Prophylactic Resveratrol and Telmisartan Combination Ameliorates Experimentally-Induced Diabetic Nephropathy in Rats, Focus on the Pro-Sclerotic Cytokine, Transforming Growth Factor-β1 (TGF-β1)

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Abstract

Objectives: Prevention or retardation of diabetic nephropathy (DN) has become a major goal in biomedical research. Local renal renin-angiotensin system (RAS) activation and increased oxidative stress have been implicated in the pathogenesis of diabetic nephropathy, this study tested the prophylactic effect of resveratrol (Res), as a natural antioxidant, or telmisartan (Tel), an angiotensin II receptor antagonist, and their combination on the development and progression of diabetic nephropathy (DN) in experimentally-induced diabetes in albino rats.

Material and Methods: 48 male albino rats equally divided into 6 groups; 2 negative control groups and 4 diabetic groups. Diabetes was induced by single dose of streptozotocin (50 mg/kg i.p.). Diabetic rats received; no prophylaxis (DN group), prophylaxis with Tel or Res alone or their combination. At the end of 12 weeks, systolic blood pressure and body weight were determined. Blood glucose, serum urea and creatinine were estimated. Also, 24-h urinary proteins and creatinine clearance (Ccr) were determined. Fresh urine samples were collected for estimation of urinary Transforming Growth Factor-β1 (TGF-β1). Cortical total RNA was extracted and the mRNA expression of TGF-β1 was determined by a “two-step” RT-PCR.

Results: Prophylaxis therapy either by Tel or Res alone induced significant attenuation of DN, the combined therapy was additive, with significant reduction in blood pressure and proteinuria. Single-line prophylaxis therapy reduced urinary TGF-β1 and renal tissue TGF-β1 mRNA expression levels significantly. However, combination therapy with Tel+Res induced superior effect compared to each used alone.

Conclusion: Resveratrol showed a renoprotective effect manifested as significant reduction of TGF-β1 with its fibrogenic effect and being an herbal drug could be advised for early-stage diabetics without evident manifestations of nephropathy as a prophylactic therapy and due to the additive beneficial effects of its combination with telmisartan, both could be used as combination therapy for patients developed manifestations.

Key Words: Resveratrol – Diabetic nephropathy – Rat – Telmisartan – TGF-β1

Introduction

DIABETIC nephropathy (DN) is the leading cause of end stage renal disease (ESRD), and the incidence of DN continues to rise, [1]. Persistent proteinuria is the hallmark of DN, a condition that is characterized by rise in BP, a deterioration of Glomerular Filtration Rate (GFR), and a dramatic increase in cardiovascular events. The awareness of the important role of proteinuria has improved, leading to a more aggressive therapy of hypertension and blockade of the renin-angiotensin system (RAS). Current first-line therapies include blockade of the RAS with angiotensin-converting enzyme inhibitors (ACEIs) and/or angiotensin receptor blockers (ARBs); these treatments reduce proteinuria and delay time to ESRD in type 1 and type 2 DN; importantly, reduction in proteinuria is associated with an improved cardiovascular outcome in patients with DN, [2,3]. However, there is still a large need to improve prevention of DN and reduce its progression to ESRD and associated cardiovascular events, [4]. Novel and multimodal intervention strategies targeting pathogenic pathways other than angiotensin II are required to afford renoprotection in overt diabetic nephropathy.

Several manifestations of diabetic nephropathy may be a consequence of altered production and/or response to cytokines or growth factors. Transforming growth factor-beta (TGF-β) is one such factor because it promotes renal cell hypertrophy and regulates the production of extracellular matrix molecules. In addition, high ambient glucose increases TGF-β1 mRNA and protein level in cultured proximal tubular cells and glomerular epithelial

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and mesangial cells. Neutralizing anti-TGF-β1 antibodies or antisense TGF-β1 oligodeoxynucleotides prevents the hypertrophic effects of high glucose and the stimulation of matrix synthesis in renal cells. Several reports have described overexpression of TGF-β in the glomeruli and tubulointerstitium of experimental and human diabetes mellitus, [5-7].

