

Effects of water extracts of *Rehmannia glutinosa* on antioxidant system of Nrf2 in paraquat-induced insulin resistance diabetic rat model

LEI REN¹, YAPEI XU², GUIJUN QIN¹, CONG LIU³ and SHOUJUN WANG¹

¹Department of Endocrinology, The First Affiliated Hospital of Zhengzhou University, Zhengzhou, Henan 450002;

²Department of Nephrology, The Third People's Hospital of Zhengzhou, Zhengzhou, Henan 450000;

³Department of Endocrinology, Shengjing Hospital of China Medical University, Shenyang, Liaoning 110022, P.R. China

Received May 18, 2017; Accepted September 19, 2017

DOI: 10.3892/etm.2017.5302

Abstract. The objective of this study was to observe the effects of water extracts of *Rehmannia glutinosa* on the antioxidant system of Nrf-2 in diabetic mice induced by paraquat, and to provide the basis for its further development. Thirty male mice were randomly divided into the control group, model group and observation group. The mice in the model group and the observation group were treated with paraquat to induce insulin resistance, with the control group injected with the same volume saline. After the model establishment, the mice in observation group was given 1.2 g/kg-day with water extract of *Rehmannia glutinosa*, and the other groups were given equal volume of 1% hydroxymethyl cellulose sodium. After 7 days, the glucose tolerance was detected and the body weight was measured before and after the treatment. The body weight of the mice in the model group was significantly decreased ($P<0.05$), but the body weight of mice in the observation group was significantly higher than that in the model group ($P<0.05$). After 7 days of model establishment, the glucose tolerance of mice was damaged, with the blood sugar increased, but the level of blood sugar was significantly decreased when treated with water extracts of *Rehmannia glutinosa*. The water extract of *Rehmannia glutinosa* increased the level of phosphorylation of PKB significantly compared to the model group with the inhibition of PTEN. The level of malondialdehyde in mitochondria and muscle tissue was significantly increased after treated with water extracts of *Rehmannia glutinosa* ($P<0.05$). With decreased NQO-1 protein expression and the nuclear translocation of Nrf-2 in the model group, the water extract of *Rehmannia*

glutinosa could reverse the injury effectively. Similarly, the water extract of *Rehmannia glutinosa* significantly increased the expression of IKB α , which was significantly decreased in the model group. In conclusion, water extracts of *Rehmannia glutinosa* effectively reversed the glucose metabolism disorder in insulin resistance mice induced by paraquat, and effectively activated the level of Nrf-2 to enhance the muscle insulin signal while alleviating the insulin resistance in mice.

Introduction

Type 2 diabetes has a high incidence of the chronic metabolic diseases, the pathogenesis of which is complex (1). Studies have pointed out that insulin resistance plays an important role in the occurrence and development of type 2 diabetes (2). Paraquat as an organic contact heterocyclic herbicide and defoliant is widely used at this stage. Paraquat can effectively induce mitochondria to produce large amounts of peroxide, leading to blood glucose metabolism disorders and insulin resistance, and can be used as a drug for the insulin resistance type 2 diabetes model. Nrf2, an important transcription factor, can effectively regulate the NQO-1 level to participate in the insulin signal system and inflammation signal regulation. *Rehmannia glutinosa* can effectively enhance the body immunity, reduce blood sugar levels, and protect the renal function by Traditional Chinese medicine theory, which is a commonly used for treatment of type 2 diabetes. However, the biological mechanism of the regulation of blood sugar is still rarely reported (3). The water extract of *Rehmannia glutinosa* has a high retention of active ingredients and is easy to prepare. In this study, the water extract of *Rehmannia glutinosa* was selected as the research object, used to detect the effect of the water extract of *Rehmannia glutinosa* on the Nrf-2 antioxidant system of diabetic mice induced by paraquat and to provide a basis for its further development.

Materials and methods

Materials. Glucose, hydroxymethylcellulose sodium (CMC Na) and paraquat were purchased from Sigma. Insulin was obtained from Novo Nordisk, Inc. (Bagsvaerd, Denmark).

