ORIGINAL ARTICLE
Iran J Allergy Asthma Immunol
In press.

Beneficial Effects of Hydroalcoholic Extract of Saffron in Alleviating Experimental Autoimmune Diabetes in C57bl/6 Mice

Shole Faridi, Norouz Delirezh, and Seyyed Meysam Abtahi Froushani

Department of Microbiology, Faculty of Veterinary Medicine, Urmia University, Urmia, Iran

Received: 2 March 2018; Received in revised form: 29 July 2018; Accepted: 30 July 2018

ABSTRACT

Streptozocin (STZ) is a strong alkalizing agent which is capable of destroying the beta cells of the pancreatic islets. Multiple low doses (40 mg/kg, intraperitoneally for 5 consecutive days) prescription of STZ to mice can lead to the T cell-dependent immune response and induction of autoimmune diabetes (AD) with complete similarity to the human type 1 diabetes (T1D). This study has evaluated the effects of hydroalcoholic extract of saffron on the clinical and immunological profile of experimental autoimmune diabetes in C57BL/6 mice.

After the establishment of the AD, mice were treated orally with hydroalcoholic extract of saffron (500 mg/kg) for 3 weeks. The results with $p<0.05$ were considered significant.

Obtained data showed that treatment with the hydroalcoholic extract of saffron significantly reduced the incidence of hypoglycemia and restored insulin secretion and histopathological changes in pancreas sections. In addition, treatment with saffron reduced lymphocyte proliferation index in the cells isolated from the pancreas of diabetic mice. Also, the extract of saffron markedly decreased the production of pro-inflammatory interleukin-17 (IL-17) increased anti-inflammatory IL-10 and transforming growth factor-β in the pancreatic cell population. Moreover, the production of proinflammatory nitric oxide and reactive oxygen substances were down-regulated by the saffron extract.

It seems that the hydroalcoholic extract of saffron can be considered as a useful strategy in the treatment of type 1 diabetes.

Keywords: Crocus sativus.L; Inflammation; Saffron; Type 1 diabetes mellitus

INTRODUCTION

Type 1 diabetes or insulin dependent diabetes mellitus (T1D or IDDM) is a chronic autoimmune disease characterized by the penetration of immune cells into pancreatic islets and the destruction of insulin-producing beta cells.[1,2]

T1D is the most common autoimmune disorder in childhood, but it can be manifested at any age, even in the elderly.[3] The prevalence of T1D has increased dramatically throughout the world over the past two decades. Recent statistical studies showed that more than 231 million people worldwide suffer from this

Corresponding Author: Seyyed Meysam Abtahi Froushan:PhD; Department of Microbiology, Faculty of Veterinary Medicine, Urmia University, Urmia, Iran. P.o.Box: 571531177, Tel: (+98 913) 3000 470, E-mail: sm.abtahi@urmia.ac.ir

Published by Tehran University of Medical Sciences (http://ijaai.tums.ac.ir)

disease and are expected to reach 371 million in 2025.[4] Various animal models have been used to study diabetes, including NOD mice and induction of diabetes in BALB/cByJ and C57/BL6 mice.[5] Also, genetically modified mice that are more predisposed to diabetes have also been used for this purpose. However, the disease trend in these models is not entirely similar to that occurring in humans.[6] This fact has led to efforts to create similar models with the human type 1 diabetes, including self-immune diabetes induced by cyclophosphamide or multiple low doses of streptozotocin.[7-9] In the present study, inbred C57/BL6 mice were used which have an inherent predisposition to autoimmune diabetes. Several low-dose streptozotocin (STZ) is used in order to induce type 1 diabetes, similar to what has been said in previous studies of diabetes preclinical models.[10] Multiple low doses of STZ can induce inflammation of the pancreatic islets by the participation of macrophages and T cells, with subsequent destruction of β cells followed by progressive hyperglycemia within a few days in a very similar scheme with human disease.[6] Of note, one high dose of STZ cannot induce an immunologically induced diabetes with a similar appearance with a human.[6]

The cause of the disease has not been well defined, but it seems that the attack of immune cells, especially T-lymphocytes against beta cells, appears to be due to some environmental and genetic factors.[11] No specific treatment for this condition has been made available so far and only insulin has been used to control the underlying cause of the disease that has increased blood sugar levels.[1,6] Different therapies such as the use of herbal medicine as glucose- and lipid-lowering agents were used to treat or control diabetes. For example, Indian potato extract, Chinese hibiscus, Aloe Vera and Dill reduce the serum total cholesterol and triglyceride levels and also improve the insulin sensitivity of diabetic male rats.[1] One of the most effective herbs used to control blood glucose is saffron. Saffron with the scientific name of *Crocus sativus L.* is from the family Iridaceae.[12,13] Saffron is a domestic plant in Iran which has been applied for a long time as the most expensive traditional remedy and spice[13]. Recent studies have been investigated saffron and crosteen, a major source of saffron, as an effective ingredient in lipidemic and glycemic indices.[13,14] Saffron is considered as an anti-depressant plant in traditional Iranian medicine.[14,15] Saffron is likely to exert its effect through the effect on the level of norepinephrine.[16] Also, saffron in traditional medicine is used as a therapeutic agent for skin disorders.[16,17] A previous document reported that saffron is effective in preventing the symptoms of experimental autoimmune encephalomyelitis (EAE), an animal model of multiple sclerosis, by preventing oxidative stress and leukocyte infiltration into the CNS.[18] Given the similarity of the pathophysiology of EAE and diabetes, it is hoped that the hydroalcoholic extract of saffron will also be involved in the treatment of this case. Nonetheless, there is no or limited data about the possible beneficial effects of saffron on the T1D. Therefore, in this study, after induction of T1D in male C57BL/6 mice, the effects of administration of the hydroalcoholic extract of saffron on the immunological profile of experimental AD in these mice were evaluated.