While multiple pathophysiological mechanisms contribute to the development of diabetic nephropathy, there is ample evidence to implicate oxidative stress and gluco-oxidative stress in the pathogenesis of this complication. It stands to reason, therefore, that agents which reduce oxidative stress may provide renoprotective effect, [8].

In recent years, considerable focus has been given to an intensive search for novel type of antioxidants from numerous plant materials. Plants with antidiabetic activities provide useful sources for the development of drugs in the treatment of diabetes mellitus and its complications, [9].

Resveratrol is a natural polyphenolic compound, it has been detected in more than 70 plant species, including grapes, peanuts, berries, and pines. Fresh grape skin contains about 50 to 100 µg of resveratrol per gram wet weight, which contributes to a relatively high concentration of resveratrol in grape juice. Resveratrol, 3,4',5 trihydroxystilbene and 3,4',5-stilbenetriol, exists in cis-and trans-stereoisomeric forms. It is the parent molecule of a family of polymers called viniferins. Cis-and trans-resveratrol occur naturally as do their glucosides, [10]. In human clinical trials, high dosages of resveratrol have had few, if any, side effects, and no toxicity, [11]. Growing evidence suggests that resveratrol may play an important role in the prevention of many human diseases. Many of the biological actions of this polyphenol have been attributed to its antioxidant properties, [12].

Resveratrol, as an antioxidant, was tried experimentally as a prophylactic modality for variety of insults; Sogutlu, et al., [13] found that introduction of resveratrol into the peritoneal cavity at the time of surgery reduced adhesion formation effectively; it probably acts through reduction of lipid peroxidation products. Karabulut, et al., [14] investigated the effect of resveratrol on spleen and ileum tissues subjected to hepatic ischemia-reperfusion in rats and reported that resveratrol has a protective effect on spleen and ileal mitochondrial oxidative stress in rats subjected to ischemia-reperfusion. Doganay, et al., [15] investigated if resveratrol can prevent sodium selenite-induced experimental cataract model in rats and found the prevention of the oxidative stress in selenite cataract development by resveratrol support the possibility that high natural consumption of resveratrol in food can help prevention of human senile cataract.

The present study was designed to evaluate the effect of prophylactic administration of resveratrol or an angiotensin II receptor antagonist (telmisartan) and their combination on development and progression of diabetic nephropathy in streptozotocin-induced diabetes in albino rats. With a focus on their effect on TGF-β1 level in urine and TGF-β1 mRNA expression in the renal tissue.

Material and Methods

Animals:

Adult male Sprague-Dawley rats weighing 200-250g were purchased from Helwan farm (VAC-SERA), Egypt. The animals were housed (4 per cage) in the animal facility of the Pharmacology department, Faculty of Medicine, Benha University, Egypt, for one week for acclimatization. Rats were kept under the standard laboratory conditions (12h light/dark cycle at 25±2°C) with free access to standard balanced diet and fresh-water supply.

Induction of diabetes:

Diabetes mellitus (DM) was induced by intra-peritoneal injection with a single dose of streptozotocin (STZ) (Sigma) after an overnight fast in a dose of 50mg/kg body weight dissolved in 0.2ml of citrate buffer (pH 4.5), [16]. On the third day of injection, the animals were checked for the presence of glucose in the urine using enzymatic test strips as STZ induces diabetes within 3 days by destroying the beta cells, [17]. Confirmation was done by measuring fasting blood glucose levels; by taking a drop of blood from the rat-tail using the One Touch Blood Glucose Monitoring System (Life Scan, Milpitas, CA). Rats had blood glucose levels of ≥200mg/dl were considered diabetic, [16].

Drugs:

Telmisartan (Boehringer Ingelheim, Germany) and Resveratrol (Sigma) pure powder were suspended in 0.5% carboxymethylcellulose (vehicle) so that 1ml of the vehicle contained the desired dose. The doses used in this study are within the therapeutic doses of human and were calculated to rat dose using conversion table devised by Paget and Barnes, [18]. These doses were also used in previous studies, [19,20]. Drugs were given by the oral route through an orogastric catheter.