Correspondence to: Dr Guijun Qin, Department of Endocrinology, The First Affiliated Hospital of Zhengzhou University, 1 Jianshe Road, Erqi, Zhengzhou, Henan 450002, P.R. China
E-mail: v9b873@163.com

Key words: water extracts of *Rehmannia glutinosa*, paraquat, diabetes, insulin resistance, Nrf2, antioxidant system

Physiological saline was purchased from Beijing Jimei Biotech Co., Ltd. (Beijing, China). A nuclear extraction kit, mitochondria extraction kit, cell lysates and BCA Protein Concentration Quantitative kit were purchased from Shanghai Biyuntian Biotechnology Co., Ltd. (Shanghai, China) and the blood glucose meter was from Roche (Basel, Switzerland). The primary antibodies of histone-3, PKB, PKBSer473, NQO-1, I κ B α , β -actin and Nrf were obtained from Cell Signaling Technology, Inc. (Danvers, MA, USA) (cat. nos. 8135, 4691, 9611, 62262, 9242, 8457 and 69432), and the mouse anti-rabbit secondary polyclonal antibody was from Santa Cruz Biotechnology, Inc. (Philadelphia, PA, USA) (cat. no. sc-2357).

Preparation of water extracts of *Rehmannia glutinosa*. *Rehmannia glutinosa* (35 g) was soaked in 500 ml water for 30 min at first, after the boiling simmered for 60 min, filtered for juice, then repeated 2 times, the three juices were mixed, and 80°C water bath concentrated to 0.4 g/ml liquid, autoclaving.

Animal treatment. In this study, 30 6-week-old male 16-19 g mice were randomly divided into control group, model control group and observation group (n=10). Mice in the model control group and the observation group were injected with 5 mg/kg of paraquat intraperitoneally for 7 days to induce insulin resistance, and glucose tolerance was measured on the 8th day according to the literature (4). Meanwhile mice in the control group was injected with the same volume of saline. After model establishment, the mice in the observation group were treated with 1.2 g/kg-day of the water extracts of *Rehmannia glutinosa*, while equal volume of 1% CMC Na was given to the control group. After 7 days treated with water extracts of *Rehmannia glutinosa*, the glucose tolerance was measured again and the body weight of mice was measured before and after treatment. The study was approved by the Ethics Committee of the First Affiliated Hospital of Zhengzhou University.

Extraction of mitochondria and nuclei. Mouse muscle tissue (70 mg) was used for extraction of mitochondria and nuclei strictly in accordance with the manufacturer's instructions.

Determination of malondialdehyde. The level of malondialdehyde in mitochondria and muscle tissue was detected strictly in accordance with the manufacturer's instructions.

Protein extraction and western blotting. In this study, muscle tissue was obtained and the protein was extracted strictly in accordance with the manufacturer's instructions. The protein level was determined using the BCA Protein Concentration Quantitative kit. Protein samples (50 μ g) was loaded into SDS-PAGE gel electrophoresis, the protein was electrophoretically separated. After the electrophoresis, the protein was transferred to the PVDF membrane, the membrane was blocked for 2 h with 5% bovine serum albumin, and incubated with rabbit anti-mouse histone-3, PKB, PKBSer473, NQO-1, I κ B α , β -actin and Nrf polyclonal antibodies (1:500) overnight at 4°C, respectively. Then the membrane was washed, the HRP labeled mouse anti-rabbit secondary polyclonal antibody (1:1,000) was incubated at room temperature for 2 h, and washed again in TBST. The protein bands on the membrane

Table I. Body weight changes of mice.

Groups	Before intervention	After intervention
Control	28.61 \pm 0.91	29.13 \pm 1.23
Model	26.37 \pm 0.84	23.94 \pm 1.02 ^{a,c}
Observation	26.48 \pm 0.96	26.99 \pm 1.15 ^{a,b}

^aP<0.05 compared with the control group, ^bP<0.05 compared with the model group, ^cP<0.05.

were detected by enhanced chemiluminescence, and the gray scale was scanned by automatic gel imaging analyzer, then the relative gray value was calculated as the expression level of the protein.

Statistical analysis. In the study, the ImageJ software was used to scale the gray of bands in western blotting. SPSS 19.0 statistical analysis software (SPSS, Inc., Chicago, IL, USA) was used to evaluate the differences of the data in the different groups. Measurement data were expressed as means \pm standard deviation and the parallel variance analysis was performed. A P-value <0.05 was considered to indicate a statistically significant difference.

Results

Body weight changes of mice. The results showed that the body weight of the mice in both the model control group and the observation group was significantly lower than that in the control group before treatment (P<0.05). There was no significant difference in the body weight before and after treatment in the control group (P>0.05), but the body weight was lower when the modeling time was extended (P<0.05). There was no significant difference in body weight before and after treatment (P>0.05), but the body weight of the observation group was significantly higher than that in model control group after treatment (P<0.05) (Table I).

