Iran Red Crescent Med J. 2018 October; 20(10):e74365. doi: 10.5812/ircmj.74365.

Published online 2018 October 9. **Research Article**

The Effects of Natural Nano-Sized Clinoptilolite and Metformin on the Levels of Serum Glucose, Lipid Profile, and Minerals in Rats with Type 2 Diabetes Mellitus

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Received 2018 May 06; **Revised** 2018 August 16; **Accepted** 2018 August 24.

Abstract

Background: Natural nano-sized Clinoptilolite (NCLN) as a conventional treatment for Type 2 diabetes mellitus may reduce glucose concentration and improve levels of minerals and lipid profile (LP).
Objectives: The aim of this study was to compare the effects of NCLN with Metformin (Met) on fasting blood glucose (FBG), LP, levels of the several minerals, and body weight (BW) in rats with high-fat-diet (HFD)/Streptozotocin (STZ)-induced diabetes.
Methods: In this experimental study, 33 male Wistar rats were divide into four groups of A) healthy (n = 6), B) diabetic: (1) NCLN (n = 9) 2% of food, (2) diabetic receiving Met (n = 9) 100 mg/kg BW/day and (3) diabetic control (DC) (n = 9). The diabetic subgroups were fed with an HFD for one month and injected with a single dose of intra-peritoneal STZ (35 mg/kg BW). At the end of the 7th week, FBG, insulin, homeostatic model assessment of insulin resistance (HOMA-IR), LP, and serum levels of some minerals were evaluated.
Results: There were no significant differences in FBS, total cholesterol (TC), triglycerides (TG), and low-density lipoprotein cholesterol (LDL-c) among the groups, however, high-density lipoprotein cholesterol (HDL-c) level was significantly higher in the DM (32.92 $\pm$ 1.14) and NCLN (32.63 $\pm$ 0.73) groups in comparison with the DC group (31.64 $\pm$ 0.78) (P = 0.01). The mean score of Cu level in the DM group was significantly different from that in the DC group (2.15 $\pm$ 0.48 vs. 1.54 $\pm$ 0.21; P = 0.03). Moreover, Met significantly increased serum level of Ca as compared to the DC group (86.65 $\pm$ 10.03; P = 0.01). Also, treatment with Met favorably prevented severe weight loss until the last week of treatment.
Conclusions: Natural nano-sized Clinoptilolite and/or Met did not significantly affect levels of serum glucose, minerals, and lipid profile, but a significant increase in HDL-c was observed as a result of NCLN and Metformin treatment, and Cu and Ca levels increased only in the Metformin group.

Keywords: Diabetes Mellitus, Hyperglycemia, Lipid, Metformin, Minerals, Nano, Rats, Type 2, Zeolites

1. Background

Diabetes mellitus (DM) is one of the most common chronic diseases worldwide, which is characterized by hyperglycemia due to the deficiency in insulin secretion and/or failure in insulin action with impaired carbohydrate, lipid, and protein metabolism (1). Based on the latest estimates by the International Diabetes Federation (IDF), 415 - 422 million people (one adult out of every 11 people) are diabetic, which will increase to 642 million (one adult out of 10 persons) by 2040 (2-4).

Several studies have pointed to the role of some minerals in the pathogenesis of some metabolic disorders such as insulin resistance. Zinc (Zn), magnesium (Mg), chromium (Cr), calcium (Ca), and copper (Cu) ions play a role in glucometabolic disorders (5, 6). Increase/decrease in the serum levels of these minerals can lead to impaired glucose metabolism in diabetic patients, thus, by examining serum levels of each of these minerals, the symptoms of diabetes would be better controlled (6).

