



Tropisetron ameliorates oxidative stress in type-1 diabetic experimental rats

Shiva Gholizadeh-Ghaleh Aziz¹, Roya Naderi^{2,3*}, Naser Khalaji³, Nima Mahmodian³

¹ Assistant Professor of Molecular Medicine, Department of Biochemistry, Faculty of Medicine, Urmia University of Medical Sciences, Urmia, Iran

² Assistant Professor of Physiology, Nephrology and Kidney Transplant Research Center, Urmia University of Medical Sciences, Urmia, Iran

³ Department of Physiology, Faculty of Medicine, Urmia University of Medical Sciences, Urmia, Iran

*Corresponding authors: Roya Naderi, Address: Department of Physiology, Faculty of Medicine, Urmia University of Medical Sciences, Urmia, Iran, Email: r_naderi_s@yahoo.com, Tel: +984432780801

Abstract

In this study, we evaluated the effect of tropisetron on the oxidative biomarkers in the plasma of type 1 diabetes mellitus (DM). Twenty-one male Wistar rats were randomly divided into three experimental groups (n=7): control, diabetic and diabetic + tropisetron. Diabetes type 1 was induced by streptozotocin (STZ) (50 mg/kg; i.p). Tropisetron (3 mg/kg; i.p) as a 5-HT₃ antagonist was given once daily for 2 weeks. At the end of experiment animals were euthanized and blood samples were collected for further analysis including superoxide dismutase (SOD), glutathione peroxidase (GPx) activities and malondialdehyde (MDA), total antioxidant capacity (TAC) levels. We found that tropisetron attenuated MDA levels and increased SOD, GPx and TAC contents in the plasma of diabetic rats. Our findings indicate that tropisetron improved oxidative stress in the plasma of STZ induced-diabetic rats.

Key words: Diabetes, Tropisetron, Oxidative stress, Rat

Received 10 Mar 2018; accepted for publication 14 May 2018

Introduction

Type 1 diabetes mellitus (T1DM) is a metabolic disorder which disturbed metabolism of carbohydrate, fat, and protein (1,2). It is well known that hyperglycemia is remarkable evidence that may induce oxidative stress (3). Oxidative stress is defined as the free radical accumulation can cause diabetic complications such as retinopathy, neuropathy, nephropathy, cardiovascular complications (4). It means chronic hyperglycemia impairs antioxidant defense system enzymes including catalase, glutathione

peroxidase, and superoxide dismutase in different organs (5). Peroxidation or glycation of lipids, proteins, and DNA, reduction of antioxidants defenses and progression of tissues inflammations are related to oxidative stress (6). Several observations demonstrate that reduction of oxidative stress could be favorable and has been able to mitigate the development of diabetic complication (7). So targeting oxidative stress is assumed to provide an opening window for discovering novel strategies against diabetic complications (8). Tropisetron is a 5-HT₃ receptor antagonist is a safe drug

is widely used as an effective and well-tolerated agent to counteract chemotherapy-induced emesis (9). It was known that tropisetron has antioxidant and anti-inflammatory properties (10, 11). As best of our knowledge, no such study has been performed regarding the protective effect of tropisetron in plasma oxidative biomarkers. Therefore the present study was done in order to investigate the ameliorative effect of tropisetron on plasma antioxidant status in T1DM.

Material & methods

Animals and experimental design:

All experimental design was conducted under the approval of the Ethic Committee for Animal Experiments of Urmia University of Medical Sciences. Male Wistar rats (200–250 g) were housed four per cage and maintained on a 12 h light–dark cycle in an air-conditioned constant temperature (22 ± 1 °C) room. Food and water were made available ad libitum. Twenty-one rats were randomly assigned into three experimental groups ($n = 7$):

1. Control: animals received saline i.p
2. Diabetic: STZ (50 mg/kg, i.p) injected in a single dose to induce diabetes
3. Diabetic + tropisetron: tropisetron (3 mg/kg, i.p) administered to diabetic animals for 2 weeks.

Following 1 week adaptation, experimental diabetes was induced by injection STZ (Sigma Chemical Co., St Louis, MO) was dissolved in saline (50 mg/kg) To confirm the induction of diabetes, 3 days after the STZ injection, blood glucose levels were measured from blood samples obtained by tail prick using a strip-operated glucometer (12).

(Elegance, CT-X10, Frankenberg, Germany). Animals with blood glucose levels higher than 300 mg/dl were selected. The control animals were injected with 0.4 mL of 0.9% saline. Tropisetron was dissolved in saline and administered (3mg/kg, i.p) once a day for 2 weeks (13).

