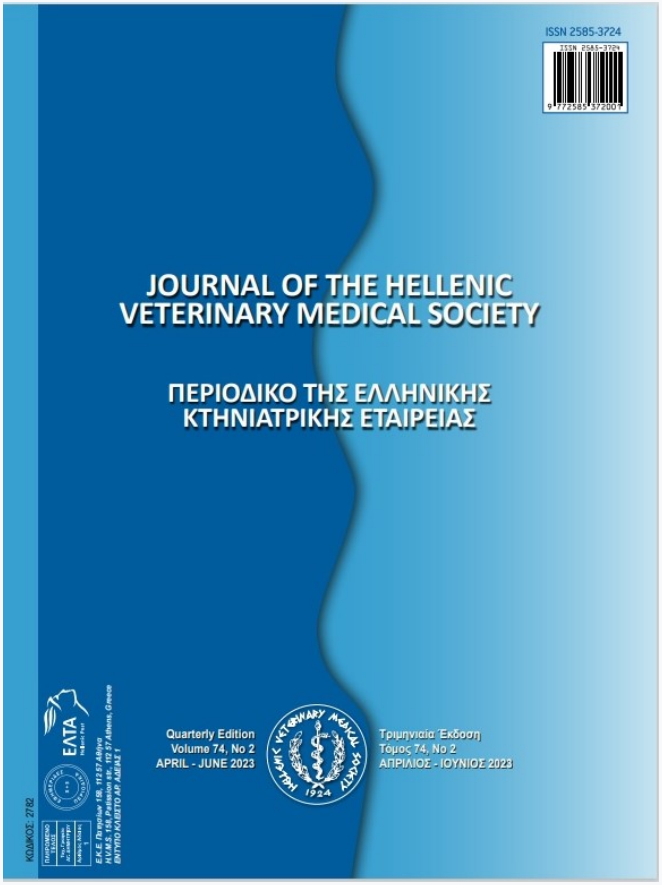
ePublishing

ΕΘΝΙΚΟ ΚΕΝΤΡΟ ΤΕΚΜΗΡΙΩΣΗΣ
EKT NATIONAL DOCUMENTATION CENTRE

Journal of the Hellenic Veterinary Medical Society

Vol 74, No 3 (2023)



Troxerutin as an anti-inflammatory and anti-oxidative drug could ameliorate Type 1 diabetes complications in C57BL/6 mice

Y Jafari-Khataylou, S Ahmadiafshar, N Mirzakhani

doi: 10.12681/jhvms.31426

Copyright © 2023, Y Jafari-Khataylou, S Ahmadiafshar, N Mirzakhani

This work is licensed under a Creative Commons Attribution-NonCommercial 4.0.

To cite this article:

Jafari-Khataylou, Y., Ahmadiafshar, S., & Mirzakhani, N. (2023). Troxerutin as an anti-inflammatory and anti-oxidative drug could ameliorate Type 1 diabetes complications in C57BL/6 mice. *Journal of the Hellenic Veterinary Medical Society*, *74*(3), 6257–6267. https://doi.org/10.12681/jhvms.31426

https://epublishing.ekt.gr | **e-Publisher:** EKT | **Downloaded at:** 25/05/2026 07:38:12

J HELLENIC VET MED SOC 2023, 74 (3): 6257-6267
ΠΕΚΕ 2023, 74 (3): 6257-6267

Research article
Ερευνητικό άρθρο

Troxerutin as an anti-inflammatory and anti-oxidative drug could ameliorate Type 1 diabetes complications in C57BL/6 mice

Y. Jafari-Khataylou[1], S. Ahmadiafshar[2,*], N. Mirzakhani[3]

[1]Assistant Professor of Immunology, Department of Pathobiology, Faculty of Veterinary Medicine, University of Tabriz, Tabriz, Iran

[2]Phd in Immunology, Department of Microbiology, Faculty of Veterinary Medicine, University of Urmia, Urmia, Iran

[3]Assistant Professor of Pathology, Department of Pathobiology, Faculty of Veterinary Medicine, Amol University of Special Modern Technologies, Amol, Iran

ABSTRACT: Type 1 diabetes (T1D) is a chronic autoimmune disease that even with insulin therapy, inflammatory complications will develop in the long term. 40 inbred C57BL/6 mice were randomly divided into four groups (n=10): Control group consisted of healthy mice receiving citrate buffer, Diabetic group included a group of diabetic mice, Diabetic+TX group was a group of diabetic mice treated with troxerutin (TX), and TX group was a group of healthy mice treated with TX. Two weeks after the final dose of streptozotocin (STZ), The cytokine levels were measured using ELISA in the culture supernatants of spleen cells after 72 hours. Radioimmunoassay was used to measure insulin and c-peptide levels. The fasting blood sugar (FBS) was measured by an automatic glucometer device. lymphocyte proliferation index was evaluated using MTT assay, myeloperoxidase (MPO) level was measured in serum and pathologic studies of the kidney and liver were performed. The levels of interleukin 1(IL-1), IL-17, tumor necrosis factor (TNF)-α and interferon(IFN)-γ as well as MPO, FBS levels and proliferation index was significantly decreased in the treated diabetic group compared to the diabetic mice ($p<0.05$). Plasma C-peptide and insulin significantly increased in treated diabetic group than in the diabetic mice ($p<0.05$). Histologically, in diabetic animals treated with TX, inflammatory and degenerative processes in both kidney and liver tissues were alleviated significantly ($p<0.05$). According to the results, it was supported the anti-diabetic and anti-inflammatory effects of TX, however, more studies are needed to investigate the effects of TX and the dose-response relationship in this disease.

Keywords: Anti-diabetic anti-inflammatory, anti-oxidative, diabetes type 1, C57BL/6 mice, troxerutin.

Corresponding Author:
Somayeh Ahmadiafshar, Iran, Urmia city, 11km SERO Road, postal code5756151818
E-mail address: Somayehahmadiafshar@gmail.com

Date of initial submission: 16-09-2022
Date of acceptance: 13-02-2023

INTRODUCTION

Type 1 diabetes (T1D) is one of the most common autoimmune diseases in the world, which results from the destruction of insulin-producing pancreatic cells (M.A. and G.S., 2001). The prevalence of this disease has been increasing worldwide in recent decades (relative increase in annual incidence is 1.8%) (Mayer-Davis *et al.*, 2017). During the disease, leukocytes infiltrate into the pancreatic islets and eventually cause activation of inflammatory cascades in these islets. As a result, this inflammation, termed insulitis, gradually destroys the *beta cells* of the *pancreatic islets*. Destruction of beta cells in the pancreas leads to insulin deficiency and eventually diabetes (Chang *et al.*, 2007). Insulin is a hormone that aids cells in utilizing glucose for energy. It causes glucose to be deposited as glycogen in liver cells and accordingly decreases glucose levels. Therefore, in T1D the body is unable to process glucose due to lack of insulin and this causes raise in level of glucose and blood sugar (Rother, 2007).