Glucose-lowering agents such as Metformin (Met), sulfonylureas, and insulin are used for the management of type 2 diabetes, and for adequate glycemic control, multiple treatments are usually required (7). The use of such

hypoglycemic pharmacologic agents is often limited and they may lose their effect on glycemic control over time or may lead to adverse effects such as hypoglycemia and weight gain (8, 9). One of the main complications in diabetes is tissue damage, and these agents are incompetent in controlling these complications (10). Therefore, traditional medicine has persuaded researchers to investigate drugs with fewer side effects. Different sources like plants, animals, and microorganisms are natural agents that are powerful candidates for substituting pharmaceutical drugs (11)

Clinoptilolites (CLNs) are hydrated natural or synthetic microporous crystals containing AlO_4 and SiO_4 connected through the common oxygen atoms (12). Several CLNs have been previously used in medicine as antidiarrheal, bactericidal, and antifungal medications for wound healing (13). Some scientific evidence proposed that CLNs could lower blood glucose in non-diabetic animal models (14). Previous studies have suggested that CLNs may have glucose absorbing characteristics. Therefore, they can be used in diabetic patients. CLNs may reduce blood glucose by preventing β-cell destruction and thus, enhancing insulin production (15). Our previous works showed the promising effects of nano-sized CLN (NCLN) on the prevention or treatment of diabetes mellitus (16-18).

Health concerns of CLNs may be interference with the absorption of essential minerals, including Ca, Zn, and Mg; however, some studies have reported the inconsistent effects of zeolites on serum levels of Ca, Zn, and Mg (13, 19).

2. Objectives

Considering the promising but inconsistent results regarding CLNs supplementation and given the lack of its impact on diabetes mellitus in comparison with Met, this study aimed to compare the effects of NCLN powder with Met as the first-line treatment on the levels of fasting blood glucose (FBG), lipid profile (LP), and several minerals in rats with high-fat-diet (HFD)/streptozotocin (STZ)-induced type 2 diabetes.

3. Methods

This experimental study was conducted in Tabriz University of Medical Sciences, Tabriz, Iran, in 2016. Streptozotocin (STZ, Sigma Chemicals, and St. Louis, MO, USA), Metformin (hydrochloride) powder (Cayman, England), diethyl ether, and other solvents, and buffers (Merck, Germany) were used in this project. One of the tools that were used in this research was a 0.01 -gram digital scale, calibrated using known weight. An inductively coupled plasma-optical emission spectrometer (ICP/OES, Model 730-ES, Varian, USA) was another device, which was calibrated as follows: A wavelength calibration needs to be performed the first time the instrument is run and when a new software is loaded. It is recommended that a wavelength calibration be performed weekly thereafter. The hardware calibration step of this procedure only needs to be performed every six months.

3.1. Animals

Male Wistar rats weighing 250 < grams were acquired from Animal Breeding Center of Tabriz University of Medical Sciences. The studied groups were as follows: A) healthy (n = 6), B) diabetic: (1) nano zeolite (DN) (n = 9), (2) metformin (DM) (n = 9), and (3) diabetic control (DC) (n = 9). The rats were allocated to these groups using the simple randomization method. The inclusion criteria for selection of the rats included: a) rats of the same age (about five months), race, and sex (male-Wistar), b) FBG > 250 mg/dL, and c) proof of developing type 2 diabetes with an oral glucose tolerance test (OGTT). The exclusion criteria included: a) FBG < 250 mg/dL, b) death or too much weight loss before the end of the study period, and c) baseline weight less than 200 gr.

Every three rats were kept in one cage and fed normal pellet diet (NPD (14% total energy from fat, 58% carbohydrate, and 28% protein) and drinking water, with the controlled temperature of 23 - 25°C, humidity of 30% - 50%, and light cycle (12/12 light cycle). All the ethical considerations related to the storage and handling of these animals were observed.

Duration of intervention in this study was seven weeks. At the end of the study (7th week), all the parameters were assessed from 5 mL blood samples drawn from animals' hearts. The serum levels of Cu, Zn, Ca, Mg, and P were measured by an ICP/OES in Sharif University of Technology, Iran. Serum concentrations of glucose and LP (T cell count (TC), high-density lipoprotein cholesterol (HDL-c), and triglycerides (TG)) were measured by the enzymatic method, and low-density lipoprotein cholesterol (LDL-c) concentration was calculated by using the Fried-Wald formula. Food intakes were measured at baseline and the end of the 7th week, and the weights of the rats in each group were measured at the same time.

