



Troloxerutin, a Bioflavonoid, Improves Oxidative Stress in Blood of Streptozotocin-Induced Type-1 Diabetic Rats

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Abstract

Type 1 diabetes is a chronic disease characterized by the body's inability to produce insulin due to destruction of the beta cells. There is increasing evidence that reactive oxygen species (ROS) play a major role in the development of diabetic complications. The purpose of this study is to investigate the effects of troloxerutin administration on oxidative stress markers in blood of STZ-induced diabetic rats. Male Wistar rats were divided into 4 groups as: control (con), control-troloxerutin (CON-TRX), diabetes (Dia), diabetic-troloxerutin (DIA-TRX). Type 1 diabetes was induced by injection of streptozotocin (STZ) (i.p, 55mg/kg) and lasted for 10 weeks. Animals received oral administration of troloxerutin (150 mg/kg) for 4 weeks. At the end of study, malondialdehyde (MDA, the main product of lipid peroxidation), activity of antioxidant enzymes superoxide dismutase (SOD), glutathione peroxidase (GPX), and catalase (CAT) were measured spectrophotometrically. Induction of diabetes with STZ resulted in increased MDA levels and decreased blood antioxidant capacity as compared with those of controls ($P < 0.05$). Pre-treatment of diabetic rats with troloxerutin significantly decreased the levels of MDA ($P < 0.01$) and increased the activity of antioxidant enzymes SOD, GPX, and CAT compared to untreated-diabetic groups. Troloxerutin had no significant influence on non-diabetic rats. These findings showed that troloxerutin may prevent oxidative complications of diabetic circumstances by elevating antioxidant enzymes activities and reducing lipid peroxidation.

Keywords: diabetes, troloxerutin, oxidative stress, antioxidant, malondialdehyde, superoxide dismutase, glutathione peroxidase, catalase

1. Introduction

Diabetes mellitus is major causes of morbidity and mortality worldwide. It has been accounted that the number of adults

affected by diabetes in the world will enhance from 135 million in 1995 to 300 million in the year 2025 [1, 2]. Hyperglycemia is the most main risk factor for diabetic

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complications, resulting from uncontrolled glucose regulation [3]. Numerous studies demonstrated that hyperglycemia-induced production of free radicals and generation of oxidative stress, contributes to the expansion and development of diabetes and related contributions [4]. Hyperglycemia can progress an enhancement in oxidative stress markers such as membrane lipid peroxidation. The level of lipid peroxidation in cells was directly relative to the glucose amount [5]. The formation in the lipid peroxidation was avoidable after the control of glycemia in streptozotocin-treated diabetic rats [6]. Oxidative stress is defined in overall as addition formation and/or insufficient removal of extremely reactive molecules such as reactive oxygen species (ROS)[7]. ROS highly reactive and can injury protein, RNA, and DNA structure.

However, they are mostly reduced by internal antioxidant defense mechanisms in order to reserve a stable intracellular milieu[8]. Oxidative stress occurs when the amount of ROS surpasses the levels of antioxidants. Reactive species can be removed by a number of antioxidant mechanisms. Superoxide dismutase (SOD), a plentiful free radical

scavenger, converts $\bullet\text{O}_2^-$ to H_2O_2 , which is then detoxified to water either by catalase or by glutathione peroxidase. SOD delivers the body's important defense against the highly damaging free radicals and is thereby a main protective mechanism against damage induced by oxidative stress [9]. Thus, in the diabetic cases, the increase of oxidative stress is most possibly a result of abnormal metabolism of glycemia. Therefore, considerable attention has focused on the application of protective agents in various diseases which can decrease these free radicals and thereby improve the oxidative damage to biomolecules. Numerous studies have reported that active dietary ingredients, such as phytochemicals, have promising cytoprotective effects in many pathological conditions. These phytochemicals comprise phenolic compounds, alkaloids, lectins, terpenoids, isoprenoids, and quinones [10]. Thus, it became clear that improving oxidative stress through treatment with antioxidants might be a beneficial strategy for decreasing diabetic complications.

