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The Effect of Combination Therapy (Thermal Therapy and Oxymel) on Insulin Resistance and Langerhans Islands in Diabetic Rats

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Abstract

Background: Although thermal therapy is recently suggested as a safe promising adjuvant therapy for insulin resistance and diabetes, its combination with oxymel has been claimed more effective by Traditional Persian Medicine (TPM).
Objectives: This study was to examine the effect of thermal therapy plus oxymel on insulin resistance and Langerhans islands in diabetic rats.
Methods: This experimental study was performed in the Experimental Animal Unit of Qom University of Medical Science in Iran in 2018. Sixty-four male rats were divided into 8 groups (using block randomization): four Normal (Nl), Diseased without treatment (D), Oxymel (OXM) and Sauna (Sauna) groups and one major SINA group (oxymel plus sauna) subdivided into four groups of different intervention frequency of 1 (SINA1d), 3 (SINA3d) and 5 days (SINA5d2m) a week for 8 weeks and 5 days a week for 4 weeks (SINA5d1m). Diabetes was induced using a high-fat diet (milk butter) and low-dose streptozotocin. Measurement of serum levels of glucose, insulin, lipid profile, and glycosylated hemoglobin and histological examination of liver, pancreas, heart, and kidney was done at the end of the study.
Results: The count of Langerhans Islands significantly increased in the groups of SINA3d (6.25 $\pm$ 0.94), (P = 0.05) and SINA5d2m (7 $\pm$ 0.36), (P = 0.008) in comparison to the D group (4 $\pm$ 0.44), and cell organization became nearly normal, but serum insulin and glucose levels did not change. On the other hand, despite the high-fat diet, lipid profile and histological findings did not support fatty deposition and insulin resistance context.
Conclusions: Sauna-oxymel combination (SINA) therapy, which was derived from TPM philosophy to increase systemic and pancreatic blood perfusion, was shown significantly effective in inducing regeneration of Langerhans Islands, but owing to the lack of adequate ectopic fat accumulation, its effect on insulin resistance and beta-cell functions remain uncovered. More suitable studies are needed to approve or disapprove the idea.

Keywords: Diabetes Mellitus, Diet, Experimental, High-Fat, Insulin Resistance, Langerhans Islets, Oxymel, Pancreas, Regeneration, Traditional Medicine, Thermal Therapy

1. Background

In recent years, owing to side effects and costs of modern medicine, the integrative use of complementary and alternative medicine as a treatment for many chronic diseases has become prevalent and also is recommended by the World Health Organization (1). One of the abovementioned diseases is diabetes, which has an increasing incidence. Today, 387 million people suffer from the disease, and 179 million people are not even aware of their illness and remain undiagnosed. The number of people affected is estimated to reach 592 million by 2035 (2, 3). In such conditions, besides current treatments of diabetes, lifestyle modifications, and new adjuvant modalities may be beneficial (4).

Type 2 diabetes is the most common type of the disease, and it is characterized by insulin resistance and beta-cell dysfunction (5). The role of ectopic fat accumulation in the pathogenesis of insulin resistance and type 2 diabetes has been highlighted in many studies (6-8). Although fat is a source for energy storage and part of the cell's structure

and intracellular and intercellular messaging system (9), its ideal storage or usage may be altered pathologically due to various reasons, including over intake of fat or calories (9, 10). The result is the deposition of ectopic fat in various tissues and organs, such as the heart, liver, skeletal muscle, pancreas, and kidney, causing many complications and dysfunctions (11). This problem results in the insulin resistance of skeletal muscles and liver and fat accumulation both around and inside the pancreas, causing abnormalities in this organ where the most important of which is beta cell dysfunction (12-14).