This research was approved by the Clinical Research Ethics Committee in Tabriz University of Medical Sciences, Iran (Ethical code: IR.TBZMED.REC.1395.69).

3.2. Induction of Type 2 Diabetes in Rats

Type 2 diabetes was induced by administering an HFD for one month (32% of total energy from fat, 48% carbohydrate, and 20% protein; Table 1) and then intraperitoneal injection of a single dose of 35 mg/per kg of body

Table 1. Elemental Composition of Normal Pellet Diet and High-Fat-Diet Regimens

Variables	Value
NPD, %	
Crude protein	22.5 - 23.5
Crude fat	3.5 - 4.5
Crude fiber	4 - 5
Lysine	1.15 - 1.2
Methionine	0.33 - 0.37
Methionine + cysteine	0.63 - 0.65
Threonine	0.72 - 0.75
Tryptophan	0.25 - 0.32
Ash	Up to 10
Calcium	0.95 - 1
Phosphorus	0.70
Salt	0.5 - 0.55
HFD, %	
Powdered NPD	50
Rump oil	25
Sucrose	12
Chickpea flour	1
Wheat flour	10
Cholice acid	1
Cholesterol	1

Abbreviations: HFD, high fat diet; NPD, normal pellet diet.

weight (BW) of STZ. One week after the injection, blood samples were collected from the orbital sinus (1 - 2 drops) for measurement of FBG and diagnosis of diabetes. Rats with BG levels above 250 mg/dL were selected and enrolled as diabetes. Oral glucose tolerance test (OGTT) was performed to ensure the development of type 2 diabetes. For this purpose, a solution containing 20% glucose (2 g/ kg BW) was administered to the rats by gavage. Blood samples were obtained from the tail to measure BG and insulin concentrations (Figure 1). Xylazine 10 mg/Kg or Ketamine 50 mg/Kg were used intraperitoneally for anesthesia. This formula calculated the homeostatic model assessment (HOMA IR): HOMA-IR = Fasting insulin (μU/mL) x Fasting glucose (mg/dL) /405.

3.3. Preparation of Therapeutic Diets

The NCLN particle was produced from CLN by the glow discharge plasma method (20), which is a novel Fe-impregnated nanocatalyst for the heterogeneous Fenton process, in Research Institute for Applied Physics and Astronomy at University of Tabriz. Particle analysis of NCLN as compared to CLN is presented in Table 2. The intervention groups were prescribed NCLN powder at a dose of 2% in rats' standard food and Met at a dose of 100 mg/kg BW/day was dissolved in water. The exact amount of the prescribed supplements varied based on foods consumed. During the study, animals had free access to food and water.

3.4. Statistical Analysis

In this study, the data were presented as the mean ± standard deviation (SD). In case of normal distribution of data, one-way analysis of variance (ANOVA) test was used for comparison of the average amount of each parameter in the studied groups. Otherwise, Kruskal-Wallis test and an appropriate follow-up test were used. Tukey's post hoc test was employed to examine differences between groups. Repeated measures ANOVA test was used to compare weight means at different times. Data analysis was performed using IBM SPSS, Statistics for Windows, version 21.0 (IBM Corp., Armonk, N.Y., USA), and a P value less than 0.05 was considered significant.

