Endocrine (2012) 42:592–598
DOI 10.1007/s12020-012-9670-7

ORIGINAL ARTICLE

Radix Astragali lowers kidney oxidative stress in diabetic rats treated with insulin

Y. Gao · R. R. Zhang · J. H. Li · M. Ren · Z. X. Ren · J. H. Shi · Q. Z. Pan · S. P. Ren

Received: 18 January 2012 / Accepted: 4 April 2012 / Published online: 20 April 2012

Abstract Fluctuations in glucose levels in diabetic patients can result in oxidative stress, resulting in an increased risk for diabetic complications. We investigated whether antioxidation would protect the kidney from oxidative stress in diabetic rats treated with insulin and provide evidence for the efficacy of antioxidant treatment in diabetes management. Diabetes was induced by injection of Streptozotocin intraperitoneally in male Wistar rats. Diabetic rats received either insulin, both insulin and Radix Astragali (RA), RA, or no treatment. The levels of malondialdehyde (MDA), interleukin 6 (IL-6), tumor necrosis factor α (TNF-α) and activities of superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) in kidney were determined. The changes of blood glucose levels and body weight were monitored. The levels of serum creatinine (Scr) were determined. The expression of PKCα was determined by western blot. NF-κB activation in kidney was assessed using EMSA. Compared to diabetic rats treated with insulin alone, the diabetic rats treated with combination of insulin and RA showed: (1) significantly lower levels of MDA, IL-6, TNF-α, and Scr ($p < 0.05$); (2) significantly higher SOD and GSH-Px activities ($p < 0.05$); (3) significantly lower NF-κB activation and lower expression levels of PKCα ($p < 0.05$); (4) significantly smaller kidney-to-body weight ratio ($p < 0.05$). RA is an effective agent in lowering oxidative stress in diabetic rats treated with insulin. Antioxidation is beneficial in reducing the risk of kidney damage due to oxidative stress in diabetic patients.

Keywords Radix Astragali · Oxidative stress · Kidney damage · NF-κB

Y. Gao · R. R. Zhang
Department of Endocrinology, First Hospital of Jilin University, Changchun 130021, Jilin Province, China

J. H. Li · M. Ren · Z. X. Ren · J. H. Shi · Q. Z. Pan · S. P. Ren (✉)
Department of Toxicology, School of Public Health, Jilin University, Changchun 130021, Jilin Province, China
e-mail: rensp@jlu.edu.cn

Introduction

Diabetes mellitus is a life-long disease which is characterized by persistent elevated blood glucose levels. One of the consequences of high blood glucose is oxidative stress which is one of the mechanisms of diabetes complications [1]. Local oxidative stress is suggested to be an important factor in the development of diabetic nephropathy [2]. Many diabetic patients and undiagnosed diabetic adults have a certain extent of kidney damage which, if not controlled properly, will result in kidney failure [3]. Diabetic nephropathy accounts for increasing morbidity and mortality in developed countries [4]. Exogenous insulin dominates the current approach to lowering blood glucose levels in diabetes. Although it is successful in reducing blood glucose levels, it cannot completely mimic physiologic secretion of insulin endogenously [5]. Marked fluctuations in glucose levels in diabetic patients will result in oxidative stress even in patients being treated with insulin [6, 7]. Vitamin C and vitamin E do not always work for all diabetic patients [6, 8].

Radix Astragali (RA) is a traditional Chinese herbal medicine which has been used in treating diabetes and kidney abnormalities caused by diabetes [9–12]. RA has the capacity to remove free radicals and help ameliorate proteinuria adriamycin nephropathy [13]. The aim of the study is to explore whether RA can complement insulin

treatment by protecting the kidney from the damage caused by oxidative stress and provide a new, preventive therapy in diabetes treatment.

Materials and methods

Diabetes induction

Diabetes was induced by a single injection of streptozotocin (STZ) (GIBCO, America) at the concentration of 60 mg/kg body weight in a 0.1 M citrate buffer (pH 4.0) intraperitoneally in male Wistar rats (purchased from Animal center of Jilin University, China). The rats had body weights of 200 ± 20 g, free access to food and water, and were kept in light/darkness conditions (12 h each). The control rats received an equivalent amount of citrate buffer. Diabetic rats having a blood glucose level of >16.7 mmol/l at 2 days following STZ-injection were included in this study. Blood samples were obtained from the cut tip of the tail and blood glucose levels were confirmed using a strip operated reflectance meter (Free style K-F095-33749, U.S.).

