70°C.

148 **B) RT-PCR experiments:**

149 RT-PCR was done using the extracted RNA for detection of gene expression as
150 follows: RNA was reverse transcribed using 12-15ml oligo (dT) 18 primer and was
151 denaturated at 70°C for 2 min, the denaturated RNA was placed on ice for 5 min. 6.5 ml of
152 reverse transcription mixture was prepared containing :50 mM Tris Hcl pH 8.3, 50 mM kcl,
153 1-5 mM mgcl_2 , 0.5 mM d NTPs, 1 unit / ml RNase inhibitor, 200 unit of Moloney murine
154 leukemic virus reverse transcriptase (MMLV).

155 The mixture was added to the RNA and subjected to the following cycling
156 condition needed for reverse transcription and synthesis of cDNA from RNA:

- 157 • 42°C for 1 hour.
- 158 • 95°C for 5 min to stop the reaction.

159 Then the PCR reaction was performed by adding PCR mix to a final volume of
160 100 ml, the PCR mix contain: 10 m Mol/L Tris Hcl pH 8.3, 50 m Mol KCl, 1.5m Mol
161 mgCl_2 , 0.001% gelatin, 250 μ Mol dNTPs mix, 2.5 unit Taq polymerase, 100 ml Mol of
162 each primer

- 163 • Specific primer sequence for ACE2 (gene bank number NM 001012006):

5' CGCTGTCACCAGACAAGAA 3' (sense)

5' GCCATTATTTCTGTTCAATCC 3' (antisense)

164 The final PCR product was 139 bp in size.

- 165 • Specific primer sequence for AT2R (gene bank number NM 0124943):

5' GGA GCG AGC ACA GAA TTG AAA GC 3' (sense)

5' TGC CCA GAG AGG AAG GGT TGC C' (antisense)

The final PCR product was 445 bp in size.

- 166 • Specific primer sequence for b-actin (used as house keeping gene) (gene bank
167 accession number : NM 001014725.2)

168

169 5'-TTC-TACAATGAGCTGCGTGTGGC-3' (sense)

170 5'-AGAGGTCTTTACGGATGTCAACG-3'; (antisense)

171

172 The reaction mixture was then subjected to 40 cycles of:

173 • 95°C for 1 min.

174 • 55°C for 1min.

175 • 72°C for 1min.

176 After the last cycle, a final extension at 72°C for 1min. was done.

177 **C) Agarose gel electrophoresis:**

178 All PCR products were electrophoresed on 2% agarose gel stained with ethidium
179 bromide and visualized by ultra-violet transilluminator.

180 **D) Gel documentation:**

181 The PCR products were semiquantitated using the gel documentation system (Bio
182 Doc Analyze) supplied by Bio metra, using standard DNA with different concentration to
183 make curve from which concentration of PCR products was detected.

184 **2.2.10. Estimation of MDA levels in rat heart:**

185 This was done by the thiobarbituric acid (TBA) test. *In brief*, 2.5 ml of 20 mg/dl trichloroacetic
186 acid is added to 0.5 ml of tissue homogenate. The tube is left to stand for 10 min at room
187 temperature. After centrifugation at 3500 rpm for 10 min the supernatant is washed once
188 with 0.05 M sulfuric acid. 2.5 ml of sulfuric acid and 3 ml of TBA in 2 Mol sodium sulfate are
189 added to this precipitate and the coupling of lipid peroxide with TBA is carried out by heating
190 in a boiling water bath for 30 min. After cooling in cold water, the resulting chromogen is
191 extracted with 4 ml of n-butyl alcohol by vigorous shaking, separation of organic phase is
192 facilitated by centrifugation at 3000 rpm/min for 10 min and its absorbance is determined at
193 the wave length of 530 nm. The standard curve was linear up to 21.2 nMol/ml. The
194 concentration of sample was determined from standard curve. [14]

195 **Statistical Analysis:**

196 The results were analyzed using SPSS computer software package, version
197 10.0(Chicago-IL, USA). Data were presented as mean $\pm$ S.D. Differences among the three
198 groups were compared by one-way ANOVA. To study the relationship between the
199 variables, Pearson's correlation coefficient was calculated. The results were considered
200 statistically significant at $P < 0.05$.

