

Resveratrol attenuates nephropathy in streptozotocin-induced diabetic rats

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Abstract: Diabetes mellitus is characterized by hyperglycemia and is associated with disturbances in carbohydrate, protein and fat metabolism which occurs secondary to an absolute (type I) or relative (type II) lack of insulin. The aim of present study was to evaluate the effects of resveratrol on nephropathy subsequent diabetes mellitus in rats. In this study, Forty eight healthy male Wistar rats (about 180–200 g body weight) were randomly divided into 4 groups (12 rats each): group 1: normal control; group 2: diabetic rats; group 3: diabetics received resveratrol and group 4: non-diabetics received no treatment. After 4 month, animals of different groups were sacrificed under light anesthesia (ketamine 80mg/kg) 1 day after the end of the treatment. The kidneys fixed in a 10% neutral-buffered formalin solution were embedded in paraffin and were used for histopathological examination. Results showed that there was statistically significant difference between control and treatment group in term of mentioned injuries ($P < 0.005$). Also, there was statistically significant difference between control and treatment group in term of vasculopathy ($P < 0.05$). In conclusion, the present study provides evidence that RSV reduced plasma glucose and creatinine, oxidative stress, proinflammatory cytokines and up-regulated AMPK proteins in diabetes which may contribute to its renoprotective effects in the early stage of DN.

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1. Introduction

Diabetes mellitus (DM), mainly characterized by recurrent hyperglycemia, had become one of the chronic disorders derived from insulin deficiency or resistance in the developed countries. As the high blood glucose level in diabetes persisted and progressed without appropriate medical care, relative secondary disorders involving atherosclerosis, retinopathy, nephropathy, neuropathy, stroke, and foot ulcer would individually develop with an insidious onset, which could eventually be life-threatening. Diabetic nephropathy (DN), the second most prevalent diabetes-associated complication inferior to cardiovascular disorders, impaired the renal function of DM patients and therefore cost appreciable medical labor and resource for DN management annually. Histologically featured by thickening of basement membrane, expansion and nodular aggregation of mesangial matrix (the Kimmelstiel-Wilson lesions) and sclerosis in glomeruli, DN could be multifactorial in the pathogenesis. In these risk factors, hyperglycemia was currently regarded as one of the leading causes in the progression of DN. Accumulating evidence also suggested the development of DN was associated with the activation of several stress-sensitive signal pathways, including nuclear factor kappa B (NF- κ B) and mitogen-activated protein kinase (MAPK) (Nishikawa et al., 2000; Brownlee, 2001; Joseph et al., 2002; Chen et al., 2008). Additionally, it was reported that both oxidative stress (Dhaunsi and

Bitar, 2004; Araujo and Welch, 2006; Fujii et al., 2010; Xiao et al., 2009) and proinflammatory cytokines (Vlassara et al., 2009; Wu et al., 2009) detrimentally accelerated the pathological process of DN. Adenosine monophosphate-activated protein kinase (AMPK), a regulator of cellular energy homeostasis, was recently identified to play an important role in DN (Lee et al., 2007). Decreased phosphorylation of AMPK was contributed to hyperglycemia-associated renal enlargement. Further studies indicated that suppression of AMPK activity was linked with oxidative stress (Wang et al., 2010) and inflammatory response (Ko et al., 2009). Reversion of AMPK activity could ameliorate oxidative damage (Ceolotto et al., 2007) and inflammation (Jeong et al., 2009). Thus, attention has been drawn to the modulation of AMPK signal transduction to attenuate DM-affected renal dysfunction. Resveratrol (trans-3,4',5-trihydroxyestilbene, RSV), one naturally existing polyphenolic compound rich in grapes and several plants, was characterized as a potently free radical scavenger and antioxidative agent. Besides, RSV was pronounced to possess both cardioprotective (Hung et al., 2004; Lin et al., 2008; Chan et al., 2008) and antidiabetic benefits (Su et al., 2006; Chi et al., 2007). A vast majority of reports also supported that RSV displayed a hypoglycemic effect on DM animal models via AMPK stimulation (Baur et al., 2006; Zang et al., 2006; Penumathsa et al., 2008; Um et al., 2010). In DN studies, RSV was proved to mitigate

renal dysfunction and oxidative stress in type 1 diabetic rats (Sharma et al., 2006; Dhaunsi and Bitar, 2004). The aim of present study was to evaluate the effects of resveratrol on nephropathy subsequent diabetes mellitus in rats.