Grouping and dosing: A total of 48 male albino rats were randomly divided into:
Control groups: Consisted of 2 negative control groups (n=8 for each), one received no medications and the other animals injected intraperitoneally with one injection of citrate buffer and received 1ml/rat of 0.5% carboxymethylcellulose orally for 12 weeks. The third control group included 8 rats with STZ induced type 2 DM and started daily therapeutic regimen (Positive control group).

The study group: Included 24 animals had induced T2DM and started daily therapeutic regimen for 12 weeks once diagnosis of DM was assured, [21] by estimation of blood glucose level. It was divided into the following equal groups (n=8):

A- Group 1: Rats were administered telmisartan (Tel) in a dose of (5mg/kg/day p.o.), [19] for a period of 12 weeks.

B- Group 2: Rats were administered resveratrol (Res) in a dose of (10mg/kg/day p.o.), [20] for a period of 12 weeks.

C- Group 3: Rats were administered combination of telmisartan (5mg/kg/day p.o.) and resveratrol (10mg/kg/day p.o.) for a period of 12 weeks.

At the end of 12-weeks after induction of diabetes, body weight and systolic blood pressure were assessed. Systolic blood pressure (SBP) was recorded non-invasively in restrained conscious rats by tail cuff plethysmography (Harvard, UK) at the same time of day. The mean of three successive measurements of each rat was recorded. Blood samples, withdrawn from the tail vein, were obtained; the plasma was separated by centrifugation and used for estimation of glucose by glucose oxidase method, [22].

For collection of 24-h urine, animals were housed individually in metabolic cages. Blood samples were collected and allowed to clot then serum was separated by centrifugation at 3000rpm for 10min. Serum and urinary creatinine were determined by measuring rate of formation of creatinine-picrate colored complex in alkaline solution using spectrophotometer (Jaffe method), [23]. Creatinine clearance rate (Ccr) was calculated and expressed as milliliters per minute, [24]. Serum urea was estimated using modified Berthelot reaction, [25]. Urinary protein was measured using a pyrogallol red microtiter plate technique (Bradford method); proteinuria was expressed as milligrams of protein per 24h. [26].

A fresh urine sample was collected at the end of 12-weeks after induction of diabetes for estimation of urinary TGF-ß1. Collected urine samples were centrifuged at 1500rpm for 10min to remove particulate matter, a 2.0ml aliquot of clear supernatant was then immediately stored at 80°C and thawed for assay on the same day. The urinary total TGF -ß1 levels were determined by enzyme-linked immunosorbent assays (ELISA) using commercially available kits (TGF -ß1 immunoassay; Quantikine; R and D system, Minneapolis, MN) according to the manufacturers’ instructions. Briefly, urine samples were incubated with 1.0N HCl for 10min to activate latent TGF -ß1 to the immunoreactive form and then neutralized with 1.2 N NaOH/0.5M HEPES buffer (pH 7.2-7.6). Absorbance was measured at 450nm using a plate reader. TGF -ß1 values are presented as picograms (of TGF-ß1) per mg of creatinine (pg/mg cr), to correct for variations in urine amount. [27].

Quantitative determination of cortical TGF -ß1 mRNA expression:

Cortical total RNA was extracted with TRIzol reagent (Gibco BRL, Berlin, Germany), according to the manufacturer’s instructions. The mRNA expression of TGF -ß1 was determined by a “two-step” RT-PCR, as previously described, [28]. A cDNA copy was created with reverse transcriptase from an RNA PCR Core kit (Roche Applied Biosystems). Real-time PCR was performed using the Light Cycler System and SYBR Green I as double-stranded DNA binding dye (Roche Diagnostics, Mannheim, Germany) using the following primer pair (annealing temperature in parentheses):

sense 5’-GGTGGCGAGGGAGCGCCTGA-3’
antisense 5’-GGCATGGTAGCCCTTGGGCT-3’.

