Chinese Herbal Medicines 12 (2020) 73–78

Contents lists available at ScienceDirect

Chinese Herbal Medicines

journal homepage: www.elsevier.com/locate/chmed

Original Article

Attenuation of inflammation in streptozotocin-induced diabetic rabbits by *Matricaria chamomilla* oil: A focus on targeting NF-κB and NLRP3 signaling pathways

Saeid Saghahazrati[a], Seyed Abdulmajid Ayatollahi[a,b,c,*], Farzad Kobarfard[a,d], Bagher Minaii Zang[e]

[a] Phytochemistry Research Center, Shahid Beheshti University of Medical Sciences, Tehran 19839-63113, Iran
[b] Department of Pharmacognosy, School of Pharmacy, Shahid Beheshti University of Medical Sciences, Tehran 19839-63113, Iran
[c] Department of Chemistry, Richardson College for Environmental Science Complex, University of Winnipeg, Winnipeg R3C1X6, Canada
[d] Department of Medicinal Chemistry, Shaheed Beheshti School of Pharmacy, Tehran 19839-63113, Iran
[e] Department of Histology, Faculty of Medicine, Tehran University of Medical Sciences, Tehran 1416753955, Iran

ARTICLE INFO

Article history:
Received 27 May 2019
Revised 3 July 2019
Accepted 13 August 2019
Available online 24 December 2019

Keywords:
chamomile oil
inflammation
NF-κB protein
NLRP3 protein
type 1 diabetes mellitus

ABSTRACT

Objectives: The present study was conducted to investigate the protective effects of chamomile oil from *Matricaria chamomilla* against type 1 diabetes mellitus (T1DM) and its potential mechanisms.

Methods: T1DM was established in male New Zealand white rabbits via a single intraperitoneal infusion of streptozotocin (STZ) (80 mg/kg body weight^{-1}, dissolved in 0.2 mL of normal saline). Different doses of chamomile oil (25, 50 and 100 mg/kg) were orally administrated to STZ induced diabetic rabbits for 21 consecutive days. The expression of pro-inflammatory cytokines was determined using ELISA assay. The expression of NF-κB and NLRP3 was measured using Western blot assay.

Results: Compared with normal rabbits, STZ-induced diabetic rabbits exhibited significant increased levels of blood glucose and decreased levels of serum insulin that were reversed using middle and high tested dose of chamomile oil. Likewise, STZ-induced diabetic rabbits showed a significant increased expression of NF-κB and NLRP3 proteins in the pancreas tissue that was reversed by high tested dose of chamomile oil.

Conclusion: Collectively, our findings demonstrated that chamomile oil possesses anti-hyperglycemic, and anti-inflammatory activities in STZ-induced diabetic rabbits by targeting inflammatory cytokines and NF-κB and NLRP3 signaling pathways.

1. Introduction

Studies have shown that all types of diabetes represent an ever-growing prevalence around the world in current years (Kankala et al., 2018; Kasputis et al., 2018; Liu, Shi & Lee, 2019; Rak & Bronkowska, 2019; Saghahazrati, Ayatollahi, Kobarfard & Zang, 2020). The leading cause of type 1 diabetes mellitus (T1DM) is the severe destruction and death of insulin producing beta cells in the pancreas through infiltration of macrophages and T lymphocytes into islets of langerhans (Assmann, Brondani, Bouças, Canani & Crispim, 2015). This autoimmune disease includes about 5%−10% of all cases of diabetes in worldwide and is characterized by absolute lack of insulin and chronic hyperglycemia. The current treatment option is substitutive chronic use of exogenous insulin (Götze et al., 2018). At the variance with considerable advances, chronic use of exogenous insulin has been challenged owing to lack of effectiveness and limitations to prevent neurological and vascular complications (Chatenoud, 2010). Inflammation plays an important role in diabetes and other degenerative diseases (Amani et al., 2019d; Imam et al., 2019; Janzadeh et al., 2017; Mehrjerdi et al., 2015; Sireesh, Dhamodharan, Ezhilarasi, Vijay & Ramkumar, 2018). The initiation of inflammatory responses by acute phase proteins can lead to diabetes-preventing or diabetes-

* Corresponding author.
E-mail address: majid_ayatollahi@yahoo.com (S.A. Ayatollahi).