MATERIALS AND METHODS

Reagent

Fetal calf serum and Dulbecco's Modified Eagle's Medium (DMEM) were procured from GIBCO/Life Technologies Inc. Saffron and other reagents were obtained from Sigma-Aldrich.

Extraction

To prepare the hydroalcoholic extract of saffron, 14 grams of saffron were ground and mixed with 500 cc ethanol 98%, stored in a shaker incubator for 24 hours at 42°C. Then filtered and concentrated and kept at 37°C until the alcohol evaporated completely.

Animals and Experimental Design

Six to eight weeks male C57BL/6 mice (Pasteur Institute, Iran) were divided into 3 groups (n=10): 1) Healthy mice, 2) diabetic mice without treatment 3) diabetic mice with daily treatment started at the day of 7 when diabetes was established and continue for 21 constitutive days. Mice were kept at constant temperature (23°C) in a 12 h light/dark cycle and received food and water ad libitum. Diabetes was induced as described in.[19] Briefly, mice received intraperitoneal (I.P.) injection of 40 mg/kg STZ (Sigma, Germany) in 200 µL fresh citrate buffer (pH: 4.5) for 5 days. Before every injection of STZ, the mice fasted for 4 hours (all groups). The hydroalcoholic extract of saffron (500 mg/kg every day) was orally administrated into the treatment group from the seventh

day after the initiation of the study and continued throughout the study. This dosage was selected in accordance with the previous study on the mouse model of multiple sclerosis. [18] The blood glucose levels of mice were evaluated on days 0, 7, 14, 21 and 28 with Roche Diagnostics ACCU-CHEK Active Glucose Monitor. The tail tip clipping was used to collect a small drop of blood. The accuracy of the glucometer was adjusted by using a standard glucose colorimetric assay kit (Ziest Chem Diagnostics, Iran). The levels of triglyceride and cholesterols were determined at day 28 (Ziest Chem Diagnostics, Iran). Insulin level was determined by an ELISA kit at day 28 (Merck Millipore). Furthermore, mice Animals were weighed daily.

Animal welfare was observed throughout the investigation in compliance with the constitution of the Ministry of Health, IR Iran confirmed by the Medical Ethics Committee of the Urmia University for Animal Exams (512/TD.T/3).

Isolation of Pancreatic Cells

Multiple low doses of STZ can induce inflammation of the pancreatic islets followed by infiltration of auto-pathogenic Th17 and Th1 and subsequent activation of macrophages.[6,11,20] Therefore, we isolated pancreatic islet cells and monitored them for lymphocyte proliferation, cytokine assay and inflammatory activity of the macrophage population.

At day 28, the mice were perfused (with 40 mL PBS/mouse) under deep anesthesia (Ketamine 100 mg/kg, xylazine 10 mg/kg) and pancreatic tissues were aseptically removed. A single cell suspension by cutting up tissues using scissors and placing into freshly digestion buffer (1 mg/mL of collagenase Type IV, 10 U/mL of DNAse I, 1% BSA and DMEM) was prepared. Tissues were digested for 20 min at 37ºC at 150 rpm submerged in shaking water bath.[21]

Lymphocyte Proliferation and Cytokine Assay

Pancreatic isolated cells were cultured in 96-well plate at the concentration of 1×10^6 cells/ml with and without 50 µL phytohemagglutinin (PHA) (1 mg/mL) and incubated at 37ºC for 72 hours. 25 µl of the MTT solution (5 mg/mL) added and incubated again for 4 hours and after adding 100 µL of DMSO the optical densities at 490 nm were determined. The data are reported as the proliferation index according to the ratio of OD490 of stimulated cells with PHA to OD550 of non-stimulated cells.[22]

To monitor the ex vivo cytokines profile, 2×10^6 cells/mL were cultured in 24-well plates and pulsed with PHA (1 mg/mL). The supernatants were removed after 72 h. Interferon gamma (IFN-γ), interleukin-17 (IL-17), transforming growth factor-β (TGF-β) and IL-10 production were evaluated by ELISA according to the manufacturer's instructions (PeproTec, UK).

Evolution of Reactive Oxygen Substances

In brief, 2×10^6 cells/mL was incubated for 20 minutes with 100 ng/mL TPA and 0.1% NBT. Finally, 400 µL of N-N-dimethylformamide was added to each well and centrifuged at 800 g for 10 minutes at 4 °C and 200 µL of supernatant were transferred to 96-well plate to quantitation of optical density at 540 nm.[23]

Nitric Oxide Measurement

Nitric oxide is extremely unstable and converted to nitrate and nitrite by oxidation, which can be determined by the method of Grease. In brief, 2×10^6 cells/mL were pulsed with LPS (10 pg/mL) for 24 hours. The cell-free supernatants (50 µL) were removed and combined with 50 µL Griess reagent (0.1% sulfanilamide, 3% phosphoric acid, and 0.1% naphthyl ethylenediamine). The mixture was incubated at room temperature for 10 minutes. Finally, the absorbance was recorded at 540 nm on a microplate reader (Dynatech, Denkendorf, Germany). The nitrite concentration was calculated based on the standard curve.[23]

Pathological Examination of the Pancreas

To confirm the effect of saffron, the pancreases of the mice were placed in separate formalin-containing tubes and embedded in paraffin. 5 µm sections were cut using microtome and stained by hematoxylin and eosin. Sections were monitored under an optic microscope (Olympus BX40) and digital images were captured and analyzed by Zeiss software (AxioVision 40 4.7). The average size of the pancreatic islets for each slide was calculated according to the ratio of the area of pancreatic islets to the total area of each slide.