Step “RT-PCR, as previously described, [28]. A cDNA copy was created with reverse transcriptase from an RNA PCR Core kit (Roche Applied Biosystems). Real-time PCR was performed using the Light Cycler System and SYBR Green I as double-stranded DNA binding dye (Roche Diagnostics, Mannheim, Germany) using the following primer pair (annealing temperature in parentheses):

sense 5’-GATGGTGCGAGGGAGCGCCTGA-3’
antisense 5’-GGCATGGTAGCCCTTGGGCT-3’ (64°C).

Statistical analysis:

All data were expressed as mean ± S.E.M. and analyzed with statistical package SPSS (Version 10, 2002) for Windows. One-way analysis of variance (ANOVA) was used to determine statistically significant differences among the groups, and means of every two different groups were detected with Student’s t-test. p<0.05 was considered statistically significant.

Results

Estimated variables showed a non-significant (p>0.05) difference between negative and vehicle control groups, so all statistical analysis of study groups were conducted versus negative control group.

At 12-w after induction of diabetes, body weight measures of studied animals were significantly (p<0.05) lower compared to the negative control group. However, body weight measures of animals
received prophylactic therapy were significantly ($p<0.05$) higher compared to positive control animals (DN) with the difference being non-significantly ($p>0.05$) higher in the group received combined therapy, (Table 1).

Blood glucose concentration of rats with DN was significantly ($p<0.05$) higher than that of the negative control but was significantly ($p<0.05$) reduced in diabetic animals received prophylaxis. Diabetic animals received combined prophylaxis showed non-significantly ($p>0.05$) lower plasma glucose level compared to those received either telmisartan or resveratrol alone, (Table 1).

At 12-w after induction of diabetes, diabetic non-treated animals exhibited significantly ($p<0.05$) higher blood pressure measures compared to negative control group. Telmisartan (Tel) used alone significantly ($p<0.05$) reduced blood pressure compared to positive control animals and to animals received resveratrol (Res) alone. Moreover combination of telmisartan with resveratrol induced significant ($p<0.05$) lowering of blood pressure compared to resveratrol alone on contrary, the blood pressure controlling ability of resveratrol was weak and resulted in non-significant ($p>0.05$) difference compared to positive control group. There was insignificant ($p>0.05$) difference between combination therapy versus telmisartan alone, (Table 1).

<table>
<thead>
<tr>
<th>Groups</th>
<th>Body weight (g)</th>
<th>Blood glucose (mg/dl)</th>
<th>Blood pressure (mmHg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control groups</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>354±6.12</td>
<td>81±9.2</td>
<td>118±5.3</td>
</tr>
<tr>
<td>Vehicle</td>
<td>355±4.12</td>
<td>78±8.4</td>
<td>121±5.4</td>
</tr>
<tr>
<td>Positive (DN)</td>
<td>182.25±5.8*</td>
<td>279.72±22.5*</td>
<td>145.8±6.4*</td>
</tr>
<tr>
<td>Single therapy groups</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tel</td>
<td>206.3±8.3‡</td>
<td>193.3±9.4†</td>
<td>116.4±5.3‡</td>
</tr>
<tr>
<td>Res</td>
<td>203.5±7.1†</td>
<td>183.8±8.2†</td>
<td>142.6±6.8*</td>
</tr>
<tr>
<td>Combination therapy</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tel + Res</td>
<td>208.7±6.3*†‡</td>
<td>178.79±9.6*†</td>
<td>118.5±5.6†‡</td>
</tr>
</tbody>
</table>

$*$ : Significant difference versus negative control group.
† : Significant difference versus positive control group (DN).
‡ : Significant difference versus resveratrol alone.
DN : Diabetic nephropathy group.
Tel : Diabetic rats treated with telmisartan (5mg/kg/day p.o. for 12w).
Res : Diabetic rats treated with resveratrol (10mg/kg/day p.o. for 12w).