201 **3.RESULTS**

202 Table 1 shows that untreated diabetic rats (group 2) had significantly higher level of fasting
203 blood glucose (mmol/L), fasting plasma insulin (uIU/ml) and HOMA-IR compared to non
204 diabetic control rats group 1. However, these levels decreased significantly in protected
205 groups: pioglitazone protected diabetic rats group 3, vit E protected diabetic rats group 4 and
206 metformin protected diabetic rats group 5 compared to untreated diabetic group 2 with no
207 significant difference between the three treated groups. However, none of the measured
208 parameters in the protected groups returned to control values.

209 **Table 1. Levels of fasting blood glucose , fasting insulin and HOMA test in the**
 210 **studied groups.**

Calculated parameters	Group 1	Group 2	Group 3	Group 4	Group 5	P
Glucose (mmol/L)	4.81 ± 0.51 a	12.14 ± 1.44 b	9.45 ± 1.27 c	8.68 ± 1.79 cd	7.08 ± 0.47 d	0.00
Insulin (uIU/ml)	10.58 ± 0.57 a	18.2 ± 2.76 b	14.71 ± 1.78 c	15.13 ± 1.54 c	13.81 ± 0.46 c	0.00
Homa	2.21 ± 0.21 a	9.79 ± 2.01 b	6.10 ± 1.00 c	5.80 ± 1.28 cd	4.35 ± 0.28 d	0.00

211

212 *Values are mean ± Standard deviation (SD)*

213 *Groups bearing the same initials are not statistically significant from each other at P<0.05.*

214 As shown in table 2:

215 The unprotected diabetic rats group 2 showed significant increase in the level of
 216 AT₂R and ACE₂ gene expression in cardiac tissue (µg/ml homogenized tissue) compared to
 217 control group 1. Pioglitazone treatment of diabetic rats almost doubled the level of AT₂R and
 218 ACE₂ gene expression in cardiac tissue compared to the untreated diabetic group and the
 219 other two protected groups (Vit E and metformin).

220 As regards the level of MDA in cardiac tissue (nmol/mg ptn): The unprotected
 221 diabetic rats group 2 showed significant increase in the level of MDA in cardiac tissue
 222 (nmol/mg ptn) compared to control group 1. Moreover, the protected groups: PPARγ
 223 protected diabetic rats group 3, vit E protected diabetic rats group 4 and metformin protected
 224 diabetic rats group 5 showed significant decrease in level of MDA in cardiac tissue
 225 compared to untreated diabetic group 2 although the level of the MDA remained significantly
 226 elevated compared to control group 1.

227 **Table 2. AT₂R and ACE₂ expression and level of MDA in cardiac tissue in the studied**
 228 **groups.**

Calculated parameters	Group 1	Group 2	Group 3	Group 4	Group 5	P
AT ₂ R	0.18 ± 0.04 a	0.508±0.06 b	0.84 ± 0.106 d	0.408±0.07 b	0.503±0.06 1 b	0.00
ACE ₂	0.108±0.03 1 a	0.36± 0.085 b	0.72±0.12 d	0.35±0.026 b	0.39±0.018 b	0.00
MDA nmol/mg ptn	10.93±1.36 a	23.01±2.68 b	16.28±1.97 c	14.91±1.88 c	17.45±0.52 c	0.00

229

230

Values are mean± SD.

231

Groups bearing the same initials are not statistically significant from each other at

232

P<0.05.

233

As shown in table 3:

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The unprotected diabetic rats group 2 showed significant decrease in the level of LVDP (mmHg) and dp/dt (mmHg/sec) compared to control group 1 but there was no significant difference in the level of HR (b/min) between the 2 groups.

237

Both pioglitazone protected diabetic rats (group 3) and vit E protected diabetic rats (group 4) showed significant improvement in LVDP and dp/dt but still not reaching control values of the nondiabetic rats. However, there was no significant difference between the metformin protected diabetic rats (group 5) and the unprotected diabetic rats (group 2).

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Table 3. Left ventricular performance (developed pressure), heart rate, and contractility index (dp/dt) during the initial 30 minutes of perfusion in the studied groups.

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243

Calculated parameters	Group 1	Group 2	Group 3	Group 4	Group 5	P
LVDP mmHg	114.56 ±9.47 a	58.44 ±5.43 b	79.44 ±6.32 d	79.67 ±3.39 d	59.44 ±5.43 b	0.00
HR b/min	248.78 ±109.11 a	224.00 ±46.56 a	161.33±1 8.98 b	162.44 ±9.15 b	225.00 ±46.56 a	0.00
dp/dt mmHg/sec	116.44 ±7.126 a	65.78 ±4.969 b	97.89 ±7.688 cd	95.22 ±8.228 d	66.78 ±4.969 b	0.00

244

245

Values are mean± SD.