https://doi.org/10.1016/j.chmed.2019.12.003

promoting effects. In fact, excessive production of the nitric oxide by pro-inflammatory cytokines such as tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β) and interleukin-6 (IL-6) results in suppression of insulin synthesis, secretion, cytocidal actions to pancreatic islets, as well as apoptotic and necrotic death of pancreatic β-cells (Alexandraki et al., 2008). These pathological cascades can be linked to the development and progression of serious short-term and long-term complications (Massaro et al., 2019). It has been reported that intracellular double-stranded (ds) RNA and extracellular dsRNA secreted by damaged or dying cells can be bonded to Toll-like receptor 3 (TLR3) and trigger a sequential chain of pathological cascades including activation of the transcription factors such as nuclear factor-κB (NFκB) and interferon regulatory factor 3 (IRF-3), overproduction of cytokines and chemokines, and subsequently β-cell death (Eizirik, Colli & Ortis, 2009). On the other hand, it has recognized that inflammasomes, a diverse class of cytosolic protein complexes, play a pivotal role in the development and progression of T1DM through production of mature IL-1β. Nuclear localization leucine-rich-repeat protein 1 (NLRP1) and nuclear localization leucine-rich-repeat protein 3 (NLRP3) inflammasomes can be bonded to the protein apoptosis-associated speck like protein which, in turn, result in the mature and the release of pro-IL-1β in the form of IL-1β and subsequently progression of inflammation (Liu et al., 2017). Currently, many new methods and therapeutic agents have induced to treat degenerative disorders (Amani et al., 2019a, 2017b, Amani et al., 2018; Amani, Kazerooni, Hassanpoor, Akbarzadeh, & Pazoki-Toroudi, 2019; Javedan et al., 2016). In recent years, researchers have introduced many new therapeutic agents and methods to treat diabetes and other diseases (Amani et al., 2019b, 2019d; Javdan et al., 2019; Zhao, Jin, Chen, & Han, 2015). Herbal medicines and natural products have been used to prevent or treat various diseases (Choudhary et al., 2012); Chen, Liao, Qin, & Li, 2019; (Shamsabadipour et al., 2013). Chamomile (*Matricaria chamomilla* L.) is a well-known ancient medicinal herb which belongs to the daisy family (Asteraceae/Compositae). Chamomile has been posed as star among medicinal species that is widely used for cosmetics and medicinal purposes (Al-Dabbagh et al., 2019; Srivastava, Shankar & Gupta, 2010). In recent years, chamomile has been used as anti-oxidant, anti-cancer, and anti-inflammatory agents because it possesses different classes of bioactive ingredients such as flavonoids and terpenoids (Al-Dabbagh et al., 2019). Some previous studies have shown that chamomile can contribute to inhibition of oxidative stress in patients with type 2 diabetes mellitus and differentiation of mesenchymal stem cells (MSCs) into insulin-producing cells (Saghahazrati, Ayatollahi, Kobarfard & Minaei, 2019; Zemestani, Rafraf & Asghari-Jafarabadi, 2016). Here, we went on to explore the protective effects of chamomile on streptozotocin (STZ)-induced diabetic rabbits and its potential molecular mechanisms.

2. Material and methods

2.1. Reagents

Chamomile (*Matricaria chamomilla* L.) oil, RIPA buffer, bovine serum albumin (BSA), Immobilon®-FL Polyvinylidene Fluoride (PVDF) membrane were purchased from Sigma (Sigma Aldrich Chemical Co, USA). NF-κB p65 antibody was obtained from Cell Signalling Technology, Inc. (CST) (Danvers, Massachusetts). NLRP3 Antibody was purchased from Novus Biologicals Company (Novus Biologicals, Littleton, Co.). Rabbit Insulin ELISA Kit was obtained from MyBioSource Company (San Diego, USA). Glucometer elite and glucometer strips were purchased from Bayer Company (Leverkusen, Germany).

2.2. Animals and ethical statement

Twenty-five male New Zealand white rabbits with a mean weight of 2.5 kg were obtained from Razi Institute, Iran. The animals were housed in a controlled room temperature [(22 ± 1) °C], on a 12/12 cycle with humidity of (60 ± 5)% and free access to standard chow pellets and water ad libitum. All the experiments followed the guidelines established by The Institutional Animal Ethical Committee of Shahid Beheshti University of Medical Sciences, Tehran, Iran.

2.3. Experimental design

The animals were randomly assigned into three major groups with five rabbits in each group: normal control group (Control), streptozotocin (STZ)-induced diabetic group, and chamomile oil-treated diabetic group which the animals were treated with different doses of chamomile oil (25, 50 and 100 mg/kg). T1DM was induced via a single intraperitoneal injection of STZ (80 mg/kg body weight^{-1}) dissolved in 0.2 mL of normal saline. Chamomile. oil was orally administrated 1 h after injection of STZ and for 21 consecutive days. At 72 h after intraperitoneal injection of STZ, blood glucose levels were measured to confirm the diabetes induction in animals. To this end, a glucometer was used to read the strip containing a drop of blood. Rabbits with blood glucose levels of 220 mg/dL with glycosuria were considered as diabetic and entry to study.

2.4. Glucose tolerance test (GTT)

Rabbits were fasted overnight prior to the experiment. Glucose with dose of 4 g/kg was orally administrated to all the groups. Blood glucose measurements was performed at 1 h after intraperitoneal injection of STZ and 1, 7, 14, and 21days following administration of different doses of chamomile oil (25, 50 and 100 mg/kg).

2.5. Tissue and serum collection

To obtain the serum samples, blood samples from all the groups were collected and centrifuged at 3000 rpm for 10 min. Then, serum samples were kept at −80 °C prior to ELISA assay. At the end of treatment period, the abdominal regions was opened, pancreas tissues were rapidly excised from the body on ice. After washing with cold saline solution, the tissues were kept at −80 °C prior to assessments. All the steps were performed at −4 °C to prevent the destruction of protein.