Experimental procedures

Diabetic rats were divided into four groups of 20 rats each. The DM rats received no treatment; the insulin-treated rats received insulin (Jiangsu Wanbang Biochemical Pharmaceutical Corporation Limited, China) injected intramuscularly once a day based on the determination of blood glucose levels. The insulin dose was adjusted based on the determination of blood glucose levels. Radix Astragali (RA, aqueous extract of Radix Astragali, Harbin Zhenbao Pharmaceutical Corporation limited, China) rats received RA through gastric injection at the dose of 10 g RA per kg body weight, one injection each day. The insulin + RA rats received insulin injection and RA administration with the same dose and route as the insulin-treated and RA rats. Healthy rats were used as controls. Insulin and RA were administered to the diabetic rats for 60 days. After the 60 days, the rats from all five groups were killed and blood samples were collected. Kidneys were dissected, weighed, and frozen in liquid nitrogen. Serum and kidney were stored at −80 °C freezer for further analyses.

Serum creatinine levels determination

Blood samples were stood for 30 min at 4 °C. After centrifugation (3,000 rpm for 10 min at 4 °C), the supernatants were collected and assayed for serum creatinine (Scr). Scr levels were determined by Automatic Biochemistry Analyzer.

Body weight changes monitoring

Body weight of all rats was weighed every 2 days to monitor the changes of body weight in different groups.

Blood glucose levels monitoring

Blood glucose levels in rats from different groups were determined using a strip operated reflectance meter every 2 days and insulin dose was adjusted to keep the blood glucose levels below 8 mmol/l.

Preparation of kidney homogenates

100 mg kidney from cortex was put in 1 ml RIPA lysis buffer (Beyotime Institute of Biotechnology, China) containing 50 mM Tris (pH 7.4), 150 mM NaCl, 1 % Triton X-100, 1 % sodium deoxycholate, 0.1 % SDS, and PMSF (final concentration 1 mM). After homogenization, the lysates were centrifuged for 5 min at 14,000 rpm at 4 °C; the supernatant was removed and stored at −80 °C.

Determination of MDA levels in kidney

Measurements of total malondialdehyde (MDA) levels were performed by the thiobarbituric acid reacting substances (TBARS) test using a commercial kit from Nanjing Jiancheng Bioengineering Corporation (100T Assay, China). Two hundred microliters of kidney homogenates were used for measurements of total MDA. The absorbance of chromogenic product was measured at 532 nm (Spectrophotometer Uvikon 752) and compared with the absorbance in corresponding MDA standards. The total MDA in kidney was expressed as nM per milligram protein.

Determination of glutathione peroxidase activity in kidney

Glutathione peroxidase (GSH-Px) activity determination was carried out by the DTNB colorimetric assay [14]. The enzyme assay tubes were incubated at 37 °C and contained 0.5 ml of 2.0 mM GSH, 0.5 ml of 0.40 M sodium phosphate buffer (pH 7.0) in 20 μl of sample. The reaction was initiated by addition of 0.5 ml of 1.25 mM H_2O_2 after a 5 min preincubation. Two milliliters of metaphosphoric acid solution was then added 5 min later. GSH in the protein-free supernatant was measured by mixing 1.0 ml of supernatant with 1.0 ml of DTNB, and absorbance was recorded at 412 nm. A blank (with buffer substituted for enzyme source) was carried through the incubation simultaneously since non-enzymatic GSH oxidation occurred during incubation. GSH-Px activity was expressed as units/mg protein, and one unit was defined as a

decrease in GSH of 1 mM min^{-1} after the decrease in GSH per minute of the non-enzymatic reaction was subtracted.

Determination of superoxide dismutase activities in kidney

Total superoxide dismutase (SOD) activities were determined by a commercial kit manufactured by Nanjing Jiancheng Bioengineering Corporation, China. The assay used xanthine–xanthine oxidase system to produce superoxide anions, which react with 2-(4-iodophenlyl)-3-(4-nitrophenol)-5-phenyltetrazoliomchloride to form a red formazan dye and the absorbance at 550 nm was determined. The values were expressed as units per milligram of protein, where one unit of SOD activity was defined as the amount of enzyme that inhibits the rate of reaction by 50 % at 25 °C.