C-peptide is a byproduct of insulin production pathway which is produced by β-cell of the pancreas and secretes into the blood and has a longer half-life than insulin, so they are a good indicator of insulin levels (Kuhtreiber *et al.*, 2015). The balance between cytokines produced by T helper (Th)1 and Th2 cells secreted by immune cells in the islets of Langerhans is important in diabetes. For, the collapse of the balance and the tendency toward cytokines such as IFN-γ and TNF-α cause the development of inflammatory insulitis and diabetes. destruction of β cells in diabetes are associated with increased pro-inflammatory cytokines expression such as IL-1, TNF-α and IFN-γ and Th1 cytokines such as IFN-γ, TNF-α, IL-2 and IL-12. (Khataylou *et al.*, 2020; Xiao *et al.*, 2014). In addition to Th1, Th17 cells have been shown to be involved in T1D pathology. Because some Treg cells are defective in T1D patients, they lose control of Th17 expansion. Recently, some preliminary evidences suggest that Th17 cells may be an effective factor in the onset and development of T1D. (Emamaullee *et al.*, 2009; S. *et al.*, 2012).MPO is the most toxic enzyme in neutrophil azurophil granules, monocyte and neutrophil lysosomes. MPO kills pathogens by producing hypochlorous acid (HClO) during infections. However, this hypochlorous acid may also cause oxidative damage in host tissue. Increase in the level and activity of MPO has been observed in a number of autoimmune diseases including diabetes (Heilman *et al.*, 2009; Strzepa *et al.*, 2017). In general, the extend of leukocytes migration and aggregation can be measured by assessing the level of MPO.

Since chemical drugs have many side effects, herbal drugs today are preferred for control of diabetes complications. One of these herbal drugs is TX, a tri - hydroxyethylated derivative of natural flavonoids. TX is also known as vitamin P4 which is found in tea, coffee, cereal grains, some fruits and vegetables(Fan *et al.*, 2009). This substance has many biological and pharmacological advantages such as anti-oxidative, anti-inflammatory, anti-fatigue, anti-thrombolytic and anti-hyperglycemic properties (Yu and Zheng, 2017) .Based on descriptions provided above, the present study was evaluated the effects of TX administration during T1D on inflammatory cytokines, plasma C-peptide and insulin levels, FBS, MPO, lymphocyte proliferation index, inflammatory and degenerative processes in both kidney and liver tissues.

MATERIALS AND METHODS

Animals and treatment

This study was conducted on C57BL/6 inbred male mice aged 6 to 8 weeks purchased from the Pasteur Institute of Iran. The mice were kept under standard conditions with temperature (24°C) and humidity (60%) and a 12h light/dark schedule and given free access to normal food and water. After one week of acclimatization, mice were randomly divided into four groups (10 mice in each group): Control group which is treated with saline, diabetic mice and diabetic mice treated with TX (Diabetic + TX group (130 mg/kg body weight; intraperitoneal (i.p)) and TX group treated with TX (130 mg/kg body weight; i.p). The TX used in this study were based on previously published studies (Gui *et al.*, 2015; Shan *et al.*, 2017). The experimental method was confirmed by University of Tabriz Animal Ethical Committee (NUM: IR.TABRIZU.REC.1398.002). All experiments were conducted in accordance with ARRIVE guidelines (https All://arriveguidelines.org) for the care and use of Laboratory animals.

Diabetes Induction in C57BL/6 Mice and Treatment Protocol

Induction of diabetes was carried out through streptozotocin (STZ),(Sigma, Germany) at 50 mg/kg for 5 consecutive days, intraperitoneally, and the mice were fasted for 4 hours before induction of each STZ dose. 10 minutes before STZ injection, 50 mg/kg of this product was dissolved in 200 μL citrate buffer

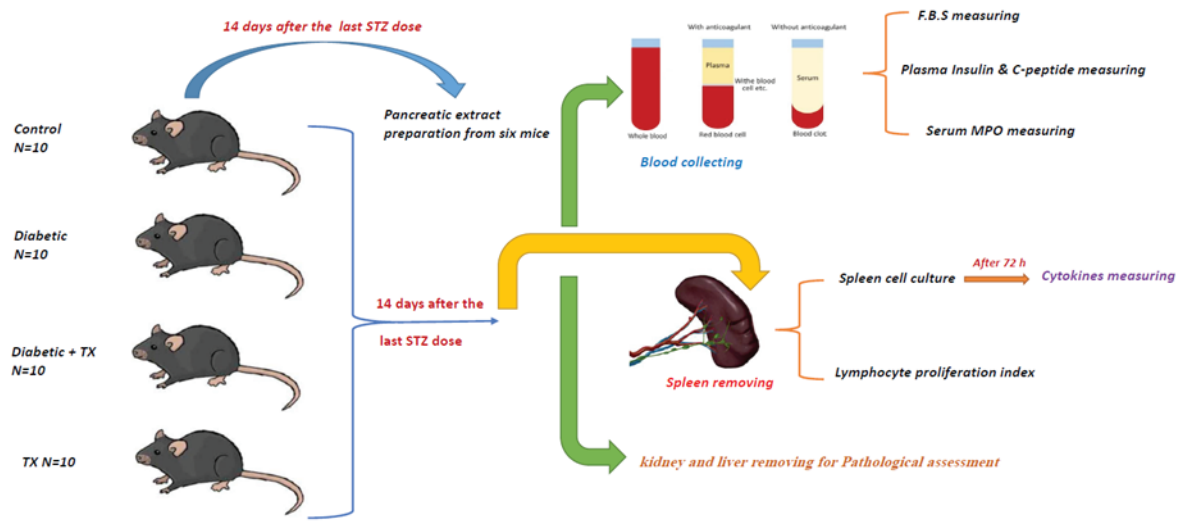


Figure1: Schematic diagram of sampling method and conducting experiments related to the study

with PH=4.5. When FBS (fasting blood sugar reached 300 mg/dL, the mice were considered diabetic. One day after the last induction dose of STZ (the sixth day), Troxerutin (Sigma, Germany) was injected i.p at a dose of 130 mg/kg for 14 days (Faridi *et al.*, 2019; Lenzen, 2008).

Evaluation of Diabetes in Mice

14 days after the last induction dose of Troxerutin, the FBS level in venous tail blood was measured using a Glucometer (ACCU-CHEK, compact plus, Ireland). To measure plasma insulin and C-peptide, a commercial kit (Rat Insulin ELISA kit, CAT # 1NSKR020, Crystal chem. Inc, Chicago, IL) and a radio- immuneassay commercial kit (cat # RCP-21K, Linco Res. Inc., st.charles, MO) were used according to the manufacturer's protocol (the sensitivity was 1.0 ng/mL for insulin and 0.5 ng/mL for C-peptide).

Pancreatic extract

To prepare pancreatic extracts as stimulating antigen, spleen cells of six C57BL/6 mice (mice in control group) were sacrificed by cervical dislocation, their pancreas was collected immediately afterward and homogenized in cold Phosphate-buffered saline Containing protease inhibitor (Sigma Co, Germany) and weighed, then homogenized tissue was placed in two stages in a centrifuge, the first phase 3000 rpm for 10 min and the second phase 12,000 rpm for 20 minutes at 4°C. The supernatant was collected. Bradford method was used to measure protein concentration (Kruger, 1994).

Spleen Cell Culture

To cultivate the spleen cells and cell culture supernatant used for Cytokines measuring six mice of each group were sacrificed two weeks after the last dose of STZ, then mice spleens were collected under sterile conditions and were moved to Petri dishes containing 5ml medium (Sigma America) RPMI-1640 containing 10% fetal bovine serum (FBS) (Gibco German company). Samples were completely sliced into small pieces and obtained tissue was passed through wire mesh with 1.0 mm diameter to prepare cell suspension and removal of excess tissue blocks. The obtained cell suspension (3 mL) was placed in pipes centrifuges and 3ml ficoll (Sigma, Germany) was slowly added so that ficoll was placed below the cell suspension and then 15 minutes at 4°C in 600g and centrifuged. The obtained cell pellet was washed two times in PBS and cultured in 24 houses plate (the number of 2×10^6 cell/ml from each sample) containing RPMI-1640 medium enriched by 10% FBS in the presence and absence of pancreas extract as stimulating antigen with concentration of 50 µg/mL for 72 hours in an incubator containing 5% CO2.