Troxerutin is a derived of the natural bioflavonoid rutin, hydroxylethylated ($-\text{CH}_2-\text{CH}_2-\text{OH}$) at the 3', 4' and 7th locations on the rutin skeleton (Figure1). Troxerutin is a yellow powder that dissolves in water and its solubility increases with increasing temperature [11]. It has been confirmed that troxerutin have antioxidative, anti-inflammatory, antihyperlipidemic, and nephroprotective effects, and it is revealed as an effective agent in the treatment of

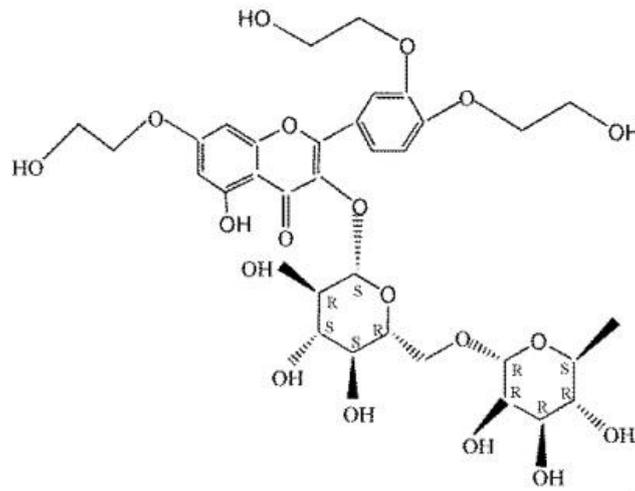


Figure 1. The chemical structure of troloxerutin.

cardiovascular diseases [12-14]. Recent studies have shown that troloxerutin administration effectively reduce blood glucose level, advanced glycation end products and reactive oxygen species levels in high cholesterol and fructose diet-fed mice [15, 16]. Troloxerutin by improving the expression of insulin signaling molecules and increasing glucose uptake in cells play an important role in management of diabetes mellitus [17]. The antioxidative activity of troloxerutin in type 1 diabetes has not been investigated so far. Therefore, this study was designed to evaluate the antioxidative effect of troloxerutin in blood of streptozotocin-induced type 1 diabetic rats.

2. Materials and Methods

2.1. Animals

Male wistar rats (200-250g) were used in this experimental study. The animals were housed at a constant room temperature (24°C),

a relative humidity for 50%, and a 12 h dark/light cycles that had access to food and water *ad libitum*. This study was approved by the Animal Ethics Committee in accordance with the instruction for the care and use of laboratory animals prepared by Tabriz University of Medical Sciences.

2.2. Animal Grouping

Thirty two rats were randomly divided into four groups as following (n=8/each):

- 1- Control (CON): vehicle treated healthy rats
- 2- Control-Troloxerutin (CON-TRX): healthy rats were treated with troloxerutin (150 mg/kg, orally) once a day for 30 days.
- 3- Diabetic (DIA): vehicle treated diabetic rats
- 4- rats
- 5- Diabetic-Troloxerutin (DIA-TRX): diabetic rats were treated with troloxerutin (150 mg/kg, orally) once a day for 30 days.

2.3. Development of Type 1 Diabetic Rats

Diabetes was induced by intraperitoneal injection of streptozotocin (STZ; 55mg/kg/body weight; from Sigma, Germany) dissolved in 0.2 ml of 0.1 M citrate buffer, pH 4.5. Control rats were injected with the vehicle (citrate buffer) alone. Three days after STZ injection, development of diabetes was confirmed from tail vein blood glucose sampling. Rats with blood glucose levels of 300 mg/dl and higher were considered as type 1 diabetic rat. Four weeks after diabetes induction, troxerutin (150 mg/kg; from Sigma, Germany) was administered once a day orally through the gavage tube for 4 weeks [15, 17, 18].

2.4. Blood Collection and Sample Processing

At the end of experiment, the rats were sacrificed under anesthesia with ketamine/xylazine (60/10 mg/kg, i.p.). Blood samples were collected from the inferior vena cava and were centrifuged at 1400g at 4 C for 10 min. The supernatants were used for the biochemical evaluation of serum.

2.5. Lipid Peroxidation Measurement Malondialdehyde

Lipid peroxides are unstable and decompose to produce a series of compounds including reactive carbonyl compounds. Polyunsaturated fatty acid peroxides generate malondialdehyde (MDA) and its measurement has been used as indicator of lipid peroxidation, which is examined by measuring

thiobarbituric acid reactive substances (TBARS) in homogenates. Briefly, the samples (250 μ L) were mixed with 1 mL 10% trichloroacetic acid (TCA) and 1 mL of 0.67% thiobarbituric acid. Then, the samples were heated in a boiling water bath for 15 minutes and n-butylalcohol (2:1 v: v) was added to the solution. After centrifugation (5 minutes, 3500 g) at room temperature, TBARS was approved from the absorbance at 535 nm, using a spectrophotometer (Pharmacia Biotech; England) and the values obtained were expressed as nmol per 100 mg tissue protein [19, 20]. The intra- and inter-assay coefficient of variations on measurements done on the same sample was less than 5% and 7%, respectively.