At 12-w after induction of diabetes; estimated serum urea levels were significantly ($p<0.05$) higher in all diabetic animals compared to negative control group. Diabetic animals received prophylactic therapy using either resveratrol or telmisartan alone or in combination showed significantly ($p<0.05$) lower serum urea levels compared to positive control group (DN). However, serum urea levels showed non-significant ($p>0.05$) difference among animals received prophylactic therapy, (Table 2). Furthermore, estimated serum creatinine levels were significantly ($p<0.05$) higher in all diabetic animals compared to negative control group. Diabetic animals received prophylactic therapy showed significantly ($p<0.05$) lower creatinine levels compared to positive control group. However, serum creatinine levels showed non-significant ($p>0.05$) difference among animals received single prophylactic therapy. Animals received telmisartan and resveratrol combination showed significantly ($p<0.05$) lower serum creatinine levels compared to resveratrol alone, and there was insignificant ($p>0.05$) difference between combination therapy versus telmisartan alone, (Table 2).

As regards creatinine clearance rate, all diabetic animals showed significantly ($p<0.05$) lower rates compared to negative control group. Animals received telmisartan prophylaxis, either alone or combination, showed significantly ($p<0.05$) higher clearance rates compared to positive control group (DN), while resveratrol prophylaxis showed non-significantly ($p>0.05$) higher clearance rates compared to positive control group. There was non-significant ($p>0.05$) difference among groups received mono-therapeutic prophylaxis, (Table 2).

| Table (1): Mean (±SE) body weight, blood glucose and blood pressure reported in all studied groups (n=8). |

<table>
<thead>
<tr>
<th>Groups</th>
<th>Body weight (g)</th>
<th>Blood glucose (mg/dl)</th>
<th>Blood pressure (mmHg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control groups</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>354±6.12</td>
<td>81±9.2</td>
<td>118±5.3</td>
</tr>
<tr>
<td>Vehicle</td>
<td>355±4.12</td>
<td>78±8.4</td>
<td>121±5.4</td>
</tr>
<tr>
<td>Positive (DN)</td>
<td>182.25±5.8*</td>
<td>279.72±22.5*</td>
<td>145.8±6.4*</td>
</tr>
<tr>
<td>Single therapy groups</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tel</td>
<td>206.3±8.3‡</td>
<td>193.3±9.4†</td>
<td>116.4±5.3‡</td>
</tr>
<tr>
<td>Res</td>
<td>203.5±7.1†</td>
<td>183.8±8.2†</td>
<td>142.6±6.8*</td>
</tr>
<tr>
<td>Combination therapy</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tel + Res</td>
<td>208.7±6.3*†‡</td>
<td>178.79±9.6*†</td>
<td>118.5±5.6†‡</td>
</tr>
</tbody>
</table>

| Table (2): Mean ± SE serum urea and creatinine levels and creatinine clearance rates of studied animals (n=8). |

<table>
<thead>
<tr>
<th>Groups</th>
<th>Serum urea (mg/dl)</th>
<th>Serum creatinine (mg/dl)</th>
<th>Cr. Clearance (ml/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control groups</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>22±0.88</td>
<td>0.66±0.06</td>
<td>1.3±0.05</td>
</tr>
<tr>
<td>Vehicle</td>
<td>23.9±0.81</td>
<td>0.69±0.05</td>
<td>1.4±0.05</td>
</tr>
<tr>
<td>Positive (DN)</td>
<td>56.9±2.05*</td>
<td>1.85±0.07*</td>
<td>0.86±0.07*</td>
</tr>
<tr>
<td>Single therapy groups</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tel</td>
<td>44.2±2.01†‡</td>
<td>1.48±0.06*†</td>
<td>1.1±0.04*†</td>
</tr>
<tr>
<td>Res</td>
<td>47±2.12*†</td>
<td>1.62±0.06†</td>
<td>0.92±0.13*</td>
</tr>
<tr>
<td>Combination therapy</td>
<td></td>
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<tr>
<td>Tel + Res</td>
<td>42.9±1.9†</td>
<td>1.36±0.04†‡</td>
<td>1.2±0.05†‡</td>
</tr>
</tbody>
</table>

* : Significant difference versus negative control group.
† : Significant difference versus positive control group (DN).
‡ : Significant difference versus resveratrol alone.
DN : Diabetic nephropathy group.
Tel : Diabetic rats treated with telmisartan (5mg/kg/day p.o. for 12w).
Res : Diabetic rats treated with resveratrol (10mg/kg/day p.o. for 12w).
All diabetic animals showed significantly ($p < 0.05$) higher urinary protein excretion compared to negative control group. Animals received prophylaxis showed significantly ($p < 0.05$) lower urinary protein levels compared to positive control animals (DN). There was non-significant ($p > 0.05$) difference as regards the extent of proteinuria among animals received mono-therapeutic prophylaxis. However, animals received combination of telmisartan with resveratrol showed significantly ($p < 0.05$) lower proteinuria compared to those received telmisartan or resveratrol alone, (Table 3).