Cell culture supernatant Cytokines measuring

Supernatant of cell culture which was collected after 72h, were used to measure cytokines levels (TNF-α, IL-6, IL-1, IFN-γ) by Sandwich ELISA according to instructions of commercially available kit (Bender Med Co., Austria). The 96 wells plates were coated with specific mouse antibodies for IL-1, TNF-α, IFN-γ, IL-6 cytokines. Standards and samples were added in duplicates to the wells and incubated for 2h at room temperature. Then detection antibodies

were added for 1h and after washing the plates in PBS (phosphate buffer saline), Avidin- HRP (Horseradish peroxidase) was added and incubated for 30 min. Again, the wells were washed. Color was developed by adding TMB (Tetramethyl benzidine) as a substrate of HRP enzyme. 2 N sulphuric acid was used to stop the reaction. Absorbance was measured at 450 nm by ELISA reader. The cytokine levels of samples were calculated from the standard curve. The sensitivity was 1.6 pg/mL for IL-17, and 5.3 pg/mL for IFN-γ and TNF-α and 2.3 pg/ml for IL-1 (Chiswick *et al.*, 2012).

Lymphocyte proliferation index

After bleeding from the mice (all groups), , the spleens were removed under sterile conditions. Each spleen tissue was sliced and crushed in 5 ml of RPMI-1640 medium containing 05% FBS. The spleens were passed through a filter with a swelling of 0.02 ml and the cell suspension was centrifuged at 2000 rpm for 10 minutes. In order to remove the red blood cells, 5ml of ACK (Ammonium-Chloride-Potassium) buffer [containing 8.29g ammonium chloride, 1g potassium bicarbonate and 32.2g EDTA] was added to the cellular deposition. After the cell counting process, the cell suspension was prepared in 1×10^6 cell/ml of each sample. For each sample, three replicates were considered in the presence of 50µl of phytohemagglutinin (1 mg/ml) and three replicates without phytohemagglutinin. In the three wells, RPMI empty environment was used as blank. After 72 hours' incubation of the samples with 5% CO2, 25 µl of MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) (5 mg/ml) was added to each well. After adding the 100µl of DMSO (dimethyl sulfoxide), the color intensity was determined at 495 nm, and the stimulation index was calculated according to the following equation: stimulation index=OD (with the presence of Phytohemagglutinin (PHA)) -OD (blank)/OD (without the presence of PHA)-OD (blank) (Tim Mosmann, 1983).

Myeloperoxidase (MPO) activity measurement

The activity of MPO enzyme in collected sera was determined by method Pulli et al (2013). Briefly, 10 µL of serum sample was added to 80 µL H_2O_2, and 0.75 mM 3,3′,5,5′-Tetramethylbenzidine (TMB) (2.9mM TMB, 14.5% DMSO, 150 mM phosphate buffer, pH 5.4). After incubation for 5 minutes at 37 °C, the change in absorbance was measured at 450 nm. The outcomes were presented as U/L over 5 minutes, whereby one unit of the enzyme was described as the content of MPO degrading 1 nM H2O2 per minute at 37°C.

Histological examination

To evaluate the pathologic variations, kidneys and livers of mice were removed and kept in cold 10.00% buffered formalin. After preparing paraffin embedded blocks, lamination was done and prepared sections were stained by Hematoxylin and Eosin (H & E) method. Then they were studied in terms of hyperemia, infiltration of the inflammatory cells and degeneration in the kidney tissue by a pathologist under a light microscopy (YS100; Nikon, Tokyo, Japan). The severity of the lesion was determined by the following scores: 0= no lesion; 1= low; 2= medium; 3= severe(Lee *et al.*, 2010).

Statistical Analysis

Statistical analyses were conducted using the SPSS software 19.0 and one-way ANOVA. Additionally, the mean comparison was performed using Tukey HSD for parametric tests and non-parametric tests (repeated measure) for F.B.S data. Histopathological data were performed using the GraphPad Prism (8.0.1) software (San Diego, CA, USA). Because of semi-quantitative nature of data obtained from histopathological changes, Kruskal-Wallis and post hoc Mann-Whitney tests were performed. All values were expressed as the Mean ± SEM. Significance at $p<0.05$ has been given receptive in all testes.

RESULTS

Measurement of cytokines levels

Our results provided the levels of TNF-α, IL-17, IL-1, IFN-γ in spleen cell culture. Supernatant of diabetic group after 72h was markedly higher than that of the healthy control group with significant ($p<0.05$) and after treatment with troxerutin in diabetic + TX group the level of all these cytokines decreased significantly compared to diabetic group ($p<0.05$). No significant difference was observed in group of healthy mice treated with TX group compared to the control group (Figure 2)

TX effect on MPO activity and Lymphocyte proliferation index

Induction of diabetes with STZ (diabetic group) resulted in a significant increase in the activity of MPO compared to control group and after treatment with troxerutin (Diabetic + TX group), the level of MPO significantly decreased compared to the dia-

betic group (P < 0.05) (Figure3). Our findings presented marked increase in lymphocyte proliferation in diabetic group compared to the control group (P < 0.05). Furthermore, lymphocyte proliferation was significantly decreased in TX treated diabetic group compared to the diabetic group (P < 0.05). No significant difference was observed in group of healthy mice treated with TX compared to the control group. (Figure4).

Plasma C-peptide and Insulin Measuring

As our results indicated, Induction of diabetes with STZ (Diabetic group) resulted in a significant decrease in plasma insulin and C-peptide levels compared to control group (P < 0.05) and after treatment with troxerutin (Diabetic + tx group). Plasma insulin and C-peptide levels significantly increased compared to the diabetic group (P < 0.05). No significant difference was observed in group of healthy mice treated with TX compared to the control group. (Figure 5).

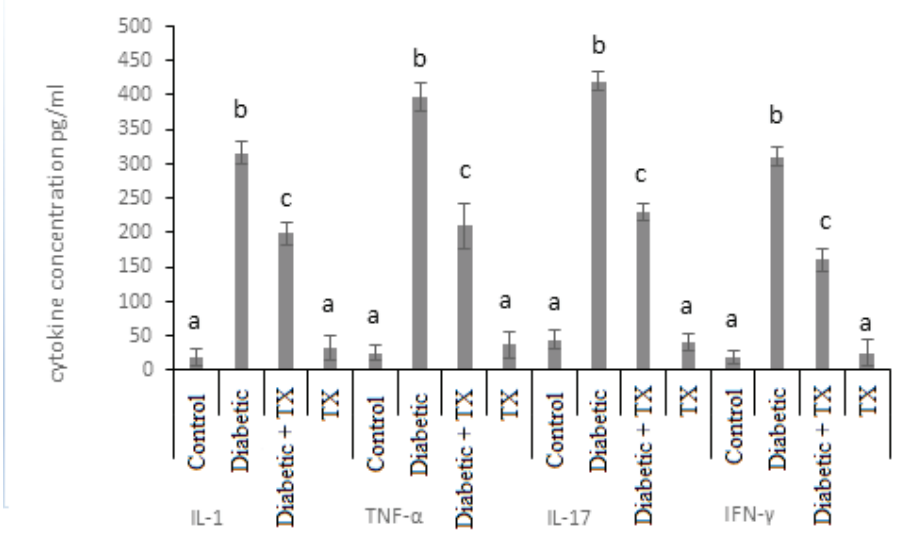


Figure 2: Cytokine concentration in the spleen cell culture supernatant after 72h. the values are shown as mean±SEM. columns with no common superscript letter differ significantly (P < 0.05).