IL-6 and TNF-α level determination in kidney

The levels of interleukin 6 (IL-6) and tumor necrosis factor α (TNF-α) in kidney were determined by ELISA (Kits from GBD, America) using kidney homogenates. Proteins in kidney homogenates were measured by enhanced BCA protein assay kit (Beyotime Institute of Biotechnology, China).

Western blot

Western blot analysis was performed as followed, proteins in kidney homogenates were measured by enhanced BCA protein assay kit (Beyotime Institute of Biotechnology, China). Equal amounts of protein (20 μg/well) were loaded and separated by 10 % SDS–polyacrylamide gel electrophoresis and transferred to polyvinylidene difluoride (PVDF) membranes. The membrane was incubated with the first antibodies (mouse anti-PKCα) and second antibody (rabbit anti-mouse IgG conjugated with horseradish peroxidase). Detection was done by measuring the chemiluminescence of ECL reagent (PIERCE, USA). The photographs generated were quantitatively analyzed with a Quantity One image densitometer. Protein levels were standardized by comparison with anti-GAPDH antibody.

Preparation of nuclear extract

Nuclear extract from kidney was performed using nuclear extract kits from Sangon Biotech Co., Ltd (Product No. BSP009, Shanghai, China). The extract procedure was carried out according to the manufacturer's protocol. 100 mg kidney was homogenized with 0.6 ml cold hypotonic buffer and the homogenates were transferred into a 1.5 ml centrifuge tube and incubated on ice for 10 min. The homogenates were centrifuged at 3,000 rpm, 4 °C for 5 min, after the supernatant was removed and 0.4 ml cold hypotonic buffer was added to the pellets and vortexed for 30 s, then centrifuged at 5,000 rpm, 4 °C for 5 min. The pellets were resuspended in 0.2 ml lysis buffer (5 μl phosphatase inhibitor, 10 μl PMSF, and 1 μl DTT were contained in 1 ml lysis buffer) and vortexed, incubated on ice for 20 min, and centrifuged at 15,000 rpm 4 °C for 10 min; the supernatant was collected and stored at −70 °C.

Electrophoretic mobility shift assay (EMSA)

NF-κB consensus oligonucleotide (5′-AGTTGAGGGGAC TTTCCCAGGC-3′), the biotin-labeled DNA probe, was purchased from Beyotime Institute of Biotechnology, China. All of the reactions were loaded onto precast, non-denaturing 5 % polyacrylamide gels. The gels containing either DNA, protein, or both DNA and protein were stained using an EMSA kit (PIERCE, USA). The mixtures were loaded onto 5 % non-denaturing polyacrylamide gels and run at 100 V in 0.5× TBE running buffer, and the gels were run until the bromophenol blue dye front reached the bottom. After the electrophoresis, the mixtures were transferred to a nylon membrane and crosslink for 10–15 min with the membrane face down on a transilluminator equipped with 312 nm bulb. Biotin-labeled DNA was detected by chemiluminescence. The photographs generated were quantitatively analyzed with a Quantity One image densitometer.

Statistical analysis

The levels of MDA, SOD, GSH-Px, IL-6, TNFα, Scr, and kidney-to-body weight ratio were expressed as the mean ± standard deviation and analyzed by one-way ANOVA by SPSS 13.0 software.

Results

RA did not affect the body weight significantly

There was not a significant difference in body weight between diabetic rats treated with RA + insulin and those treated with insulin alone ($p > 0.05$). Hence, RA did not affect the body weight changes significantly (Fig. 1).

The changes of blood glucose levels in diabetic rats treated with RA and insulin

Every 2 days, fast blood glucose levels were determined and insulin dose was adjusted to keep blood glucose levels below 8 mmol/l (Fig. 2).

