

Supplemental Effect of Zinc Oxide Nanoparticles and *Mentha spicata* butanol Extract on Blood Glucose of Diabetic Wistar Rats

Maryam Rakebizadeh, Mahshid Zahedizadeh, Yadolah Edalat Panah*

Department of Biology, Islamic Azad University Dehdasht, Dehdasht, Iran

ARTICLE INFO

Article history:

Received 26 March. 2018

Accepted 22 May 2018

Published 21 July. 2018

Keywords:

Diabetes Mellitus, Zinc Oxide Nanoparticles, Mentha Spicata Ferulacea, Glucose, Rats

ABSTRACT

Diabetes mellitus is one of the most common diseases affecting the endocrine system. It has been estimated that the prevalence of diabetes mellitus will be increasing in the human population. It is a metabolic disease characterized by chronic escalation of blood glucose and disrupted metabolism in carbohydrates, fats and proteins. This was an experimental study conducted on 40 male Wistar rats. Diabetes was induced in the animals through Streptozotocin (STZ). After three days, blood glucose levels were measured. The rats were diagnosed with diabetes when blood glucose was more than 250. The data were analyzed through SPSS 21. Moreover, the independent t-test was used to examine the relationship between variables. Results of statistical analysis in relation to body weight of rats showed that there was a significant relationship between all groups at $P < 0.05$ except for diabetic group and diabetic group by *Mentha spicata* Butanol extract as well as diabetic group by *Mentha spicata* butanol extract and zinc oxide nanoparticles. Concerning the glucose levels, the statistical analysis indicated that there was a significant relationship between all groups at $P < 0.05$. When applied as a supplement, zinc oxide nanoparticles and *Mentha spicata* butanol extract can have anti-diabetic properties, curtailing blood glucose, revealing that nanoparticles can be used in the future as a treatment for diabetes.

1. Introduction

Diabetes mellitus is one of the most common diseases affecting the endocrine system. It has been estimated that the prevalence of diabetes mellitus will be increasing in the human population. It is a metabolic disease characterized by chronic escalation of blood glucose and disrupted metabolism in carbohydrates, fats and proteins. Diabetes is accompanied with long-term complications such as retinopathy, nephropathy, neuropathy and cardiovascular diseases. It is estimated that more than 3.6% of the world's population are currently suffering from diabetes mellitus. In the process of diabetes, long episodes of hyperglycemia can lead to production of free radicals, especially ROS. It represents the oxidation of glucose and protein glycation, where the undesirable conditions in all tissues can impair the balance between ROS production and cell defense mechanism. This imbalance leads to cell damage and changes in the cell function and tissue damage, especially pancreas [9]. There is currently a great interest of medical and food industry in the detection of antioxidant compounds with pharmacological potential without any side effects or at least with minimal side effects [3]. Due to the high cost and side effects of chemical drugs today, it has become a top priority to study plants used in traditional medicine so as to achieve further progress in medical sciences. Herbal remedies are natural substances with lower risk of side effects. Many of these herbs provide a rich source of natural antioxidants that can curtail the side effects of oxidants and some diseases. Belonging to genus *Apiaceae*, *Mentha spicata* grows across different areas of Iran including the

Alborz slopes, Azerbaijan, Kurdistan, Kerman and Fars. Many of its species can be found in India, Anatolia, Central Asia, Iran and the Pacific [4]. *Mentha spicata* is a valuable herb used in traditional medicine for the treatment of many diseases. Numerous experimental studies have proven its therapeutic properties. In traditional medicine, *Mentha spicata* has been applied as a carminative, laxative, stomach tonic, relieving the nervous system, anti-inflammatory, anti-virus, anti-parasitic, anti-fungal and anti-bacteria substance [6].

This plant grows in April and develops all through May. Afterwards, it initiates the reproductive stage and produces its fruit in late May. The seed ripen in early July and at the same time, the plant begins to completely wilt away. At the beginning of August, the plant undergoes a dormant phase and there are usually no remains on the ground. The green stems of *Mentha spicata* are edible. In late April and early May, this plant grows on. It can be used as food after drying. The leaves of *Mentha spicata* are applied for treatment of digestive diseases, without exposing to toxicity [2]. Phytochemical studies have isolated and identified a variety of coumarins, alkaloids, flavonoids and terpenoids in *Mentha spicata* species. Recent studies have shown that this herb is rich in antioxidants [1]. Previously phytochemical studies indicated the presence of coumarins, alkaloids, flavonoids and terpenoids in the plant. Furthermore, some important compounds such as Emilyferon, frandol, froliden, prangon and pentylcoumarin have been identified at the root of *Mentha spicata ferulacea*. Previous studies have shown that antioxidant compounds in

*Corresponding author: edalatpanah1367@gmail.com

DOI: <https://doi.org/10.24200/jrset.vol6iss03pp1-5>

Mentha spicata are important for reduction of oxidative stress [10].