2.6. Superoxide Dismutase Measurement

Superoxide dismutase (SOD) activity was determined using a RANSOD kit (RandoxCrumlin, UK). SOD activity was measured at 505 nm by a spectrophotometer. In this method, xanthine and xanthine oxidase were used to generate superoxide radicals able to react with 2-[4-iodophenyl]-3-[4-nitrophenol]-5-phenyl tetrazolium chloride (ITN) to form a red formazan dye. Concentrations of substrates were 0.05 mmol/L for xanthine and 0.025 mmol/L for ITN. Superoxide dismutase activity was assessed by the degree of inhibition of this reaction. After calculating the percent of inhibition using related formula, SOD activity was calculated by comparing with the standard curve and was

expressed as U/mg protein [21, 22]. The inter-sample intra-assay variation and coefficient of variation were less than 8%.

2.7. Glutathione Peroxidase Measurement

Glutathione peroxidase (GPX) activity was determined using a RANSEL kit (Randox Crumlin, UK). GPX catalyzes the oxidation of glutathione (at a concentration of 4 mmol/L) by cumene hydroperoxide. In the presence of glutathione reductase (at a concentration ≥ 0.5 units/L) and 0.28 mmol/L of NADPH, oxidized glutathione is immediately converted to the reduced form with concomitant oxidation of NADPH to NAD⁺. The decrease in absorbance at 340 nm (37°C) was measured using a spectrophotometer, and then GPX concentration was calculated and expressed as U/mg protein. The coefficient of variation for intra-assay and inter-assay were 7%, and 8%, respectively [23].

2.8. Catalase Measurement

CAT activity was assayed by the method of Aebi(24). Briefly, to a quartz cuvette, 0.65 ml of the phosphate buffer (50 mmol/l; pH 7.0) and 50 μ l sample were added, and the reaction was started by addition of 0.3 ml of 30 mM hydrogen peroxide (H₂O₂). The decomposition of H₂O₂ was monitored at 240 nm at 25 °C CAT activity was calculated as nM H₂O₂ consumed/min/mg of tissue protein.

2.9. Statistical Analysis

All values were expressed as means \pm SEM. The between-group parameters were

analyzed using *one-way ANOVA* followed by *Tukey* post-hoc test. Differences were considered statistically significant when $P < 0.05$.

3. Results and Discussion

3.1. Results

3.1.1. Effect of Troloxerutin on Lipid Peroxidation

As shown in Figure 2, the level of lipid peroxides increased significantly in DIA group in comparison with CON group ($p < 0.001$). After 4 weeks of treatment of diabetic rats with troloxerutin, the level of MDA decreased in plasma of diabetic rats as compared with DIA group ($p < 0.01$).

3.1.2. Effect of Troloxerutin on Glutathione Peroxidase

One-way ANOVA showed that the glutathione peroxidase levels were significantly lower in non-treatment diabetic rats than in control rats ($p < 0.001$) and troloxerutin-treated control rats ($p < 0.001$). A comparison between the diabetic rats treated with troloxerutin and non treated diabetic group exhibited significantly difference among these groups. Data showed that troloxerutin could improve glutathione peroxidase activity in diabetic rats ($p < 0.05$) (Figure 3).

3.1.3. Effect of Troloxerutin on Superoxide Dismutase

Results demonstrated that induction of diabetes reduced superoxide dismutase activity in diabetic group as compared with treated