Sampling:

At the end of 2th week, all rats were deeply anesthetized with ketamine (60mg/kg) and xylazine

(6mg/kg). Blood samples were collected from rat hearts by syringe from fasted animals mixed with ethylenediamine tetra-acetic acid (EDTA) as anticoagulant and centrifuged at 3000 rpm for 10 min and plasma stored at deep freeze (-70 °C) for determination of SOD, GPX, MDA, and TAC levels.

Determination of the plasma MDA, TAC levels and antioxidant enzyme activity (GPX, SOD):

The MDA levels, as a marker of oxidative stress, were measured by thiobarbituric acid reactive substances (TBARS) method and expressed as nmol/mL. SOD and GPx activities in the supernatants were determined using RANSOD kits (Randox Laboratories Ltd, United Kingdom) according to the manufacturer's instructions and expressed as U/g Hb in plasma (14).

TAC (nmol/mL) was assessed by a Randox (Crumlin, County Antrim, United Kingdom) total antioxidant status kit in which 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfanate) (ABTS) is incubated with a peroxidase and H₂O₂ resulting in the radical cation ABTS⁺ production. This has a stable blue-green color, which is measured at 600 nm (15).

Statistical Analysis:

Evaluation of normality was performed using the Kolmogorov-Smirnov test. Data were statistically analyzed using SPSS software (version 16) by one-way analysis of variance (ANOVA) followed by Tukey's test. The significant level was set at $p < 0.05$. Results are expressed as means \pm SEM.

Results

Lipid Peroxidation and Total Antioxidant Capacity:

Our results demonstrated a significant ($p < 0.001$, fig1) increase in MDA levels and a significant ($p < 0.001$, fig2) decrease in the TAC level in the plasma of diabetic group when compared with control group. However, tropisetron treatment significantly ($p < 0.001$) attenuated MDA levels and increased TAC content as compared to the diabetic group.

Antioxidant enzymes:

As shown in fig3 and 4, diabetes attenuated SOD and GPx ($p < 0.001$) levels in plasma versus the control

group. However, tropisetron remarkably enhanced the activity of these enzymes compared to the diabetic group ($p < 0.001$).

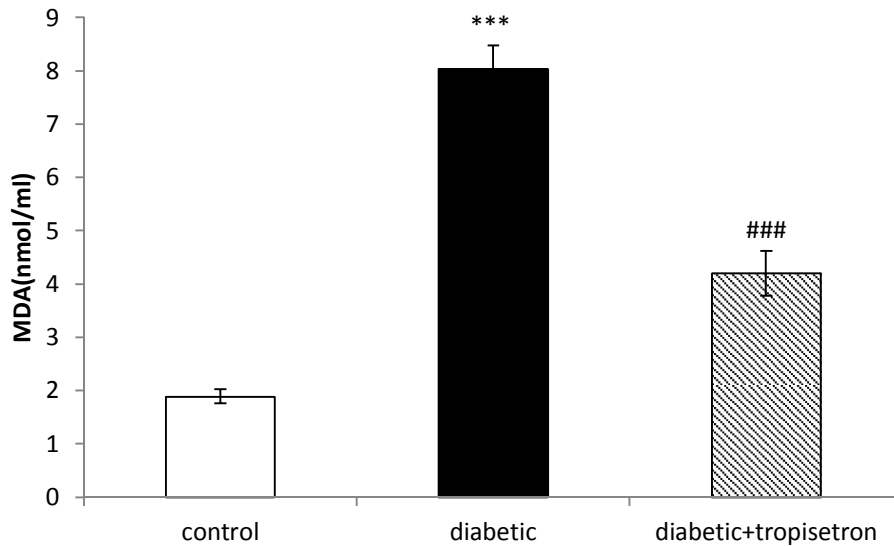


Fig.1: Effect of tropisetron on plasma MDA (malondialdehyde) level in different groups (means \pm SEM). *** $p < 0.001$ versus control group. ### $p < 0.001$ versus Diabetic group.