Statistical Analysis

Data were analyzed by IBM SPSS Statistics for Windows, version 23 (IBM Corp., Armonk, N.Y., USA) and analyzed by One Way ANOVA (POSTHOC: TUKEY ALPHA). The normal

distribution of data was confirmed with the Kolmogorov-Smirnov test. The results with *p*-value<0.05 were considered significant.

RESULTS

Blood glucose, Body weight, Insulin, Triglyceride, and Cholesterol

The most important factor in diabetes is the change in blood glucose and insulin. As expected, STZ injections showed a significant increase in blood glucose levels in mice at day 7 after initiation of the study. Receiving the hydroalcoholic extract of saffron after stabilization of diabetes caused a slight and non-significant decline in blood glucose level in mice at day 21. Although the level of blood glucose in mice receiving the hydroalcoholic extract of saffron after diabetes induction showed a significant reduction at days 21 and 28 (p<0.05, Figure 1 A).

As shown in Table1, weight gaining in diabetes C57bl/6 mice was hindered and the mean body weight of diabetic animal markedly lowered, compared to the normal animal. The extent of weight loss significantly prevented in diabetic C57bl/6 mice received a hydroalcoholic extract of saffron, compared to vehicle-treated diabetic mice (p<0.05, Table 1).

The circulating insulin level of mice received multiple low doses of STZ was markedly decreased compared to normal mice (p<0.05, Figure 1 B). Extract therapy led to a significant improvement in the circulating insulin level of diabetic mice (p<0.05, Figure 1 B).

The levels of blood triglycerides and cholesterol in diabetic mice showed a significant increase compared to normal mice (p<0.05, Figure 1 C and D). Treatment with hydroalcoholic extract of saffron significantly reduced the level of triglyceride and cholesterol in diabetic mice, so that triglyceride in treated diabetic mice returned to the range of healthy mice (p=0.7, Figure 1 C and D).

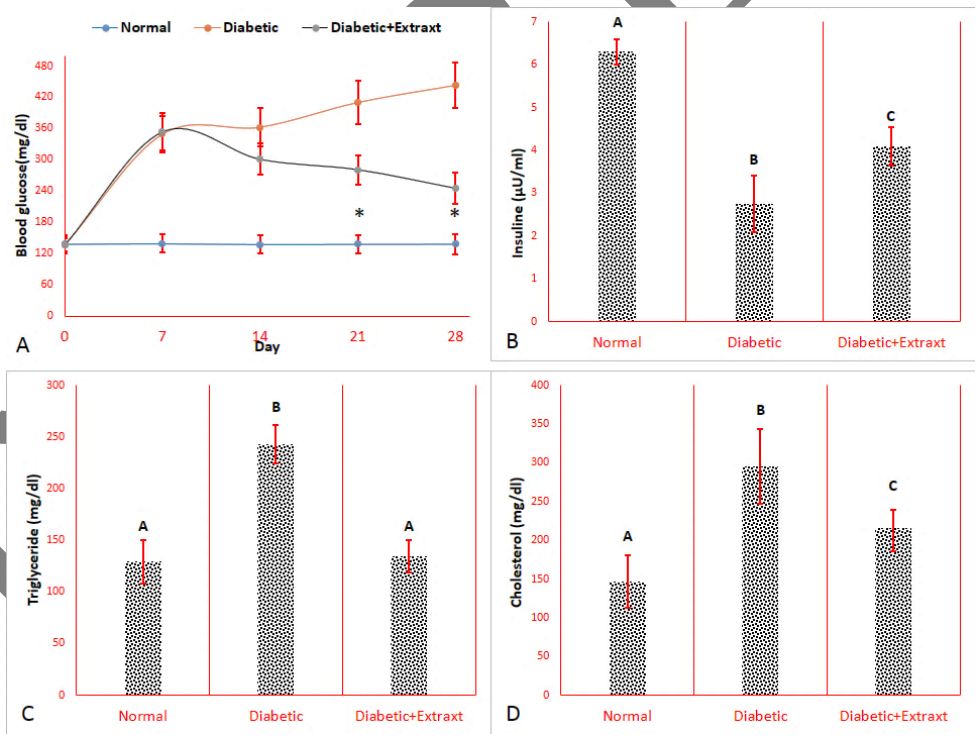

Figure 1. Evaluation of Blood glucose (A), Insulin (B), Triglyceride (C), and Cholesterol (D) in the serum of C57Bl/6. After the establishment of the AD, mice were treated orally with hydroalcoholic extract of saffron (500 mg/kg) for 3 weeks. The blood glucose levels of mice were evaluated on days 0, 7, 14, 21 and 28. The levels of insulin, triglyceride, and cholesterols were determined at day 28. Data were presented as mean ± SD (*p< 0.05 versus diabetic mice, Different letters represent a significant difference at p<0.05).

Table 1. Mean body weight evaluation of normal and diabetic mice during 28 days of study.

Treatment group	Mean body weight (mean±SD)
Normal	21.92 ± 0.36^{A}
Diabetic	16.37 ± 0.42^{B}
Diabetic+Extract	18.56 ± 0.74^{C}

The hydroalcoholic extract of saffron (500 mg/kg every day) was orally administrated into the treatment group from the seventh day after the initiation of the study and continued throughout the study.. The mean body weight of the diabetic mice significantly reduced compared to normal animals. The extent of weight loss significantly prevented in diabetic C57bl/6 mice received a hydroalcoholic extract of saffron, compared to vehicle-treated diabetic mice (Different letters represent a significant difference at $p<0.05$).

