Progress in Nutrition 2019; Vol. 21, Supplement 1: 147-153 DOI: 10.23751/pn.v21i1-S.5833

Original Article

The effects of natural nano-sized clinoptilolite and *Nigella sativa* supplementation on blood glucose and lipid profile in rats with type 2 diabetes mellitus

Hossein Omidi[1], Sirous Khorram[2], Mehran Mesgari[3], Mohammad Asghari-Jafarabadi[4], Ali Tarighat-Esfanjani[5]

[1]Master of Science in Nutrition Sciences, Student Research Committee, , Tabriz University of Medical Sciences, Tabriz, I. R. Iran; [2]Assistant Professor, Experimental Physics, Materials Physics, Materials Science, University of Tabriz, Tabriz, I. R. Iran; [3]Drug applied research center, Tabriz University of Medical Sciences, Tabriz, I. R. Iran; [4]Associate Professor of Biostatics, Department of Statistics and Epidemiology, Tabriz University of Medical sciences, Tabriz, I. R. Iran; [5]Associate Professor in Nutrition Sciences Nutrition Research Center, School of Nutrition and Food Sciences, Tabriz University of Medical sciences, Tabriz, I. R. Iran - E-mail: tarighata@tbzmed.ac.ir

Summary. *Objectives:* Hyperglycemia and hyperlipidemia have major roles in incidence or development of complications diabetes mellitus. Regarding side effects of diabetes treatment, patients seek natural, economical, and more effective treatments. In current study we investigated the effects of separate and concurrent supplementation of natural nano-sized clinoptilolite (NCLN) and *Nigella sativa* (NS) on blood glucose and lipid profile (LP) in high-fat-diet (HFD)/streptozotocin (STZ)-induced diabetic rats. *Methods:* In this experimental study, 42 male Wistar rats divided into two groups as diabetic and non-diabetic. Diabetic group fed with HFD for 1 month, and then injected intraperitoneal single dose STZ (35 mg/kg BW). After a week, oral glucose tolerance test and homeostatic model assessment of insulin resistance (HOMA-IR) test was carried out to confirm diabetes. Diabetic group was divided to 4 subgroups: (1) control (n=9), (2) NS 1g/kg (n=9), (3) NCLN 2%/food (n=9), (4) NS 1g/kg + NCLN 2%/food (n=9). At end 7th week, fasting blood glucose, triglyceride, total cholesterol, low density lipoprotein cholesterol and high density lipoprotein cholesterol were tested. Data analysis was performed using SPSS software version 16 and P <0.05 was considered significant. *Results:* Results showed that hyperglycemia is reduced significantly in NS (P<0.05) and NS + NCLN (P<0.05) groups. In addition, supplementation with NS reduced HOMA-IR near to normal range. Any of supplementation had no significant effect on LP. *Conclusions:* Based on the results of this study, NS exerts significant hypoglycemic effect in HFD/STZ-induced diabetic rats while simultaneous supplementation of NS and NCLN had no synergistic effect on hyperglycemia.

Key words: Hyperglycemia, lipid profile, *Nigella sativa*, Zeolite, Rats.

Introduction

Type 2 diabetes mellitus (T2DM) is a multifactorial chronic disease that characterized by hyperglycemia and increasing globally (1, 2). By up-regulating insulin secretion, b cells can compensate insulin insensitivity, although this may be insufficient. Defect in peripheral glucose utilization and thus gluconeogenesis by liver result in hyperglycemia. This devastating illness leads to pancreas, liver, kidney, heart and nerve disorders (3, 4). Combination of high-fat diet (HFD) and low-dose streptozotocin (STZ) in animal models is appropriate for testing anti-diabetic agents in T2DM. Recent animal studies represented that the HFD before STZ injection had increased insulin, free fatty acids (FFA), and triglycerides (TG) concentrations and induce insulin resistance (IR). On the other hand, low dose STZ caused the b-cell dysfunction or destruction and the frank hyperglycemia in normal rat model (5).