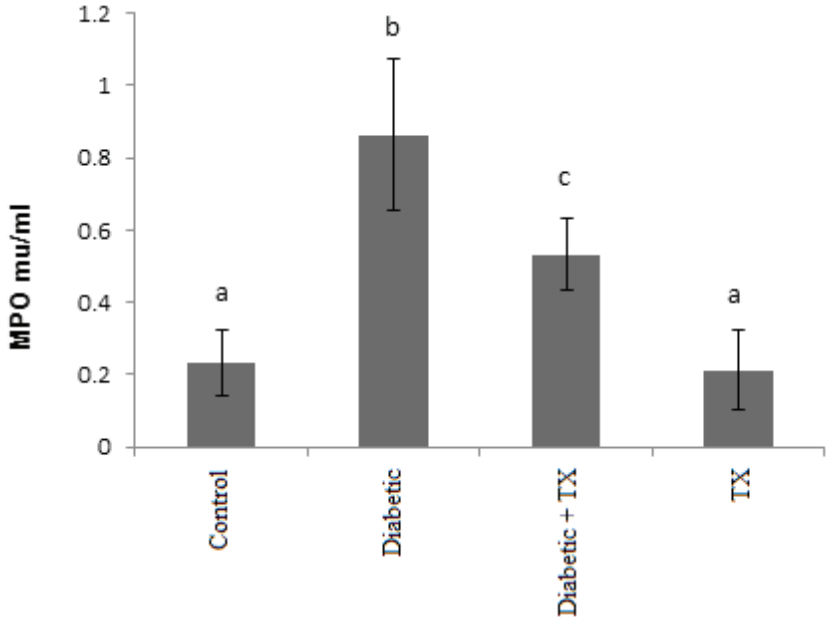


Figure 3: Effects of TX administration on MPO level. The values are shown as mean±SEM. columns with no common superscript letter differ significantly (P < 0.05).

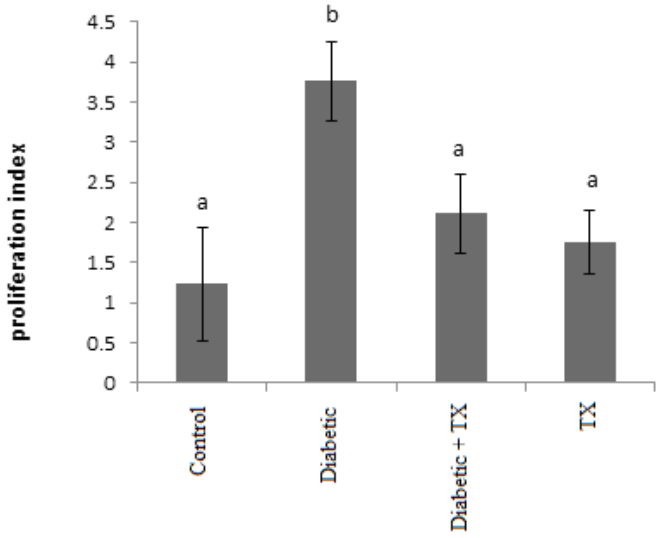


Figure 4: Effects of TX administration on proliferation index. The values are shown as mean±SEM. columns with no common superscript letter differ significantly ($p<0.05$).

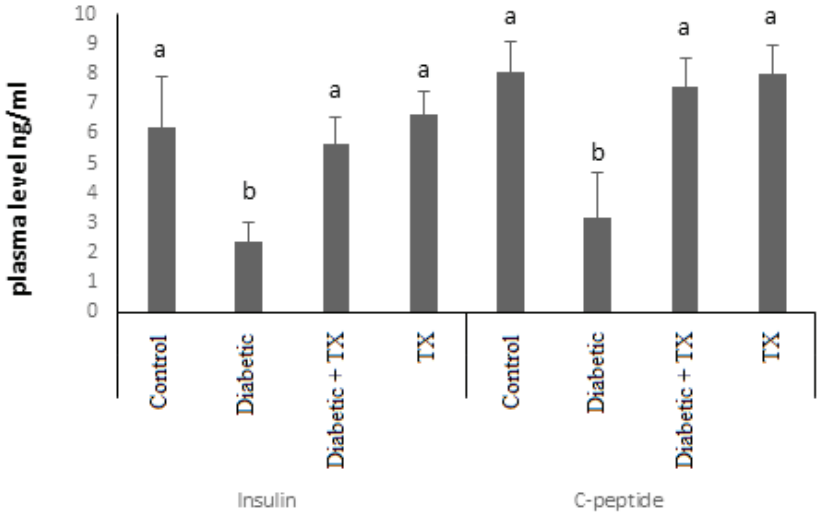


Figure 5: Effects of TX administration on Plasma C-peptide and Insulin. The values are shown as mean±SEM. columns with no common superscript letter differ significantly ($p<0.05$).

Measurement of FBS

Our data showed that the FBS level increased in diabetic group compared to the healthy control group; the difference was significant ($P<0.05$). However, the FBS level was significantly lower in diabetic mice treated with troxerutin (Diabetic + TX group) than in the untreated diabetic group ($P<0.05$). No significant difference was observed in group of healthy mice treated with TX compared to the control group (Figure 6).

Histopathological results:

The architecture of kidney and liver in control group were normal, with regular appearance of the tissue structure and absence of any inflammatory process or injury throughout the tissues (Figure 6). In diabetic group, kidney tissue revealed intense inflammatory cell infiltration along with hydropic degeneration of epithelial cells in the proximal tubules of the kidney. Besides, leakage of protein in lumen of the renal tubules due to glomerular injury were observed (Figure 6 and 7). In the liver tissue of diabetic animals, massive infiltration of mononuclear cells (MNC) were observed throughout the liver tissue along with notable fatty degeneration of hepatocytes and necrosis in some hepatocytes with pyknotic nuclei (Figure 6 and 8). In diabetic animals treated with TX (Diabetic+TX) inflammatory and degener-

ative processes in both kidney and liver tissues were alleviated significantly compared with diabetic group (Figure 6,7 and 8). In animals treated with TX only, no lesion was observed in kidney and liver tissues and they resembled completely to control group (Figure 7,8 and 9).

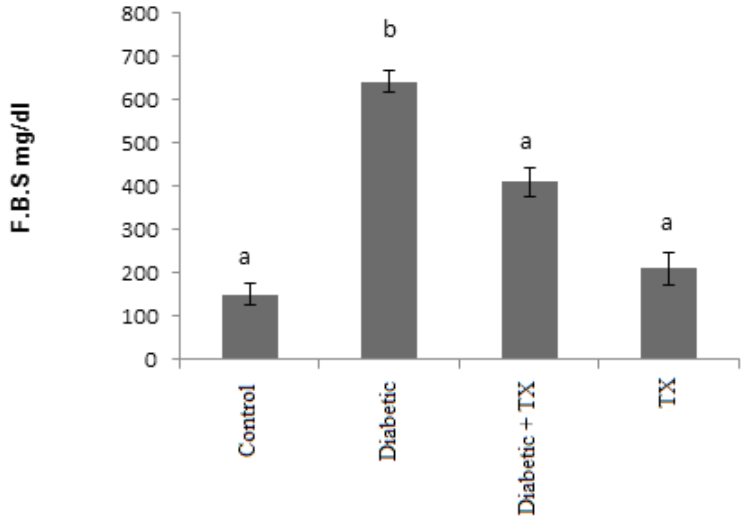


Figure 6: Effects of TX administration on FBS. The values are shown as mean±SEM. columns with no common superscript letter differ significantly ($p<0.05$).