Immunological Changes

Ex vivo stimulation of pancreatic cells with PHA showed that the index of lymphocyte proliferation was significantly increased in diabetic mice compared to normal mice ($p<0.05$, Figure 2). Obtained data exhibited that treatment with saffron reduced lymphocyte proliferation index in the cells isolated from the pancreas of diabetic mice compared to diabetic rat without treatment, so that lymphocyte proliferation index in treated diabetic mice returned to the range of healthy mice($p=0.23$, Figure 2). Moreover, multiple low doses of STZ caused a meaningful increase in the production of the pro-inflammatory IFN-γ and IL-17 and simultaneously caused a significant decline in the production of the anti-inflammatory TGF-β and IL-10 in the isolated pancreatic cells from diabetic mice compared to the normal mice ($p<0.05$, Figure 3). Treatment with extract of saffron markedly decreased the production of pro-inflammatory IL-17 and increased anti-inflammatory IL-10 and TGF-β in the pancreatic cell population of diabetic mice compared to the vehicle-treated diabetic mice ($p<0.05$, Figure 3).The level of the pro-inflammatory IFN-γ was down-regulated in the lymphocyte population of the pancreatic cell isolated from diabetic mice after extract therapy. Nevertheless, there was no significant difference between the treatment group and diabetic mice ($p=0.15$, Figure 3).

Obtained findings also indicated that multiple low doses of STZ could significantly up-regulate respiratory burst and NO production by innate cell population of the pancreatic cell isolated from diabetic mice compared to respiratory burst and NO production by innate cell population of the pancreatic cell isolated from normal mice ($p<0.05$, Figure 3, Figures 4 A and B). Moreover, the production of proinflammatory nitric oxide and reactive oxygen substances were reduced by saffron extract ($p<0.05$, Figures 4 A and B).

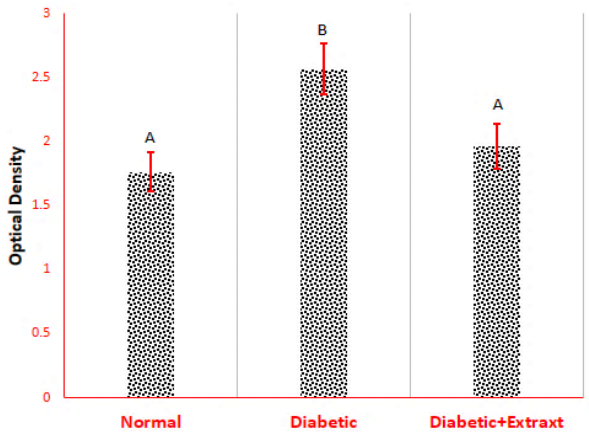

Figure 2. Evaluation of treatment with a hydroalcoholic extract of saffron on the proliferation index of pancreatic cells. The pancreatic cells of mice were isolated from mice with autoimmune diabetes on day 21 after the establishment of autoimmune diabetes. The cells were cultured phytohemagglutinin (1 mg/mL).The proliferation index was determined by reduction tetrazolium dye as described under Materials and Methods. Results were presented as mean ± SD (Different letters represent a significant difference at $p<0.05$).

Published by Tehran University of Medical Sciences (http://ijaai.tums.ac.ir)

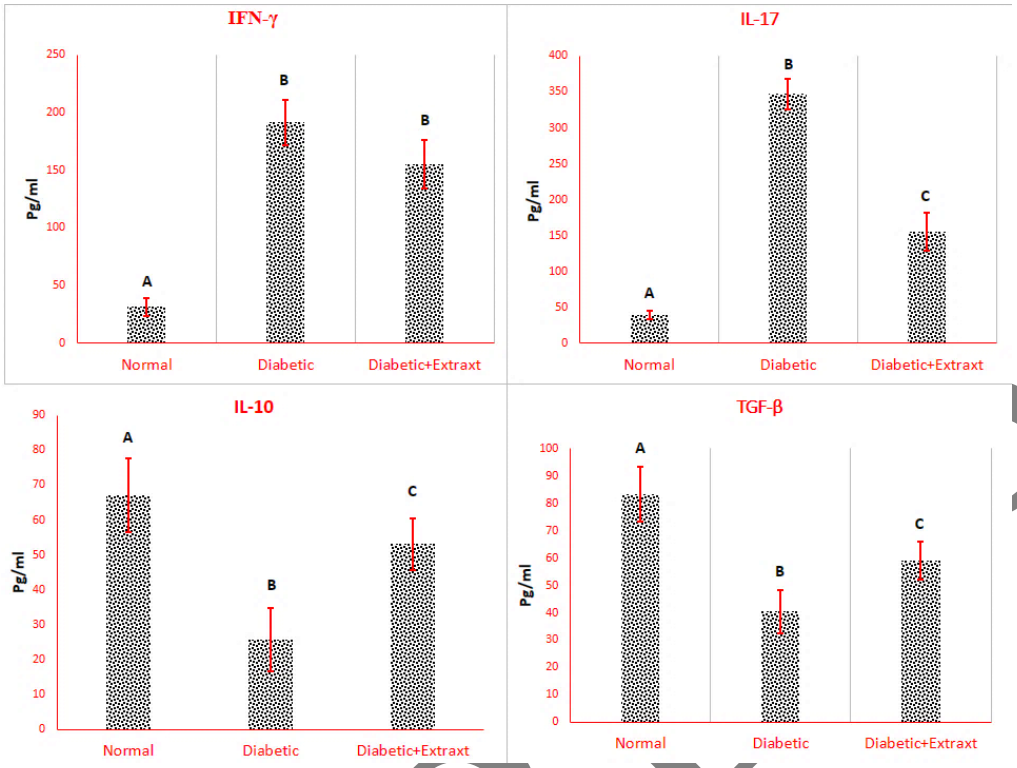

Figure 3. Ex vivo assessment of cytokine production in the pancreatic cell population. After the establishment of autoimmune diabetes, mice were treated orally with hydroalcoholic extract of saffron (500 mg/kg) for 3 weeks. To evaluate the ex vivo cytokines profile, 2×10^6 pancreatic cells/mL were cultured in 24-well plates and pulsed with phytohemagglutinin (1 mg/mL). The supernatants were removed after 72 h. Interferon-gamma (IFN-γ), interleukin-17 (IL-17), transforming growth factor-β (TGF-β) and IL-10 production was evaluated by the ELISA kits.The values were presented as mean ± SD (Different letters represent a significant difference at $p< 0.05$).