4. Results

4.1. Glycemic Control and Serums Lipid Profile

The mean and SD of the measured parameters are summarized in Table 3. There were no statistically significant differences in means of FBG, insulin, TC, TG, and LDL-C among the groups ($P > 0.05$). Administration of HFD/STZ significantly increased HOMA-IR values ($P = 0.00$) in the diabetic subgroups. Supplementation of Met decreased HOMA-IR values, but they were not significantly different from those of the DC group. When we used HDL-C as a dependent variable, there was a statistically significant difference between the groups ($P = 0.01$). Tukey's post hoc test revealed that HDL-C level was significantly higher in the DM (32.92 ± 1.14) and NCLN (32.63 ± 0.73) groups in comparison with the DC group (31.64 ± 0.78; $P = 0.01$). There was no statistically significant difference between the other groups. Altogether, these results suggest that Met and NCLN probably have beneficial effects on the HDL-C level.

4.2. Mineral Levels in Serum

As shown in Table 4, Met significantly increased Cu levels ($F(3, 29) = 4.782$, $P = 0.03$). Post hoc comparisons using Tukey's HSD test indicated that the mean score for the Cu level in the DM group (2.15 ± 0.48) was significantly different from the Cu level in the DC group (1.54 ± 0.21). There was no statistically significant difference between the other groups. Similar results were seen for Ca level

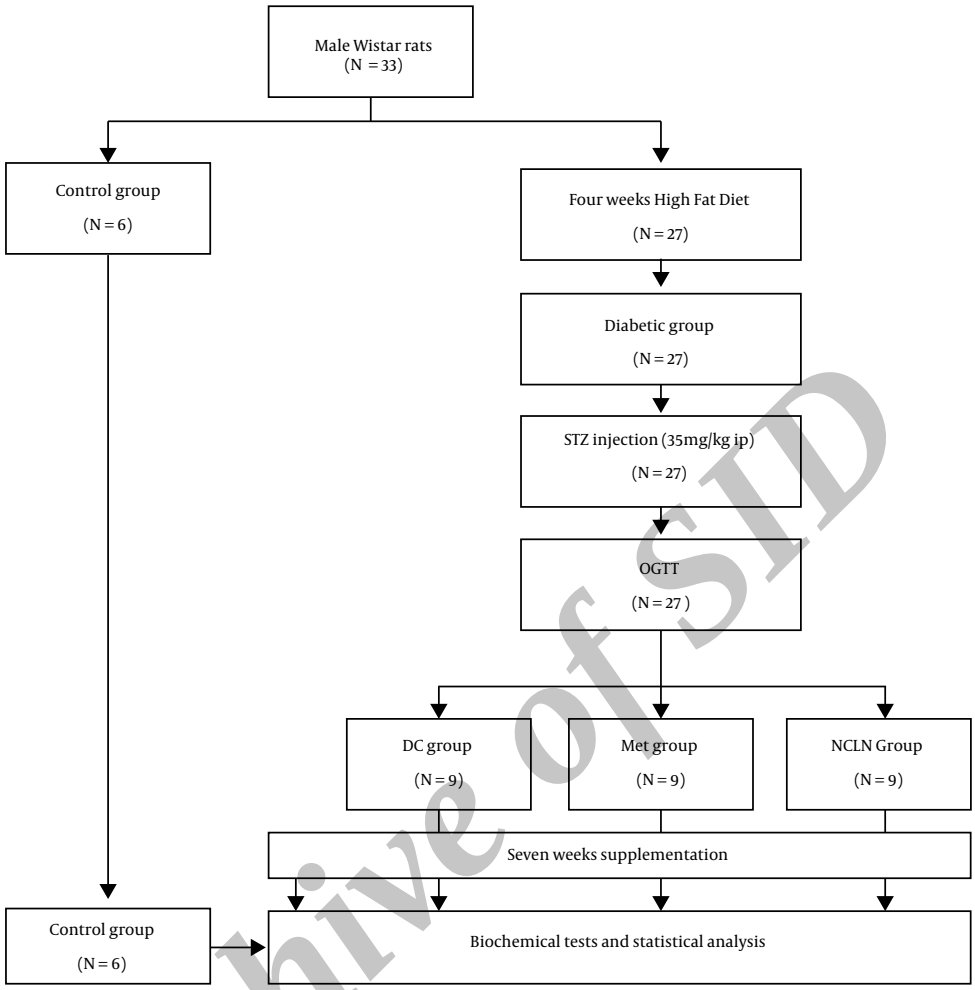

Figure 1. Diagram of study's steps. Abbreviations: STZ, Streptozotocin; OGTT, Oral Glucose Tolerance Test; DC, Diabetic control; Met, Metformin; NCLC, Nano-sized Clinoptilolite.