2.6. Evaluation of serum insulin

On the 21th day, serum insulin was determined using ELISA kit according to instruction of manufacturers.

2.7. Evaluation of pro-inflammatory cytokines

At the end of treatment period, the serum levels of pro-inflammatory cytokines including TNF-α, IL-1β and IL-10 were determined using ELISA Kits according to instruction of manufacturers.

2.8. Western blot assay

First, 100 mg, pancreas tissue was ground in liquid nitrogen. Then, 1 mL RIPA lysis buffer was added to it and homogenized. In the next step, homogenized solution was centrifuged at 13 000 rpm for 20 min and the supernatant was separated. The concentration of protein was obtained using a nanodrop

spectrophotometer. The loading buffer was added to each lysate and boiled for 5 min. To separate protein, 50 μL of resulting solution was electrophoresed in sodium dodecyl sulfate (SDS)-polyacrylamide gels and then transferred into the PVDF sheets. To block non-specific sites, the PVDF sheets were incubated in BSA in 0.1% Tris-buffer saline/Tween 20 overnight. Then, the PVDF sheets firstly were incubated with primary antibodies including NF-κB P65 antibody, NLRP3 antibody and then with appropriate peroxidase-conjugated secondary antibodies. To visualize reactive bands, chemiluminescence reagent and Kodak X-OMAT films were used. κ-actin was used as an internal control to ensure equal loading.

2.9. Statistical analysis

All data are expressed as mean ± standard deviation (SD). Prism Software, version 5 (CA, USA) was used to analyze data. The normal distribution of all data was determined using Shapiro-Wilk and Kolmogorov-Smirnov tests. One-way ANOVA with post hoc test was used to compare three or more groups. A value of $P < 0.05$ was accepted to be statistically significant.

3. Results

3.1. Effect of chamomile oil on blood glucose and serum insulin levels

As shown in Fig. 1A and B, STZ-induced diabetic rabbits showed an increased level of blood glucose compared with control group. Post-treatment with low dose of chamomile oil (25 mg/kg b.w) did not reduce blood glucose levels. Middle and high doses of chamomile oil (50 and 100 mg/kg b.w) significantly reduced blood glucose levels compared with STZ group. The high dose exhibited higher efficiency compared to middle dose. Serum insulin level was found to be reduced in STZ-induced diabetic rabbits when compared to control group. In chamomile oil (100 mg/kg b.w)-treated diabetic rabbits, significant increased levels of serum insulin were found. Similar to blood glucose levels, the low dose of chamomile oil (25 mg/kg b.w) did not increase the insulin levels compared to STZ group. Furthermore, insulin levels were higher in diabetic rabbits treated by chamomile oil (50 mg/kg) compared to STZ group, although difference did not reach a significant level. The high dose (100 mg/kg b.w) demonstrated stronger efficiency compared to middle dose (50 mg/kg b.w).

3.2. Effects of chamomile oil on expression of pro-inflammatory cytokines

Compared to the control group, the STZ group exhibited significant increased levels of pro-inflammatory cytokines including TNF-α and IL-1κ (Fig. 2A and B). Post-treatment with low dose of chamomile oil (25 mg/kg b.w) did not reduce levels of TNF-α and IL-1κ. In chamomile oil (50 and 100 mg/kg b.w) treated diabetic rabbits, significant decreases in the level of TNF-α and IL-1β were found relative to STZ-induced diabetic rabbits. High tested dose of chamomile oil (100 mg/kg b.w) demonstrated more efficiency compared to middle tested dose (50 mg/kg b.w).

Likewise, although the protein expression of IL-10 as an anti-inflammatory cytokine increased in STZ-induced diabetic rabbits, difference did not reach a significant level (Fig. 2C). In chamomile oil (50 and 100 mg/kg b.w) treated diabetic rabbits, a significant elevated level of IL-10 level was found relative to STZ-induced diabetic rabbits. Low dose of chamomile oil (25 mg/kg) did not change the expression level of IL-10 compared to STZ-induced diabetic rabbits. There were significant differences between high tested dose (100 mg/kg b.w) and middle tested dose (50 mg/kg b.w) of chamomile oil.

3.3. Effects of chamomile oil on expression of NF-kB and NLRP3 protein

To explore possible mechanisms of chamomile oil and obtain greater insights on its protective effects against T1DM, we evaluated the expression of NF-κB and NLRP3 protein by Western blot assay. The STZ-induced diabetic rabbits exhibited the elevated expression levels of NF-κB and NLRP3 proteins compared to control group. The expression level of NF-κB and NLRP3 proteins were markedly down-regulated in chamomile oil (100 mg/kg b.w)-treated diabetic rabbits (Fig. 3).