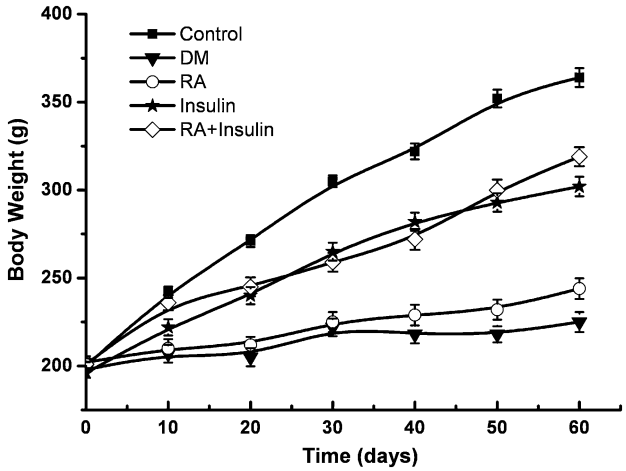


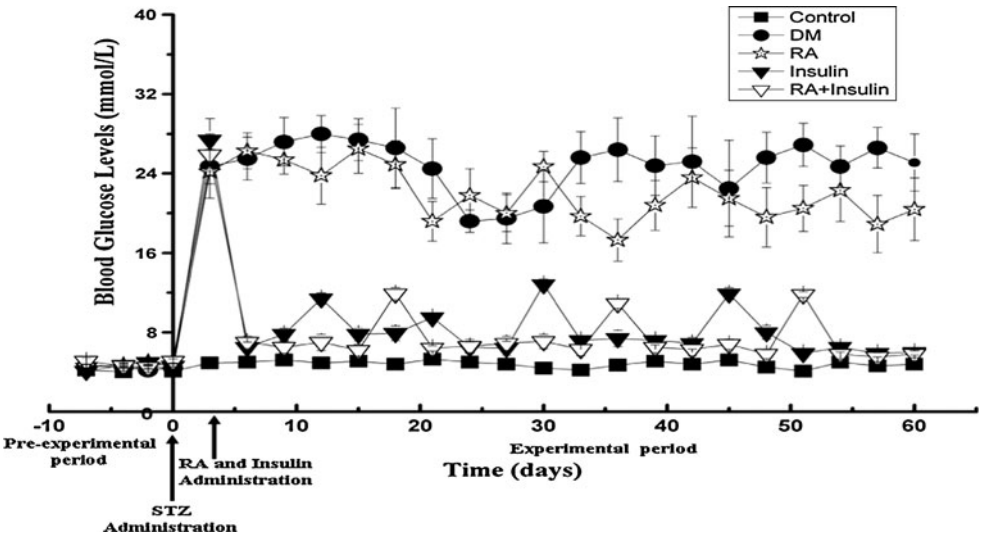
Fig. 1 Body weight changes in diabetic rats treated with insulin and RA, in combination or alone

RA helps lower oxidative stress in diabetic rats treated with insulin

MDA, SOD, and GSH-Px are markers of oxidative stress. To test whether RA can help lower oxidative stress in diabetic rats, the levels of MDA and activities of SOD and GSP-Px in kidney were determined.

MDA levels in kidney from rats treated with both RA and insulin were significantly lower than those treated with insulin alone ($p < 0.05$). The activities of GSH-Px and SOD in rats treated with both RA and insulin were significantly higher than those treated with insulin alone ($p < 0.05$). MDA levels in kidney from rats treated with insulin were significantly lower than those treated with RA or no treatment ($p < 0.05$), and the activities of SOD and GSH-Px were significantly higher ($p < 0.05$) (Fig. 3).

Fig. 2 The changes of blood glucose levels in diabetic rats treated with RA and insulin



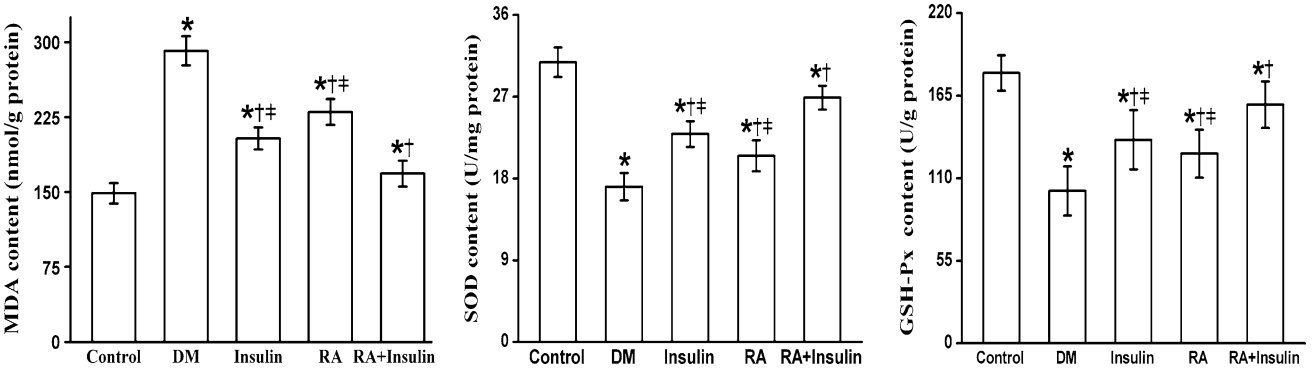


Fig. 3 Radix Astragali helps lower oxidative stress in diabetic rats treated with insulin. Diabetic rats received either insulin alone, both insulin and RA, or RA alone for 60 days. On day 60, rats were killed and kidneys were dissected and homogenates prepared. Levels of MDA and activities of SOD and GSH-Px in kidney were determined. Results are means ± SD determined from six rats performed in triplicate. * $p < 0.05$, significantly different from control; † $p < 0.05$, significantly different from DM; ‡ $p < 0.05$, significantly different from insulin + RA; one-way ANOVA