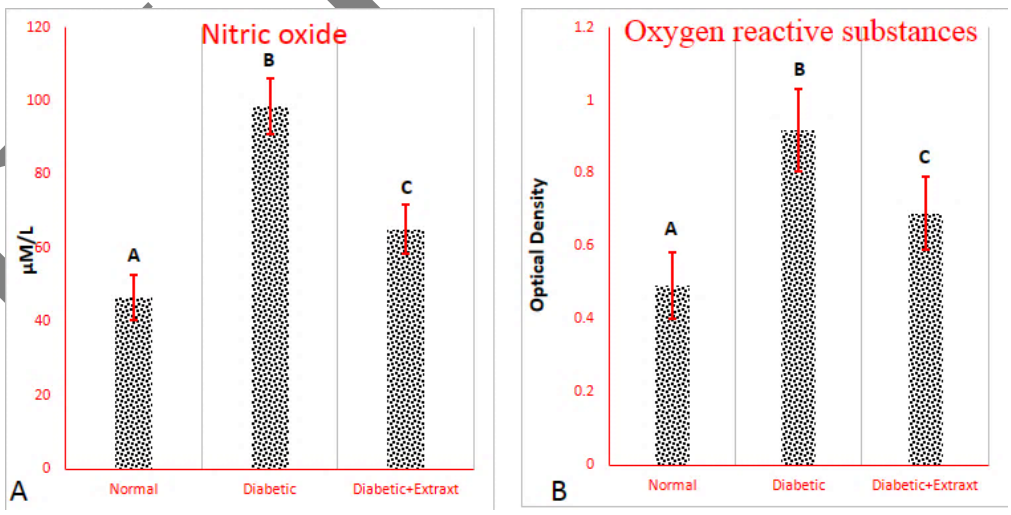

Figure 4. Modulation of production of nitrogen (A) and oxygen (B) reactive substances in the pancreatic cell population. After establishment of autoimmune diabetes and 3 weeks treatment of rats by a hydroalcoholic extract of saffron, the pancreatic cells of mice were isolated and the potential of cells for production of free radicals was evaluated. The data were expressed as mean ± SD (Different letters represent a significant difference at $p<0.05$).

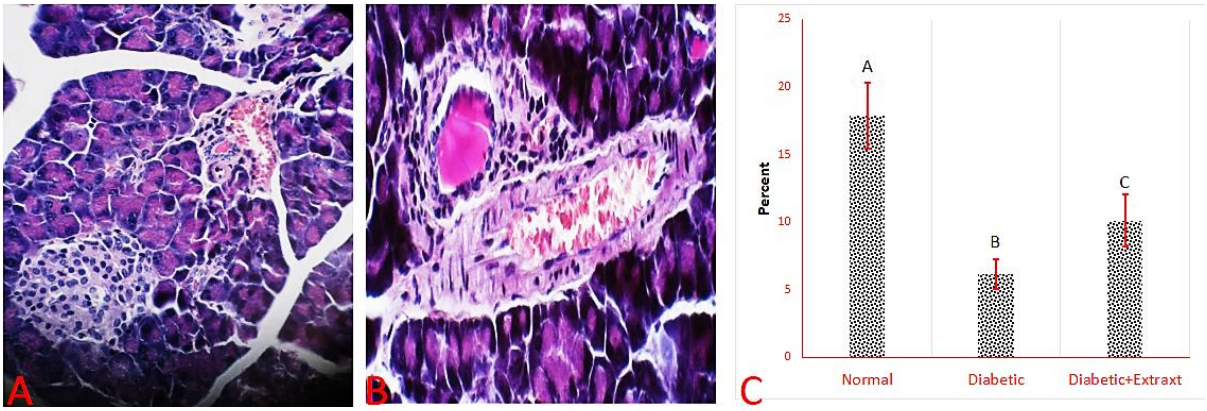

Figure 5. Histopathological exanimation of pancreatic sections. After the establishment of autoimmune diabetes, mice were treated orally with hydroalcoholic extract of saffron (500 mg/kg) for 3 weeks. To confirm the effect of saffron, the pancreases of the mice were removed and used for pathological examination. A) The section of the pancreas in the control group. The healthy structure of the pancreatic islands is visible. (Hematoxylin and eosin (H&E) staining at 400X). B). A section of the pancreas in the diabetic group. STZ has reduced the structure of the islands and its size has decreased (H&E staining at 400X). C). The average size of the pancreatic islets for each slide was calculated according to the ratio of the area of pancreatic islets to the total area of each slide. The data were expressed as mean ± SD (Different letters represent a significant difference at $p<0.05$).