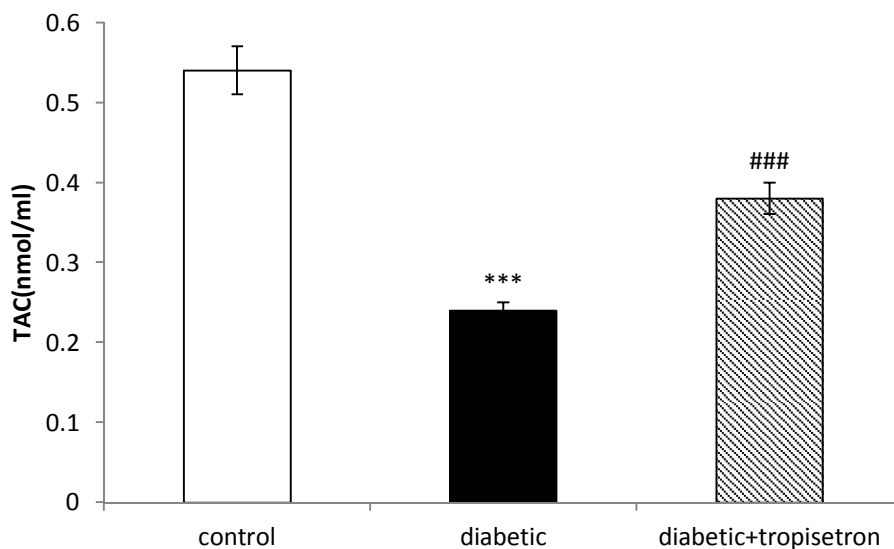


Fig.2: Effect of tropisetron on plasma TAC (total antioxidant capacity) level in different groups (means \pm SEM). *** $p < 0.001$ versus control group. ### $p < 0.001$ versus Diabetic group.

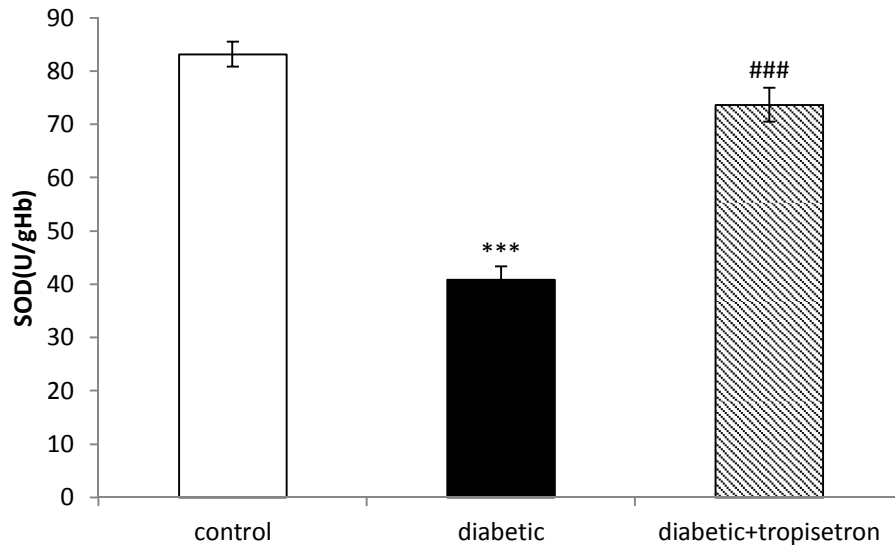


Fig.3: Effect of tropisetron on plasma SOD (superoxide dismutase) level in different groups (means \pm SEM). *** $p < 0.001$ versus control group. ### $p < 0.001$ versus Diabetic group.

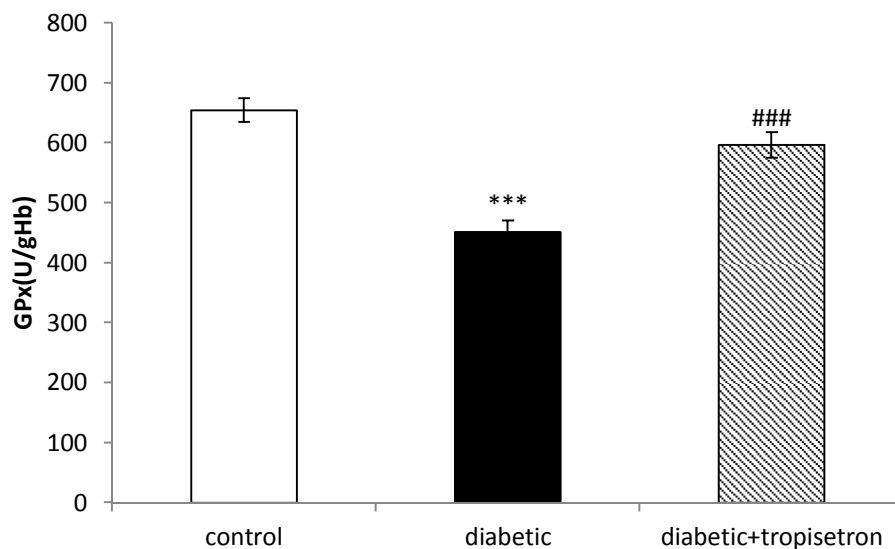


Fig.4: Effect of tropisetron on plasma GPx (glutathione peroxidase) level in different groups (means \pm SEM). *** $p < 0.001$ versus control group. ### $p < 0.001$ versus Diabetic group.