246

Groups bearing the same initials are not statistically significant from each other at P<0.05.

247

As shown in table 4:

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The percentage recovery of the left ventricle performance following ischemia-reperfusion (LVDP (mmHg), contractility index dp/dt (mmHg/sec) and heart rate(b/min) was significantly decreased in the unprotected diabetic rats (group 2) compared to control rats (group 1).

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Regarding the protected groups of diabetic rats; PPAR γ protected diabetic rats (group 3) showed significantly higher values of percentage recovery of LVDP and dp/dt compared to vit E protected diabetic rats (group 4), metformin protected diabetic rats (group 5) and the untreated diabetic rats (group 2). Interestingly, pioglitazone prevented the effect of diabetes on % recovery of left ventricular performance after I/R that even returned to non diabetic control levels.

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258 **Table 4. The percentage recovery of the left ventricle performance following**
 259 **ischemia-reperfusion (developed pressure), heart rate and contractility index (dp/dt)**
 260 **in the five studied groups.**

Calculated parameters	Group 1	Group 2	Group 3	Group 4	Group 5	P
LVDP mmHg	78.144± 1.93 ab	63.456± 4.39 c	76.38±2. 54 b	64.28±3. 72 c	69.36±0. 71 d	0.00
HR b/min	77.100± 1.90 a	65.98±7. 14 b	78.21±3. 02 a	67.63±2. 16 b	69.83±0. 83 b	0.00
dp/dt mmHg/sec	77.256± 2.83 a	71.55±4. 14 b	79.56±1. 43 a	66.87±2. 90 d	69.68±0. 91 bd	0.00

261

262 Values are mean± SD.

263 Groups bearing the same initials are not statistically significant from each other at

264 P<0.05.

265 When Pearson correlation was performed in studied groups, significant positive
 266 correlation was found between the level of AT2R expression in cardiac tissue and the
 267 percentage recovery of the left ventricle performance following ischemia-reperfusion "LVDP
 268 (r=.412**), heart rate(r=.546**) and contractility index dp/dt (r=.610**) & also, there was
 269 significant positive correlation between the level of ACE2 expression in cardiac tissue and
 270 the percentage recovery of the left ventricle performance following ischemia-reperfusion
 271 "LVDP (r= .465**) and contractility index dp/dt (r=.603**) and the percentage recovery the
 272 heart rate (r = .624**). In addition, there was significant negative correlation between the level
 273 of MDA in cardiac tissue and the percentage recovery of the left ventricle performance
 274 following ischemia-reperfusion "LVDP (r = -.439**), heart rate(r= -.363**) and contractility
 275 index dp/dt (r=-.353**)" (Table 5)

276 **Table 5. Correlations between studied parameters**

		AT2	ACE2	MDA
MDA	Pearson Correlation	.088	.115	
	Sig. (2-tailed)	.491	.371	
	N	63	63	
LVDP % recovery	Pearson Correlation	.412**	.465**	-.439**
	Sig. (2-tailed)	.001	.000	.000

	N	63	63	63
	Pearson Correlation	.546**	.624**	-.363**
HR % recovery	Sig. (2-tailed)	.000	.000	.003
	N	63	63	63
	Pearson Correlation	.610**	.603**	-.353**
Dp/dt % recovery	Sig. (2-tailed)	.000	.000	.003
	N	63	63	63

Correlation is significant at the 0.05 level (2-tailed).

4.DISCUSSION

The protective role of PPAR- γ agonist and vitamin E in diabetes was confirmed in the present study. However, the glucose level was significantly reduced in the metformin group compared to other protected groups.

A recent study found that tocopherols dramatically enhanced adiponectin expression and that this effect was mediated through a PPAR- γ -dependent process. These findings illustrate a possible mechanistic link between vitamin E and insulin sensitivity [15] It is likely that the diabetic milieu and associated changes in the myocardium sensitize the diabetic heart to dysfunction after ischemic injury[16].

Vitamin E-protected rats showed significantly higher values of left ventricular performance and contractility index.

Increased ROS generation may activate maladaptive signaling pathways, which may lead to cell death, which could contribute to the pathogenesis of diabetic cardiomyopathy [17] Increased ROS-mediated cell death could promote abnormal cardiac remodeling, which ultimately may contribute to the characteristic morphological and functional abnormalities that are associated with diabetic cardiomyopathy[18] Increased ROS also might contribute to mitochondrial uncoupling, which could impair myocardial energetics in diabetes. Strategies that either reduce ROS or augment myocardial antioxidant defense mechanisms might have therapeutic efficacy in improving myocardial function in diabetes mellitus [19].