Regarding new discoveries in medicinal plants, world health organization (WHO) recommended study of anti-diabetic herbs such as *Nigella sativa* (NS) in diabetic subjects (6, 7). NS, also known as black seed or black cumin, is from the botanical family of Ranunculaceae that has been used in folk medicine. As a natural remedy, recent investigations have represented anti-diabetic (8, 9), Anti-oxidative, anti-inflammatory (10), hypotensive (11), anti-bacterial, bronchodilator (12), carcinogenesis inhibitory, immunopotentiating, analgesic antimicrobial activity in-vitro and in-vivo. Also, it has been reported that the thymoquinone/NS oil decrease TG, total cholesterol (TC) concentration and lipid-peroxidation (13) and normalize high density lipoprotein cholesterol (HDL-C) and augment hepatocytes insulin sensitivity (14). It was reported that NS oils in dose response manner decrease the elevated fasting blood glucose (FBG), 2 hour plasma glucose (2hPG) and glycosylated hemoglobin (HbA1c) without change in body weight (15). Feeding powder or oil of *NS* to diet-induced hypercholesterolemic (HC) rabbits; result in decreasing in the TC and low-density lipoprotein cholesterol (LDL-C) levels and improvement of HDL-C levels (16). High dose thymoquinone can be result in respiratory, kidney, heart and liver disorders (17).

Zeolites are Nano-porous crystals that are now widely used in catalytic, adsorption, and separation processes (18). Medical applications of zeolites include removing of urea in uremia, hemodialysis material, osteoporosis treatment, and as antioxidant and immunostimulatory agent (19). As an anti-diabetic agent, it has prevented initiation of diabetes in young BB rats (20) and reduced blood glucose in non-diabetic animal models (21). In our earlier study NCLN decreased FBG levels in type1diabetes (T1DM) wistar rats (Tarighat et al, unpublished data). This effect may be because its adsorption and ion exchange properties (22). Ca2+-zeolite usage in combination with insulin in T1DM mice could modify hyperglycemia through blockage of sodium- glucose transporter (SGLT) and preserves the periphery of glucose excess (23). Superantigenic action of silicate materials (zeolites components), and thus autoimmune diseases, mesothelioma, and lung fibrosis have been reported (18).

Concurrent supplementation of NCLN and NS can cause synergistic decrease in blood glucose and improve LP while preventing allergic reaction and other side effects on vital organs. Considering our earlier promising evidence of NCLN on the blood glucose in T1DM, lack of study to assess the effect of NCLN on T2DM, uncertainty of the NS's effective type and dose in T2DM, this study aimed to test synergistic effects of these two materials in blood glucose levels and LP in T2DM rats.

Methods

Animals

A total of 44 (case=36 and control=6) male Wistar rats weighing >250 grams with mean age of 5-6 months, were purchased from laboratory animal breeding center of Tabriz University of Medical Sciences. All animals were fed on standard ad-libitum and normal drinking water and were kept to acclimate to conditions of temperature, humidity and light (cycle dark/light 12 hours) 4 or 5 rats in each cage. At the end of the study (8th week), FBG and LP was assessed through the final blood sampling (5 mL) of the animals. Moral laws were respected related to the maintenance and operation of these animals and ethical approval was obtained from the ethics committee of the Tabriz University of Medical Sciences.

Grouping of animals

The diabetic rats were divided into four groups: group 1 (n=9) received only NS diet, group 2 (n=9) received only NCLN diet, group 3 (n=9) received both NS and NCLN diet, and group 4 (n=9) received standard rat's diet (as diabetic control) and healthy control one group (n=6).

Induction of T2DM in rats

Induction of T2DM was carried out by administering a month HFD (48% carbohydrate, 32% of energy from fat, 20% protein), followed by intraperitoneal injection of 35 mg/kg BW using a single dose STZ in 0.1 M sodium citrate buffer, and pH 4.5. One week after injection, for the diagnosis of diabetes, blood glucose levels were obtained from the orbital sinus (1-2 drops). Blood glucose levels were determined using an Accu-Chek glucometer (Roche, Germany) and rats

with high blood sugar of 250 mg/dl was selected and studied as diabetic.

Oral glucose tolerance test (OGTT) and homeostatic model assessment (HOMA-IR)

To ensure of the T2DM induction, OGTT with 12 hours fasting, was carried out. A solution containing 20% glucose (2 g/kg BW) was prescribed by oral gavage to the animals. Blood samples for measurement blood glucose after 0, 30, 60 and 120 minutes and serum insulin level before OGTT by rat insulin ELISA kit (East biopharm co, china) were taken from the animal's orbital sinus. At the end of the 7th weeks of treatment, IR estimation performed using HOMA-IR, by following formula: Plasma glucose (mg/dL) × fasting plasma insulin (mU/L) divided by 405 (24).