Discussion

Tropisetron is an antiemetic drug in clinical practice for the prevention of nausea and vomiting (16). It was known that tropisetron has an organ-protective function by attenuating inflammatory and apoptotic response (17). However, little is known about the role of tropisetron in diabetes-induced stress oxidative in plasma.

The present study revealed that tropisetron as a 5HT₃ antagonist attenuated oxidative stress biomarkers as manifested by decreased MDA content and increased SOD, GPx and TAC levels in plasma of diabetic animals.

Diabetes mellitus is a metabolic and common endocrine disorder which is associated with oxidative stress (12). Glucose oxidation, nonenzymatic glycation

of proteins, and the subsequent oxidative degradation of glycosylated proteins occurs as a result of accumulation-free radicals in diabetic animals (18).

Moreover, disturbances in the metabolism of glucose and oxidative stress are considered to be the main factors in various complications in non-treated diabetic animals (19). Lipid peroxidation is a key marker of oxidative stress which causes tissue damage in a diabetic situation (20). SOD is indicated as an important antioxidant enzyme, for the elimination of reactive oxygen species. Therefore, the reduction of the activity of this enzyme may cause accumulation of superoxide radicals and hydrogen peroxide (16).

In all, hyperglycemia, the main factor of the pathogenesis of diabetes, not only produces more free radicals, such as superoxide and hydrogen peroxide but also attenuates antioxidative mechanisms through glycation of the scavenging enzymes including SOD and CAT (21, 22). This is in agreement with our findings, as diabetic rats exhibited a marked increase in plasma MDA content and a decrease in TAC level and the activity of SOD and GPx. A good strategy for attenuating oxidative markers and increasing antioxidant capacity may contribute to improvement of various disorders induced by diabetes (14).

Moreover, several studies indicated that antioxidant therapy could obviate diabetic complications (14, 23). Previously it was indicated that tropisetron prevented enhanced oxidative stress and TNF- α level in early diabetic nephropathy (13). Amin Zadeh et al reported that pretreatment with tropisetron significantly improved ROS (reactive oxygen species) production, lipid peroxidation (LPO) levels and total antioxidant power (TAP) in PC12 culture cells so protected PC12 cells against hyperglycemia (24). These results are consistent with our finding.

To our best knowledge, there is no report about tropisetron-mitigated oxidative stress in plasma of diabetic animals in literature. In this study, we demonstrated that tropisetron could alleviate plasma oxidative status induced by diabetes.

Conclusion

In conclusion, our results present the beneficial properties of tropisetron in an experimental

model of STZ-induced diabetic condition, in part, through the improvement of oxidative stress. Future investigations can dissolve the detailed mechanisms of tropisetron-induced protection in diabetes.

Acknowledgments

This study was supported by a grant from the Urmia University of Medical Science.

Conflict of interests

The authors declare no conflict of interests.

References

1. Giugliano D, Ceriello A, Paolisso G. Oxidative stress and diabetic vascular complications. *Diabetes Care* 1996;19(3):257-67.
2. Ghiasi R, Ghadiri Soufi F, Somi MH, Mohaddes G, Mirzaie Babil F, Naderi R, et al. Swim Training Improves HOMA-IR in Type 2 Diabetes Induced by High Fat Diet and Low Dose of Streptozotocin in Male Rats. *Adv Pharm Bull* 2015;5(3):379-84.
3. Parveen K, Ishrat T, Malik S, Kausar MA, Siddiqui WA. Modulatory effects of Pycnogenol in a rat model of insulin-dependent diabetes mellitus: biochemical, histological, and immunohistochemical evidences. *Protoplasma* 2013;250(1):347-60.
4. Vessby J, Basu S, Mohsen R, Berne C, Vessby B. Oxidative stress and antioxidant status in type 1 diabetes mellitus. *J Internal Med* 2002;251(1):69-76.
5. Kamuren ZT, McPeck CG, Sanders RA, Watkins JB, 3rd. Effects of low-carbohydrate diet and Pycnogenol treatment on retinal antioxidant enzymes in normal and diabetic rats. *J Ocul Pharmacol Ther* 2006;22(1):10-8.
6. Rains JL, Jain SK. Oxidative stress, insulin signaling, and diabetes. *Free Radical Biol Med* 2011;50(5):567-75.
7. Naderi R, Mohaddes G, Mohammadi M, Ghaznavi R, Ghyasi R, Vatankhah AM. Voluntary Exercise Protects Heart from Oxidative Stress in Diabetic Rats. *Adv Pharm Bull* 2015;5(2):231-6.