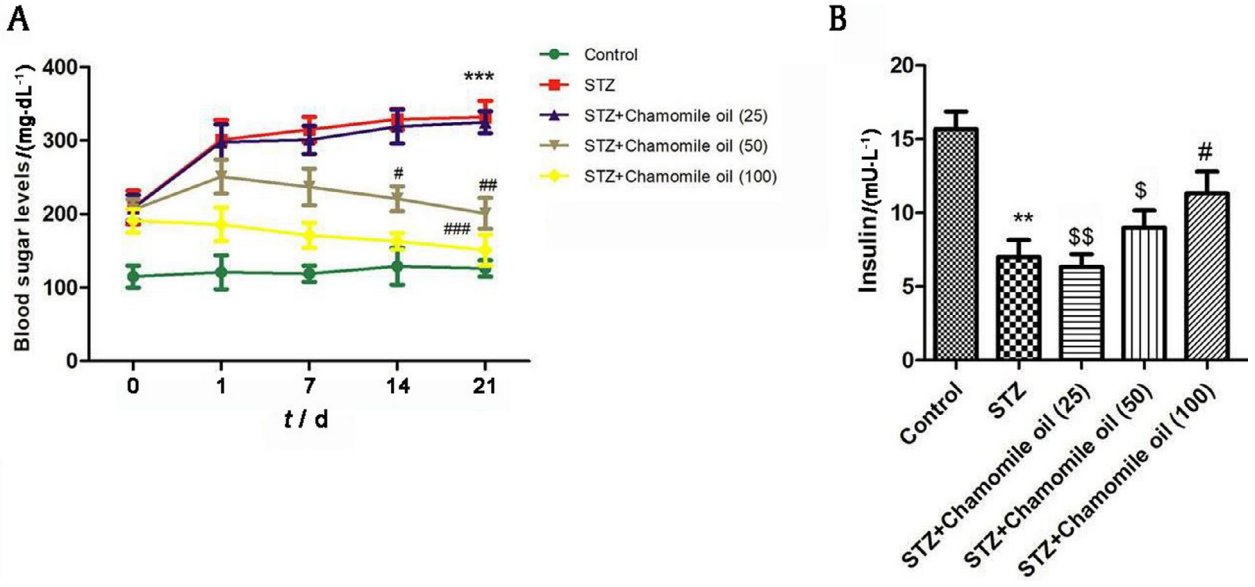

Fig. 1. Chamomile oil post-treatment for 21 d significantly reduced blood glucose levels (A) and increased serum insulin levels (B).
Note: For Blood glucose levels: $^{***}P < 0.001$ *vs* control for all days, $^{\#}P < 0.05$ and $^{\#\#}P < 0.01$ *vs* STZ and STZ+ chamomile oil (25 mg/kg) for 14 days and 21 days, respectively; $^{\#\#\#}P < 0.001$ *vs* STZ and STZ+ chamomile oil (25 mg/kg) for all days; For serum insulin levels: $^{**}P < 0.01$ *vs* control, $^{\#}P < 0.05$ *vs* STZ, $^{\$}P < 0.05$ and $^{\$\$}P < 0.01$ *vs* control). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