Preparation of therapeutic diets

Seeds of NS were purchased from Tabriz city's local market, and natural clinoptilolite (CLN) from Tehran (Afrazand Co., Tehran, Iran). Microparticles of CLN converted to nanorods by glow discharge plasma (25) in Research Institute for Applied Physics and Astronomy at University of Tabriz. Then CNLN powdered using mixer grinder. Prescription of NS and NCLN powders in the pelleted form was 1,000 mg/kg BW and 2% respectively for 8 weeks. The main constituents of NCLN that used in this study are shown in Table1.

Statistical analysis

The data were presented as mean ± SD. Kolmogorov-Smirnov test (KS) took place to test the normal data distribution. For measurement and comparison of the LP and FBG mean values in the studied groups, ANOVA test was used in the cases of having normal distribution. Otherwise, Kruskal-Wallis and appropriate post-hoc test was applied. Data analysis was conducted by using SPSS software version 16 and P <0.05 was considered significant. The mean of each blood parameter from both tested groups was compared to its corresponding parameter in the control groups using unpaired Student's t-test.

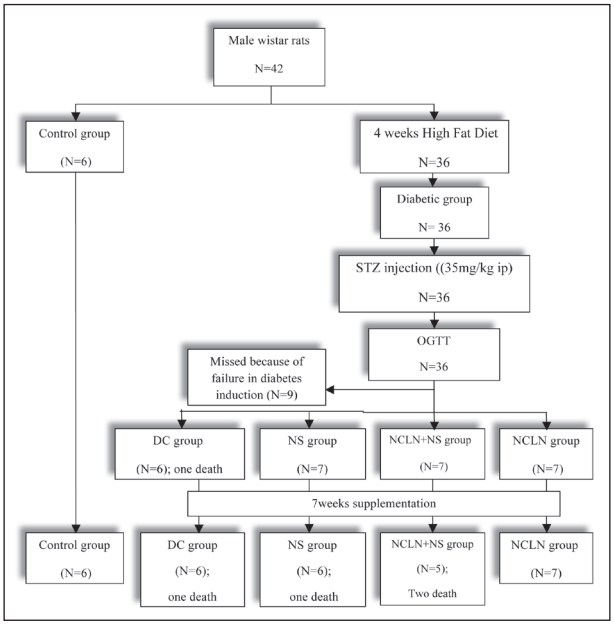

Figure 1. A diagram of study's steps

Results

Blood glucose

Table 2 shows the effect of prescribed diets on FBG concentration. After 8 weeks of treatment, diabetic rats fed with 1000 mg/Kg BW NS seed (group1) powder showed significantly lower blood glucose as compared to rats fed NCLN (Pv=0.004) group

Table 1. Elemental composition of clinoptilolite and nano-sized clinoptilolite

	Weight (%)				mole/ratio		
	Na	Al	Si	K	Si/Al	Na/Al	K/Al
CLN	3.58	7.07	60.33	0.72	8.28	1.25	0.15
NCLN	8.86	4.81	44.27	10.94	8.88	4.52	3.23

CLN= clinoptilolite; NCLN= nano-sized clinoptilolite; Na=Sodium; Al=Aluminium; Si= Silicon; K=Potassium
Adapted with permission from Table 2 in "Khataee, A., Bozorg, S., Khorram, S., Fathinia, M., Hanifehpour, Y., & Joo, S. W. (2013). Conversion of natural clinoptilolite microparticles to nanorods by glow discharge plasma: a novel Fe–impregnated nanocatalyst for the heterogeneous Fenton process. Industrial & Engineering Chemistry Research, 52(51), 18225-18233."

and diabetic control (DC) group (Pv=0.006). Also NCLN+NS group as compared to NCLN and DC groups showed significantly (Pv=0.041) lower blood glucose level. This test demonstrated higher blood glucose concentration in NCLN group as compared to NCLN+NS (Pv=0.041), NS (Pv=0.004) and control (Pv=0.012) groups.