Table 2. Elemental Composition of Cinoptilolite 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

Abbreviations: Al, Aluminium; CLN, Clinoptilolite; K, Potassium; Na, Sodium; NCLN, Nano-sized Clinoptilolite; Si, Silicon.

(F (3, 29) = 3.99, P = 0.01). Post hoc comparisons using Tukey's HSD test indicated that the mean score of Ca level in the DM group was significantly higher than the Ca level in the DC group (102.82 ± 13.63 vs. 86.65 ± 10.03; Figure 2). There was no statistically significant difference between the other groups. In sum, these results suggest that Met can affect Cu and Ca levels.

4.3. Body Weight (BW)

As shown in Figure 3, there was no significant difference in BW between the groups before STZ injection and prior to the Oral Glucose Tolerance Test (OGTT). In comparison with the NC group, a significant decrease ($P < 0.05$) in mean BW was observed in the DC group after the 3rd week and in the DN group during weeks 3 - 5. There was no significant difference between the diabetic subgroups

Table 3. Mean and Standard Deviation of Glycemic Control and Lipid Profile[a]

Variables	DC (N = 9)	DN (N = 9)	DM (N = 9)	NC (N = 6)	P Value[b]
FBS, mg/dL	215.9 (216.85)*	323.5 (157.95)*	340.0 (182.60)*	187.8 (56.15)	0.06
Insulin, MIU/L	9.23 ± 0.46	9.15 ± 1.02	8.50 ± 0.81	8.96 ± 0.92	0.44
HOMA-IR	7.14 ± 1.17**	7.69 ± 1.08**	6.91 ± 1.66**	3.99 ± 0.91	0.00
TC, mg/dL	100.42 ± 35.62	95.77 ± 10.65	110.62 ± 27.51	99.63 ± 19.55	0.65
TG, mg/dL	71.64 ± 37.22	70.00 ± 25.50	69.47 ± 30.45	62.76 ± 11.53	0.94
LDL-C, mg/dL	54.44 ± 34.07	49.14 ± 10.14	60.68 ± 20.99	55.43 ± 20.00	0.77
HDL-C, mg/dL	31.64 ± 0.78	32.63 ± 0.73***	32.92 ± 1.14***	31.65 ± 0.86	0.01

Abbreviations: DC, Diabetic control; DM, Diabetic + Metformin; DN, Diabetic + Nano-sized Clinoptilolite, NC, Normal control; SD, Standard deviation.
[a] *Median (IQR), **P < 0.01 as compared to control and ***P < 0.05 as compared to DC and control groups.
[b] One-way ANOVA was used for comparison of the variables.

Table 4. Mean and Standard Deviation of Mineral's Levels in Serum[a]

Variables	DC (N = 9)	DN (N = 9)	DM (N = 9)	NC (N = 6)	P Value[b]
Mg, mg/dL	23.63 ± 3.55	20.42 ± 3.10	23.37 ± 6.67	19.38 ± 2.96	0.18
Ca, mg/dL	86.65 ± 10.03	95.14 ± 11.27	102.82 ± 14.63*	87.07 ± 7.19	0.01
Cu, μg/dL	1.54 ± 0.21	1.75 ± 0.41	2.15 ± 0.48**	1.59 ± 0.29	0.03
P , μg/dL	108.59 ± 45.97	96.38 ± 15.52	116.61 ± 29.55	100.88 ± 2.45	0.39
Zn, μg/dL	1.51 ± 0.27	1.67 ± 0.24	1.66 ± 0.36	1.37 ± 0.17	0.17

Abbreviations: DC, Diabetic control; DM, Diabetic + Metformin; DN, Diabetic + Nano-sized Clinoptilolite; NC, Normal control; SD, Standard deviation.
[a] * and ** P < 0.05 as compared to other groups.
[b] One-way ANOVA was used for comparison of the variables.