Generally, deposition of lipids can produce critical elements in the pathophysiological cascade that are detrimental to cell health and tissue function, causing intracellular dysfunction, chronic inflammation, and also dysfunctions in energy, metabolism, vital responses, and finally, cell death. All these issues reflect the importance of lipid deposition in the treatment of diabetes and its complications. Studies focusing on Traditional Persian Medicine (TPM) and diabetes have indicated that increase of fat and fluid in the body may lead to diabetes. According to this etiology, some modalities such as thermal therapy, massage, dry cupping, and herbal medicine has been suggested as the treatment (15-17). In addition, some traditional literature prescribes thermal therapy and herbal drugs such as oxymel to decrease excess fat and fluid and to control diabetes (15, 18-20).

Warming the individual's environment can be considered the equivalent of thermal therapy in the current medicine, which its effects on type 2 diabetes have been shown in both animal and human studies. These studies began in 1999 by Hooper by whom the effect of sauna in patients with diabetes was investigated. In the following, Kokura exposed diabetic rats to infrared and checked the efficacy. Chung studied the treatment role of heat shock proteins (HSPs) in diabetic rats (21-23). Altogether thermal therapy, in numerous animal and human studies, has led to significant reductions in insulin resistance, fasting blood glucose, and glycated hemoglobin (HbA1C) in diabetic samples (24-27).

The mechanisms currently known for these findings include an increase in the production and induction of nitric oxide synthase (NOS), as well as the induction of HSPs. The increase in the levels of nitric oxide (NO), HSP70, 50AMP-activated protein kinase (AMPK), and endothelial nitric oxide synthase (eNOS) improves insulin signaling, body composition, while decreases endothelial dysfunction and low grade inflammation (25, 28-30). On the other hand, oxymel is a Traditional Iranian drink; a baked mixture of vinegar and honey or other sugary materials which is used as a treatment for many diseases in TPM (31). The mixture can target the accumulated fat and also may make blood thinner causing better penetration of blood and reduction of insulin resistance, and more insulin bioavailability. Meanwhile, according to one study, this mixture does not affect the fasting blood sugar levels of healthy people (32); thus is safe in diabetes.

Based on the aforementioned content, we designed a novel combinational protocol composed of a new modified thermal therapy method where its temperature is close to normal core body temperature and the ingestion of oxymel. Regarding the previously discussed effects of each of the two interventions alone, it seems that their combination may be more helpful for patients with diabetes. This combination therapy-derived from principles of TPM-is called SINA therapy (33) aimed to make better blood perfusion and penetration, to increase of insulin bioavailability and sensitivity by creating greater cardiac outputs, to make the blood thinner, and to degrade obstructing fat accumulation. In this study, the effect of combination therapy (thermal therapy and oxymel) was investigated in type 2 diabetic rats.

2. Methods

2.1. Animals and Diets

This experimental block randomization study was conducted in the Experimental Animal Unit of Qom University of Medical Sciences in Iran, in 2018. We used a sampling formula for comparing the two means, and the study power was considered 80%. However, owing to low variance in our study population (a specific type of rat in the laboratory setting), the minimum sample size for each group was considered 6. We increased the sample number for resolving the attrition or missing values. For this study, 8 week-old male Wistar rats weighing 220 - 180 g were prepared from the Pasteur Institute of Iran and were kept under controlled conditions (temperature range of 22 $\pm$ 1°C, humidity 40% - 45%, and illumination conditions of 12 hours of light and 12 hours of darkness). Maintenance protocols and animal experiments were carried out according to the Ethics Committee of Qom University of Medical Sciences (Ethical code: IR.MUQ.REC.1396.15).

Rats had free access to water and food (normal or high fat). The study was conducted after a week of adaptation to the environment. The composition of the diet was 72% carbohydrate, 22% protein, and 5.7% fat in the normal diet, and 27.5% carbohydrate, 22.5% protein, and 58.8% fat in the high-fat diet. Casein was used to supply protein. Aside cysteine deficiency for which DL-methionine was necessary to be added, the amino acid content was adequate (34). The content of the high-fat diet (g/kg) was composed of: Normal pellet diet: 585.4, cow milk Butter: 310.9, Casein: 73.2, Mineral mix: 24.6, Vitamin mix: 4.1, and DL-methionine: 1.8.