In recent years, however, the structure of inorganic nanoparticles have been the subject of much speculation in terms of physical, chemical and biological properties. Moreover, the high potential of nanomaterials in biology and pharmacology has attracted many researchers. There are several applications of nanomaterials such as treatment of HIV [15]. The nanoparticles can be used in drug delivery applications, production of high quality microscopic images, treatment of cancer and diseases [7]. Other applications of nanotechnology can be found in drug delivery to treat cancer involving liposomes [3].

In this regard, a study by Mandade et al. (2013) showed that consumption of *Mentha spicata* hydroalcoholic extract in the treated diabetic group decreased serum creatinine and urea nitrogen levels compared to the control group. Antioxidant properties of *Mentha spicata* can strengthen the antioxidant system and resistance against oxidative stress in rats, thus curtailing the risk of kidney damage. Urea and creatinine rise by increasing renal function in diabetic rats [13]. In a study by Kafash et al. (2012), the anti-diabetic properties of *Mentha spicata* were examined for the first time, revealing that diabetic rats treated with the hydroalcoholic extract of *Mentha spicata* roots significantly reduced blood glucose, total cholesterol, triglyceride and glycosylated hemoglobin and significantly increased HDL levels and adjusting the white blood cells (WBC) to normal level [11]. In a study by Farokhi et al. (2013), *Mentha spicata* extract significantly improved the tissue lesions in kidney, liver and pancreas and minimized most unfavorable changes in tissues, including necrotic foci, tissue atrophy, increased lymphocyte attack and glomerulonephritis renal lesions in some areas to a large extent similar to the healthy subjects, mainly due to an increase in body weight of rats under treatment [5]. In a study by Zareh et al. (2012) on the effect of *Mentha spicata* hydroalcoholic extract on blood indices of kidney and liver functions among male diabetic rats showed that levels of blood glucose, aspartate amino transferase, alanine amino transferase and creatinine in groups treated with *Mentha spicata* hydroalcoholic extract significantly decreased compared to the diabetic group. Levels of alkaline phosphatase, urea, urea nitrogen and albumin decreased, but not significantly. Concerning the anti-diabetic effect of ZnO nanoparticles, there have not been any specific research [18]. In another study by Mahdavi Rad et al. (2014) in connection with the anti-cancer effects of zinc oxide nanocomposites and its combination with silver nanoparticles on melanoma malignant cancer cells. They concluded that zinc nano-oxide and its composition with nano-silver can induce cell death in cancer cells at micromolar concentrations of melanoma [14]. Another study by Heidarinejad et al. (2014) examined the acute toxicity of zinc nano-oxide on serum biochemical liver function in white rats, where the results indicated that zinc nano-oxide can be toxic on liver and should be consumed with caution [8]. The aim of this study was to evaluate the supplemental effect of zinc oxide nanoparticles with *Mentha spicata* extract on blood glucose levels in diabetic Wistar rats.

MATERIAL AND METHODS:

This was an experimental study where *Mentha spicata* was harvested in May 2014 from Jokar in Boyrahmad, located 120 kilometers from Yasouj, Iran. In order to prepare two separate hydro-alcoholic extracts, the roots of the plant were individually and the stems and green leaves together were harvested at a weight ratio of 1:1. Having been collected, the samples were identified by professors of botany at University of Yasouj. Then, they were labeled and stored in the herbarium. The samples were then dried and crushed into powder in the mill. At the next stage, 100 grams of powdered roots, as well as 100 grams of powder mixture stems and leaves separately were poured into 1-liter flasks, to which 96% ethanol was added, so that it covered the surface of the powder. After 24 hours, the solutions were filtered. Next, 75% alcohol was added to the remaining pulp. After 24 hours, the solutions were filtered again and then concentrated by vacuum distillation at 50 °C, 70 rpm was to one-third of the original volume. Solutions obtained from the last step in the autoclave (IranTolid medical equipment) were dried at 40 °C under sterile conditions. In this procedure, the dry powder extracts were prepared after a few days and were kept at 4 °C [16]. The Zinc oxide nanoparticles were supplied by TECONAN, Spain (diameter 20-25 nm). Purity of zinc oxide nanoparticles was more than 99.98%. Zinc oxide nanoparticles were dissolved in double sterile distilled water and sonicated so as to be scattered forming the same colloidal suspension. All tests involved a freshly prepared colloidal suspension system. Then, the solution of 8 mM zinc oxide nanoparticles was applied to examine the anti-diabetic effects [16].