Serums lipid profile

The effects of interventions on LP at the 7th weeks of treatment are shown in Table2. There was no significant difference in TG, TC, LDL-C and HDL-C between groups. Level of TC in NS group was lower than DC group; (85.46 (9.60) vs 91.65 (25.75)), and HDL-C level in NCLN group was higher than other groups (Pv=0.053); although these differences were non-significant.

Serum insulin concentration

As presented in Table 3, serum insulin levels after a week of STZ injection showed a significant reduction (Pv= 0.001) in diabetic groups compared to the control group. At the end of study, there were no significant differences in serum insulin levels between groups but were higher than pre-intervention.

HOMA-IR

Administration of HFD/STZ in rats increased (Pv=0.025) HOMA-IR values (figure 2). Separate supplementation of NS and concurrent use of it with NCLN, decreased HOMA-IR values but NCLN had no significant effect compared to DC group. Supplementation with NS compared to other interventions, could make more reduction in the HOMA-IR index.

Discussion

In this study, T2DM associated with IR induction was made by HFD for 4 weeks, with a low dose of STZ (35 mg/kg). This model was reported to be similar to the T2DM in humans (26). In our study, FBG levels increased and serum insulin levels decreased following HFD/STZ. Our experiment showed that NS in 1000 mg/kg BW for 7 weeks had a marked ameliorative effect on the FBG; but despite of non-significant decrease in TC levels, had no statistically significant effect on LP.

The hypoglycemic effect of NS reported in current study is in agree with previous reports in normal and alloxan-induced diabetic rabbits (8), STZ-induced diabetic rats (27) and in human subjects (15). But our

Table 2. Mean (SD) of FBG and LP levels in rats after interventions

Groups	Treatment	FBG (mg/dL)	TC (mg/dL)	TG (mg/dL)	LDL-C (mg/dL)	HDL-C (mg/dL)
1	DC (n=6)	314.3 (49.9)	91.65 (25.75)	84.71(38.26)	42.55 (22.38)	31.23 (0.27)
2	NCLN+NS (n=5)	195.0 (94.0)*	99.38 (16.72)	59.08 (19.25)	55.88 (17.01)	31.70 (1.29)
3	NS (n=5)	156.2 (96.7)*	85.46 (9.60)	63.28 (28.6)	41.00 (12.15)	31.80 (0.78)
4	NCLN (n=7)	314.5 (54.6)	95.98 (12.28)	72.90 (28.30)	48.86 (11.52)	32.52 (0.57)
5	NC (n=6)	180.0 (33.4)	99.63 (19.55)	62.76 (11.53)	55.43 (20.00)	31.60 (0.86)

*DC= Diabetic control, NCLN= Nano-sized clinoptilolite, NS= Nigella. Sativa, NC= Normal control. FBG= fasting blood glucose; LP=lipid profile. ANOVA followed by Post-Hoc and Sidak tests. * Lower blood glucose (P <0.05) compared to group 1, 4.*

Table 3. Mean (SD) serum insulin levels (MIU/L) before and after interventions in rats

Groups	Before DI	Treatment	After DI
Diabetic (n=36)	7.05 (1.12)*	DC (n=6)	9.23 (0.46)**
		NCLN+ NS (n=5)	9.92 (1.87)**
		NS (n=5)	9.20 (0.58)**
		NCLN (n=7)	9.15 (1.02)**
Control (n=6)	8.88 (0.78)	NC	8.96 (0.92)

*DI= Dietary interventions, DC= Diabetic control, NCLN= Nano-sized clinoptilolite, NS= Nigella Sativa, NC= Normal control. ANOVA followed by Post-Hoc and Sidak tests. * P<0.05 compared to control group. ** Pv <0.05 compared to before DI. No significant differences in serum insulin levels between groups.*