2.2. Induction of Diabetes

Type 2 diabetes was induced by 3 weeks of high-fat diet followed by a single dose intraperitoneal injection of streptozotocin (STZ) (Sigma St. Louis. MO. USA) (35 mg/kg), dissolved in 0.1 mm citrate buffer (Sigma, Aldrich, USA) and (pH = 4.5) after 6 - 8 hours of fasting. Seventy-two hours after STZ injection, the blood glucose was measured by using a glucometer and rats with fasting blood glucose (FBS) level values higher than 150 mg/dL were considered to be diabetic (35).

2.3. Experimental Design

Rats were randomly divided into 8 groups (8 rats in each group): (1) The healthy control group (Nl); receiving normal diet and a single dose of citrate buffer as STZ solvent on the first day of the fourth week of the study; (2) Diabetic group (D) without any treatment; (3) The diabetic group treated by sauna 5-days a week for 8-weeks (Sauna); (4) The diabetic group receiving 1 mL of oxymel via gavage (5-days a week for 8-weeks) (OXM); (5 - 7) Diabetic groups under SINA Therapy, receiving both oxymel and sauna for one (SINA1d), three (SINA3d), and five days (SINA5d2m) a week for 8-weeks, (8) The diabetic group under SINA Therapy for 5-days a week for 4-weeks and then without intervention for the next 4-weeks (SINA5d1m). All diabetic groups received a high-fat diet until the end of the study.

Sauna Condition: During the treatment, rats were placed in the sauna chamber-a digitally time and temperature controlled incubator for 30 minutes at 37°C and based on their groups, this process was repeated for one, three or five days a week. Oxymel preparation method: first, one kilogram of sugar was boiled with 500 mL of hot water, and then 300 g of vinegar (5% acetic acid) was added. To prepare a gavage solution, 0.2 ml of the syrup was dissolved at 0.8°C of water, which first reached 100°C, and then cooled to 50°C.

SINA Therapy is the simultaneous reception of oxymel solution and exposure to the sauna condition. Weighing the rats and testing their FBS level (using a glucometer, Byer, Germany) were performed and recorded at the beginning, middle, and at the end of the study period in all groups. At the end of the treatment and after 12 to 14 hours of fasting, the rats were killed by the painless method and tissue samples from the heart, kidneys, liver, pancreas, and a blood sample were collected.

2.4. Biochemical Analyses of Serum Parameters

The blood obtained from the heart was centrifuged at a speed of 5,000 rpm for 10 minutes. Then serum insulin concentrations were measured by using an ELISA kit (Eastbiopharm, China). Lipid profile, including total serum cholesterol (TC), triglyceride (TG), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C), as well as creatinine (Cr), were measured by appropriate kits and according to manufacturers' instructions. Also, the index of insulin resistance was calculated by the HOMA-IR model (Homeostasis Model Assessment of Insulin Resistance), according to the following formula at the end of the treatment:

HOMA-IR = Fasting insulin (μu/mL) $\times$ Fasting glucose (mg/dL)/22.5

In addition, the level of HbA_1C was measured in EDTA-blood samples with a commercial kit (Pars Azmon, Iran). All equipment and kits were calibrated before use.

2.5. Histological Evaluation

Liver, heart, kidney, and pancreas were removed from the body of the rats and were kept in a 10% formaldehyde solution for at least 48 hours. Then the tissue preparation steps were performed, according to standard protocols. The specimens were dehydrated, embedded in paraffin, serially sectioned, and stained with hematoxylin and eosin (H&E). The slides were then examined under a light microscope.