Water extracts of *Rehmannia glutinosa* improves the insulin resistance induced by paraquat. The glucose tolerance of mice in both the model control group and experiment group was impaired significantly after modeling for 7 days, as shown in Fig. 1. In the mice treated with water extracts of *Rehmannia glutinosa* for 7 days, the blood glucose level was significantly decreased (P<0.05), as shown in Fig. 2. The results of western blotting in Fig. 3 shows that the phosphorylation level of PKB was significantly decreased, and the PTEN was also inhibited significantly in the model control group. The phosphorylation level of PKB was significantly increased in the observation group treated with water extracts of *Rehmannia glutinosa*.

Effects of water extracts of *Rehmannia glutinosa* on malondialdehyde levels in mitochondria and muscle tissue of mice. As shown in Fig. 4, the levels of malondialdehyde (MDA) in mitochondria and muscle tissue of the model group were significantly higher than that in the control group (P<0.05).

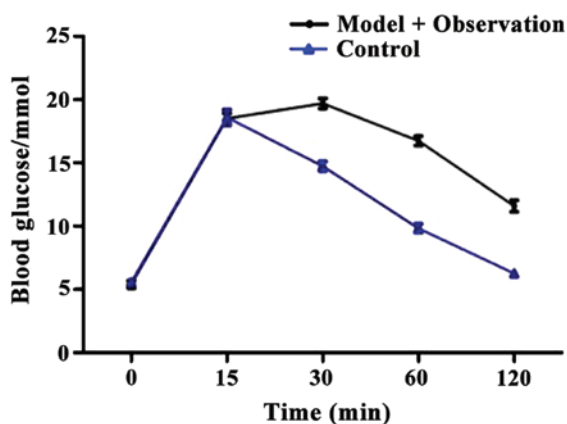


Figure 1. Determination of glucose tolerance (before intervention).

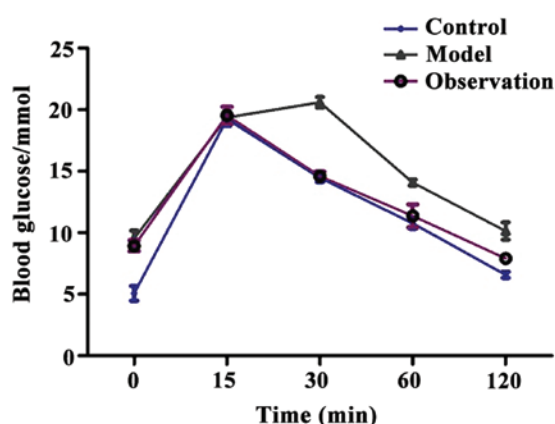


Figure 2. Determination of glucose tolerance (after intervention).

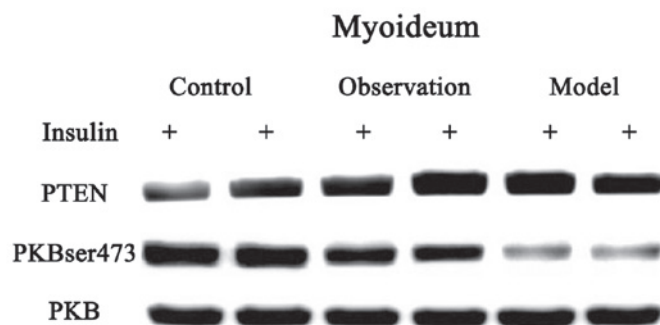


Figure 3. Muscle insulin signaling detected by western blotting. Phosphorylation level of PKB was significantly decreased in the model group.

There was no significant difference between the control group and the observation group ($P > 0.05$).

Expression of proteins related to Nrf-2 system and inflammation. As shown in Fig. 5, the NQO-1 protein expression and the nuclear translocation of Nrf-2 in the model group was significantly decreased, and the water extract of *Rehmannia glutinosa* could reverse the damage effectively. In addition, the inflammatory inhibitory protein of I κ B α was significantly reduced in the model group, which was also reversed in the observation group.