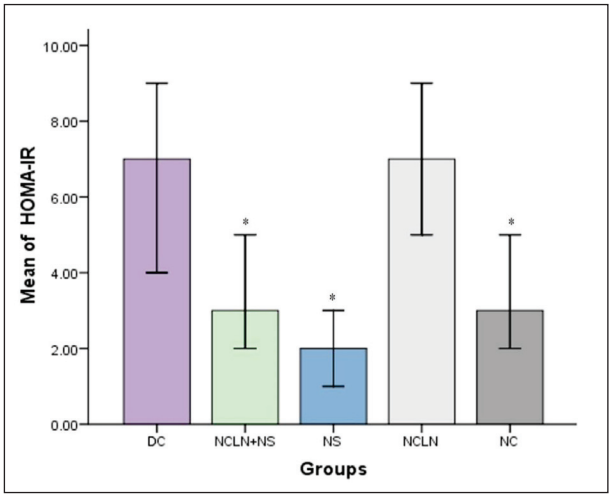

Figure 2. Insulin resistance index, HOMA-IR (homeostasis model assessment) in normal control (NC), diabetic control (DC), *Nigella sativa* (NS), Nano-sized clinoptilolite (NCLN), and NS+ NCLN groups. Data are shown as Mean ± SD. *Pv< 0.05 vs. DC and NCLN group.

results seem to be in conflict with Al-Awadi and Gumaa findings. They reported no significant change in FBG level when NS (40 mg/day) was administered to normal and STZ-induced diabetic rats, probably due to low dose NS supplementation (28).

The exact mechanism of NS action on decreasing the glucose levels in DM is unknown. Earlier experiments suggest that hypoglycemic effect of NS seeds might be due to the enhancement of peripheral metabolism of glucose, partial regeneration /proliferation in the pancreatic b–cells, insulin secretion stimulation, increase in insulin and adrenaline effect and/or induce a dose-dependent inhibition of sodium dependent d-glucose absorption (1, 24, 29). Some studies showed beneficial effects of NS on serum LP by reducing TC, LDL-C, TG and VLDL-C and increasing HDL-C in diet-induced hypercholesterolemic (HC) rabbits (16). In this study, the HFD/STZ treated rats were not exerted hyperlipidemia; therefore, we cannot conclude that the interventions did not any positive effect on lipid profile. It seems be due to pre-diabetic condition (HFD/STZ approach) since it failed to exert significant unfavorable effect on lipid profile.

In current experiment, we did not see hypoglycemic effect of NCLN in T2DM rats; although, decrease of FBG levels in T1DM rats (30) was observed. Also, non-insignificant decrease in TC and TG levels and increase in LDL-C and HDL-C levels were seen. The hypoglycemic effect of zeolites might be attributed to silica. Silica may execute inhibition of the development of spontaneous diabetes and hyperglycemia in young BB-rats (20). Hence, the lack of a hypoglycemic effect of NCLN in T2DM seems to be due to the inefficacy in improving IR. Alexopoulos et al indicated an elevated serum glucose concentration by administration of CLN-rich tuff in normal pigs (31). Prvulović et al reported that the addition of dietary CLN to pig's basal diets, deceased TC and increased TG concentration (32). Demirel et al reported similar results by dietary natural CLN administration to rats (33). In contrast, Harvey et al reported increase of TC concentration (although non-significant) by dietary supplementation of zeolitic ores in chickens (34).

The contradictory effects of zeolite on LP in earlier studies probably can be attributed to the difference in type, concentration, and purity of CLN, and animal species. For explanation of serum cholesterol reduction, recent studies suggests that CLN probably adsorbs bile acid salts on its surface in the digestive tract, thereby influence the need for their synthesis *de novo* of cholesterol (31).

Simultaneous supplementation of NCLN and NS decreased FBG levels significantly. Decrease of FBG in this group to near normal range can be considered advantage of concurrent supplementation, because separate supplementation of NS decreased FBG to lower than normal range. Also, a statistically non-significant decrease in TG levels was seen, which may have clinical importance.

The main limitations of this study seem to be relatively short time course between T2DM induction and start of intervention and lack of LP measurements before intervention. The continuation of HFD in all of study period may result in lipoprotein disorders and may appear more clearly the effects of supplementations on LP.

In conclusion, current study indicated that dietary administration of NS either separate or with NCLN could represent hypoglycemic effect in HFD/STZ induced T2DM rats. None of NS and NCLN could not show significant impact on LP, because lack of LP disorders in any of groups. Further studies are needed to clarify antidiabetic beneficial effects of NS and NCLN and mechanisms of their action.