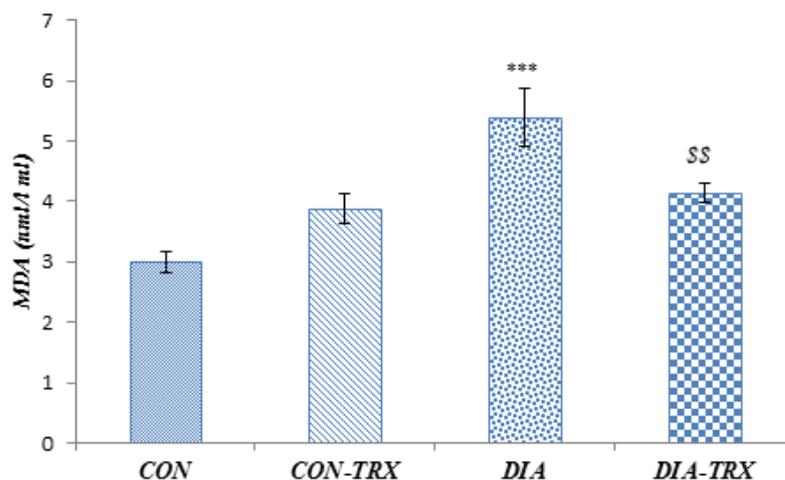


Figure 2. The effect of troxerutin on MDA level in plasma of diabetic and non-diabetic rats. Data are shown as mean \pm S.E.M. *** $p < 0.001$ indicated significantly change compared with control (CON) group and §§ $p < 0.01$ indicated significantly change compared with DIA group.

($p < 0.001$) and non-treated ($p < 0.001$) controls. As in Figure 4, troxerutin administration significantly increased superoxide dismutase levels in the blood of diabetic rats as compared to non treated diabetic group ($p < 0.05$).

3.1.4. Effect of Troxerutin on Catalase

Figure 5 shows that induction of type 1 diabetes resulted in a significant decrease in catalase activity in the blood of diabetic rats compared with CON group ($p < 0.001$). Furthermore, troxerutin administration significantly increased the levels of catalase in DIA-TRX group as compared with untreated diabetic group ($p < 0.001$).

3.2. Discussion

The oxidative stress and reduced antioxidant capacity as one of the main manifestations of diabetes mellitus were apparent in blood of diabetic rats in our study.

However, administration of troxerutin in type 1 diabetic rats restored significantly the blood levels of antioxidant enzymes and plasma levels of lipid peroxidation.

Type 1 diabetes is accompanied with the hyperglycemia-induced raised levels of reactive oxygen species (ROS) [1, 25]. Blocking ROS generation has been shown to prevent hyperglycemia-related damages [26]. These reactive species can be removed by a number of endogenous enzymatic and non-enzymatic antioxidant mechanisms. However, there are evidences that the antioxidant defense is finished in diabetic patients, proposing a disturbed capacity of scavenging of harmful free radicals [27]. Therefore, increased production of free radicals and impaired antioxidant defense capabilities in diabetic conditions designate a central contribution of ROS in the onset, progression, and pathological consequences

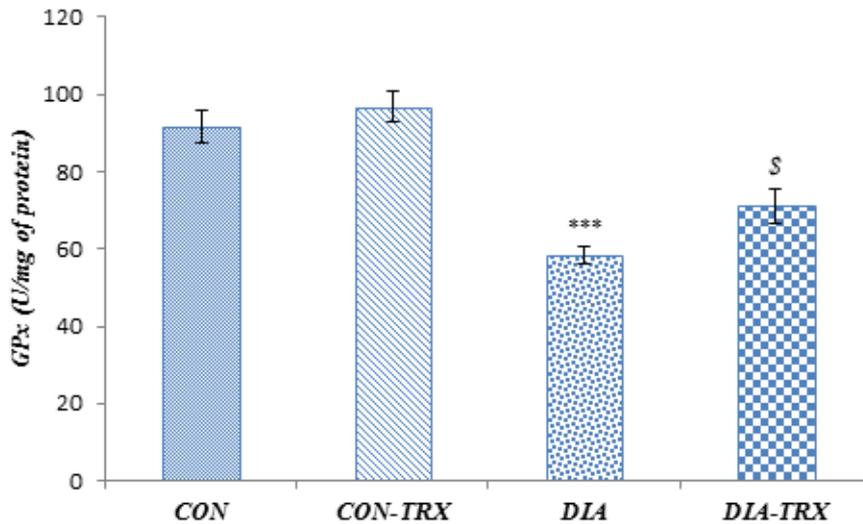


Figure 3. The effect of troloxerutin on glutathione peroxidase level. Data are shown as mean \pm S.E.M. for=8 animals, *** p <0.001 indicated significantly change compared with control group and \$ p <0.05 indicated significantly change compared with DIA group.