Histopathological Finding

The H&E stained sections of the pancreatic islets are shown in Figures 5 A and B. Destruction of Langerhans islets is very well seen in the diabetic group following the administration of STZ (Figures 5 A and B). The histopathological evaluation showed that the average size of the pancreatic islets was significantly regressed in mice with T1D compared to normal mice ($p<0.05$, Figure 5 C). Fortunately, daily treatment with the hydroalcoholic extract of saffron could markedly restore the average size of the pancreatic islets in diabetic rats ($p<0.05$, Figure 5 C).

DISCUSSION

The purpose of this study was to evaluate the effects of administration of the hydroalcoholic extract of saffron on the immunological profile of experimental autoimmune diabetes in C57BL/6 mice. It is clear that the hydroalcoholic extract of petals and the stomachs of saffron has been shown to have analgesic effects as well as significant anti-inflammatory properties in cases of acute or chronic inflammation.[16,17] Moreover, according to studies, saffron extract reduces inflammatory cells and eosinophils in animal models of allergies.[24] Comparison of blood glucose, insulin level and histopathologic results between multiple low doses STZ administered and control groups was consistent with previous studies confirming the efficacy of streptozotocin and the success of autoimmune diabetes induction in this study.

The bitter taste of saffron is due to the presence of a substance called picrocrocin. This material is converted into aromatic aldehyde by the thermal or enzymatic degradation during the processing of the plant. Crocins, which contain glycosides of carotenoids called croscine, and sugars, are responsible for the color of saffron. Crocin, croscine, and safranal are the main ingredients of saffron.[25] Medical benefits of saffron are contributed to its active ingredients, including safranal, crocin, picrocrocin, and crocetin.[26] For example, some documents have revealed that saffron and crocin dose-dependently decreased the levels of liver enzymes in male rats with fatty liver complication.[27] Previous findings were also exhibited that saffron can protect the liver and kidney from environmental toxins.[27] Of note, saffron is a safe spice with the low toxicity on the normal cells.[26] Our findings showed that diabetic mice who received therapy had significantly lower levels of blood glucose, total cholesterol, and triglyceride. Recent studies have also verified the blood lipid- and

glucose-lowering effects of saffron in metabolic syndrome and have shown that insulin resistance has been reduced by saffron. Furthermore, hydroalcoholic extract of saffron led to a significant improvement in the circulating insulin level of diabetic mice.[28] It has found that saffron and crosteen, a major component of saffron, as a factor affecting lipids and glycemic indices.[29] Interestingly, it has been reported that saffron may reduce glucose absorption enzymes and as a result can improve diabetes control.[30]

It is suggested that IFN-γ producing T helper lymphocytes (Th1) and IL-17 producing T helper cells (Th17) cells effectively participate in the pathogenesis of T1D.[31] On the other hand, it has been revealed that inflammatory process in the early stages of T1D is initiated by IL-17 which leads to huge infiltration of multiple inflammatory cells such as Th1 into the pancreas.[31] Collectively, it clears that Th17 lymphocytes are more pathogenic than Th1 cells in the induction of autoimmune diseases like EAE and T1D.[31,32] Our results showed that the levels of Il-17 in the treated diabetic mice were significantly lower than untreated diabetic mice. However, the level of IFN-γ did not show any significant differences between treated and untreated diabetic mice. Saffron stigma includes polar carotenoids.[24] Previous reports indicated that carotenoids can reduce the secretion of the inflammatory cytokines.[33] Furthermore, it is reported that inflammatory-cytokines have an essential role in the development and progression of metabolic syndrome. Interestingly, consumption of 100 mg/day *crocus-sativus* for 12 weeks could decrease the serum concentrations of the pro-inflammatory cytokines in patients with metabolic syndrome.[28] An in vitro study also indicated that saffron extract could change the ratio of IFN-γ to IL-4 toward anti-inflammatory IL-4.[34]

Also, recent studies have noted that $CD4^+CD25^+Foxp3^+$ regulatory T cells (Tregs) play a key role in controlling autoimmune diseases, such as T1D, as they can inhibit the responses of autoreactive cytotoxic T cells and might have roles in the preventing and treatment of autoimmune diseases.[35] T1D may be caused by an imbalance between Tregs and IL-17 producing Th17 cells.[31,35] IL-10 and TGF-β are the main anti-inflammatory cytokine produced by Tregs.[35] Attained data in this study showed that IL-10 and TGF-β level in the saffron treated diabetic mice was significantly higher than the untreated diabetic mice.

Saffron has been reported to inhibit the synthesis of DNA and RNA and therefore possesses anti-proliferative benefits. The anticancer effects reported for saffron are mainly due to the presence of carotenoids and carotene.[29] The observed increase in pancreatic lymphocyte proliferation in diabetic mice compared to healthy mice indicates an increase in the number of auto-reactive lymphocytes in diabetic mice (autoimmune diabetes) compared to the control group. Fortunately, the results of this study showed that the use of the hydroalcoholic extract of saffron specifically significantly reduced the proliferation of these lymphocytes. With all these interpretations, the plant used in this study does not appear to have severe and undesirable immunosuppressive effects, as the lymphocyte response in the group receiving the extract is similar to that of healthy mice.