References

1. Meddah B, Ducroc R, Faouzi M, Eto B, Mahraoui L, Benhaddou-Andaloussi, et al. *Nigella sativa* inhibits intestinal glucose absorption and improves glucose tolerance in rats. Journal of Ethnopharmacology 2009; 121: 419–24.
2. Chen L, Dianna JM & Paul ZZ. The worldwide epidemiology of type 2 diabetes mellitus present and future perspectives. Nature Reviews Endocrinology 2012; 8: 228-36.
3. Paul SJ. Metabolic consequences of hyperglycemia and insulin resistance. Clinical Cornerstone 2007; 7: 30-42.
4. Krause MV, Mahan LK, Escott-Stump S, Raymond JL. Krause's food & the nutrition care process. Elsevier Health Sciences 2012**.**
5. Januškevičus A, Januškevičene G, Plungyte K. Use of clinoptilolite in rations of adult dogs. Veteriin Zootechnika (Vet Med Zoot) 2013; 64 (86): 3-10
6. Al-Hader A, Aqel M & Hasan Z. Hypoglycemic effect of the volatile oil of *Nigella sativa* seeds. International Journal of Pharmacognosy and Phytochemical Research 1993; 31: 96–100.
7. Fararh KM, Atoji Y, Shimizu Y, Shiina T, Nikami H, Takewaki T. Mechanisms of the hypoglycaemic and immunopotentiating effects of *Nigella sativa* L. oil in streptozotocin-induced diabetic hamster. Research in Veterinary Science 2004; 77: 123–9.
8. Meral I, Yener Z, Kahraman T, Mert N. Effect of *Nigella sativa* on Glucose Concentration, Lipid Peroxidation, Anti-Oxidant Defence System and Liver Damage in Experimentally-Induced Diabetic Rabbits. Blackwell Wissenschafts-Verlag Berlin J Vet Med 2001; 48: 593-9.
9. Kaleem M, Kirmani D, Asif M, Ahmed Q and Bilqees B. Biochemical effects of *Nigella sativa* seeds in diabetic rats. Indian Journal of Experimental Biology 2006; 44(9):745-8.
10. Al-Ghamdi MS. The anti-inflammatory, analgesic and antipyretic activity of *Nigella sativa*. Jornal of Ethnopharmacology 2001; 76: 45– 8.
11. Zaoui A, Cherrah Y, Lacaille-Dubois MA, Settaf A, Amourouch H, Hassar M. Effets diurétiques et hypotenseurs de *Nigella sativa* chez le rat. spontanément hypertendu. Thérapie 2000; 55: 379–82.
12. El-Tahir KE, Ashour MM, Al-Harbi MM. The cardiovascular actions of volatile oil of the black seeds (*Nigella sativa*) in guinea-pigs: elucidation of the mechanism(s) of action. Gen Pharmacol 1993; 24:1123–31.
13. El-Saleh SC, Al-Sagair OA, Al-Khalaf MI. Thymoquinone and *Nigella sativa* oil protection against methionine-induced hyperhomocysteinemia in rats. International Journal of Cardiology 2004; 93: 19– 23.
14. Le PM, Benhaddou-Andaloussi A, Elimadi A, Settaf A, Cherrah Y, Haddad PS. The petroleum ether extract of *Nigella sativa* exerts lipidlowering and insulin-sensitizing actions in the rat. Journal of Ethnopharmacology 2004; 94: 251–9.
15. Bamosa AO, Kaatabi H, Lebda Fatma M, El Abdul-Muhssen A, Al-Sultan A. Effect of *nigella sativa* seeds on the glycemic control of patients with type 2 diabetes mellitus. Indian Journal of Physiology and Pharmacology 2010; 54(4): 344-54.
16. Al-Naqeep G, Al-Zubairi AS, Ismail M, Amom ZH and Esa NM. Antiatherogenic Potential of *Nigella sativa* Seeds and Oil in Diet-Induced Hypercholesterolemia in Rabbits. Evidence-Based Complementary and Alternative Medicine 2010; 1-8.
17. Badary OA, Al-Shabanah OA, Nagi MN, Al-Bekairi AM, Almazar MMA. Acute and subchronic toxicity of thymoquinone in mice. Drug Develop Research 1998; 44: 56–61.
18. Davis ME. Ordered porous materials for emerging applications. Nature 2002; 417: 813–21.
19. Pavelic K, Hadzija M. Medical applications of zeolites. In: Auerbach SM, Carrado KA, Dutta PK (eds). Handbook of Zeolite Science and Technology. New York: Dekker 2003; 1143-74.
20. Oschiliewski U, Kiesel U, Kolb H. Administration of Silica Prevents Diabetes in BB-Rats. Diabetes Research Institute 1985; 34.
21. Zarkovic N, Zarkovic K, Kralj M, et al. Anticancer and antioxidative effects of micronized zeolite clinoptilolite. Anticancer Research 2003; 23:1589-95.
22. Srinivasan K, Viswanad B, Asrat L, Kaul CL, Ramarao P. Combination of high-fat diet–fed and low-dose streptozotocin–treated rat: a model for type 2 diabetes and pharmacological screening. Pharmacological Research 2005; 52: 313-20.
23. Marijana PH. Inhibition of SGLT using Ca2+-zeolite and preserve the periphery of glucose excess. Endocrine abstracts 2014; 35: 494.
24. Ghiasi R, Ghadiri Soufi F, Somi MH, et al. Swim Training Improves HOMA-IR in Type 2 Diabetes Induced by High Fat Diet and Low Dose of Streptozotocin in Male Rats. Advanced Pharmaceutical Bulletin 2015; 5(3): 379-84.
25. Khataee A, Bozorg S, Khorram S, Fathinia M, Hanifehpour Y, & Joo SW. Conversion of natural clinoptilolite microparticles to nanorods by glow discharge plasma: a novel Fe-impregnated nanocatalyst for the heterogeneous Fenton process. Industrial & Engineering Chemistry Research 2013; 52(51): 18225-33.
26. Zhang M, Lv XY, Li J, Xu ZG, Chen L. The characterization of high-fat diet and multiple low-dose Streptozotocin induced type 2 diabetes rat model. Experimental Diabetes Research 2008; 704045.
27. Kanter M, Meral I, Yener Z, Ozbek H, Demir H. () Partial regeneration/proliferation of the beta-cells in the islets of Langerhans by *Nigella sativa* L. in streptozotocin-induced diabetic rats. The Tohoku Journal of Experimental Medicine 2003; 20: 213–9.
28. Al-Awadi FM, Gumaa KA. Studies on the activity of individual plants of an antidiabetic plant mixture. Acta Diabetologica Latina 1987; 24: 37–41.
29. Nehar SH, Kauser H, Rani P and Alam I. Effects of *Nigella sativa* Seed Extract on Insulin Resistant Non-insulin-Dependent Diabetic Guinea Pigs. American Journal of Ethno-