In this experimental study, 40 Wistar rats in the weight range of 250 to 300 grams were supplied by an Animal House at Yasuj University of Medical Sciences. Animals were exposed to a cycle of 12 hours of light and 12 hours of dark at 20 degrees centigrade and relative humidity of 25 to 30 percent, along with a standard diet of pellets and municipal water. In order to achieve a compromise with the environment, all the tests were performed after at least 10 days since the establishment of animals in the nest. Ethics of research was exercised regarding minimal harassment. In this study, 40 rats were divided randomly into 5 groups of 8 each, including:

- 1- Control group receiving normal saline only during the experiment.
- 2- Streptozotocin-induced diabetic control group only receiving normal saline.
- 3- Streptozotocin-induced diabetic rats receiving 100 mg/kg of *Mentha spicata* extract.
- 4- Streptozotocin-induced diabetic rats receiving 8 mM of zinc nano-oxide solution.
- 5- Streptozotocin-induced diabetic rats received zinc oxide nanoparticles and *Mentha spicata* butanol extract as a supplement [12].

After 12 hours of fasting, the experimental model of diabetes mellitus type 1 was employed in rats with a single intra peritoneal injection of streptozotocin at 120 mg per kg of body weight. Symptoms of diabetes included polydipsia, urination and weight loss after 5 days. The blood glucose levels of diabetic rats were ensured through collecting blood

samples from tails and direct lancet from the tails controlled by glucometer [17].

The treatment duration was 4 weeks, in which the plant extracts, 8-Molar zinc nano-oxide and normal saline were administered orally on a daily basis. Blood samples were collected in the first and second stages, so as to determine the blood glucose level through the tail vein and direct lancing of the tail. The blood samples were poured in test tubes and then transferred to the laboratory for biochemical tests. The data were analyzed through SPSS 19. Moreover, the independent t-test was used to examine the relationship between the groups.

RESULTS

Weight of the experimental rats

In relation to the weight of the rats, Figure 1 indicates the diabetic group was 263.4 and 320.7 before and after the test, respectively. The weights for the diabetic group together with intake of zinc oxide nanoparticles before and after were 261.8 and 304.9, respectively. The results show that streptozotocin-induced diabetic rats experienced higher body weight. For diabetics receiving *Mentha spicata*, the weights before and after the test were 260.6 and 289.8, respectively. As for the diabetics receiving ZnO nanoparticles and *Mentha spicata ferulacea*, the weights before and after the test were 260.1 and 290.25, respectively.

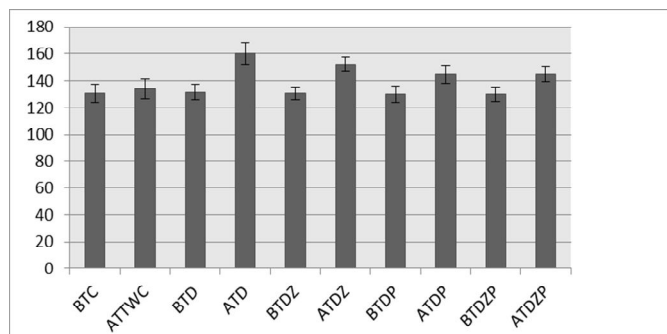


Figure 1: Weight levels in experimental rats

BTC(Before Test Control)

ATTWC (After Two Week Control)

B TD (Before Test Diabetic)

ATD(After Test Diabetic)

BTDZ (Before Test Diabetic ZnO)

ATDZ(After Test Diabetic ZnO)

BTDZP (Before Test Diabetic *Mentha spicata*)

ATDZP(After Test Diabetic *Mentha spicata*)

BTDZP (Before Test Diabetic ZnO and *Mentha spicata*)

ATDZP(After Test Diabetic ZnO and *Mentha spicata*)

Table 1: The findings of the independent t-test analysis in relation to the weight of the rats under study

T	Significance	Groups
4.16	0.04	Diabetics and ZnO NP diabetic group
1.3	0.232	Diabetic group and <i>Mentha spicata</i> butanol extract diabetic group
7.6	0.0	Diabetic group and <i>Mentha spicata</i> butanol extract and zinc oxide nanoparticles diabetic group
0.021	0.984	<i>Mentha spicata</i> butanol extract diabetic group and <i>Mentha spicata</i> butanol extract and zinc oxide nanoparticles diabetic group
-6.7	0.0	ZnO NP diabetic group and <i>Mentha spicata</i> butanol extract and zinc oxide nanoparticles diabetic group

Comparison of the weight of diabetic group and zinc oxide nanoparticles diabetic group showed a significant difference at $P < 0.05$. Comparison of nanoparticle zinc oxide diabetic group and *Mentha spicata* butanol extract + zinc oxide nanoparticles diabetic group showed a significant difference at $P < 0.05$. Therefore, zinc oxide nanoparticles with *Mentha spicata* can reduce the weight of diabetic rats. Comparison of the weight of diabetic group and *Mentha spicata* diabetic group showed no significant difference at $P < 0.05$. Comparison of *Mentha spicata* diabetic group and *Mentha spicata* butanol extract + zinc oxide nanoparticles diabetic group showed no significant difference at $P < 0.05$. Therefore, *Mentha spicata* cannot reduce the weight of diabetic rats. However, it can be a factor in reducing body weight.(table1).