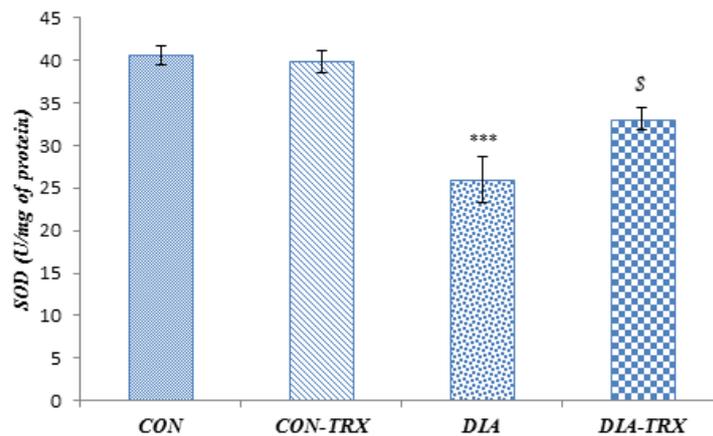


Figure 4. The effect of troloxerutin on superoxide dismutase level. Data are shown as mean \pm S.E.M. for=8 animals, *** p <0.001 indicated significantly change compared with control group and \$ p <0.05 indicated significantly change compared with DIA group.

of this disease [28, 29].As a result, successful efforts to diminish oxidative stress in diabetic models have involved the use of antioxidant supplementation [30].

Troloxerutin can be found in tea, coffee, cereal grains, and a variability of fruits and vegetables (12).It has been reported to have a powerful antioxidant effects and prevents

injury caused by oxidative stress in different tissues, e.g., liver, kidney, and brain [31-33]. Panat et al (2016) confirm that Troloxerutin protects cells against oxidative stress-induced cell death through radical scavenging mechanism [34]. Previous studies by Pingand *et al* (2012), have revealed that that troloxerutin

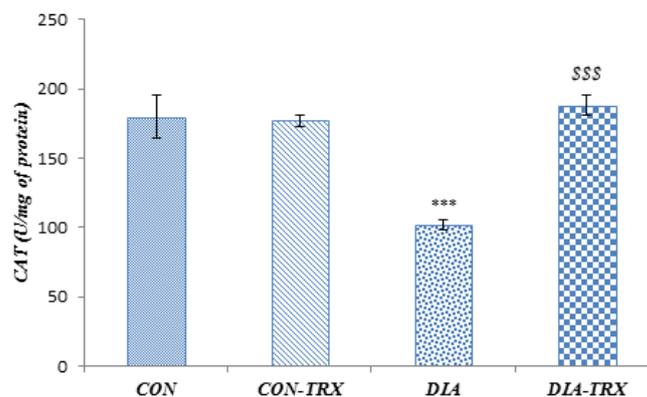


Figure 5. The effect of troxerutin on catalase level. Data are shown as mean \pm S.E.M. for=8 animals, *** p <0.001 indicated significantly change compared with control group and \$\$\$ p <0.001 indicated significantly change compared with DIA group.

protected the cells against γ -irradiation-induced cellular injury, including cell death [35].

According to the findings of the present study, pre-administration of troxerutin in dose of 150 mg/kg daily for four weeks could decrease MDA levels and significantly increased SOD, GPX, and CAT activity in blood of diabetic rats. These findings might be ascribed at least in part to the troxerutin ability to scavenge and avoid free radical generation and improved antioxidant properties.

Antioxidant enzymes can protect cellular compartments and organelles against oxidative damage. The SOD decomposes superoxide radicals (O_2^-) and generates H_2O_2 . H_2O_2 is subsequently decreased to water by CAT in the peroxisomes, or by GPX oxidizing GSH in the cytosol [36, 37]. Many studies have shown that the overproduction of ROS in diabetes would break the balance between ROS generation and antioxidant defenses.

The antioxidant enzymes were maybe exhausted, leading to the reduction of their activity [12, 38, 39]. In the present study, the activities of antioxidant enzymes, including SOD, CAT, and GPX, in blood of diabetic rats were decreased by the injection of STZ and subsequent hyperglycemia. It indicated that hyperglycemia may increase the oxidative stress by the inhibiting the activities of antioxidant enzymes. Interestingly, troxerutin could markedly induce the activities of those antioxidant enzymes in the blood of diabetic rats. This study is the first original article that suggested the protective effect of troxerutin against oxidative stress of blood in STZ-induced type 1 diabetes, by renewing the activity of antioxidant enzymes accompanied with a reduction in oxidative stress. In agreement with these experimental observations, troxerutin diminished nickel-induced oxidative stress in rats [40], improved hepatic lipid homeostasis [41], protected the

mouse brain and kidney from D-galactose injury [12, 42] and suppressed lipid abnormalities in the heart of high-fat-high-fructose diet-fed mice [15].