2. Materials and methods

In this study, Forty eight healthy male Wistar rats (about 180–200 g body weight) were purchased from Animal House, Islamic Azad University. All animals were conditioned at room temperature at a natural photoperiod for 1 week before experiment execution. A commercial balanced diet and tap water ad libitum were provided. The duration of experiment was 3 month. The rats were randomly divided into 4 groups (12 rats each) as the following: Group 1, healthy control rats received no treatment; group 2 diabetic rats received no treatment; rats of group 3 received resveratrol (5 ml/kg) orally for 3 month one week after induction diabetes (Ronald et al., 2005), rats of group 4 were non-diabetic treated with resveratrol 5 mg/kg orally. For induction the diabetes rats received streptozotocin at the dose of 50 mg/kg i.p. 15 minutes following the i.p. nicotinamide at 110 mg/kg and blood glucose more than 250 mg/dl 48 hours after induction considered as diabetic rats (Par Stephan et al., 2006). After 4 month, animals of different groups were sacrificed under light anesthesia (ketamine 80mg/kg) 1 day after the end of the treatment (Hisayo et al., 2004). The kidneys fixed in a 10% neutral-buffered formalin solution were embedded in paraffin and were used for histopathological examination. Five micrometer-thickness sections were cut, deparaffinized, hydrated, and stained with hematoxylin-eosin. The renal sections were examined blindly for tubular cell swelling, interstitial edema, tubular dilatation, and moderate to severe necrosis in all treatments. A minimum of 10 fields for each kidney slide were examined and assigned for severity of changes.

2.1. Statistical Analysis

The Statistical Package for Social Sciences (SPSS Inc., Chicago, IL, USA), version 13.0, was used for statistical analysis. All data are presented as mean \pm SEM. Before statistical analysis, all variables were checked for normality and homogeneity of variance by using the Kolmogorov-Smirnoff and Levene tests, respectively. The data obtained were tested by ANOVA followed by Tukey's posthoc multiple comparison test. The Kruskal-Wallis test, followed by Mann-Whitney U posttest, was used for the analysis of degree of histopathological kidney injury. $P < 0.05$ was considered statistically significant.

3. Results

The photomicrographs showed a diffuse glomerulosclerosis extended in renal of control group

which was obvious as diffuse increase in mesangial matrix and proliferation of mesangial with thickening glomeruli basal membrane (fig 1). Sometimes, glomeruli and periglomerular sclerosis changes were more severe. But, in treatment group, these changes were reduced at minimal levels. There was statistically significant difference between control and treatment group in term of mentioned injuries ($P < 0.005$).

Renal arteriosclerosis was obvious in diabetic rats as hyalinization of epithelium with stenosis of renal parenchyma vessels, but, in treatment group, these changes were mild or not observed (fig 2). There was statistically significant difference between control and treatment group in term of vasculopathy.

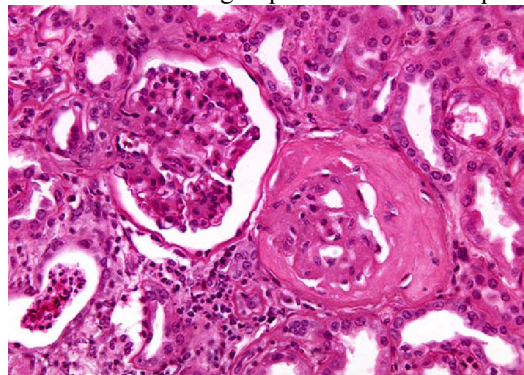


Fig 1: Photomicrograph from renal of diabetic rats. Severe glomerulosclerosis is obvious. The thickening of Bowman's capsule epithelium and severe sclerosis is clear. H&E; 20x.

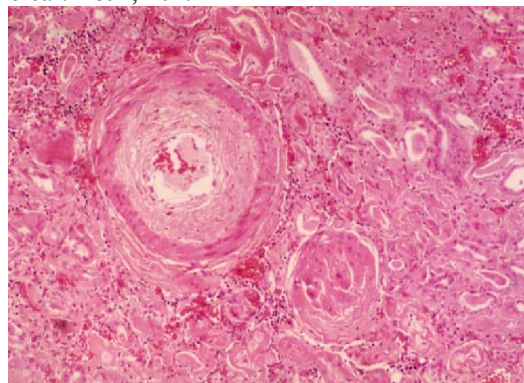


Fig 2: Photomicrograph from renal of diabetic rats. Hyaline arteriosclerosis is obvious in which the arterial epithelium got hyalinized and lumen has stenosis. H&E; 40.

Infiltration of mononuclear inflammatory cells such as macrophages and lymphocytes surround the renal parenchyma vessels with mild fibrosis were other microscopic findings. These changes were not observed in the treatment group. Inflammation and clear hypertrophy was seen in the epithelial cells of distal and proximal tubules with vascular nephrosis but

not in the treatment group, in the trace level. Severe proteinuria (fig 4) was another obvious pathologic finding which was seen as protein sediments or hyaline casts (fig 3) in the tubular duct. These changes were at the minimum level in the treatment group. Also, it must be remembered that total urine protein in treatment group was lesser than control group significantly.