Table (3): Mean ($\pm$SE) 24-hours urinary protein estimated in studied animals compared to control groups (n=8).

<table>
<thead>
<tr>
<th>Groups</th>
<th>24-h urinary protein (mg/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Control groups</strong></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>13.1±0.71</td>
</tr>
<tr>
<td>Vehicle</td>
<td>13.8±0.67</td>
</tr>
<tr>
<td>Positive (DN)</td>
<td>297±9.7*</td>
</tr>
<tr>
<td><strong>Single therapy groups</strong></td>
<td></td>
</tr>
<tr>
<td>Tel</td>
<td>168.8±7.7*†</td>
</tr>
<tr>
<td>Res</td>
<td>182.1±8.16*†</td>
</tr>
<tr>
<td><strong>Combination therapy group</strong></td>
<td></td>
</tr>
<tr>
<td>Tel+Res</td>
<td>130±8.27*†‡‡#</td>
</tr>
</tbody>
</table>

* : Significant difference versus negative control group.  
† : Significant difference versus positive control group (DN).  
‡ : Significant difference versus telmisartan alone.  
# : Significant difference versus resveratrol alone.  
DN : Diabetic nephropathy group.  
Tel : Diabetic rats treated with telmisartan (5mg/kg/day p.o. for 12 weeks).  
Res : Diabetic rats treated with resveratrol (10mg/kg/day p.o. for 12 weeks).

Quantitative PCR estimation of renal tissue TGF-$\beta_1$ mRNA expression level in diabetic animals was significantly higher compared to negative control group with significantly higher expression levels in DN group compared to all other diabetic groups. Single-line prophylaxis despite significantly decreased TGF-$\beta_1$ mRNA expression level the difference among groups was non-significant despite being in favor of resveratrol. On the other hand, combined telmisartan-resveratrol therapy significantly reduced TGF-$\beta_1$ mRNA expression level compared both to each used alone. [(Table 4) and Fig. (2)].

Table (4): Mean ($\pm$SE) urinary TGF-$\beta_1$ level (pg/mg creatinine) and renal tissue TGF-$\beta_1$ mRNA level (ng/ml) estimated in studied animals compared to control groups (n=8).

<table>
<thead>
<tr>
<th>Groups</th>
<th>Urinary TGF-$\beta_1$</th>
<th>Renal tissue TGF-$\beta_1$ mRNA level (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Control groups</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>1.6±0.3</td>
<td>45.6±4.9</td>
</tr>
<tr>
<td>Vehicle</td>
<td>1.9±0.4</td>
<td>47.5±6.2</td>
</tr>
<tr>
<td>Positive (DN)</td>
<td>39.6±4.8*</td>
<td>435±20*</td>
</tr>
<tr>
<td><strong>Single therapy groups</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tel</td>
<td>26.82±1.9 *†</td>
<td>254±15.9*†</td>
</tr>
<tr>
<td>Res</td>
<td>21.42±1.8 *†‡#</td>
<td>246±13.5*†</td>
</tr>
<tr>
<td><strong>Combination therapy group</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tel+Res</td>
<td>16.2±1.5 *†‡‡#</td>
<td>130±10*†‡‡#</td>
</tr>
</tbody>
</table>

* : Significant difference versus negative control group.  
† : Significant difference versus positive control group (DN).  
‡ : Significant difference versus resveratrol alone.  
# : Significant difference versus telmisartan alone.  
DN : Diabetic nephropathy group.  
Tel : Diabetic rats treated with telmisartan (5mg/kg/day p.o. for 12 weeks).  
Res : Diabetic rats treated with resveratrol (10mg/kg/day p.o. for 12 weeks).