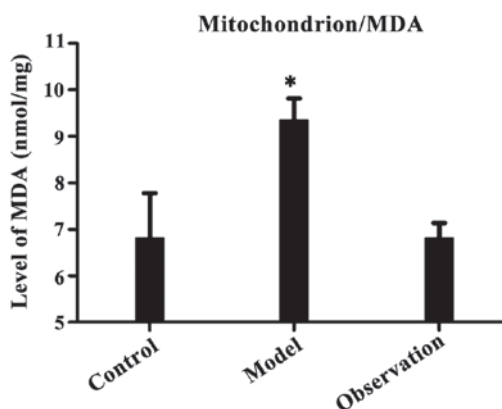
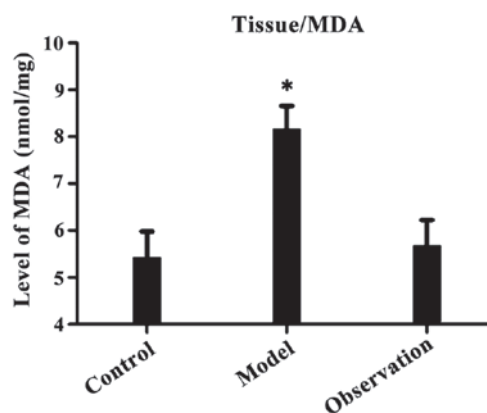


Figure 4. The level of MDA in muscle tissue and mitochondria of mice (* $P < 0.05$ vs. control).

Discussion

Paraquat is a common environmental pollutant and pesticide ingredients, which can effectively induce insulin resistance and oxidative stress (3,5). Results suggested that environmental pollution in modern industrialized societies is one of the major factors that may lead to high incidence of diabetes (6). Peripheral muscular tissue is an important part of insulin regulation, and paraquat-induced injury of glucose tolerance was achieved by damage to muscle signal transmission. The results in this study showed that the body weight of the mice in the model group was significantly lower than that in the control group, and it was confirmed that the paraquat had a higher toxicity. Compared with the model group, the water extract of *Rehmannia glutinosa* could improve the body weight of mice in the observation group. *Rehmannia* has functions of enriching blood, nourishing yin, tonifying kidney and YiJing nourishing by theory of Traditional Chinese Medicine, which could improve the toxicity of paraquat. Considerable literature has reported that oxidative stress was an important factor leading to the occurrence and development of insulin resistance, but the specific mechanism still lacks relevant reports (7,8). PTEN is a more sensitive indicator of oxidative stress activation, which can promote the inhibition of PI3 kinase activity by PTEN (9). The level of PTEN in muscle tissue in the model group was significantly increased in our research, which was regulated effectively by water extract of *Rehmannia glutinosa* and with decreasing of PTEN. The results suggested that paraquat damaged the signaling transduction of PKB, and the water

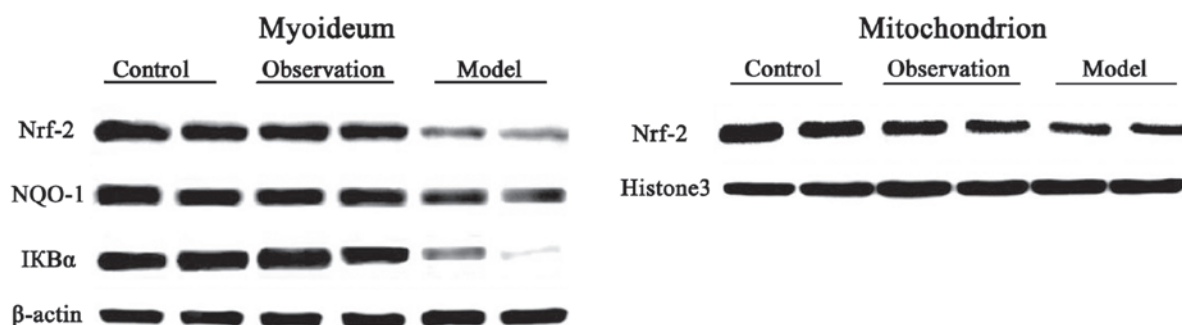


Figure 5. The expression level of proteins related to Nrf-2 system and inflammation.

extracts of *Rehmannia glutinosa* could reverse the damage effectively, which could be used as a molecular mechanism for the treatment of type 2 diabetes.