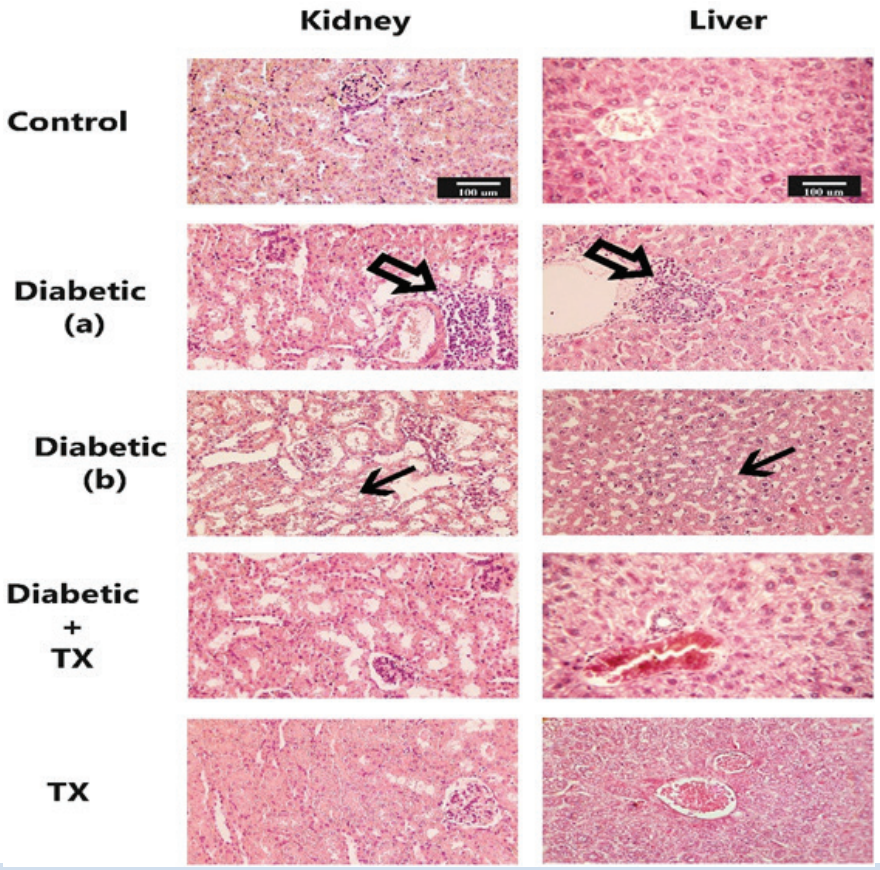


Figure 7: Photomicrograph of kidney and liver tissues of mice. In Control group normal architecture of kidney and liver tissue is revealed. In renal tissue of diabetic group, massive inflammatory cell infiltration (hollow arrow), and severe hydropic degeneration along with protein leakage in the lumen of tubules (thin arrow) are seen. Liver tissue of Diabetic group shows notable inflammatory cell infiltration (hollow arrow) and intense fatty degeneration along with necrosis of hepatocytes (thin arrow). In Diabetic+TX animals decreased degeneration in both kidney and liver tissues as well as alleviated inflammation are observed. In TX treated animal normal structure of kidney and liver are seen resembling control group (H & E, Scale bar: 100 μm of the section).

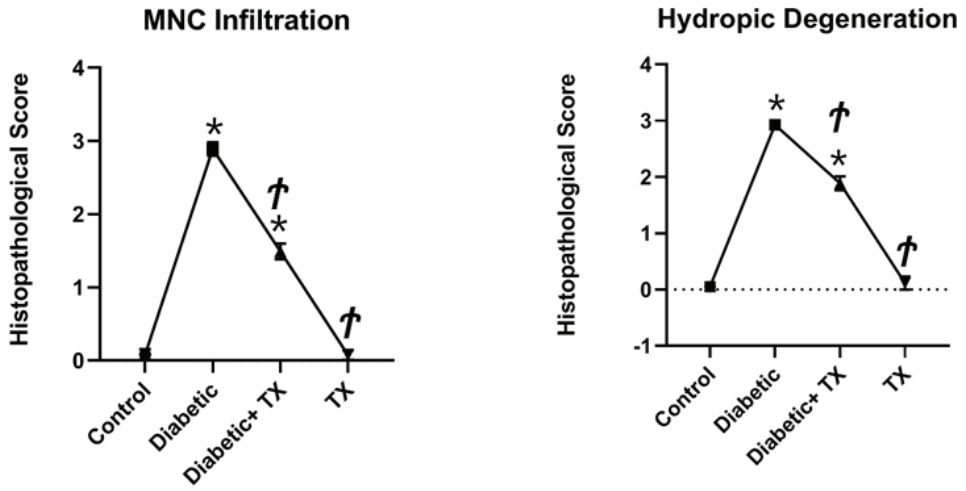


Figure 8: Severity of Mononuclear cell infiltration (MNC) and hydropic denegation in kidney tissue of experimental groups. * shows significant difference with control group and † shows significant difference with diabetes group ($p<0.05$).

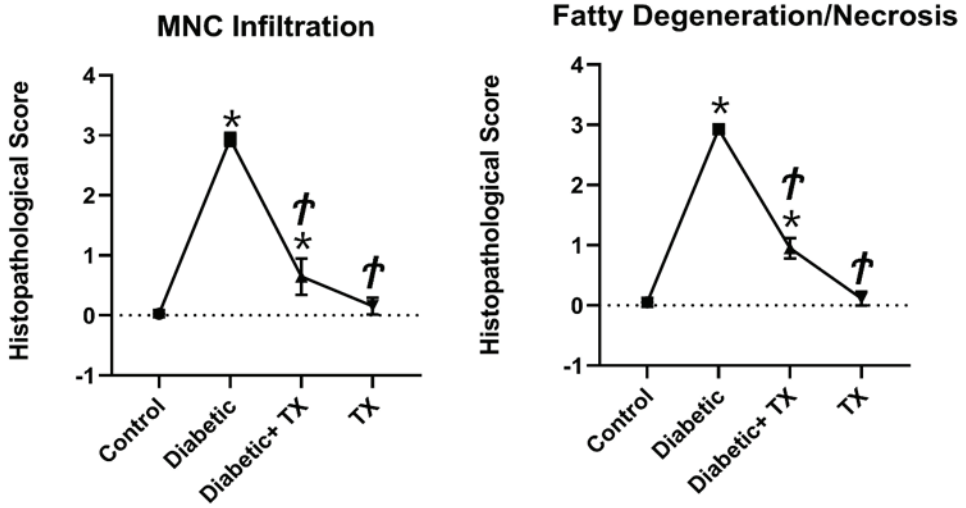


Figure 9: Severity of Mononuclear cell infiltration (MNC) and fatty denegation and necrosis in liver tissue of experimental groups. * shows significant difference with control group and † shows significant difference with diabetes group ($p<0.05$).