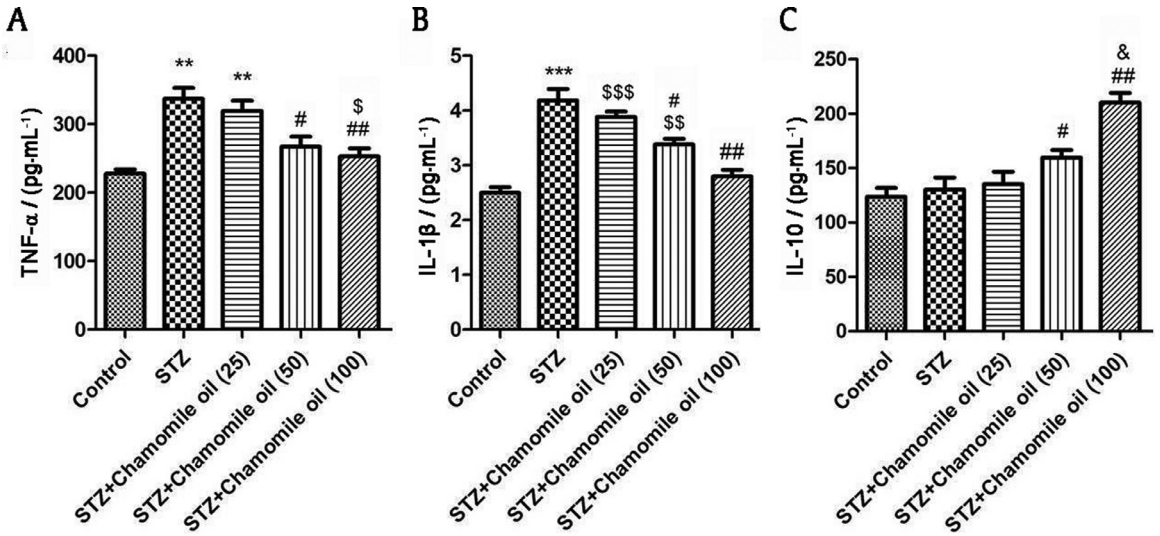

Fig. 2. Chamomile oil post-treatment for 21 days significantly reduced amount of pro-inflammatory cytokines of TNF-α (A), IL-1β (C) and increased expression of anti-inflammatory cytokines of IL-10 (C) in a dose dependent mechanism.
Note: For the expression levels of TNF-α: $^{**}P < 0.01$ *vs* control; $^{\#}P < 0.05$ and $^{\#\#}P < 0.01$ *vs* STZ; $^{\$}P < 0.05$ *vs* STZ+ chamomile oil (25); For the expression levels of IL-1β: $^{***}P < 0.001$ *vs* control; $^{\#}P < 0.05$ and $^{\#\#}P < 0.01$ *vs* STZ; $^{\$\$}P < 0.01$ and $^{\$\$\$}P < 0.001$ *vs* STZ+ chamomile oil (25); For the expression levels of IL-10: $^{\#}P < 0.05$ *vs* STZ, STZ+ chamomile oil (25), $^{\#\#}P < 0.01$ *vs* control, STZ, STZ+ chamomile oil (25); $^{\&}P < 0.05$ *vs* STZ+ chamomile oil (50).

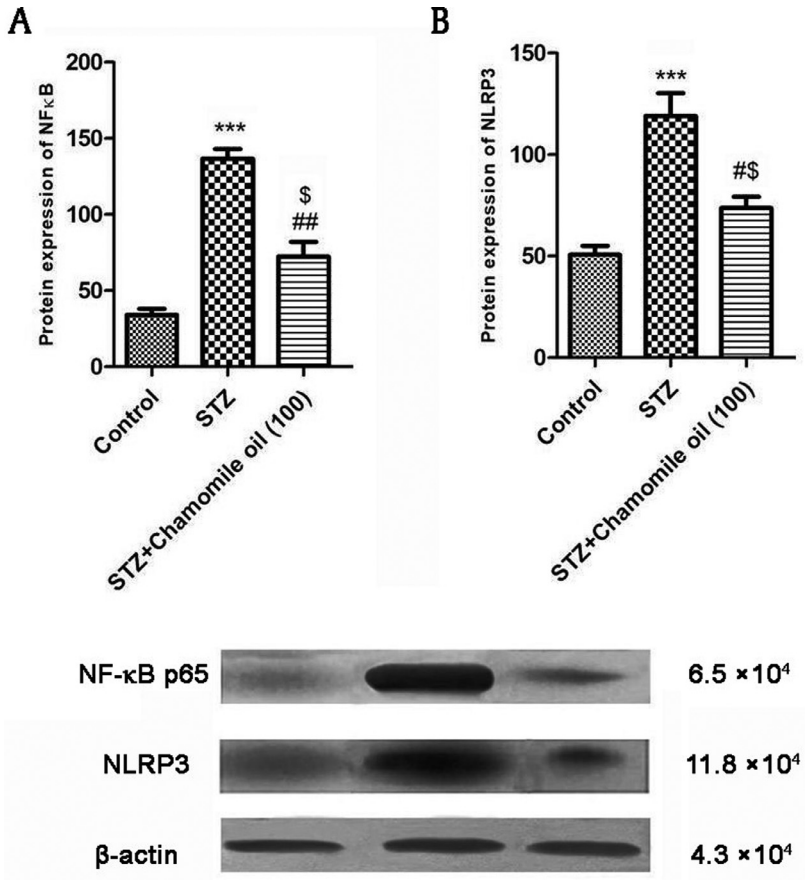

Fig. 3. Post-treatment with high tested dose of chamomile oil (100 mg/kg b.w) for 21 d significantly reduced expression levels of NF-κB (A) and NLRP3 (B) proteins in chamomile oil-induced diabetic rabbits ($^{***}P < 0.001$ *vs* control; $^{\#}P < 0.05$ and $^{\#\#}P < 0.01$ *vs* STZ; $^{\$}P < 0.05$ *vs* control).