2.6. Statistical Analysis

After collecting data and inserting them to the IBM SPSS for Windows, version 20.0 (IBM Corp., Armonk, N.Y., USA). The normality of quantitative variables was investigated using Shapiro-Wilk and Kolmogorov-Smirnov tests. In order to compare the mean of variables in the studied groups, statistical analysis of variance and post hoc Tukey test were used. In some cases, owing to the lack of assumptions for analysis of variance, the Kruskal-Wallis test was used. Owing to low sample size in some variables, the bootstrap method was used to calculate the confidence interval (CI). To investigate the variables during the follow-up, analysis of variance was performed in repeated measurements.

3. Results

3.1. Bodyweight

The total average weight of rats in different groups during zero (W.0), fourth (W.4w), sixth (W.6w), eighth (W.8w) and twelfth (W.12w) weeks are shown in Table 1. Throughout the study (0, 4, 6, 8, and 12 weeks), the bodyweights did not change significantly among the groups even in comparison with the Nl group ($P < 0.05$).

Table 1. Bodyweight and Fasting Blood Glucose (Mean ± Standard Deviation) During the Study[a]

	NL	D	Sauna	OXM	SINA1d	SINA3d	SINA5d1m	SINA5d2m
W.0	195 ± 16.83	192 ± 10.95	194 ± 19.49	205.5 ± 144.75	185 ± 4.08	202.5 ± 15	188.75 ± 10.30	199.17 ± 14.28
W.4w	256.25 ± 11.08	275 ± 22.36	253 ± 34.56	273.75 ± 26.88	256.25 ± 18.87	253.75 ± 41.50	231.25 ± 39.66	258.33 ± 43.89
W.6w	267.5 ± 9.57	285 ± 21.79	247 ± 36.33	271.25 ± 44.97	266.25 ± 27.8	253.75 ± 37.05	243.75 ± 40.28	279.17 ± 47.68
W.8w	291.25 ± 4.78	301 ± 37.98	250 ± 39.84	282.5 ± 48.39	261.25 ± 34.28	265 ± 32.4	258.75 ± 54.98	281.67 ± 55.01
W.12w	310 ± 4.08	303 ± 42.51	255 ± 42.27	287.5 ± 45.73	271.25 ± 55.43	242.5 ± 33.29	268.75 ± 64.21	275 ± 56.56
FBS.4W	95.5 ± 4.20	264 ± 58.56[b]	304 ± 43.35[b]	262.5 ± 43.49[b]	257.5 ± 79.73[b]	296.25 ± 92.86[b]	287.5 ± 82.61[b]	270 ± 107.5[b]
FBS.6W	94 ± 4.54	362 ± 147.5[b]	454 ± 23.02[b]	390 ± 132.41[b]	427.5 ± 29.86[b]	415 ± 78.5[b]	412.5 ± 94.29[b]	360 ± 120.83[b]
FBS.8W	93.75 ± 4.78	334 ± 168.01[b]	460 ± 22.36[b]	412.5 ± 168.39[b]	420 ± 20[b]	402.5 ± 70.88[b]	430 ± 109.24[b]	361.67 ± 117.88[b]
FBS.12W	94.25 ± 4.19	348 ± 160.37[b]	448 ± 17.88[b]	365 ± 131.78[b]	425 ± 28.86[b]	397.5 ± 85.39[b]	415 ± 85.04[b]	330 ± 124.57[b]

[a] NL, The healthy control group receiving normal diet and a single dose of citrate buffer as STZ solvent on the first day of the fourth week of the study; D, Diabetic group without any treatment; Sauna, The diabetic group treated by sauna 5-days a week for 8-weeks, OXM, The diabetic group receiving 1 mL of oxymel via gavage (5-days a week for 8-weeks), SINA1d, 3d, 5d, Diabetic groups under SINA Therapy, receiving both oxymel and sauna for one (SINA1d), three (SINA3d), and five days (SINA5d2m) a week for 8-weeks; SINA5d1m, The diabetic group under SINA Therapy for 5-days a week for 4-weeks and then without intervention for the next 4-weeks; W, weeks; FBS, fasting blood sugar.
[b] Significant difference compared to the NL group.