It is clear that nitrogen species (like NO) and reactive oxygen species (ROS) by innate cells contribute in host defense against infections. Nevertheless, overproduction of these substances in the non-infection condition like autoinflammatory and autoimmunity conditions participate in host pathology.[23] Direct support for a ROS role of nitric oxide in the progress of T1D includes the ability of inhibitors of NO and antioxidant materials to prevent or alleviate the development of T1D in animal models.[36] Radical scavenging activity of the bioactive constituents of *Crocus sativus L.* extract is clear.[26,44] Crocin exhibited high free radical scavenging activity (50% and 65% for 500 and 1,000 ppm solution in methanol, respectively), followed by safranal (34% for 500 ppm solution).[37] It has been reported that 30 mg/kg daily injection of crocin or saffron for 21 days regress oxidative stress in the animal tissues like kidney, liver, and brain.[26] Also, an interesting document indicated that adding the aqueous extract of saffron to aerobic training may be an appropriate strategy to reinforce the hepatic non-enzymatic antioxidant system in diabetic rats.[36] In this regards, it has been revealed that IFN-γ and IL-17 can activate the macrophages and infiltrate innate cells to produce ROS role of nitric oxide and conversely, IL-10 and TGF-β can diminish production of both.[22,35] Interestingly, our results showed that extract of saffron markedly decreased the production of pro-inflammatory IL-17 increased anti-inflammatory IL-10 and TGF-β in the pancreatic cell population concurrent with a significant decrease in the production of ROS and nitric oxide. Similarly, previous data indicated the extract of saffron could regress the levels

of total NO and nitrite in the serum of guinea-pigs sensitized by ovalbumin.[38]

To best of the knowledge, this survey is the first experimental assay of saffron in the control of autoimmune type 1 diabetes. Overall, it seems that treatment with the hydroalcoholic extract of saffron leads to several advantages in reducing diabetes so that these mice show a lower level of blood glucose than untreated diabetic ones and at the same time the consequences of diabetes, such as hypertriglyceridemia and hypercholesterolemia. Also, extract of saffron markedly decreased the production of pro-inflammatory IL-17 cytokines and increased the levels of anti-inflammatory IL-10 and TGF-β in the pancreatic cell population. Moreover, the production of proinflammatory nitric oxide and reactive oxygen substances were down-regulated by saffron extract in the pancreatic cell population. It seems that the hydroalcoholic extract of saffron can be considered as a useful strategy in the treatment of type 1 diabetes. Nevertheless, this survey is a preliminary animal study and further researches are needed to be designed.

ACKNOWLEDGEMENTS

The study procedure has been supported by Urmia University, Urmia, Iran.

REFERENCES

1. Pane JA, Webster NL, Coulson BS. Rotavirus Activates Lymphocytes from Non-Obese Diabetic Mice by Triggering Toll-Like Receptor 7 Signaling and Interferon Production in Plasmacytoid Dendritic Cells. PLoS Pathogens 2014; 10(3):e1003998.
2. Clark M, Kroger CJ, Tisch RM. Type 1 diabetes: A chronic anti-self-inflammatory response. Front Immunol 2017; 8:1898.
3. Atalay M, Oksala NK, Laaksonen DE, Khanna S, Nakao C, Lappalainen J, et al. Exercise training modulates heat shock protein response in diabetic rats. J Appl Physiol 2004; 97(2):605-11.
4. Rostambeigy P, Ghaemi SZ, Shirazi FM, Hashemi N. The Effects of Nutrition Education and Insulin Injection Training on Glycemic Control in Iranian Patients with Type 1 Diabetes. Adv Biores 2014; 5(2):143-7.
5. Rubinstein MR, Genaro AM, Wald MR. Differential effect of hyperglycaemia on the immune response in an experimental model of diabetes in BALB/cByJ and C57Bl/6J mice: Pfarticipation of oxidative stress. Clin Exp Immunol 2013; 171(3):319-29.
6. Wei L, Lu Y, He S, Jin X, Zeng L, Zhang S, et al. Induction of diabetes with signs of autoimmunity in primates by the injection of multiple-low-dose streptozotocin. Biochem Biophys Res Commun 2011; 412(2):373-8.
7. Singh R, Kaur N, Kishore L, Gupta GK. Management of diabetic complications: a chemical constituents based approach. J Ethnopharmacol 2013; 150(1):51-70.
8. Wang K, Tang Z, Zheng Z, Cao P, Shui W, Li Q, et al. Protective effects of Angelica sinensis polysaccharide against hyperglycemia and liver injury in multiple low-dose streptozotocin-induced type 2 diabetic BALB/c mice. Food Funct 2016; 7:(12):4889-4897.
9. Lee DY, Kim MH, Suh HR, Jung YS, Hwang DY, Kim KS. Comparison of the response using ICR mice derived from three different sources to multiple low-dose streptozotocin-induced diabetes mellitus. Lab Anim Res 2017; 33(2):150-6.
10. Amirshahrokhi K, Dehpour AR, Hadjati J, Sotoudeh M, Ghazi-Khansari M. Methadone ameliorates multiple-low-dose streptozotocin-induced type 1 diabetes in mice. Toxicol Appl Pharmacol 2008; 232(1):119-24.
11. Rabinovitch A, Suarez-Pinzon WL. Cytokines and their roles in pancreatic islet beta-cell destruction and insulin-dependent diabetes mellitus. Biochem Pharmacol 1998; 55(8):1139-49.
12. Malik AH, Ashraf N. Transcriptome wide identification, phylogenetic analysis, and expression profiling of zinc-finger transcription factors from Crocus sativus L Saffron. Mol Genet Genomics 2017; 292(3):619-33.
13. Razavi BM, Hosseinzadeh H. Saffron: a promising natural medicine in the treatment of metabolic syndrome. J Sci Food Agric 2017; 97(6):1679-85.
14. Boskabady MH, Farkhondeh T. Antiinflammatory, Antioxidant, and Immunomodulatory Effects of Crocus sativus L. and its Main Constituents. Phytother Res 2016; 30(7):1072-94.
15. Karimi Gh.R. Khalegh Panah P HH. Study of antidepressant effect of aqueous and ethanolic extract of crocus sativus in mice. Iran J Basic Med Sci 2001; 4(3):11-5.
16. Hosseinzadeh H, Karimi G, Niapoor M. sAntidepressant effect of Crocus sativus L. stigma extracts and their constituents, crocin and safranal, in mice. JMP 2004; 3(11):48-58.
17. Rezaee R, Hosseinzadeh H. Safranal: From an aromatic natural product to a rewarding pharmacological agent.