8. Elmarakby AA, Sullivan JC. Relationship between oxidative stress and inflammatory cytokines in diabetic nephropathy. *Cardiovasc Ther* 2012;30(1):49-59.
9. Scuderi PE. Pharmacology of antiemetics. *Int Anesthesiol Clin* 2003;41(4):41-66.
10. Fiebich BL, Akundi RS, Lieb K, Candelario-Jalil E, Gmeiner D, Haus U, et al. Antiinflammatory effects of 5-HT₃ receptor antagonists in lipopolysaccharide-stimulated primary human monocytes. *Scand J Rheumatol Suppl* 2004;119:28-32.
11. Muller W, Fiebich BL, Stratz T. New treatment options using 5-HT₃ receptor antagonists in rheumatic diseases. *Curr Top Med Chem* 2006;6(18):2035-42.
12. Naderi R, Mohaddes G, Mohammadi M, Alihemmati A, Khamaneh A, Ghyasi R, et al. The Effect of Garlic and Voluntary Exercise on Cardiac Angiogenesis in Diabetes: The Role of MiR-126 and MiR-210. *Arq Bras Cardiol* 2019;112(2):154-62.
13. Barzegar-Fallah A, Alimoradi H, Asadi F, Dehpour AR, Asgari M, Shafiei M. Tropisetron ameliorates early diabetic nephropathy in streptozotocin-induced diabetic rats. *Clin Exp Pharmacol P* 2015;42(4):361-8.
14. Naderi R, Mohaddes G, Mohammadi M, Alihemmati A, Badalzadeh R, Ghaznavi R, et al. Preventive effects of garlic (*Allium sativum*) on oxidative stress and histopathology of cardiac tissue in streptozotocin-induced diabetic rats. *Acta physiologica Hungarica* 2015;102(4):380-90.
15. Mohammadi M, Ghaznavi R, Keyhanmanesh R, Sadeghipour HR, Naderi R, Mohammadi H. Voluntary exercise prevents lead-induced elevation of oxidative stress and inflammation markers in male rat blood. *Sci World J* 2013;2013:320704.
16. Liu F-C, Lee H-C, Liao C-C, Li AH, Yu H-P. Tropisetron Protects Against Acetaminophen-Induced Liver Injury via Suppressing Hepatic Oxidative Stress and Modulating the Activation of JNK/ERK MAPK Pathways. *Biomed Res Int* 2016;2016:1952947.
17. Rahimian R, Fakhfour G, Ejtemaei Mehr S, Ghia JE, Genazzani AA, Payandemehr B, et al. Tropisetron attenuates amyloid-beta-induced inflammatory and apoptotic responses in rats. *Eur J Clin Invest* 2013;43(10):1039-51.
18. Khan AN, Khan RA, Ahmad M, Mushtaq N. Role of antioxidant in oxidative stress and diabetes mellitus. *J Pharmacognosy Phytother* 2015;3(6):217-20.
19. Giacco F, Brownlee M. Oxidative stress and diabetic complications. *Circul Res* 2010;107(9):1058-70.
20. Jain N, Vijayaraghavan R, Pant SC, Lomash V, Ali M. Aloe vera gel alleviates cardiotoxicity in streptozocin-induced diabetes in rats. *J Pharm Pharmacol* 2010;62(1):115-23.
21. Ha H, Kim KH. Pathogenesis of diabetic nephropathy: the role of oxidative stress and protein kinase C. *Diabetes Res Clin Practice* 1999;45(2-3):147-51.
22. Wolff SP, Jiang ZY, Hunt JV. Protein glycation and oxidative stress in diabetes mellitus and ageing. *Free Radic Biol Med* 1991;10(5):339-52.
23. Tanveer A, Akram K, Farooq U, Hayat Z, Shafi A. Management of diabetic complications through fruit flavonoids as a natural remedy. *Crit Rev Food Sci Nutr* 2017;57(7):1411-22.
24. Aminzadeh A. Protective effect of tropisetron on high glucose induced apoptosis and oxidative stress in PC12 cells: roles of JNK, P38 MAPKs, and mitochondria pathway. *Metab Brain Disease* 2017;32(3):819-26.