Endogenous antioxidation is an important factor in the development and progression of insulin resistance (10). Some researchers fed the high fat diet to establish the insulin resistance model in mice, which led to the significant inhibition of Nrf-2, and the activation of Nrf-2 by drug treatment alleviating insulin resistance (11). Nrf-2 system is an important protective mechanism in the human body, which can transfer from the cytoplasm into the nucleus when the oxidative stress activate and start the regulation of antioxidant enzyme expression (12). Long-term pathological oxidative stress led to damage of Nrf-2 antioxidant function, and the activation of the inflammatory signal is regulated by the redox state and is closely related to insulin resistance (13). Studies have shown that *Rehmannia* can effectively activate Nrf-2, and improve the inhibition of Nrf-2 system by high-fat intake (14). In this study, the water extract of *Rehmannia glutinosa* could effectively improve the imbalance of Nrf-2 system and oxidative stress induced by paraquat, with the mechanism of prevention of insulin resistance and activation of Nrf-2, but it still needs further clinical data support. In summary, the water extract of *Rehmannia glutinosa* could effectively reverse the disorder of glucose metabolism in mice induced by paraquat, which could effectively activate Nrf-2 level and enhance the muscle insulin signal while alleviating the insulin resistance in mice.

References

- Meo SA, Rouq FA, Suraya F and Zaidi SZ: Association of ABO and Rh blood groups with type 2 diabetes mellitus. *Eur Rev Med Pharmacol Sci* 20: 237-242, 2016.
- de Luis DA, Perez Castrillon JL, Aller R, Izaola O and Bachiller C: Response of osteocalcin and insulin resistance after a hypocaloric diet in obese patients. *Eur Rev Med Pharmacol Sci* 19: 2174-2179, 2015.
- Sun B and Chen YG: Advances in the mechanism of paraquat-induced pulmonary injury. *Eur Rev Med Pharmacol Sci* 20: 1597-1602, 2016.
- Hillian AD, McMullen MR, Sebastian BM, Roychowdhury S, Kashyap SR, Schauer PR, Kirwan JP, Feldstein AE and Nagy LE: Mice lacking Clq are protected from high fat diet-induced hepatic insulin resistance and impaired glucose homeostasis. *J Biol Chem* 288: 22565-22575, 2013.
- Qiaoli S, Yi S, Jie Z and Deyun C: KLF2 and caveolin-1 as early indicators of acute lung injury induced by paraquat. *Eur Rev Med Pharmacol Sci* 20: 138-145, 2016.
- Meo SA, Memon AN, Sheikh SA, Rouq FA, Usmani AM, Hassan A and Arian SA: Effect of environmental air pollution on type 2 diabetes mellitus. *Eur Rev Med Pharmacol Sci* 19: 123-128, 2015.
- Alsultan AI, Seif MA, Amin TT, Naboli M and Alsuliman AM: Relationship between oxidative stress, ferritin and insulin resistance in sickle cell disease. *Eur Rev Med Pharmacol Sci* 14: 527-538, 2010.
- Castillo-Quan JI, Li L, Kinghorn KJ, Ivanov DK, Tain LS, Slack C, Kerr F, Nespital T, Thornton J, Hardy J, *et al*: Lithium promotes longevity through GSK3/NRF2-dependent hormesis. *Cell Rep* 15: 638-650, 2016.
- Gong P, Lu Z, Xing J, Wang N and Zhang Y: Traditional Chinese medicine Xuebijing treatment is associated with decreased mortality risk of patients with moderate paraquat poisoning. *PLoS One* 10: e0123504, 2015.
- Nishimura C and Kuriyama K: Alteration of lipid peroxide and endogenous antioxidant contents in retina of streptozotocin-induced diabetic rats: Effect of vitamin A administration. *Jpn J Pharmacol* 37: 365-372, 1985.
- Jiang LJ, Zhang SM, Li CW, Tang JY, Che FY and Lu YC: Roles of the Nrf2/HO-1 pathway in the anti-oxidative stress response to ischemia-reperfusion brain injury in rats. *Eur Rev Med Pharmacol Sci* 21: 1532-1540, 2017.
- Tan Y, Wang Q, She Y, Bi X and Zhao B: Ketamine reduces LPS-induced HMGB1 via activation of the Nrf2/HO-1 pathway and NF-κB suppression. *J Trauma Acute Care Surg* 78: 784-792, 2015.
- Zhou Y, Zhang Y, Wei J, Zhang Y, Li J, Wang W, Duan H and Chen J: Cloning and analysis of expression patterns and transcriptional regulation of RghBNG in response to plant growth regulators and abiotic stresses in *Rehmannia glutinosa*. *Springerplus* 4: 60, 2015.
- Mohamed EM and Samak MA: Therapeutic potentials of mesenchymal stem cells on the renal cortex of experimentally induced hypertensive albino rats: Relevant role of Nrf2. *Tissue Cell* 49: 358-367, 2017.