Iran J Basic Med Sci 2013; 16(1):12-26.
18. Ghazavi A1, Mosayebi G, Salehi H, Abtahi H. Effect of ethanol extract of saffron (Crocus sativus L.) on the inhibition of experimental autoimmune encephalomyelitis in C57bl/6 mice. Pak J Biol Sci 2009; 12(9):690-5.
19. Najemnikova E, Rodgers CD, Locke M. Altered heat stress response following streptozotocin-induced diabetes. Cell Stress Chaperones 2007; 12(4):342-52.
20. Dardalhon V, Korn T, Kuchroo VK, Anderson AC. Role of Th1 and Th17 cells in organ-specific autoimmunity. J Autoimmun 2008; 31(3):252-6.
21. Green EA, Choi Y, Flavell RA. Pancreatic lymph node-derived CD4(+)CD25(+) Treg cells: highly potent regulators of diabetes that require TRANCE-RANK signals. Immunity 2002; 16(2):183-91.
22. Abtahi Froushani SM, Galeh HEG. New insight into the immunomodulatory mechanisms of tretinoin in NMRI mice. Iran J Basic Med Sci 2014; 17(9):632-7.
23. Shushtari N, Abtahi Froushani SM. Caffeine Augments The Instruction of Anti-Inflammatory Macrophages by The Conditioned Medium of Mesenchymal Stem Cells. Cell j 2017; 19(3):415-24.
24. Papandreou MA, Kanakis CD, Polissiou MG, Efthimiopoulos S, Cordopatis P, Margarity M, et al. Inhibitory activity on amyloid-β aggregation and antioxidant properties of Crocus sativus stigmas extract and its crocin constituents. J Agric Food Chem 2006; 54(23):8762-8
25. Christodoulou E, Kadoglou NP, Kostomitsopoulos N, Valsami G. Saffron: a natural product with potential pharmaceutical applications. J Pharm Pharmacol 2015; 67: 1634-49 .
26. Tamaddonfard E, Farshid AA, Hosseini L. Crocin alleviates the local paw edema induced by histamine in rats. Avicenna J Phytomed 2012; 2(2):97-104.
27. Milajerdi A, Jazayeri S, Bitarafan V, et al. The effect of saffron (Crocus sativus L.) hydro-alcoholic extract on liver and renal functions in type 2 diabetic patients: A double-blinded randomized and placebo control trial. J Nut and Int Meta 2017; 9:6-11.
28. Kermani T, Zebarjadi M, Mehrad-Majd H, Mirhafez SR, Shemshian M, Ghasemi F, et al. Anti-inflammatory effect of Crocus sativus on the serum cytokines levels in subjects with Metabolic Syndrome: A Randomized, Double-blind, Placebo - controlled trial. Curr Clin Pharmacol 2017; 12(2):122-6.
29. Abdullaev FI. Cancer chemopreventive and tumoricidal properties of saffron (Crocus sativus L.). Exp Biol Med 2002; 227(1):20-5.
30. Loizzo MR, Marrelli M, Pugliese A, Conforti F, Nadjafi F, Menichini F, et al. Crocus cancellatus subsp. damascenus stigmas: chemical profile, and inhibition of α-amylase, α-glucosidase and lipase, key enzymes related to type 2 diabetes and obesity. J Enzyme Inhib Med Chem 2016; 31(2):212-8.
31. Li Y, Liu Y, Chu CQ. Th17 Cells in Type 1 Diabetes: Role in the Pathogenesis and Regulation by Gut Microbiome. Mediators Inflamm 2015; 2015:63847.
32. Abtahi Froushani SM, Delirezh N, Hobbenaghi R, Mosayebi G. Synergistic effects of atorvastatin and all-trans retinoic acid in ameliorating animal model of multiple sclerosis. Immunol Invest 2014; 43(1):54-68.
33. Kurtys E, Eisel ULM, Verkuyl JM, Broersen LM, Dierckx R, de Vries EFJ. The combination of vitamins and omega-3 fatty acids has an enhanced anti-inflammatory effect on microglia. Neurochem Int 2016; 99:206-214.
34. Feyzi R, Boskabady Mh, Seyed hosseini Tamijani SM, Rafatpanah H, Rezaei SA. The Effect of Safranal on Th1/Th2 Cytokine Balance. Iran J Immunol 2016; 13(3):263-273.
35. Garetto S, Trovato AE, Lleo A, Sala F, Martini E, Betz AG, et al. Peak inflammation in atherosclerosis, primary biliary cirrhosis and autoimmune arthritis is counter-intuitively associated with regulatory T cell enrichment. Immunobiology 2015; 220(8):1025-9.
36. Peeri M, Mosalman Haghighi M, Azarbayjani MA, Atashak S, Behrouzi G. Effect of aqueous extract of saffron and aerobic training on hepatic non enzymatic antioxidant levels in streptozotocin-diabetic rats. Archives Des Sciences 2012; 65(10):525-32.
37. Assimopoulou AN, Sinakos Z, Papageorgiou VP. Radical scavenging activity of Crocus sativus L. extract and its bioactive constituents. Phytother Res 2005; 19(11):997-1000.
38. Boskabady MH, Byrami G, Feizpour A. The effect of safranal, a constituent of Crocus sativus (saffron), on tracheal responsiveness, serum levels of cytokines, total NO and nitrite in sensitized guinea pigs. Pharmacol Rep 2014; 66(1):56-61.