DISCUSSION

In this study, it appears that TX reduces the expression of inflammatory cytokines and decreases proliferation index and MPO levels in diabetic rats, causing anti-inflammatory effects and reducing β-cell destruction in pancreatic islets of Langerhans. These findings support the positive effects of TX as an anti-diabetic and anti-inflammatory agent. Previous studies have shown that TX has beneficial effects in various diseases including cognitive dysfunction and diabetes (Badalzadeh *et al.*, 2015; Farajdokht *et al.*, 2017). These beneficial effects of troxerutin include increased antioxidant activity, decreased lipid peroxidation levels (Badalzadeh *et al.*, 2015), anti-diabetes (Sampath and Karundevi, 2014), anti-inflammatory (Fan *et al.*, 2009) and anti-apoptotic effects (Mokhtari *et al.*, 2015). In this study, the significant reduction in spleen cell culture supernatant cytokines of diabetic mice is provided as an evidence of the anti-inflammatory activity of TX

Many studies have noted to the important role of cytokines in regulation complex multicellular interactions between β cells and immune cells in the development of type 1 diabetes (T1D), therefore cytokines could be considered potential immunotherapy targets for this disorder. It is thought that cytokines such as IL -6, IL -17, IL -21 and TNF-α, which increase the differentiation and function of immune cells, lead to the onset and progression of T1D. Therefore, reduction in inflammatory cytokines with immunosuppressive drugs is a possible means of preventing β cell

failure (Langrish *et al.*, 2005). Key pro-inflammatory cytokines in T1D are IL- 1β, TNF-α and IFN-γ. IL-17 is another cytokine that is considered to be one of the main causes of inflammation in this autoimmune disease.

Islet-infiltrating immune cells are thought to secrete these cytokines in the vicinity of beta cells at high concentrations in T1D. These cytokines synergize to produce cytotoxic effects, which eventually destroy β cells in the islets of Langerhans by accelerating apoptosis (Miller and Raison, 2016; Rabinovitch, 1998).The positive therapeutic effect of TX has been shown in many other autoimmune diseases. The results of a survey In 2020 showed that treatment with TX in a mouse model of MS caused a decrease in the levels of cytokines IL-17, IL-1, TNF-α and also a decrease in nitric oxide production, which resulted in improved symptoms (Jafari-Khataylou and Ahmadiafshar, 2020). In another study, in the same year, it was found that TX significantly reduce the inflammatory cytokines and enhances the anti-oxidant activity in septic mice. Further, TX significantly decreased the MPO activity and lymphocyte infiltration. TX was found safe at administered dose with potent anti-inflammatory and antioxidant activity. Treatment with TX caused high survival rates compared to untreated septic mice. Findings of this study indicated that TX is a potential therapeutic agent in the management of sepsis (Jafari-Khataylou et al., 2021). In line with these studies, the present study showed that the levels of pro-inflammatory cytokines IL-1, IL-17, TNF-α and IFN-γ increased significantly in diabetic mice and treatment with troxerutin, significantly reduced the levels of pro-inflammatory cytokines IL-1, IL-17, TNF-α and IFN-γ.

Another study was conducted to investigate the protective effects of this drug on rat liver against oxidative damage caused by D-Galactose stress, in which troxerutin was shown to improve the symptoms of type 2 diabetes, including fasting blood sugar, through an anti-inflammatory mechanism and significantly inhibits the inflammatory response in rat liver (Zhang *et al.*, 2015).

We measured the anti-proliferative effect of TX on splenocyte proliferation ex vivo. Finally, it was found that proliferation index increased significantly in diabetic mice and TX significantly decreased proliferation index in diabetic mice. In line with this result, Jafari-Khataylou et al. (2020), measured the anti-proliferative effect of TX on LPS induced splenocyte proliferation ex vivo and demonstrated that Splenocyte proliferation caused by LPS significantly reduced in TX + LPS group. In addition, it was investigated the cytotoxic effect of TX on peritoneal macrophages and splenocytes by MTT method, which is a common method for monitoring of extracts and natural compounds, and no cytotoxic effect on the macrophages and splenocytes were observed in treated groups with TX (Jafari-Khataylou *et al.*, 2021).

MPO, a potent oxidative mediator, is found mainly in the primary granules of neutrophils and contains approximately 5% of all neutrophil proteins. MPO is associated with oxidative stress because it catalyzes the formation of ROS, which could facilitate atherogenesis and alter fats and proteins. Increased MPO activity has been shown to indicate inflammation(Li *et al.*, 2018). A number of other studies have shown that serum MPO concentrations in diabetic children are significantly increased compared to the healthy control group (Heilman *et al.*, 2009; Olza *et al.*, 2012) also Increased levels and activity of MPO have been observed in a number of other autoimmune diseases including multiple sclerosis (MS) (Gray *et al.*, 2008) and rheumatoid arthritis (RA) (Odobasic *et al.*, 2014; Strzepa *et al.*, 2017). HCLO (Hypochlorous acid) produced by MPO cause oxidative damage in host tissue and MPO activity could be inhibited in diabetic patients, thereby reducing the phagocytic activity of neutrophils. MPO is also measured to evaluate leucocyte infiltration. Our findings showed that induction of diabetes in mice resulted in a significant increase in MPO activity compared with controls.

In TX-treated diabetic mice, a significant decrease in MPO activity was observed compared to the diabetic group ($P < 0.05$). In addition, as mentioned above, the rate of proliferation of inflammatory cells in the TX-treated group significantly decreased. Consistent with the findings of this study, it has been shown in previous studies that MPO activity is significantly reduced in mice with TX-treated sepsis. In addition, the infiltration of inflammatory cells in the TX-treated group was significantly reduced, which was confirmed by histological examination of the liver (Jafari-Khataylou *et al.*, 2021) and also Dadpisheh *et al.*, (2020) showed that TX decreased the activity of MPO, MDA, and ROS in the sciatic nerve ischemia-reperfusion injury, which is consistent with the results of this study. In the present study, we also treated diabetic mice with troxerutin, which showed a decrease in glucose levels and an increase in insulin and C-peptide levels

that in all cases, the resulting changes were significant ($p<0.05$). Similar to our results, Zavvari Oskuye et al. (2019) Showed that troxerutin significantly reduced the blood sugar in the diabetic group (Sampath and Karundevi, 2014). Similarly, another study examining the role of troxerutin in the type-1 diabetes-induced testicular disorders has shown that troxerutin plays a key role in the management of the disease by lowering blood glucose and modulating apoptosis (Ghadiri et al., 2020). Chen et al. In a 2014 study examined the effects of a number of natural flavonoids on diabetes. The results of this study in many flavonoids are similar to the results of the present study

Many studies have shown decreased glucose, anti-inflammatory, anti-apoptotic and antioxidant effects of troxerutin in other models (Sampath and Karundevi, 2014; Yu and Zheng, 2017). Geetha *et al.*, 2017 have shown that TX administration improved insulin resistance and decreased serum glucose levels in diabetic mice. In another study done by Qadiri et al. (2017) chronic administration of TX to type 1 diabetic adult male mice significantly reduced blood glucose levels and significantly increased insulin levels and C-peptide. Moreover, TX is successful in improving the histopathological changes of the kidney and liver. It seems that the TX can be considered as a useful agent in the treatment of type 1 diabetes.

CONCLUSION

Although this study supports the anti-inflammatory role of troxerutin due to the significant effect of this flavonoid on the level of pro-inflammatory cytokines and MPO, more studies are required to confirm the role of TX in reducing the complications of autoimmune T1D as well as the relationship between dose and response.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENT

We would like to express our thanks to the university of Tabriz and faculty of veterinary medicine for financial support.

Permission has been obtained for use of copyrighted material from other sources.

The experimental method was confirmed by University of Tabriz Animal Ethical Committee (NUM: IR.TABRIZU.REC.1398.002). All experiments were conducted in accordance with National Institutes of Health guide for the care and use of Laboratory animals (NIH Publications No. 8023, revised 1978).

REFERENCES

Abuohashish H.M., Al-Rejaie S.S., Al-Hosaini K.A., Parmar M.Y. & Ahmed M.M. 2013. Alleviating effects of morin against experimentally-induced diabetic osteopenia. *Diabetol Metab Syndr*, 5.