3.2. Fasting Blood Glucose

The mean of the FBS in the fourth (Fbs.4w), sixth (Fbs.6w), eighth (Fbs.8w), and twelfth (Fbs.12w) week is shown in Table 2. Comparison of fasting blood glucose in the fourth week showed that all groups had a significant increase with (P = 0.000) compared to the NL group. Also, fasting blood glucose in all groups at sixth, eighth, and twelfth weeks was significantly higher than the NL group (P = 0.000). Additionally, during these weeks, the groups did not show any significant increase or decrease in FBS in comparison to each other (Table 1).

3.3. HbA1C, Insulin, HOMA

The mean of HbA_1C in the D (P = 0.04), OXM (P = 0.03) SINA1d (P = 0.003) SINA3d (P = 0.000), SINA5d1M (P = 0.000) and SINA5d2M (P = 0.000) groups were significantly higher than the NL group, but there was no significant difference in the Sauna group. Similarly, there was no significant difference in insulin levels in any of the groups compared to the NL group. Except for the Sauna (P = 0.000) group, the insulin resistance index (HOMA-IR) was not significantly increased in the other groups in comparison to the NL group (Table 3).

3.4. Lipid Profile

Table 3 also shows the mean serum levels of TG, TC, HDL-C, and LDL-C in the groups. Mean comparison of TG, LDL-C, and TC among the groups showed that there were no significant differences between any of the groups compared to each other and in comparison to the NL group (P > 0.05). HDL-C levels were significantly increased in the SINA3d group compared to the NL group (P = 0.004), while in the other groups there was no significant difference compared to the NL and also compared to the other groups (P > 0.05).

3.5. Histological Assessment

3.5.1. Pancreas

In the histological evaluation, the number of Langerhans Islands, as well as the presence or absence of vacuoles in Langerhans Island cells, were investigated. In Table 2, the mean number and diameter of Langerhans islands are presented. The SINA3d (P = 0.05) and SINA5d2m (P = 0.008) groups showed a significant increase in the number of Langerhans Islands in comparison to the D group.

In Figure 1, a sample set of pancreatic tissue structure photograph of study groups is shown with 40× magnification. In the NL group, the tissue had a completely normal appearance. The cells were seen together in order and tidy rows. Cell's nuclei and the boundaries of each nucleus were quite clear, and there was no tissue disorganization in 90% of the samples. No intercellular space was present in any sample. Cell density was normal in all samples (100% of samples). No vacuoles were seen. Changes in other groups are shown in Table 4.

3.5.2. Liver

The liver tissue was examined for the structure of classical lobules, the presence of vacuoles in hepatocytes, the dilatation of sinusoids, and the number of lymphatic cells. Measurement and statistical analysis of these factors did not show any significant difference among the groups; therefore, the tables and comparison charts are not shown.

Table 2. The Mean Number and Diameter of Langerhans Islands (Mean ± SEM)[a]

	NL	D	Sauna	OXM	SINA1d	SINA3d	SINA5d1m	SINA5d2m
Number	6.25 ± 0.69	4.4 ± 0.40	5 ± 0.40	5.25 ± 0.25	4.5 ± 0.28	6.25 ± 0.94[b]	4.5 ± 0.86	7 ± 0.36[b]
Diameter	2.17 ± 0.31	1.15 ± 0.05	1.11 ± 0.11	1.05 ± 0.03	1.23 ± 0.32	1 ± 0.1	0.87 ± 0.1	1.29 ± 0.7

[a] NL, The healthy control group receiving normal diet and a single dose of citrate buffer as STZ solvent on the first day of the fourth week of the study; D, Diabetic group without any treatment; Sauna, The diabetic group treated by sauna 5-days a week for 8-weeks, OXM, The diabetic group receiving 1 mL of oxymel via gavage (5-days a week for 8-weeks), SINA1d, 3d, 5d, Diabetic groups under SINA Therapy, receiving both oxymel and sauna for one (SINA1d), three (SINA3d), and five days (SINA5d2m) a week for 8-weeks; SINA5d1m, The diabetic group under SINA Therapy for 5-days a week for 4-weeks and then without intervention for the next 4-weeks; W, weeks; FBS, fasting blood sugar.
[b] Significant difference compared to D group.