Furthermore, it is clear that hyperglycemia is the main cause of diabetic complications. Hyperglycemia stimulates cellular ROS production by various major mechanisms including elevated levels of circulating advanced glycation end-products (AGEs), activation of multiple isoforms of PKC, stimulated endoplasmic reticulum stress, increased mitochondrial dysfunction [43, 44]. One of the main causes of hyperglycemia-related injuries is activation of PKC and its downstream target such as NF- κ B [45, 46]. It is demonstrated that activation of PKC have been associated with tissue damages in the diabetic models [46]. It can be supposed that troloxerutin may inhibit over-activation of PKC or its targets in diabetic condition. In this case, it has been shown that that troloxerutin has significant inhibitory effects on NF- κ B in renal and hepatic tissues of diabetic and healthy rats [12, 47] and in hippocampus of high cholesterol-fed mice [16, 32, 42]. Thus, troloxerutin, through above-mentioned mechanisms, is capable to reverse the oxidative potential during STZ-induced type 1 diabetes. Although as limitations of the present study, we could not perform direct measurement of ROS and oxidant anions generation instead of antioxidative enzymes and did not investigate the mechanisms of troloxerutin-induced reduction in oxidative stress; however, direct contribution of above-

mentioned mechanisms in antioxidative influences of troloxerutin warrants further investigation.

4. Conclusion

In conclusion, the present results confirmed that troloxerutin administration had protective influences in type 1 diabetes mellitus. Marked reduction in lipid peroxidation (indicated by MDA) and an enhancement in antioxidant enzymes levels following troloxerutin administration indicated its antioxidant activity in this model. Therefore, troloxerutin could be introduced as a therapeutic agent in oxidative stress-related diseases.

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References

- [1] Rolo AP, Palmeira CM. Diabetes and mitochondrial function: role of hyperglycemia and oxidative stress. *Toxicology and applied pharmacology* (2006) 212(2):167-178.
- [2] King H, Aubert RE, Herman WH. Global burden of diabetes, 1995–2025: prevalence, numerical estimates, and projections. *Diabetes care* (1998) 21(9):1414-1431.
- [3] Brownlee M. Biochemistry and molecular cell biology of diabetic complications. *Nature* (2001) 414(6865):813-820.
- [4] Ceriello A, Motz E. Is oxidative stress the pathogenic mechanism underlying insulin resistance, diabetes, and cardiovascular disease? The common soil hypothesis revisited. *Arteriosclerosis, thrombosis, and vascular biology* (2004) 24(5):816-823.