4. Discussion

We have examined whether chamomile oil can attenuate the diabetic complications induced by STZ because the detailed mechanisms of the anti-diabetic effects of this herbal medicine is not available. During T1DM, insufficient secretion of insulin results in increased glucose concentration in blood that is associated with the inhibition of glycogenesis (Nain, Saini, Sharma, & Nain, 2012). Current studies have shown that chamomile oil possesses many pharmacological activities including antimicrobial (Göger, Demirci, Ilgın & Demirci, 2018), wound healing (Gad, El-Rahman & Hamdy, 2019), antioxidant and anticancer (Al-Dabbagh et al., 2019) activity. Moreover, previous studies have shown that daily consumption of chamomile tea has beneficial effects in patients with type 2 diabetes mellitus through the prevention of hyperglycemia (Kato et al., 2008; Zemestani et al., 2016). In agreement with previous reports, findings of this study showed that oral administration of chamomile oil at 50 and 100 mg/kg concentration significantly reduced the blood glucose and also increased serum insulin levels in STZ-induced diabetic rabbits relative to control. Inflammation has been reported to play an important role in T1DM. Diabetes-induced hyperglycemia triggers acute inflammatory responses and subsequently cell apoptosis (Chen et al., 2012). It has been reported that level of the inflammatory mediators such as TNF-α and IL-1β was increased in blood of STZ-induced diabetic rats (Chandirasegaran, Elanchezhiyan, Ghosh & Sethupathy, 2017). The binding of TNF-α to its receptor (TNF-R1) promoted the up-regulation of NF-κB, activation of caspases and subsequently apoptotic cell death by increasing inducible nitric oxide synthase (iNOS) and nitric oxide (NO) free radicals (Frances, Ingaramo, Ronco & Carnovale, 2013). On the other hand, the importance of NLRP3 in the pathogenesis of T1DM has been reported by Hu et al. they found that NLRP3 deficiency contributed to protection against T1DM that was attributed to reduced activation of T cell and the decrease in pathogenic T-cell migration to the islets by suppressing expression of chemokine receptors CCR5 and CXCR3 on T cells (Hu et al., 2015). To obtain greater insights on protective effects of chamomile oil, we also examined the expression of pro-inflammatory cytokines. ELISA studies revealed the fact that increased expression levels of TNF-α and IL-1β and decreased levels of IL-10 in diabetic rabbits was reversed by chamomile oil at middle and high tested concentration. In keeping with our finding, a previous study showed that *M. chamomilla* extract could contribute to the improvement of the healing of traumatic oral ulcers in diabetic rats via suppression of pro-inflammatory cytokines such as TNF-α and apoptotic cell death (Oliveira et al., 2016). To explore possible mechanisms which by chamomile oil suppress inflammation, we examined the expression of NF-κB and NLRP3 proteins. In agreement with above mentioned studies, our findings showed that increased expression of NF-κB and NLRP3 proteins in diabetic rabbits that was reversed by chamomile oil at high tested concentration.

5. Conclusion

Collectively, our results showed that chamomile oil confers protection in STZ-induced diabetic rabbits by targeting NF-κB and NLRP3 proteins and pro-inflammatory cytokines.

Declaration of Competing Interest

The authors declare no conflict of interest.

Acknowledgments

This study was financially supported by a research grant (Grant no. 77004821) from the Phytochemistry Research Center of Shahid Beheshti University of Medical Sciences.

References

Al-Dabbagh, B., Elhaty, I. A., Elhaw, M., Murali, C., Al Mansoori, A., Awad, B., et al. (2019). Antioxidant and anticancer activities of chamomile (*Matricaria recutita* L.). *BMC Research Notes, 12*(1), 3. doi:10.1186/s13104-018-3960-y.

Alexandraki, K. I., Piperi, C., Ziakas, P. D., Apostolopoulos, N. V., Makrilakis, K., Syriou, V., et al. (2008). Cytokine secretion in long-standing diabetes mellitus type 1 and 2: Associations with low-grade systemic inflammation. *Journal of Clinical Immunology, 28*(4), 314–321.

Amani, H., Arzaghi, H., Bayandori, M., Dezfuli, A. S., Pazoki-Toroudi, H., Shafiee, A., et al. (2019a). Controlling cell behavior through the design of biomaterial surfaces: A focus on surface modification techniques. *Advanced Materials Interfaces*, 1900572. doi:10.1002/admi.201900572.

Amani, H., Habibey, R., Hajmiresmail, S., Latifi, S., Pazoki-Toroudi, H., & Akhavan, O. (2017b). Antioxidant nanomaterials in advanced diagnoses and treatments of ischemia reperfusion injuries. *Journal of Materials Chemistry B, 5*(48), 9452–9476.

Amani, H., Habibey, R., Shokri, F., Hajmiresmail, S. J., Akhavan, O., Mashaghi, A., et al. (2019b). Selenium nanoparticles for targeted stroke therapy through modulation of inflammatory and metabolic signaling. *Scientific Reports, 9*(1), 6044. doi:10.1038/s41598-019-42633-9.

Amani, H., Kazerooni, H., Hassanpoor, H., Akbarzadeh, A., & Pazoki–Toroudi, H. (2019c). Tailoring synthetic polymeric biomaterials towards nerve tissue engineering: A review. *Artificial cells, Nanomedicine, and Biotechnology, 47*(1), 3524–3539.

Amani, H., Mostafavi, E., Alebouyeh, M. R., Arzaghi, H., Akbarzadeh, A., Pazoki-Toroudi, H., et al. (2019d). Would colloidal gold nanocarriers present an effective diagnosis or treatment for ischemic stroke? *International Journal of Nanomedicine, 14*, 8013. doi:10.2147/IJN.S210035.

Amani, H., Mostafavi, E., Arzaghi, H., Davaran, S., Akbarzadeh, A., Akhavan, O., et al. (2018). Three-dimensional graphene foams: Synthesis, properties, biocompatibility, biodegradability, and applications in tissue engineering. *ACS Biomaterials Science & Engineering, 5*(1), 193–214.