Table 3. HbA_1C, Insulin, HOMA, and Lipid Profile (Mean ± Standard Deviation) at the End of Study

	Nl	D	Sauna	OXM	SINA1d	SINA3d	SINA5d1m	SINA5d2m
HbA_1C	3 ± 0.00	4.6 ± 1.14[a]	4.4 ± 0.54	4.75 ± 0.95[a]	5.5 ± 1.29a	7.25 ± 0.5[a]	6.25 ± 0.95[a]	6.67 ± 2.25[a]
Insulin	5.72 ± 1.93	4.56 ± 2.28	5.36 ± 2.02	3 ± 0.81	5.47 ± 3.06	3 ± 0.81	3.77 ± 1.68	3 ± 0.94
HOMA	1.36 ± 0.52	4.17 ± 3.46	5.97 ± 2.15[a]	2.91 ± 1.52	5.75 ± 2.96	3.08 ± 1.27	3.69 ± 1.38	2.58 ± 1.49
TG	21.25 ± 2.21	105.8 ± 120.04	148.2 ± 172.12	43.5 ± 12.01	313 ± 349.82	87.25 ± 62.42	82.25 ± 46.91	66 ± 38.28
TC	55.25 ± 9.1	55.8 ± 15.36	67.2 ± 42.92	47.25 ± 11.23	107 ± 63.16	70 ± 14.87	58.5 ± 10.47	54.67 ± 5.68
LDL-C	10.75 ± 1.7	10.8 ± 5.21	8.6 ± 4.15	6.75 ± 0.5	13 ± 7.39	6.75 ± 2.5	7.5 ± 2.64	9 ± 1.09
HDL-C	36.25 ± 6.7	37.6 ± 6.76	40.4 ± 6.73	38.5 ± 7.18	48.25 ± 11.14	53 ± 9.05*	43 ± 2.94	43.5 ± 5.75

Abbreviations: HDL-C, high-density lipoprotein cholesterol; HOMA, homeostasis model assessment of insulin resistance; LDL-C, low-density lipoprotein cholesterol; TG, triglyceride; TC, total cholesterol.
[a] Significant difference compared to the NL group.

Table 4. Pancreatic Tissue Structure[a,b]

	NL	D	Sauna	OXM	SINA1d	SINA3d	SINA5d1m	SINA5d2m
Tissue disorganization	10	100	20	40	40	20	20	10
Normal cell density	100	0	75	60	55	80	70	95
Vacuole around nuclei	0	75	33	50	45	25	60	10

[a] Values are expressed as percentage.
[b] NL, The healthy control group receiving normal diet and a single dose of citrate buffer as STZ solvent on the first day of the fourth week of the study; D, Diabetic group without any treatment; Sauna, The diabetic group treated by sauna 5-days a week for 8-weeks; OXM, The diabetic group receiving 1ml of oxymel via gavage (5-days a week for 8-weeks), SINA1d, 3d, 5d, Diabetic groups under SINA Therapy, receiving both oxymel and sauna for one (SINA1d), three (SINA3d), and five days (SINA5d2m) a week for 8-weeks; SINA5d1m, The diabetic group under SINA Therapy for 5-days a week for 4-weeks and then without intervention for the next 4-weeks; W, weeks; FBS, fasting blood sugar.

3.5.3. Kidney

Three factors for kidney tissue and morphology of its cells were investigated: the number of the glomeruli, their diameter and the height of the proximal and distal ducts. None of these factors were statistically significant in any group, so the tables and graphs are not presented.

3.5.4. Heart

The myocardial cells were examined for the deposition of fat globules and other pathological change; however, all groups showed normal morphology.