Fig. (1): Urinary TGF-$\beta_1$ levels estimated in studied animals at 12 weeks after induction of diabetes. Data are presented as mean±SEM (n=8).

NC : Negative control group.  
VC : Vehicle control group.  
DN : Diabetic nephropathy group.  
Tel : Diabetic rats treated with telmisartan (5mg/kg/day p.o. for 12 weeks).  
Res : Diabetic rats treated with resveratrol (10mg/kg/day p.o. for 12 weeks).  
Tel+Res : Diabetic rats treated with combination of telmisartan and resveratrol in the same doses.
Discussion

Prevention or retardation of diabetic nephropathy (DN) has become a major goal in biomedical research. Local renal renin-angiotensin system (RAS) activation and increased oxidative stress have been implicated in the pathogenesis of diabetic nephropathy, which is clinically characterized by proteinuria and progressive renal insufficiency. Proteinuria not only indicates renal damage, but is also a powerful predictor of cardiovascular morbidity and mortality at least in patients with high cardiovascular risk and potentially pre-existing vascular damage. Management of the multiple factors for renal and cardiovascular disease is mandatory in the diabetic patient, [29,30].

In the present study, body weight measures of animals received prophylactic therapy with telmisartan or resveratrol were significantly higher compared to positive control animals despite being significantly lower compared to negative control groups. These data illustrate the effect of applied therapies on the metabolic milieu of studied animals improving insulin sensitivity and thereby improving fuel utilization and so on weight gaining. In addition, blood glucose level was significantly decreased in animals received prophylactic therapy compared to positive control (DN) animals despite being significantly lower compared to negative control groups. In support of such assumption, Huang, et al., [31] suggested that a local pancreatic renin-angiotensin system and renin-angiotensin system inhibitors may be selectively controlling pancreatic islet blood flow, augmenting insulin secretion and thereby improving glucose tolerance and concluded that the antidiabetic actions of renin-angiotensin system inhibitors might occur, in part, through this beneficial direct islet effects. Also, Cetinkalp, et al., [32] found the short-term treatment with renin-angiotensin system inhibitors is effective to decrease microalbuminuria in normotensive type-2 diabetes patients independent of its antihypertensive effect.

Furthermore, Palsamy and Subramanian [33] reported that based on histological and ultrastructural observations, oral administration of resveratrol may effectively rescue beta-cells from oxidative damage without affecting their function and structural integrity and exhibits significant antidiabetic potential by attenuating hyperglycemia, enhancing insulin secretion and antioxidant competence in pancreatic beta-cells of diabetic rats. Moreover, Palsamy, et al., [34] found oral administration of resveratrol to diabetic rats showed a significant decline in hepatic proinflammatory cytokines and notable attenuation in hepatic lipid peroxides, hydroperoxides and protein carbonyls effectively rescues the hepatocytes from hyperglycemia-mediated oxidative damage without affecting its cellular function and structural integrity.

The current experimental model of diabetic nephropathy illustrated the beneficial effects of prophylactic administration of telmisartan or resveratrol alone or in combination during the early stage of diabetes. These effects were manifested as reduction of manifestations of DN, hypertension and proteinuria with a significant difference compared to positive control animals developed DN without prophylaxis. Despite the effect of each therapeutic line was variant telmisartan alone provided the best chance for control of both hypertension and proteinuria compared to resveratrol alone. These data indicated an extent of renoprotection provided by all of the three regimens as single or combined treatment.

These data go in hand with multiple previous studies suggested such renoprotection by telmisartan; the results obtained by Masuda, et al., [35] suggested that ARB, particularly telmisartan, is effective in reducing proteinuria in hypertensive patients with overt diabetic nephropathy, partly through inhibitory effects on ambulatory short-term BP variability and sympathetic nerve activity, in addition to its longer duration of action on night time BP reduction. Also, Nakamura, et al., [36]
found angiotensin II receptor blockers have renoprotection and this effect of telmisartan appears to be more potent than that of losartan, candesartan, or olmesartan in early-stage diabetic nephropathy patients.