IL-6 and TNF-α levels were reduced in diabetic rats receiving both insulin and RA

Inflammatory cytokines IL-6 and TNF-α are products of oxidative stress and are also linked with insulin resistance. To investigate whether Radix will help lower oxidative stress in diabetic rats treated with insulin, the levels of inflammatory cytokines IL-6 and TNF-α in kidney were determined.

The levels of IL-6 and TNF-α in kidney from rats treated with both RA and insulin were significantly lower than those treated with insulin alone ($p < 0.05$). The levels of IL-6 and TNF-α in kidney from rats treated with insulin alone were significantly lower than those treated with RA or no treatment ($p < 0.05$) (Fig. 4).

PKCα expression was inhibited in diabetic rats receiving both RA and insulin

PKCα expression in kidney of diabetic rats treated with both insulin and RA was significantly inhibited compared with that of diabetic rats treated with RA, or insulin or no treatment ($p < 0.05$) (Fig. 5).

NF-κB activation was reduced in rats treated with both RA and insulin

NF-κB subunit expression in kidney of diabetic rats receiving insulin alone was increased compared with that of diabetic rats receiving both insulin and RA. Hence, NF-κB activation was reduced in rats treated with both insulin and RA (Fig. 6).

RA helps insulin protect kidney

Scr is a useful parameter of renal function. Scr and kidney-to-body weight ratio were significantly lower in diabetic rats treated with combination of insulin and RA than those treated with insulin alone ($p < 0.05$), which suggests that RA enhances the protection of kidney in diabetic rats treated with both RA and insulin (Table 1).

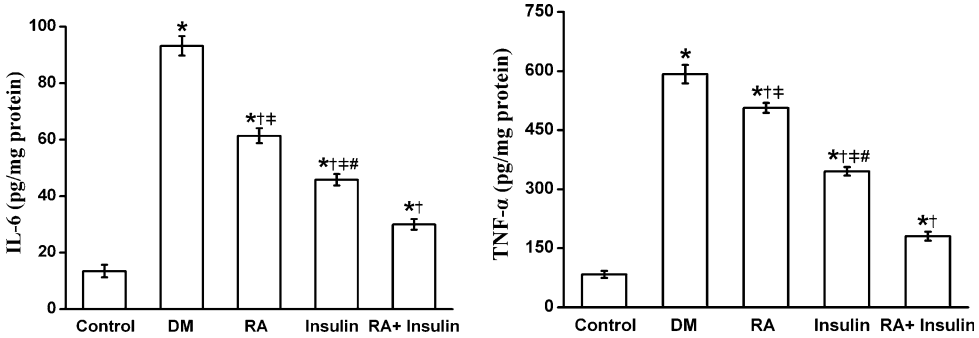


Fig. 4 Radix Astragali helps lower levels of IL-6 and TNF-α in kidney of diabetic rats. Diabetic rats received insulin and RA, in combination or alone for 60 days. On day 60, rats were killed and kidney homogenates were prepared. The levels of IL-6 and TNF-α in kidney were determined by ELISA. Results are means ± SD determined from six rats performed in triplicate. * $p < 0.05$, significantly different from control; † $p < 0.05$, significantly different from DM; ‡ $p < 0.05$, significantly different from insulin + RA; # $p < 0.05$, significantly different from RA; one-way ANOVA