4. Discussion

Considering the worldwide interest towards integrating traditional and conventional medicines and WHO guidelines in this regard (1); we approached insulin resistance and diabetes with a combination therapy (Thermal therapy and oxymel) named SINA therapy derived from TPM with the intention to increase general and pancreatic blood perfusion. According to results, owing to STZ injection, expected pathological changes occurred in the pancreatic tissue. The STZ damaged pancreatic beta cells, destroyed normal cell shape, and reduced the number of Langerhans cells (35, 36) as seen in the D group. Even though in the SINA3d and SINA5d2m groups, there was a significant improvement in the number of the Langer-

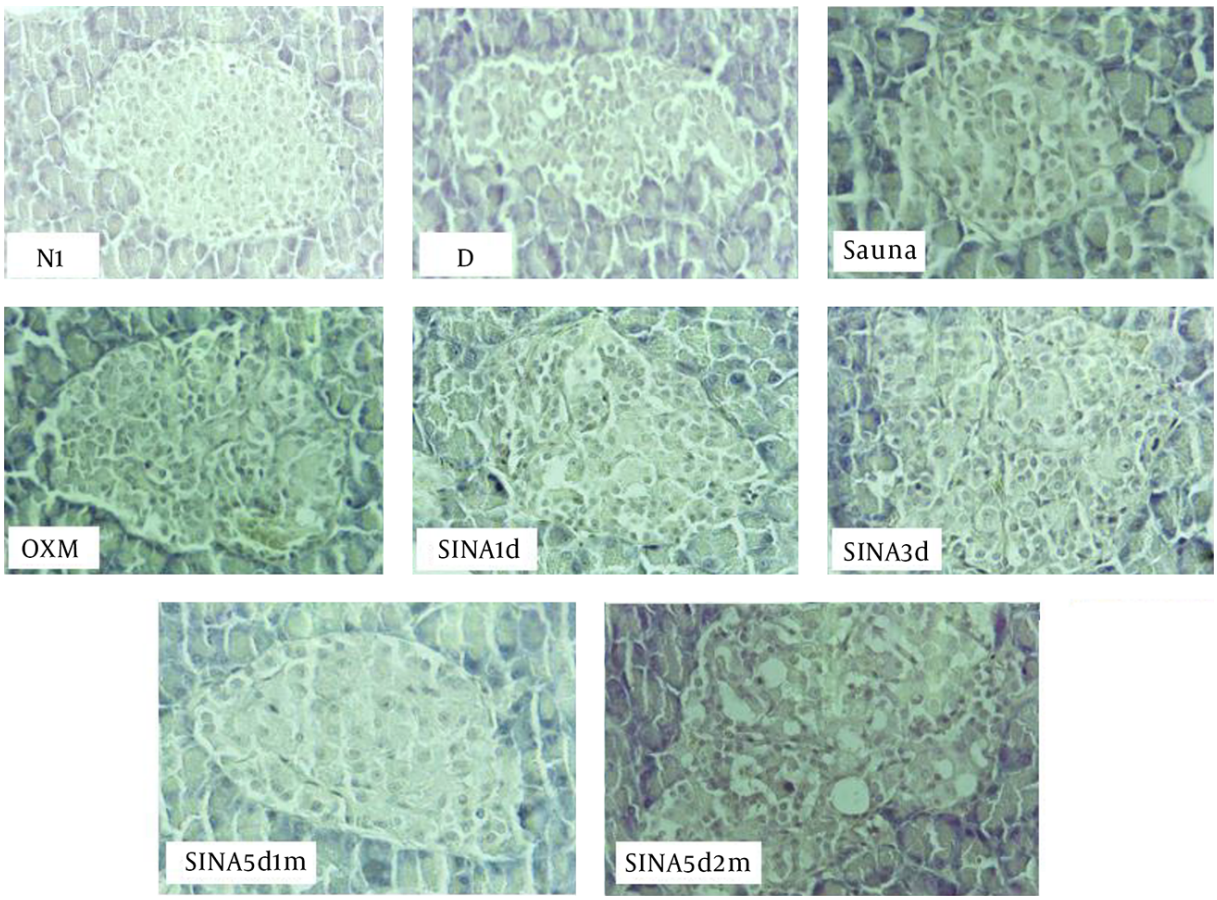

Figure 1. Sample set of pancreatic tissue structure photograph of study groups is shown with 40 × magnification (H&E staining)

hans islands and cell organization. It seems that this recovery was due to the increase in blood perfusion in the SINA methods (32).