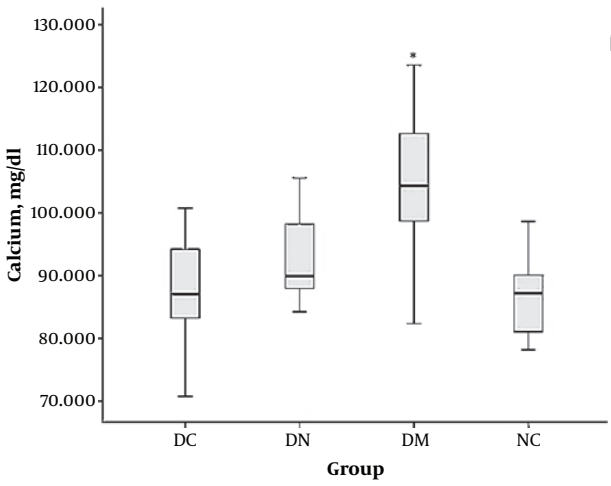

Figure 2. Calcium levels in groups based on mean ± SD. SD, Standard deviation; DC, Diabetic control; DN, Diabetic + Nano-sized Clinoptilolite; DM, Diabetic + Metformin, NC, Normal control. *P value < 0.05 as compared to DC group.

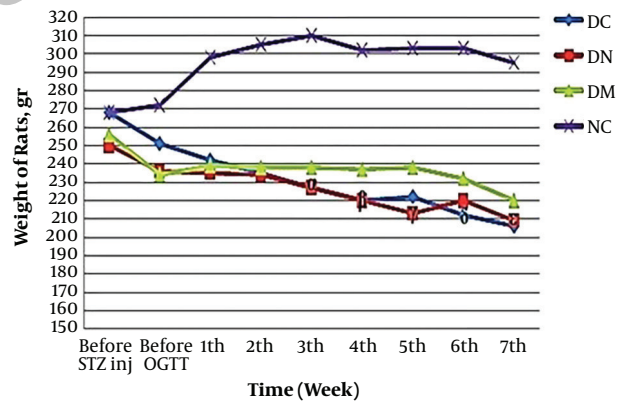

Figure 3. Body weight changes based on means ± SD. DC, Diabetic control; DN, Diabetic + Nano-sized Clinoptilolite; DM, Diabetic + Metformin; NC, Normal control; STZ, Streptozotocin; OGTT, Oral Glucose Tolerance Test α, β, δ, γ and ε P value < 0.05 as compared to NC group. Treatment by Met reduced severe weight loss until the last week of the intervention.

throughout the intervention period. Intra-group comparisons for BW showed a significant decrease in the DC group (P = 0.029) at the seventh week compared to the fifth week.

5. Discussion

In the present study, there were no significant differences in FBS, TC, TG, and LDL-c levels between the groups, but HDL-c levels were significantly higher in the DM and

DN groups compared to the other groups. It should be pointed out that HFD/STZ-treated rats did not show evidence of hyperlipidemia, which seems to be due to prediabetic condition and failure of HFD/STZ approach to exert significant unfavorable effects on LP. Therefore, we cannot conclude the positive effect of the interventions on lipid profile. In one study, Garimella et al. demonstrated that treatment of type 2 diabetes patients with Met 1500 mg/day for three months improved FBS, significantly reduced TC, TG, LDL-C, and VLDL-C levels, and increased HDL-C level (21). In contrast, dietary CLN at three doses (2, 4 and 6%) in the diet of adult male Sprague-Dawley rats for 56 days increased TG and VLDL levels, however, among treatment groups no significant differences were observed in terms of glucose, TC, LDL-c, and HDL-c (19).