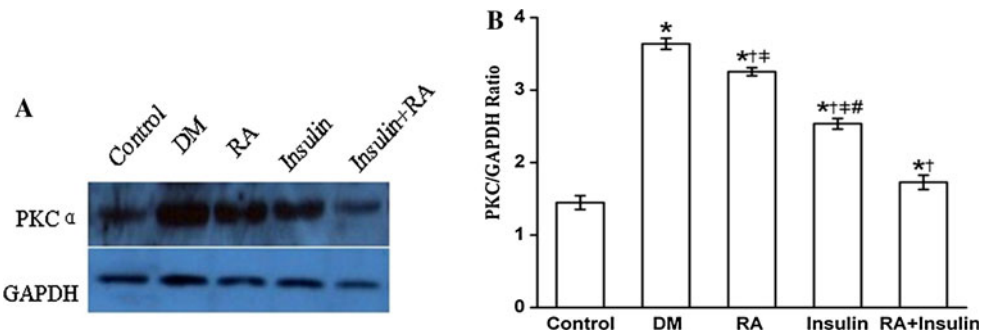


Fig. 5 PKCα expression in the kidney of diabetic rats. **a** Western blot analysis of PKCα expression in the kidney of diabetic rats treated with insulin, RA or insulin + RA. **b** The photographs generated were quantitatively analyzed for PKCα expression levels with a Quantity One image densitometer. * $p < 0.05$, significantly different from control; † $p < 0.05$, significantly different from DM; ‡ $p < 0.05$, significantly different from RA + insulin; # $p < 0.05$, significantly different from RA; one-way ANOVA

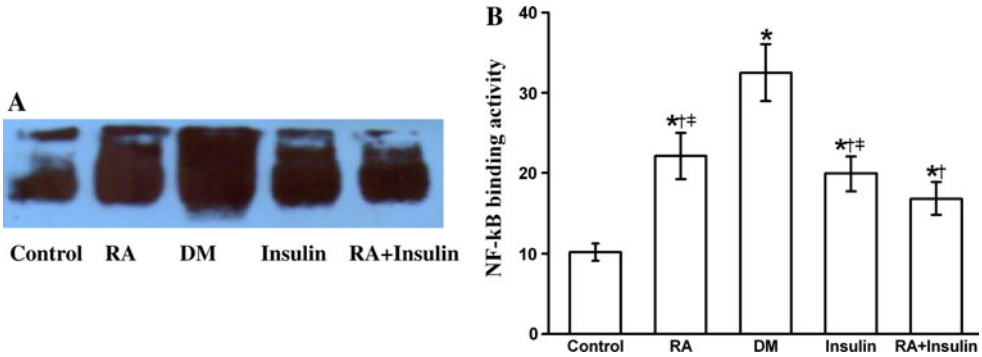


Fig. 6 NF-κB activation was reduced in kidney of rats treated with both insulin and RA. **a** Nuclear protein in kidney from rats treated with either insulin, RA or both RA and insulin was extracted and incubated with NF-κB probe. After separated by 5 % non-denaturing polyacrylamide gel, Biotin-labeled DNA was detected by chemiluminescence. **b** The photographs generated were quantitatively analyzed for NF-κB and DNA probe complex levels with a Quantity One image densitometer. * $p < 0.05$, significantly different from control; † $p < 0.05$, significantly different from DM; ‡ $p < 0.05$, significantly different from RA + insulin; one-way ANOVA

Table 1 Comparison of serum creatinine levels and kidney-to-body weight ratio in diabetic rats treated with insulin and RA ($\bar{x} \pm s$, $n = 6$)

Groups	Kidney-to-body weight ratio (%)	Serum creatinine (Scr) (μmol/l)
Control	0.59 ± 0.013	63.7 ± 4.42
DM	1.21 ± 0.016[a]	114.5 ± 6.21[a]
RA	1.09 ± 0.018[a,b,c]	98.4 ± 5.36[a,b,c]
Insulin	0.89 ± 0.014[a,b,c,d]	81.9 ± 4.55[a,b,c,d]
RA + insulin	0.78 ± 0.012[a,b]	70.6 ± 6.54[a,b]

[a] $p < 0.05$ verse control

[b] $p < 0.05$ verse DM

[c] $p < 0.05$ verse RA + insulin

[d] $p < 0.05$ verse RA; one-way ANOVA

Discussion

The major findings in the study are that RA can lower oxidative stress in diabetic rats which are receiving insulin and protect the kidney from oxidative stress.

The current major medication for diabetes is exogenous insulin injection [2], but insulin injection does not mimic the precise regulation of β-cells on glucose homeostasis and cannot maintain the optimum level of blood glucose [5]. Fluctuations in glucose levels contribute to more oxidative stress in the diabetic condition [6], hyperglycemia occurs, and with time results in the development of diabetic complications [15]. It was hypothesized that hyperglycemia-induced oxidative stress through increased polyol pathway flux, increased intracellular formation of advanced glycation end products, activation of protein kinase C, or overproduction of superoxide by the mitochondrial electron transport chain [8,16,17].