medicine 2015; 2 (1): 58-68.
30. Nia BH, Khorram S, Rezazadeh H, Safaiyan A, Tarighat-Esfanjani A. The effects of natural clinoptilolite and nano-sized clinoptilolite supplementation on glucose levels and oxidative stress in rats with type 1 diabetes. Canadian journal of diabetes 2018; 42(1):31-5.
31. Alexopoulos C, Papaioannou DS, Fortomaris P, Kyriakis CS, et al. Experimental study on the effect of in-feed administration of a clinoptilolite-rich tuff on certain biochemical and hematological parameters of growing and fattening pigs. Livestock Science 2007; 111: 230–41.
32. Prvulović D, Jovanović-Galović A, Stanić B, Popović M and Grubor-Lajšić G. Effects of clinoptilolite supplement in pig diets on performance and serum parameters. Czech Journal of Animal Science 2007; 52: 159–64.
33. Demirel R,Yokus B, Demirel DS, Ketani MA, and Baran MS. Effects of Dietary Zeolite on Serum Contents and Feeding Performance in Rats. International of journal of agriculture & Biology 2011; 13(3): 346–50.
34. Harvey RB, Kubena LF, Elissalde MH & Phillips TD. Efficacy of zeolitic ore compounds on the toxicity of aflatoxin to growing broiler chickens. Avian Diseases1993; 37: 67.

Correspondence:
Ali Tarighat-Esfanjani, Ph.D.,
Nutrition Research Center, Tabriz University of Medical Sciences, address: Attar Nishabouri St., Tabriz, I. R. Iran
Postal code: 5166614711
POBOX: 14711
Tel: 0098-41-33357582
Fax: 0098-41-33340634
E-mail: tarighata@tbzmed.ac.ir