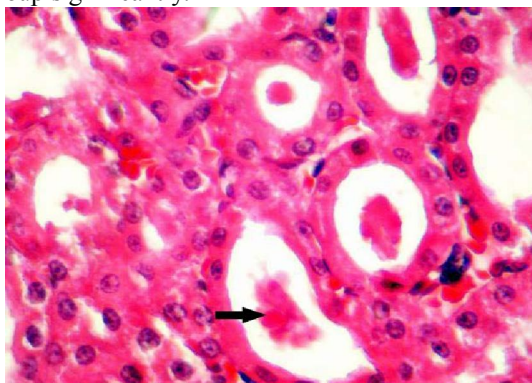


Fig 3: photomicrograph from renal of diabetic group, hyaline cast (arrow). H&E; 40x.

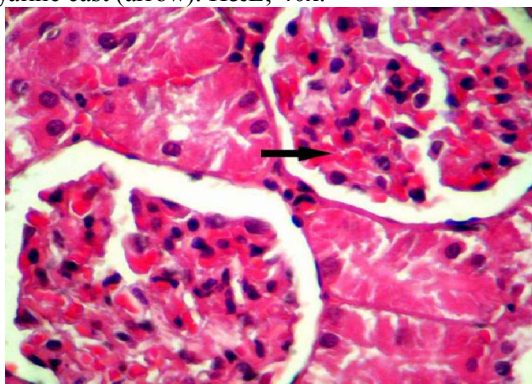


Fig 4: photomicrograph from renal of treatment group (received resveratrol), mild proteinuria without glomerular sclerosis. Thickening of capillary basal membrane (arrow) is obvious. H&E; 40x.

4. Discussion and conclusion

In the present study, we claimed that RSV significantly prevented loss of body weight, lowered plasma glucose and creatinine concentrations, and increased plasma insulin level, to moderate extents in the STZ-diabetic rats. It has been shown that hyperglycemia promoted oxidative stress in nephritic tissues, eventually leading to renal injury in diabetes. Augmentation of free radicals and impairment of key antioxidant enzymes were believed to contribute to the development of DN. The ameliorative effects of RSV on hyperglycemia-associated oxidative stress were widely recognized (Sharma et al., 2006). RSV was proved to possess an insulin-like property in vivo (Su et al., 2006).

Further, there was increasing evidence implicating that RSV alleviated oxidative stress in a variety of hyperglycemia-affected tissues, including renal (Sharma et al., 2006), neuron (Kumar et al., 2007), vascular endothelial (Ungvari et al., 2009), and pancreatic β cells (Palsamy and Subramanian, 2010). One recent study indicated that RSV prevented lipid peroxidation and increased glutathione contents and activities of SOD and catalase in STZ-induced diabetic kidneys (Sharma et al., 2006). It was also reported that RSV decreased the generation of reactive oxygen species (ROS) and nitric oxide in high glucose-exposed porcine renal proximal tubular cells (Fujii et al., 2006). However, our results suggested that RSV partially attenuated hyperglycemia-associated oxidative injury mediated by reduction of superoxide anion and protein carbonyl levels.

Attraction has been drawn to the correlation between inflammatory activity and diabetic complications. There was accumulating evidence indicating that renal inflammation played a key role in the pathogenesis of DN. It was demonstrated that diabetes increased proinflammatory cytokines including TNF- α , IL-1 β and IL-6 in the circulating (Kaul et al., 2010), renal production (Huang and Siragy, 2009; Matavelli et al., 2010), and urinary excretion (Bondar' et al., 2008).

Recently, several researches have proved that RSV did not suppress but augmented signals responsible for inflammation in the renal tissues of diabetes. Gene and protein expressions of COX, one prostaglandin synthase mainly activated in certain inflammatory conditions, were not suppressed by RSV treatment in the renal tissues of diabetic rodents (Yar et al., 2010). Instead, RSV enhanced NF- κ B activity in the renal tubular and mesangial cells exposed to cytokine mixtures (Uchida et al., 2005). Although these reports indicated the proinflammatory potential of RSV which was similar to our experimental results, they were contrastive to previous studies identifying the anti-inflammatory property of RSV in diabetes.

Under physiological circumstances, the signaling regulations of cellular energy like insulin cascades were predominated by both Akt and AMPK. It was demonstrated that suppression of AMPK was interposed by hyperglycemia and elevated Akt activity (Lee et al., 2007). In addition, a recent study revealed that reduced phosphorylation of AMPK appeared to be reversed under RSV treatment in the diabetic kidney (Ding et al., 2010).

In conclusion, the present study provides evidence that RSV reduced plasma glucose and creatinine, oxidative stress, proinflammatory cytokines and up-regulated AMPK proteins in

diabetes which may contribute to its renoprotective effects in the early stage of DN.

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