Badalzadeh R., Baradaran B., Alihemmati A., Yousefi B. & Abbaszadeh A. 2017. Troxerutin Preconditioning and Ischemic Postconditioning Modulate Inflammatory Response after Myocardial Ischemia/Reperfusion Injury in Rat Model. *Inflammation*, 40, 136-143.

Badalzadeh R., Chodari L. & Ghorbanzadeh V. 2017. Troxerutin, a bioflavonoid, improves oxidative stress in blood of streptozotocin-induced Type-1 diabetic rats. *Iran J Pharm Sci*, 13, 75-86.

Badalzadeh R., Layeghzadeh N., Alihemmati A. & Mohammadi M. 2015. Beneficial effect of troxerutin on diabetes-induced vascular damages in rat aorta: Histopathological alterations and antioxidation mechanism. *Int J Endocrinol Metab*, 13.

Chang C.L.-T., Chang S.-L., Lee Y.-M., Chiang Y.-M., Chuang D.-Y., Kuo H.-K., et al. 2007. Cytopiloyne, a Polyacetylenic Glucoside, Prevents Type 1 Diabetes in Nonobese Diabetic Mice. *J Immunol*, 178, 6984-6993.

Chen J., Mangelinckx S., Adams A., Wang Z.T., Li W.L. & De Kimpe N. 2015. Natural flavonoids as potential herbal medication for the treatment of diabetes mellitus and its complications. *Nat Prod Commun*, 10, 187-200.

Chiswick E.L., Duffy E., Japp B. & Remick D. 2012. Detection and quantification of cytokines and other biomarkers. *Methods Mol Biol*, 844, 15-30.

D. Sahu, S. Bishwal, Md. Zubbair Malik, Sukanya Sahu, S. Kaushik, Shikha Sharma, Ekta Saini, Rakesh K Arya, A. Rastogi, Sandeep Sharma, Shanta Sen, R. Singh, R. Nanda A.P. 2021. Troxerutin-mediated C9 inhibition is a disease- modifying treatment for in ammatory arthritis . *Medicine (Baltimore)*. , 2021.

Dadpisheh S., Ahmadvand H., Jafaripour L., Nouryazdan N., Babaeenezhad E., Shati H., et al. 2020. Effect of troxerutin on oxidative stress induced by sciatic nerve ischemia-reperfusion injury in rats. *J Kerman Univ Med Sci*, 27, 338-347.

Dasu M.R., Devaraj S., Zhao L., Hwang D.H. & Jialal I. 2008. High glucose induces toll-like receptor expression in human monocytes Mech-