As shown in the study, diabetic rats receiving insulin can develop oxidative stress. MDA is a marker of oxidative stress and SOD, catalase (CAT) and GSH-Px are defenses against oxidative stress. SOD will react with superoxide to generate hydrogen peroxide which will be degraded by CAT and GSH-Px [18, 19]. The levels of MDA in kidney in diabetic rats receiving insulin alone were significantly higher and activities of SOD and GSH-Px were significantly lower than those receiving both insulin and RA ($p < 0.05$). However, MDA levels from diabetic rats receiving either insulin alone or both insulin and RA were higher than those from control ($p < 0.05$); the activities of SOD and GSH-Px were lower than those from controls ($p < 0.05$), which demonstrates that diabetic rats receiving insulin treatment develop oxidative stress. In contrast, Seghrouchni et al. [7] showed that insulin-dependent diabetic patients had higher levels of SOD and GSH-Px activity than healthy subjects. However, we found that the activities of SOD and GSH-Px were significantly lower in diabetic rats receiving either insulin or both insulin and RA compared with control rats ($p < 0.05$). Oxidative stress can produce effects on inflammation and increase the levels of inflammatory cytokines. Consistent with Shams et al. [20] and Esposito et al. [21], our results show that in the diabetic state, treatment with insulin elevates the levels of the inflammatory cytokines TNF-α and IL-6.

RA helps insulin lower oxidative stress in diabetic rats and provides protection for the kidney in diabetic rats treated with insulin. MDA levels in rats receiving both insulin and RA were significantly lower and the activities of SOD and GSH-Px were significantly higher than those receiving insulin alone ($p < 0.05$). The levels of IL-6 and TNF-α in kidney as well as the Scr levels and kidney-to-body weight ratio of diabetic rats receiving both insulin and RA were significantly lower than those receiving insulin alone ($p < 0.05$).

Oxidative stress increases the expression of PKC which in turn contributes to more oxidative in diabetic condition

[22]. RA enhances the inhibition of PKCα expression in kidney of diabetic rats treated with insulin, which suggests that RA can help lower oxidative stress in diabetic rats.

Oxidative stress can stimulate the NF-κB pathway and result in production of more ROS [23, 24]. NF-κB activities were strongly inhibited in diabetic rats treated with both insulin and RA, thus less ROS were produced, decreasing the risk of oxidative damage to the kidney in diabetic rats.

Currently more than 100 constituents have been identified in RA [25]. The major active components in RA include astragalus polysaccharide (APS), flavonoids, saponins, sucrose, amino acids, and phenolic acids. APS and saponins were reported to possess the abilities of antioxidation and they produced their antioxidative effects as a free radical scavenger [25]. But how APS and saponins scavenge free radicals and how these two constituents interact with each other remain unclear and need to be investigated further.

In conclusion, antioxidation is an important complementary therapy in the management of diabetes that will have synergistic effects with insulin in diabetes treatment.

Acknowledgments We would like to thank Dr Cheryl Beseler (Colorado Injury Control Research Center, Colorado State University) for revising the manuscript for us. This research was supported by Department of Science and Technology of Jilin Province, Division of Basic Research. Grant number: 2009011-1.

Conflict of interest The authors declare that they have no conflict of interest.