In alloxan-induced diabetic mice, CLN did not significantly decrease BG level, but it could prevent or diminish some complications including polyneuropathy (22). In our previous study, treatment with NCLN significantly diminished BG levels in rats with STZ-induced type 1 diabetes (17). The lack of hypoglycemic effect of NCLN in type 2 diabetes seems to be due to the inefficacy in improving insulin resistance. Moreover, the contradictory effects of zeolites on LP and BG in previous studies probably can be attributed to the difference in type, concentration, and purity of CLN and/or animal species (18).

The comparison of the effect of Met and NCLN on micronutrients' levels indicated that both agents increased serum levels of Cu, but only this increase was statistically significant only in the Met group, which can represent a positive correlation between FBG and serum Cu level. The high levels of Cu were reported in scalp hair and blood samples from diabetic patients versus nondiabetic subjects. Cu is one of the most important trace elements involved in redox reactions as a key component of copper/zinc superoxide dismutase. On the other hand, evidence suggests that Cu may act as a prooxidant, and high serum level of Cu increases the generation of reactive oxygen species. However, the balance between Zn and Cu levels contributes to cellular redox status (23). In the present study, both Met and NCLN increased the serum level of Zn, although this change was not statistically significant.

Mg and Ca are important nutrients and cofactors for several enzymes essential for carbohydrate metabolism and glucose homeostasis (24). In our study, Ca level significantly increased in the Met group, but in the NCLN group, the increase was non-significant. Also, the serum level of Mg decreased in the NCLN group compared to the DC and increased in the Met group, none of the changes was statistically significant.

Previously, Met administration was not found to alter Mg and Ca levels in plasma in type 2 diabetes patients (25). In another study, the influence of two different concentrations (1.2 g/kg and 1.6 g/kg BW) of sodium aluminum silicate (zeolite A) for three weeks in goats revealed that plasma concentrations of phosphate (P), Mg, and 1, 25-dihydroxycholecalciferol, as well as renal excretion of phosphate were significantly lower in treatment groups compared to a control group (26). Thilsing-Hansen et al. reported that supplementation of zeolite A in pregnant cows that received 1.4 kg pellets per day (0.7 kg of pure zeolite A) during the last two weeks of pregnancy significantly increased the plasma Ca level on the day of calving, whereas plasma Mg as well as inorganic phosphate levels were suppressed (27).

Treatment with Met could favorably prevent the weight loss caused by diabetes until the last weeks. There was no significant difference in mean food intake to body weight ratio between the groups during the study period.

5.1. Strengths and Limitations

One of the strengths of this study is the innovation in the use of two substances with similar effects from two different research fields (pharmacology and plasma physics). This could provide an opportunity for linking various scientific research lines to treat various diseases. The main limitations of the present study were the short interval between induction of type 2 diabetes and initiation of interventions, and the lack of measurement of lipid profile before the intervention. Also, we were uncertain as to the optimal doses of NCLN and metformin due to lack of similar studies.

5.2. Conclusion

Although NCLN did not significantly affect levels of glucose, lipid profile, and some minerals, this study was one of the first studies that investigated the effect of NCLN on these parameters compared with Met. In future studies, it is recommended to examine the unfavorable effects of type 2 diabetes on the lipid profile and the level of minerals before any dietary or drug interventions. It seems that if the duration of intervention in this study was longer and if we examined different factors and complications, we might have achieved significant findings. Also, given that Met is the first line of treatment for type 2 diabetes, it was more favorable to investigate the effect of Met and zeolites powder simultaneously and at different doses.

Acknowledgments

This work was financially supported by a Grant from Deputy of Research of Tabriz University of Medical Sciences, Tabriz, Iran. This study was derived from an MSc thesis submitted by Habib Fallahnejad, the student of Tabriz University of Medical Sciences.

Footnote

Funding/Support: This work was financially supported by a Grant from Vice-Chancellor for Research Affairs of Tabriz University of Medical Sciences, Tabriz, Iran.

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