PPAR- γ agonists may exert their anti-inflammatory effects by negatively regulating the expression of pro-inflammatory genes induced during macrophage differentiation and activation. Several lines of evidence suggest that TZDs protect the heart and other organs against the tissue injury caused by ischemia/reperfusion (I/R) injury and shock [20]

The present study showed that PPAR- γ ligands improved cardiac performance after I/R. This agrees with Shimabukuro and colleagues (1996) [21] The authors showed that this cardioprotective effect is associated with inhibition of the JNK/AP-1 pathway [22]

305 Zhu and coworkers [23], showed that chronic troglitazone administration significantly
306 improved recovery of left ventricular systolic and diastolic function as well as increasing net
307 myocardial lactate uptake, suggesting enhanced myocardial carbohydrate oxidation [24]

308 A significant increase in the level of MDA in cardiac tissue was detected in the
309 unprotected diabetic rats. However, the MDA level was equally decreased in all protected
310 diabetic groups.

311 Under physiological states, most of the ROS generated within cells arises from
312 mitochondria. Relatively few studies to date have directly measured mitochondrial ROS
313 production in mitochondria obtained from diabetic hearts [25] ROS are produced from
314 nonmitochondrial sources such as NADPH oxidase or reduced neuronal nitric oxide
315 synthase (NOS1) activity coupled with increased activation of xanthine oxidoreductase [26]
316 Antioxidant inhibits NF- κ B activation by blocking LPS-induced I κ B degradation and nuclear
317 translocation of the cytosolic NF- κ B p65 subunit [27]

318 Potential mechanisms for cardioprotection afforded by ACE2 are unclear. They
319 include increased Ang II degradation and increased formation of Ang-(1-7) which activates
320 the G protein–coupled Mas receptor. Mas receptor activation by Ang-(1-7) opposes many of
321 the AT₁ receptor-mediated actions (vasoconstriction, hypertrophy, fibrosis), thereby
322 improving cardiac function and remodeling and attenuating heart failure. Mas receptors are
323 also localized to cardiac myocytes and activate NO production [28].

324 In the present work, % recovery of cardiac performance after I/R injury was
325 positively correlated with levels of AT2R and ACE2 gene expression and negatively
326 correlated with levels of MDA.

327 It has been shown that TZDs could inhibit the oxidative stress indirectly through the
328 inhibition of the promoter activity of the chemokine genes and the inhibition of NF- κ B
329 activation on these promoter with the inhibition of the expression of proinflammatory
330 adhesion molecules (ICAM-1, VCAM-1, E-selectin)[32]. Moreover PPAR- γ agonists prevent
331 the phosphorylation of NF- κ B p65 and inhibit the activities of NF- κ B and AP-1 and thus
332 reducing the generation of oxygen-derived free radicals in diabetic hearts [29].

333 In our study, lowering glucose level by metformin induced significant decrease in
334 MDA. Metformin prevent ROS formation by preventing the build-up of mNADH through
335 insulin-stimulated nutrient uptake and preventing the entrance of energetic substrates
336 (pyruvate, fatty acids) into the mitochondria [30]

337 Furthermore, metformin has been shown to reduce ROS levels in human leukocytes by
338 either directly scavenging the free radicals or modulating their intracellular production [31].

339 Comparing the levels of HOMA-IR and cardiac tissue MDA in our studied groups
340 confirms the role of oxidative stress in pathophysiology of diabetic hearts and also confirms
341 the antioxidant effect of PPAR- γ agonists which surprisingly enough was not different from
342 either vitamin E or metformin.

343 The unprotected diabetic rats showed significant increase in the level of AT₂R and
344 ACE₂ gene expression. This might indicate a protective role for both AT2R and ACE2 [32]

The levels of AT₂R and ACE2 gene expression in cardiac tissue were significantly elevated in pioglitazone protected groups compared to other groups.

There is good evidence that PPAR- γ agonists mediate inhibition of the AT₁R expression as well as Ang II-mediated signaling pathways which may explain the increase in the expression of AT₂R and ACE2 [33,34]

Molavi et al., (2006) [35] reported a reduction of infarct size, attenuation of myocardial AT₁R and increase in AT₂R mRNA expression in the TZDs group.

5. CONCLUSION

Taken together, these results clearly suggest a cross talk between ROS, PPAR - γ and RAS systems which interplay in the pathogenesis of diabetes and its cardiac complications.

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