References

1. R.A. Kowluru, P.S. Chan, Oxidative stress and diabetic retinopathy. Exp. Diabetes. Res. (2007). doi:10.1155/2007/43603
2. J.M. Forbes, M.T. Coughlan, M.E. Cooper, Oxidative stress as a major culprit in kidney disease in diabetes. Diabetes **57**(6), 1446–1454 (2008)
3. G.L. Bakris, Recognition, pathogenesis, and treatment of different stages of nephropathy in patients with type 2 diabetes mellitus. Mayo Clin. Proc. **86**(5), 444–456 (2011)
4. C. Maric, J.E. Hall, Obesity, metabolic syndrome and diabetic nephropathy. Contrib. Nephrol. **170**, 28–35 (2011)
5. P.K. Mishra, S.R. Singh, I.G. Joshua, S.C. Tyagi, Stem cells as a therapeutic target for diabetes. Front. Biosci. **15**, 461–477 (2011)
6. A. Ceriello, R. Testa, Antioxidant anti-Inflammatory treatment in type 2 diabetes. Diabetes Care **32**(Suppl 2), S232–S236 (2009)
7. I. Seghrouchni, J. Drai, E. Bannier, J. Rivie're, P. Calmard, I. Garcia et al., Oxidative stress parameters in type I, type II and insulin-treated type 2 diabetes mellitus; insulin treatment efficiency. Clin. Chim. Acta **321**(1–2), 89–96 (2002)
8. J.L. Rains, S.K. Jain, Oxidative stress, insulin signaling, and diabetes. Free Radic. Biol. Med. **50**(5), 567–575 (2011)
9. Y. Feng, L. Feng, Protective effect of Astragalus membranaceus on diabetic nephropathy in rats. J. Prac. Med. **22**(21), 2457–2459 (2006)
10. J.J. Liu, W.Y. La, H.F. Wang, C.Y. Bai, J. Liang, Y. Ma et al., Effects of astragalus compound particles on glucose and lipid metabolism and pancreatic function of diabetic rats. Li Shi Zhen Medicine Materia Medica Research. **22**(8), 1923–1926 (2011)
11. H. Cheng, R.H. Jia, H.Y. Liu, Effects of astragalus on renal injury of diabetic rats. J. Chinese Phys. **8**(10), 1349–1351 (2006)
12. X.X. Zhang, S. Cao, M.S. Miao, Astragalus in diabetes and its complications of application. China J. Chinese Med. **11**, 1323–1325 (2011)
13. H. You, Y. Lu, D. Gui, A. Peng, J. Chen, Y. Gu, Aqueous extract of Astragali Radix ameliorates proteinuria in adriamycin nephropathy rats through inhibition of oxidative stress and endothelial nitric oxide synthase. J. Ethnopharmacol. **134**(1), 176–182 (2011)
14. D.G. Hafeman, R.A. Sunde, W.G. Hoekstra, Effect of dietary selenium on erythrocyte and liver glutathione peroxidase in the rat. J. Nutr. **104**(5), 580–587 (1974)
15. R.C. Stanton, Oxidative stress and diabetic kidney disease. Curr. Diab. Rep. **11**(4), 330–336 (2011)
16. F.K. Ahmad, Z. He, G.L. King, Molecular targets of diabetic cardiovascular complications. Curr. Drug Targets **6**(4), 487–494 (2005)
17. M. Brownlee, Biochemistry and molecular cell biology of diabetic complications. Nature **414**(6865), 813–820 (2001)
18. S.P. Bagby, Diabetic nephropathy and proximal tubule ROS: challenging our glomerulocentricity. Kidney Int. **71**(9), 912–923 (2007)
19. H. Fujita, H. Fujishima, S. Chida, K. Takahashi, Z. Qi, Y. Kanetsuna et al., Reduction of renal superoxide dismutase in progressive diabetic nephropathy. J. Am. Soc. Nephrol. **20**(6), 1303–1313 (2009)
20. M.E. Shams, M.M. AL-Gayyar, E.A. Barakat, Type 2 diabetes mellitus-induced hyperglycemia in patients with NAFLD and normal LFTs: relationship to lipid profile, oxidative stress and pro-inflammatory cytokines. Sci. Pharm **79**(3), 623–634 (2011)
21. K. Esposito, F. Nappo, R. Marfella, G. Giugliano, F. Giugliano, M. Ciotola et al., Inflammatory cytokine concentrations are acutely increased by hyperglycemia in humans: role of oxidative stress. Circulation **106**(16), 2067–2072 (2002)
22. D.K. Singh, P. Winocour, K. Farrington, Oxidative stress in early diabetic nephropathy: fueling the fire. Nat. Rev. Endocrinol. **7**, 176–184 (2011)
23. C. Guijarro, J. Egido, Transcription factor-kappa B (NF-kappa B) and renal disease. Kidney Int. **59**(2), 415–424 (2001)
24. M. Romagnoli, M.C. Gomez-Cabrera, M.G. Perrelli, F. Biasi, F.V. Pallardó, J. Sastre et al., Xanthine oxidase-induced oxidative stress causes activation of NF-κB and inflammation in the liver of type I diabetic rats. Free Radic. Biol. Med. **49**(2), 171–177 (2010)
25. J.Q. Zhang, X.S. Xie, C. Li, P. Fu, Systematic review of the renal protective effect of Astragalus membranaceus (root) on diabetic nephropathy in animal models. J. Ethnopharmacol. **126**, 189–196 (2009)