Assmann, T. S., Brondani, L. d. A., Bouças, A. P., Canani, L. H., & Crispim, D. (2015). Toll-like receptor 3 (TLR3) and the development of type 1 diabetes mellitus. *Archives of Endocrinology and Metabolism, 59*(1), 4–12.

Chandirasegaran, G., Elanchezhiyan, C., Ghosh, K., & Sethupathy, S. (2017). Berberine chloride ameliorates oxidative stress, inflammation and apoptosis in the pancreas of Streptozotocin induced diabetic rats. *Biomedicine & Pharmacotherapy, 95*, 175–185.

Chatenoud, L. (2010). Immune therapy for type 1 diabetes mellitus—What is unique about anti-CD3 antibodies? *Nature Reviews Endocrinology, 6*(3), 149.

Chen, T. L., Xu, E. L., Lu, H. J., Xu, H., Wang, S. F., & Zhao, H. J. (2012). The influence of diabetes enhanced inflammation on cell apoptosis and periodontitis. *Advances in Bioscience and Biotechnology, 3*(06), 712.

Chen, X., Liao, D., Qin, Z., & Li, X. (2019). Synergistic interactions of catalpol and stachyose in STZ-HFD induced diabetic mice: Synergism in regulation of blood glucose, lipids, and hepatic and renal function. *Chinese Herbal Medicines, 11*(1), 70–77.

Choudhary, M. I., Hussain, A., Ali, Z., Adhikari, A., Sattar, S. A., Ayatollahi, S. A. M., & Atta-Ur-Rahman (2012). Diterpenoids including a novel dimeric conjugate from *salvia leriaefolia*. Planta Medica, *78*(3), 269–275.

Eizirik, D. L., Colli, M. L., & Ortis, F. (2009). The role of inflammation in insulitis and β-cell loss in type 1 diabetes. *Nature Reviews Endocrinology, 5*(4), 219.

Frances, D. E. A., Ingaramo, P. I., Ronco, M. T., & Carnovale, C. E. (2013). Diabetes, an inflammatory process: Oxidative stress and TNF-alpha involved in hepatic complication. *Journal of Biomedical Science and Engineering, 6*, 645–653 645-.

Gad, H. A., El-Rahman, F. A. A., & Hamdy, G. M. (2019). Chamomile oil loaded solid lipid nanoparticles: a naturally formulated remedy to enhance the wound healing. *Journal of Drug Delivery Science and Technology*. doi:10.1016/j.jddst.2019.01.008.

Göger, G., Demirci, B., Ilgın, S., & Demirci, F. (2018). Antimicrobial and toxicity profiles evaluation of the chamomile (*Matricaria recutita* L.) essential oil combination with standard antimicrobial agents. *Industrial Crops and Products, 120*, 279–285.

Götze, A., von Keyserlingk, S., Peschel, S., Jacoby, U., Schreiver, C., Köhler, B., et al. (2018). The corneal subbasal nerve plexus and thickness of the retinal layers in pediatric type 1 diabetes and matched controls. *Scientific Reports, 8*(1), 14.

Hu, C., Ding, H., Li, Y., Pearson, J. A., Zhang, X., & Flavell, R. A. (2015). NLRP3 deficiency protects from type 1 diabetes through the regulation of chemotaxis into the pancreatic islets. *Proceedings of the National Academy of Sciences, 112*(36), 11318–11323.

Imam, S., Prathibha, R., Dar, P., Almotah, K., Al-Khudhair, A., Hasan, S. A. M., et al. (2019). eIF5A inhibition influences T cell dynamics in the pancreatic microenvironment of the humanized mouse model of type 1 diabetes. *Scientific Reports, 9*(1), 1533. doi:10.1038/s41598-018-38341-5.

Janzadeh, A., Sarveazad, A., Yousefifard, M., Dameni, S., Samani, F. S., Mokhtarian, K., et al. (2017). Combine effect of chondroitinase ABC and low level laser (660nm) on spinal cord injury model in adult male rats. *Neuropeptides, 65*, 90–99.

Javdan, N., Ayatollahi, S. A., Choudhary, M. I., AlHasani, S., Kobarfard, F., Mokhtarian, K., et al. (2019). Tsc1/Tsc2 complex: A molecular target of capsaicin for protection against testicular torsion induced injury in rats. *Chinese Herbal Medicines, 11*(2), 216–221.

Javedan, G., Shidfar, F., Davoodi, S. H., Ajami, M., Gorjipour, F., Sureda, A., et al. (2016). Conjugated linoleic acid rat pretreatment reduces renal damage in ischemia/reperfusion injury: Unraveling antiapoptotic mechanisms and regulation of phosphorylated mammalian target of rapamycin. *Molecular Nutrition & Food Research, 60*(12), 2665–2677.

Kankala, R. K., Lin, X. F., Song, H. F., Wang, S. B., Yang, D. Y., & Zhang, Y. S. (2018). Supercritical fluid-assisted decoration of nanoparticles on porous microcontainers for codelivery of therapeutics and inhalation therapy of diabetes. *ACS Biomaterials Science & Engineering, 4*(12), 4225–4235.