- [5] Jain SK. Hyperglycemia can cause membrane lipid peroxidation and osmotic fragility in human red blood cells. *Journal of Biological Chemistry* (1989) 264(35):21340-21345.
- [6] Jain SK, Levine SN, Duett J, Hollier B. Elevated lipid peroxidation levels in red blood cells of streptozotocin-treated diabetic rats. *Metabolism* (1990) 39(9):971-975.
- [7] Maritim A, Sanders R, Watkins rJ. Diabetes, oxidative stress, and antioxidants: a review. *J Biochem Mol Toxicol* (2003) 17(24):38.26).
- [8] Mehta JL, Rasouli N, Sinha AK, Molavi B. Oxidative stress in diabetes: a mechanistic overview of its effects on atherogenesis and myocardial dysfunction. *The international journal of biochemistry & cell biology* (2006) 38(5):794-803.
- [9] Li G, Chen Y, Saari JT, Kang YJ. Catalase-overexpressing transgenic mouse heart is resistant to ischemia-reperfusion injury. *American Journal of Physiology-Heart and Circulatory Physiology* (1997) 273(3):H1090-H1095.
- [10] Vinod BS, Maliekal TT, Anto RJ. Phytochemicals as chemosensitizers: from molecular mechanism to clinical significance. *Antioxidants & Redox Signaling* (2013) 18(11):1307-1348.
- [11] Wang Y, Liu Y, Xu S, Liu Y, Yang P, Du S, et al. Determination and modelling of troxerutin solubility in eleven mono-solvents and (1, 4-dioxane+ 2-propanol) binary solvents at temperatures from 288.15 K to 323.15 K. *The Journal of Chemical Thermodynamics* (2017) 104:138-149.
- [12] Fan S-h, Zhang Z-f, Zheng Y-l, Lu J, Wu D-m, Shan Q, et al. Troxerutin protects the mouse kidney from d-galactose-caused injury through anti-inflammation and anti-oxidation. *International immunopharmacology* (2009) 9(1):91-96.
- [13] Gohel MS, Davies AH. Pharmacological agents in the treatment of venous disease: an update of the available evidence. *Current vascular pharmacology* (2009) 7(3):303-308.
- [14] Kessler M, Ubeaud G, Walter T, Sturm F, Jung L. Free radical scavenging and skin penetration of troxerutin and vitamin derivatives. *Journal of dermatological treatment* (2002) 13(3):133-141.
- [15] Geetha R, Yogalakshmi B, Sreeja S, Bhavani K, Anuradha CV. Troxerutin suppresses lipid abnormalities in the heart of high-fat-high-fructose diet-fed mice. *Molecular and cellular biochemistry* (2014) 387(1-2):123-134.
- [16] Lu J, Wu D-m, Zheng Z-h, Zheng Y-l, Hu B, Zhang Z-f. Troxerutin protects against high cholesterol-induced cognitive deficits in mice. *Brain* (2011):awq376.
- [17] Sampath S, Karundevi B. Effect of troxerutin on insulin signaling molecules in the gastrocnemius muscle of high fat and sucrose-induced type-2 diabetic adult male rat. *Molecular and cellular biochemistry* (2014) 395(1-2):11-27.
- [18] Zhang Z-F, Fan S-H, Zheng Y-L, Lu J, Wu D-M, Shan Q, et al. Troxerutin improves hepatic lipid homeostasis by restoring NAD⁺-depletion-mediated dysfunction of lipin 1 signaling in high-fat diet-treated mice. *Biochemical pharmacology* (2014) 91(1):74-86.
- [19] Fraga CG, Leibovitz BE, Tappel AL. Lipid peroxidation measured as thiobarbituric acid-reactive substances in tissue slices: characterization and comparison with homogenates and microsomes. *Free Radical Biology and Medicine* (1988) 4(3):155-161.
- [20] Badalzadeh R, Mohammadi M, Yousefi B, Faranjia S, Najafi M, Mohammadi S. Involvement of Glycogen Synthase Kinase-3 β and Oxidation Status in the Loss of Cardioprotection by Postconditioning in Chronic Diabetic Male Rats. *Adv Pharm Bull* (2015) 5(1).
- [21] Pari L, Monisha P, Jalaludeen AM. Beneficial role of diosgenin on oxidative stress in aorta of streptozotocin induced diabetic rats. *European journal of pharmacology* (2012) 691(1):143-150.
- [22] Ghyasi R, Sepehri G, Mohammadi M, Badalzadeh R, Ghyasi A. Effect of mebudipine on oxidative stress and lipid peroxidation in myocardial