anism of activation. *Diabetes*, 57, 3090-3098.
Elangovan P., Jalaludeen A.M., Ramakrishnan R., Amutha K. & Pari L. 2020. In-vivo and in-vitro antioxidant activity of troxerutin on nickel induced toxicity in experimental rats. *Iran J Pharm Res*, 19, 89-97.
Emamaullee J.A., Davis J., Merani S., Toso C., Elliott J.F., Thiesen A., et al. 2009. Inhibition of Th17 cells regulates autoimmune diabetes in NOD mice. *Diabetes*, 58, 1302-1311.
Fan S. hua, Zhang Z. feng, Zheng Y. lin, Lu J., Wu D. mei, Shan Q., et al. 2009. Troxerutin protects the mouse kidney from d-galactose-caused injury through anti-inflammation and anti-oxidation. *Int Immunopharmacol*, 9, 91-96.
Farajdokht F., Amani M., Bavil F.M., Alihemmati A., Mohaddes G. & Babri S. 2017. Troxerutin protects hippocampal neurons against amyloid beta-induced oxidative stress and apoptosis. *EXCLI J*, 16, 1081-1089.
Faridi S., Delirezh N. & Abtahi Froushani S.M. 2019. Beneficial effects of hydroalcoholic extract of saffron in alleviating experimental autoimmune diabetes in C57BL/6 mice. *Iran J Allergy, Asthma Immunol*, 18, 38-47.
Geetha R., Priya C.S., Anuradha C. V, Yogalakshmi B., Sreeja S., Bhavani K., et al. 2017. Troxerutin suppresses lipid abnormalities in the heart of high-fat-high-fructose diet-fed mice. *Mol Cell Biochem*, 387, 123-134.
Geetha R., Radika M.K., Priyadarshini E., Bhavani K. & Anuradha C.V. 2015. Troxerutin reverses fibrotic changes in the myocardium of high-fat high-fructose diet-fed mice. *Mol Cell Biochem*, 407, 263-279.
Ghadiri A., Bavil F.M., Hamidian G.R., Oghbaei H., Oskuye Z.Z., Ahmadi M., et al. 2020. Can troxerutin pretreatment prevent testicular complications in prepubertal diabetic male rats? *Endocr Regul*, 54, 85-95.
Gray E., Thomas T.L., Betmouni S., Scolding N. & Love S. 2008. Elevated activity and microglial expression of myeloperoxidase in demyelinated cerebral cortex in multiple sclerosis. *Brain Pathol*, 18, 86-95.
Gui Y., Li A., Chen F., Zhou H., Tang Y., Chen L., et al. 2015. Involvement of AMPK/SIRT1 pathway in anti-allodynic effect of troxerutin in CCI-induced neuropathic pain. *Eur J Pharmacol*, 769, 234-241.
Heilman K., Zilmer M., Zilmer K., Lintrop M., Kampus P., Kals J., et al. 2009. Arterial stiffness, carotid artery intima-media thickness and plasma myeloperoxidase level in children with type 1 diabetes. *Diabetes Res Clin Pract*, 84, 168-173.
Hoseindoost M., Alipour M.R., Farajdokht F., Diba R., Bayandor P., Mehri K., et al. 2019. Effects of troxerutin on inflammatory cytokines and BDNF levels in male offspring of high-fat diet fed rats. *Avicenna J Phytomedicine*, 9, 597-605.
Jafari-Khataylou Y. & Ahmadiafshar S. 2020. EFFECT OF TROXEROTIN ON PROINFLAMMATORY CYTOKINES AND LYMPHOCYTE PROLIFERATION IN AN ANIMAL MODEL OF AUTOIMMUNE EXPERIMENTAL ENCEPHALOMYELITIS. *J Urmia Univ Med Sci*, 31, 485-498.
Jafari-Khataylou Y., Emami S.J. & Mirzakhani N. 2021. Troxerutin attenuates inflammatory response in lipopolysaccharide-induced sepsis in mice. *Res Vet Sci*, 135, 469-478.
Khataylou Y.J., Ahmadiafshar S., Rezaei R., Parsamanesh S. & Hosseini G. 2020. Curcumin ameliorate diabetes type 1 complications through decreasing pro-inflammatory cytokines in C57BL/6 mice. *Iran J Allergy, Asthma Immunol*, 19, 55-62.
Kim H.J., Kim S.H. & Yun J.M. 2012. Fisetin inhibits hyperglycemia-induced proinflammatory cytokine production by epigenetic mechanisms. *Evidence-based Complement Altern Med*, 2012.
Kruger N.J. 1994. The Bradford method for protein quantitation. *Methods Mol Biol*, 32, 9-15.
Kuhtreiber W.M., Washer S.L.L., Hsu E., Zhao M., Reinhold P., Burger D., et al. 2015. Low levels of C-peptide have clinical significance for established Type 1 diabetes. *Diabet Med*, 32, 1346-1353.
Langrish C.L., Chen Y., Blumenschein W.M., Mattson J., Basham B., Sedgwick J.D., et al. 2005. IL-23 drives a pathogenic T cell population that induces autoimmune inflammation. *J Exp Med*, 201, 233-240.
Lee J., Yee S.T., Kim J.J., Choi M.S., Kwon E.Y., Seo K. Il, et al. 2010. Ursolic acid ameliorates thymic atrophy and hyperglycemia in streptozotocin-nicotinamide-induced diabetic mice. *Chem Biol Interact*, 188, 635-642.
Lee S.M., Yang H., Tartar D.M., Gao B., Luo X., Ye S.Q., et al. 2011. Prevention and treatment of diabetes with resveratrol in a non-obese mouse model of type 1 diabetes. *Diabetologia*, 54, 1136-1146.
Lenzen S. 2008. The mechanisms of alloxan- and streptozotocin-induced diabetes. *Diabetologia*, 51, 216-226.
Li Z., Gao M., Yang B., Zhang H., Wang K., Liu Z., et al. 2018. Naringin attenuates MLC phosphorylation and NF-κB activation to protect sepsis-induced intestinal injury via RhoA/ROCK pathway. *Biomed Pharmacother*, 103, 50-58.
Lu J., Wu D., Zheng Y., Hu B., Cheng W., Zhang Z., et al. 2013. Troxerutin Counteracts Domoic Acid-Induced Memory Deficits in Mice by Inhibiting CCAAT/Enhancer Binding Protein β-Mediated Inflammatory Response and Oxidative Stress. *J Immunol*, 190, 3466-3479.
M.A. A. & G.S. E. 2001. Type 1 diabetes: New perspectives on disease pathogenesis and treatment. *Lancet*, 358, 221-229.
Mayer-Davis E.J., Lawrence J.M., Dabelea D., Divers J., Isom S., Dolan L., et al. 2017. Incidence Trends of Type 1 and Type 2 Diabetes among Youths, 2002-2012. *N Engl J Med*, 376, 1419-1429.
Miller A.H. & Raison C.L. 2016. The role of inflammation in depression: From evolutionary imperative to modern treatment target. *Nat Rev Immunol*, 16, 22-34.
Mokhtari B., Badalzadeh R., Alihemmati A. & Mohammadi M. 2015. Phosphorylation of GSK-3β and reduction of apoptosis as targets of troxerutin effect on reperfusion injury of diabetic myocardium. *Eur J Pharmacol*, 765, 316-321.
Odobasic D., Yang Y., Muljadi R.C.M., O'Sullivan K.M., Kao W., Smith M., et al. 2014. Endogenous myeloperoxidase is a mediator of joint inflammation and damage in experimental arthritis. *Arthritis Rheumatol*, 66, 907-917.
Olza J., Aguilera C.M., Gil-Campos M., Leis R., Bueno G., Martínez-Jiménez M.D., et al. 2012. Myeloperoxidase is an early biomarker of inflammation and cardiovascular risk in prepubertal obese children. *Diabetes Care*, 35, 2373-2376.
Padgett L.E., Broniowska K.A., Hansen P.A., Corbett J.A. & Tse H.M. 2013. The role of reactive oxygen species and proinflammatory cytokines in type 1 diabetes pathogenesis. *Ann N Y Acad Sci*, 1281, 16-35.
Pulli B., Ali M., Forghani R., Schob S., Hsieh K.L.C., Wojtkiewicz G., et al. 2013. Measuring Myeloperoxidase Activity in Biological Samples. *PLoS One*, 8.
Qadiri A., Mirzaei Bavil F., Hamidian G., Zavvari Oskuye Z., Ahmadi M., Oghbaei H., et al. 2019. Administration of troxerutin improves testicular function and structure in type-1 diabetic adult rats by reduction of apoptosis. *Avicenna J phytomedicine*, 9, 374-385.
Rabinovitch A. 1998. An update on cytokines in the pathogenesis of insulin-dependent diabetes mellitus. *Diabetes / Metab Rev*, 14, 129-151.
Rengasamy K.R.R., Khan H., Gowrishankar S., Lagoa R.J.L., Mahomoodally F.M., Khan Z., et al. 2019. The role of flavonoids in autoimmune diseases: Therapeutic updates. *Pharmacol Ther*, 194, 107-131.
Rother K.I. 2007. Diabetes Treatment — Bridging the Divide. *N Engl J Med*, 356, 1499-1501.
S. S., F. H., Y. Y., G. Y., M. Z., X. Y., et al. 2012. Th17 cells in type 1 diabetes. *Cell Immunol*, 280, 16-21.
Sampath S. & Karundevi B. 2014. Effect of troxerutin on insulin signaling molecules in the gastrocnemius muscle of high fat and sucrose-induced type-2 diabetic adult male rat. *Mol Cell Biochem*, 395, 11-27.
Shan Q., Zhuang J., Zheng G., Zhang Z., Zhang Y., Lu J., et al. 2017. Troxerutin reduces kidney damage against BDE-47-induced apoptosis via inhibiting NOX2 activity and increasing Nrf2 activity. *Oxid Med Cell Longev*, 2017, 1-12.
Strzepa A., Pritchard K.A. & Dittel B.N. 2017. Myeloperoxidase: A new player in autoimmunity. *Cell Immunol*, 317, 1-8.
Tim Mosmann. 1983. Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. *J Immunol Methods*, 65, 55-63.
Wang H., Bloom O., Zhang M., Vishnubhakat J.M., Ombrellino M., Che J., et al. 1999. HMG-1 as a late mediator of endotoxin lethality in mice. *Science (80-)*, 285, 248-251.
Xiao J., Li J., Cai L., Chakrabarti S. & Li X. 2014. Cytokines and diabetes research. *J Diabetes Res*, 2014.
Xing C., Xiang D. & Caiying L. 2020. Effects of troxerutin on vascular inflammatory mediators and expression of microRNA-146a/NF-ΚB

signaling pathway in aorta of healthy and diabetic rats. *Korean J Physiol Pharmacol*, 24, 395-402.

Yang H., Wang H. & Andersson U. 2020. Targeting Inflammation Driven by HMGB1. *Front Immunol*, 11, 484.

Yu Y. & Zheng G. 2017. Troxerutin protects against diabetic cardiomyopathy through NF-κB/AKT/IRS1 in a rat model of type 2 diabetes. *Mol Med Rep*, 15, 3473-3478.

Zhang S., Li H., Zhang L., Li J., Wang R. & Wang M. 2017. Effects of troxerutin on cognitive deficits and glutamate cysteine ligase subunits in the hippocampus of streptozotocin-induced type 1 diabetes mellitus rats. *Brain Res*, 1657, 355-360.

Zhang Z., Wang X., Zheng G., Shan Q., Lu J., Fan S., et al. 2017. Troxerutin attenuates enhancement of hepatic gluconeogenesis by inhibiting NOD activation-mediated inflammation in high-fat diet-treated mice. *Int J Mol Sci*, 18.

Zhang Z.F., Shao-Hua F.A.N., Zheng Y.L., Jun L.U., Dong-Mei W.U., Shan Q.U.N., et al. 2009. Troxerutin protects the mouse liver against oxidative stress-mediated injury induced by d-galactose. *J Agric Food Chem*, 57, 7731-7736.

Zhang Z.F., Zhang Y.Q., Fan S.H., Zhuang J., Zheng Y.L., Lu J., et al. 2015. Troxerutin protects against 2,2',4,4'-tetrabromodiphenyl ether (BDE-47)-induced liver inflammation by attenuating oxidative stress-mediated NAD+-depletion. *J Hazard Mater*, 283, 98-109.