Kasputis, T., Clough, D., Noto, F., Rychel, K., Dye, B., & Shea, L. D. (2018). Microporous polymer scaffolds for the transplantation of embryonic stem cell derived pancreatic progenitors to a clinically translatable site for the treatment of type i diabetes. *ACS Biomaterials Science & Engineering, 4*(5), 1770–1778.

Kato, A., Minoshima, Y., Yamamoto, J., Adachi, I., Watson, A. A., & Nash, R. J. (2008). Protective effects of dietary chamomile tea on diabetic complications. *Journal of Agricultural and Food Chemistry, 56*(17), 8206–8211.

Liu, H., Xu, R., Kong, Q., Liu, J., Yu, Z., & Zhao, C. (2017). Downregulated NLRP3 and NLRP1 inflammasomes signaling pathways in the development and progression of type 1 diabetes mellitus. *Biomedicine & Pharmacotherapy, 94*, 619–626.

Liu, J., Shi, Y. C., & Lee, D. Y. W. (2019). Applications of *Pueraria lobata* in treating diabetics and reducing alcohol drinking. *Chinese Herbal Medicines, 11*(2), 141–149. doi:10.1016/j.chmed.2019.04.004.

Massaro, J. D., Polli, C. D., Silva, M. C., Alves, C. C., Passos, G. A., Sakamoto-Hojo, E. T., et al. (2019). Post-transcriptional markers associated with clinical complications in type 1 and type 2 diabetes mellitus. *Molecular and Cellular Endocrinology, 450*, 1–14. doi:10.1016/j.mce.2019.03.008.

Mehrjerdi, F. Z., Aboutaleb, N., Pazoki-Toroudi, H., Soleimani, M., Ajami, M., Khaksari, M., et al. (2015). The protective effect of remote renal preconditioning against hippocampal ischemia reperfusion injury: Role of KATP channels. *Journal of Molecular Neuroscience, 57*(4), 554–560.

Nain, P., Saini, V., Sharma, S., & Nain, J. (2012). Antidiabetic and antioxidant potential of *Emblica officinalis* Gaertn. Leaves extract in streptozotocin-induced type-2 diabetes mellitus (T2DM) rats. *Journal of Ethnopharmacology, 142*(1), 65–71.

Oliveira, B. V., BARROS SILVA, P. G., Nojosa, J., BRIZENO, L. A. C., Ferreira, J. M., & Sousa, F. B. (2016). TNF-alpha expression, evaluation of collagen, and tunel of *Matricaria recutita* L. extract and triamcinolone on oral ulcer in diabetic rats. *Journal of Applied Oral Science, 24*(3), 278–290.

Rak, K., & Bronkowska, M. (2019). Immunomodulatory effect of vitamin D and its potential role in the prevention and treatment of type 1 diabetes mellitus—A narrative review. *Molecules, 24*(1), 53. doi:10.3390/molecules24010053.

Saghahazrati, S., Ayatollahi, S. A., Kobarfard, F., & Minaei, B. (2019). The protective effects of cultured mesenchymal stem cells onto the surface of electrospun poly-*L*-lactide acid scaffolds coated with *Matricaria chamomilla* L. oil in streptozotocin-induced diabetic rabbits. *Iranian Red Crescent Medical Journal, 21*(2), e85247.

Saghahazrati, S., Ayatollahi, S. A., Kobarfard, F., & Zang, B. M. (2020). The synergistic effect of glucagon-like peptide-1 and chamomile oil on differentiation of mesenchymal stem cells into insulin-producing cells. *Cell Journal (Yakhteh), 21*(4), 371–378.

Shamsabadipour, S., Ghanadian, M., Saeedi, H., Reza Rahimnejad, M., Mohammadi-Kamalabadi, M., Ayatollahi, S. A., & Salimzadeh, L. (2013). Triterpenes and steroids from *Euphorbia denticulata* lam. with anti-herpes symplex virus activity. Iranian Journal of Pharmaceutical Research, *12*(4), 759–767.

Sireesh, D., Dhamodharan, U., Ezhilarasi, K., Vijay, V., & Ramkumar, K. M. (2018). Association of NF-E2 related factor 2 (Nrf2) and inflammatory cytokines in recent onset type 2 diabetes mellitus. *Scientific Reports, 8*(1), 5126. doi:10.1038/s41598-018-22913-6.

Srivastava, J. K., Shankar, E., & Gupta, S. (2010). Chamomile: a herbal medicine of the past with a bright future. *Molecular Medicine Reports, 3*(6), 895–901.

Zemestani, M., Rafraf, M., & Asghari-Jafarabadi, M. (2016). Chamomile tea improves glycemic indices and antioxidants status in patients with type 2 diabetes mellitus. *Nutrition, 32*(1), 66–72.

Zhao, R., Jin, R., Chen, Y., & Han, F. (2015). Hypoglycemic and hypolipidemic effects of *Lycium barbarum* polysaccharide in diabetic rats. *Chinese Herbal Medicines, 7*(4), 310–315.