So far, pancreatic beta cells have not been investigated in any previous thermal therapy studies; therefore, our finding was new and incomparable with previous studies. Despite earlier studies, which indicate thermal therapy reduces insulin resistance and serum glucose level (21-27) and despite the expected correlation between histological improvement of pancreatic cells and their insulin secretion increase, these expectations did not happen and also serum glucose levels did not decrease. On the other hand, this finding agrees with several studies that have rejected a correlation between pancreatic mass and level of insulin secretion (37-39). One reason might be that high levels of glucose in rat's blood may cause immaturity in beta cells and possibly a significant reduction in glucose level may lead to the improvement of beta-cell function. Another probability might be the down-regulation of insulin gene expression (40-42). It is also possible that more extended periods of the treatment are able to show an effect on beta-cell function aside from the structure.

In this study, we used a high-fat diet to induce insulin resistance and mimic human type 2 diabetes, but considering the insignificant change of weights and lipid profiles and the absence of fat deposition in histological examinations of the liver, heart, and kidney, one may presume that the type of fat used in our high-fat diet has influenced the obtained results. In this regard, it may be assumed that the general composition of cow milk butter, modifies the effects of its saturated fats (43). Although these results were inconsistent with a recent study in diabetic rats (33), they were consistent with the results of some studies declaring the effect of cow milk butter on the diet of normal rats. In these recent studies, not only there were no changes in cholesterol and LDL levels but also positive effects on HDL were observed (43, 44). Based on the results, we present the following suggestions:

(1) In preparing the high-fat diet to produce insulin resistance and type 2 diabetes, it is better to use other types of fats such as animal fat, corn, canola, and sunflower oils rather than milk butter (44).

(2) As it seems that the butter obtained from cow milk does not have much harmful effect, one may study about its beneficial effects, especially in diabetes.

(3) Considering the improvement of pancreas tissue in SINA3d and SINA5d2m, further studies on definite type 2 diabetic specimens are suggested to investigate their effects on beta-cell function.

4.1. Conclusions

Based on our findings, SINA improved pancreatic beta-cell structure, but its effect on insulin production was not approved, which might be due to the wrong selection of high-fat diet despite the claim of some previous studies. Nevertheless, we hope to investigate more the effects of SINA therapy for treating insulin resistance and type 2 diabetes in future studies.

Footnotes

Authors' Contribution: Study concept and design: Mahdi Alizadeh Vaghasloo, Majid Asghari and Zahra Sarbaz Hoseini. Analysis and interpretation of data: Abolfazl Mohammadbeigi, Azam Khalaj, Shima Ababzadeh, Hamid Heidari, Mahdi Alizadeh Vaghasloo, Majid Asghari and Zahra Sarbaz Hoseini. Drafting of the manuscript: Zahra Sarbaz Hoseini, Azam Khalaj, Mahdi Alizadeh Vaghasloo and Majid Asghari. Critical revision of the manuscript for important intellectual content: Mahdi Alizadeh Vaghasloo, Majid Asghari and Zahra Sarbaz Hoseini. Statistical analysis: Abolfazl Mohammadbeigi and Shima Ababzadeh.

Conflict of Interests: The authors mention that there is no conflict of interest in this study.

Ethical Considerations: Maintenance protocols and animal experiments were carried out according to the Ethics Committee of Qom University of Medical Sciences (ethical code: IR.MUQ.REC.1396.15).

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