- ischemic-reperfusion injury in male rat. *Journal of research in medical sciences: the official journal of Isfahan University of Medical Sciences* (2012) 17(12):1150.
- [23] Badalzadeh R, Yavari R, Chalabiani D. Mitochondrial ATP-sensitive K (+) channels mediate the antioxidative influence of diosgenin on myocardial reperfusion injury in rat hearts. *General physiology and biophysics* (2015).
- [24] Aebi H. [13] Catalase in vitro. *Methods in enzymology* (1984) 105:121-126.
- [25] Dave G, Kalia K. Hyperglycemia induced oxidative stress in type-1 and type-2 diabetic patients with and without nephropathy. *Cell Mol Biol (Noisy-le-grand)* (2007) 53(5):68-78.
- [26] Nishikawa T, Edelstein D, Du XL, Yamagishi S-i, Matsumura T, Kaneda Y, et al. Normalizing mitochondrial superoxide production blocks three pathways of hyperglycaemic damage. *Nature* (2000) 404(6779):787-790.
- [27] Santini SA, Marra G, Giardina B, Cotroneo P, Mordente A, Martorana GE, et al. Defective plasma antioxidant defenses and enhanced susceptibility to lipid peroxidation in uncomplicated IDDM. *Diabetes* (1997) 46(11):1853-1858.
- [28] Bonnefont-Rousselot D. Glucose and reactive oxygen species. *Current Opinion in Clinical Nutrition & Metabolic Care* (2002) 5(5):561-568.
- [29] Ceriello A. New insights on oxidative stress and diabetic complications may lead to a "causal" antioxidant therapy. *Diabetes care* (2003) 26(5):1589-1596.
- [30] Tanaka Y, Gleason CE, Tran POT, Harmon JS, Robertson RP. Prevention of glucose toxicity in HIT-T15 cells and Zucker diabetic fatty rats by antioxidants. *Proceedings of the National Academy of Sciences* (1999) 96(19):10857-10862.
- [31] Adam B, Pentz R, Siegers C, Strubelt O, Tegtmeier M. Troloxerutin protects the isolated perfused rat liver from a possible lipid peroxidation by coumarin. *Phytomedicine* (2005) 12(1):52-61.
- [32] Lu J, Wu D-m, Zheng Y-l, Hu B, Cheng W, Zhang Z-f, et al. Troloxerutin Counteracts Domoic Acid-Induced Memory Deficits in Mice by Inhibiting CCAAT/Enhancer Binding Protein β -Mediated Inflammatory Response and Oxidative Stress. *The Journal of Immunology* (2013) 190(7):3466-3479.
- [33] Zhang Z-f, Fan S-h, Zheng Y-l, Lu J, Wu D-m, Shan Q, et al. Troloxerutin protects the mouse liver against oxidative stress-mediated injury induced by D-galactose. *Journal of agricultural and food chemistry* (2009) 57(17):7731-7736.
- [34] Panat NA, Maurya DK, Ghaskadbi SS, Sandur SK. Troloxerutin, a plant flavonoid, protects cells against oxidative stress-induced cell death through radical scavenging mechanism. *Food chemistry* (2016) 194:32-45.
- [35] Ping X, Junqing J, Junfeng J, Enjin J. Radioprotective effects of troloxerutin against gamma irradiation in mice liver. *International journal of radiation biology* (2012) 88(8):607-612.
- [36] Dröge W. Free radicals in the physiological control of cell function. *Physiological reviews* (2002) 82(1):47-95.
- [37] Lee PJ, Choi AM. Pathways of cell signaling in hyperoxia. *Free Radical Biology and Medicine* (2003) 35(4):341-350.
- [38] Shan Q, Lu J, Zheng Y, Li J, Zhou Z, Hu B, et al. Purple sweet potato color ameliorates cognition deficits and attenuates oxidative damage and inflammation in aging mouse brain induced by D-galactose. *BioMed Research International* (2009) 2009.
- [39] Wu D-m, Lu J, Zheng Y-l, Zhou Z, Shan Q, Ma D-f. Purple sweet potato color repairs D-galactose-induced spatial learning and memory impairment by regulating the expression of synaptic proteins. *Neurobiology of learning and memory* (2008) 90(1):19-27.
- [40] Elangovan P, Pari L. Ameliorating effects of troloxerutin on nickel-induced oxidative stress in rats. *Redox Report* (2013) 18(6):224-232.

- [41] Zhang ZF, Fan SH, Zheng YL, Lu J, Wu DM, Shan Q, et al. Troxerutin improves hepatic lipid homeostasis by restoring NAD(+)-depletion-mediated dysfunction of lipin 1 signaling in high-fat diet-treated mice. *Biochem Pharmacol* (2014) 91(1):74-86.
- [42] Lu J, Wu D-m, Hu B, Cheng W, Zheng Y-l, Zhang Z-f, et al. Chronic administration of troxerutin protects mouse brain against D-galactose-induced impairment of cholinergic system. *Neurobiology of learning and memory* (2010) 93(2):157-164.
- [43] Vanessa Fiorentino T, Prioletta A, Zuo P, Folli F. Hyperglycemia-induced oxidative stress and its role in diabetes mellitus related cardiovascular diseases. *Current pharmaceutical design* (2013) 19(32):5695-5703.
- [44] Patel H, Chen J, Das KC, Kavdia M. Hyperglycemia induces differential change in oxidative stress at gene expression and functional levels in HUVEC and HMVEC. *Cardiovasc Diabetol* (2013) 12(1):142.
- [45] Ramana KV, Friedrich B, Srivastava S, Bhatnagar A, Srivastava SK. Activation of nuclear factor- κ B by hyperglycemia in vascular smooth muscle cells is regulated by aldose reductase. *Diabetes* (2004) 53(11):2910-2920.
- [46] Gerald P, King GL. Activation of protein kinase C isoforms and its impact on diabetic complications. *Circulation research* (2010) 106(8):1319-1331.
- [47] Zhang Z-F, Zhang Y-q, Fan S-H, Zhuang J, Zheng Y-L, Lu J, et al. Troxerutin protects against 2, 2', 4, 4'-tetrabromodiphenyl ether (BDE-47)-induced liver inflammation by attenuating oxidative stress-mediated NAD⁺-depletion. *Journal of hazardous materials* (2015) 283:98-109.