The TGF-β1 has a documented role in pathogenesis of diabetic nephropathy. In vitro, studies have shown that a range of stimuli increase TGF-β1 expression, such as hyperglycemia, and various products of oxidative stress. Indeed, TGF-β1 seems to be an important site of interaction between hemodynamic and metabolic pathways, playing a key role in the synergy between hypertension and hyperglycemia in mediating DN, [37,38]. So, the present study evaluated the urinary levels of TGF-β1 as a marker for glomerular fibrosis of experimentally-induced diabetic nephropathy. Animals developed DN without prophylaxis showed significantly higher renal tissue TGF-β1 mRNA expression and urinary TGF-β1 levels compared to negative controls and to those received prophylaxis. However, the inhibitory effect was superior with combination therapy compared to single-line therapy. The combination of telmisartan and resveratrol was the superior in inhibiting both expression of renal tissue TGF-β1 mRNA and its transcription to its protein product TGF-β1.

Consistent with the obtained data, Tsunenari, et al., [39] reported that telmisartan decreased TGF-β1 reactivity in the glomerular tissue as assessed by immunohistochemical staining thus ameliorates the progressive nephropathy in the remaining kidney in 5/6 nephrectomised rats by non-haemodynamic as well as antihypertensive actions of the drug.

The reported inhibitory effect of resveratrol on TGF-β1 levels, either mRNA or the protein product, coincided and supported that previously reported in literature illustrating the effect of resveratrol on fibrosis elsewhere in the body; Jang and Pezzuto [40] found pre-treatment of mouse skin with resveratrol prior to application of 12-O-tetradecanoylphorbol-13-acetate negated several of its-induced effects in a dose-dependent manner with selective inhibition of expression of c-fos and TGF-β1. Serrero and Lu [41] found resveratrol inhibited the expression of the autocrine growth stimulators TGF-β, PC cell-derived growth factor, and insulin-like growth factor I receptor mRNA, in addition, resveratrol significantly elevated the expression of the growth inhibitor TGF-β2 mRNA without changes in TGF-β1 and TGF-β3 expression.

Chávez, et al., [42] tried to elucidate the antifibrogenic mechanism of resveratrol, evaluating the extent of NF-kappaB activation and TGF-β production in carbon tetrachloride (CCI4) model of cirrhosis, CCl4 increased, but resveratrol abolished the changes induced in both factors especially for TGF-β1 that was increased about three fold and resveratrol decreased it under control values, so Chávez, et al., [42] concluded that resveratrol possesses a strong antifibrogenic effect probably associated with its ability to reduce NF-kappaB activation and TGF-β content.

In support of the prophylactic effect of resveratrol in diabetic nephropathy model; Losso, et al., [43] suggested that trans-resveratrol can protect the retinal pigment epithelial cells against hyperglycemia-induced low-grade inflammation and gap junction intercellular communication degradation. Li, et al., [44] investigated Smad 3 protein acetylation in renal fibrosis and found TGF-β1 stimulation of renal fibroblasts and tubular epithelial cells induced Smad3 acetylation and phosphorylation, but resveratrol, an activator of the nicotinamide adenine dinucleotide dependent protein deacetylase, reversed acetylation but not phosphorylation of Smad3 and inhibited TGF-β1–induced up-regulation of collagen IV and fibronectin mRNA levels.

Considering the urinary levels of TGF-β1 as a marker for the impact on both renal fibrogenesis and renal tissue TGF-β1 mRNA expression, resveratrol prophylaxis significantly reduced urinary TGF-β1 levels compared to telmisartan alone. Moreover, combination of resveratrol and telmisartan showed significant difference versus resveratrol alone.

Thus, it could be concluded that resveratrol showed a renoprotective effect manifested as significant reduction of TGF-β1 with its fibrogenic effect and being an herbal drug could be advised for early-stage diabetics without evident manifestations of nephropathy as a prophylactic therapy and due to the additive beneficial effects of its combination with telmisartan, both could be used as combination therapy for patients developed manifestations.

References