Glucose level of the experimental rats:

In relation to blood glucose of tested rats, Figure 2. Indicated that glucose levels in the control before and after

the test were 101.6 and 100, respectively, showing no significant difference between the rats. As for the diabetic control group, the mean glucose levels before and after the test were 104.9 and 186.25, respectively. For diabetic + zinc oxide nanoparticles group before and after the test, the mean glucose levels were 95.6 and 141.4, respectively. For diabetic + *Mentha spicata* group before and after the test, the mean glucose levels were 101 and 149.3, respectively. For diabetic + zinc oxide nanoparticles + *Mentha spicata* group, the mean glucose levels before and after the test were 96.6 and 129.1, respectively.

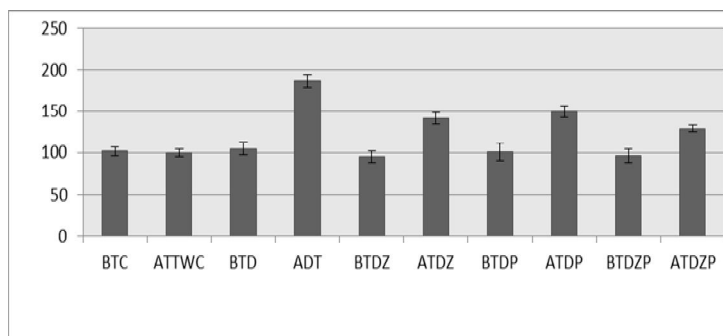


Figure 2: Glucose levels in experimental rats

Table 2. The findings of the independent t-test analysis in relation to the glucose level of the rats under study

T	Significance level	Groups
10.6	0.00	Diabetics and ZnO NP diabetic group
-3.93	0.006	Diabetic group and <i>Mentha spicata</i> butanol extract diabetic group
30.2	0.00	Diabetic group and <i>Mentha spicata</i> butanol extract and zinc oxide nanoparticles diabetic group
-7.06	0.00	<i>Mentha spicata</i> butanol extract diabetic group and <i>Mentha spicata</i> butanol extract and zinc oxide nanoparticles diabetic group
-5.3	0.01	ZnO NP diabetic group and <i>Mentha spicata</i> butanol extract and zinc oxide nanoparticles diabetic group

Comparison of the glucose level of diabetic group and zinc oxide nanoparticles diabetic group showed a significant difference at $P < 0.05$. Comparison of nanoparticle zinc oxide diabetic group and *Mentha spicata* butanol extract + zinc oxide nanoparticles diabetic group showed a significant difference at $P < 0.05$. Therefore, zinc oxide nanoparticles can reduce the glucose level of diabetic rats. Comparison of the blood glucose of diabetic group and *Mentha spicata* diabetic group showed a significant difference at $P < 0.05$. Comparison of *Mentha spicata* diabetic group and *Mentha spicata* butanol extract + zinc oxide nanoparticles diabetic group showed a significant difference at $P < 0.05$. Therefore, *Mentha spicata* can reduce the blood glucose of diabetic rats. Hence, *Mentha spicata* and zinc nano-oxide can reduce blood glucose in diabetic rats. (table 2).

DISCUSSION:

In the twenty-first century, diabetes is known as one of the most common diseases in the world and Iran [8]. There is currently no cure to diabetes and only discovered drugs can improve the disease and increase the chances of survival [7]. Nowadays, herbs are widely used to treat diseases. Moreover, a broad range of studies have been conducted in developed and the developing countries on the importance of this plant in the treatment of diseases, particularly diabetes [5]. *Mentha spicata* is known as one of the herbs that can be used in the treatment of diseases owing to its powerful antioxidant compounds [6]. Twenty-first century can be called the century of nanoparticles, which has found wide applications in various fields of nanotechnology. The advanced countries have now resorted to the use of nanoparticles for the treatment of refractory diseases [7]. In relation to the mean weight of diabetic rats, the results showed that the pre-test weight of 263.4 increased to 320.7 after the test in streptozotocin-induced diabetic rats. As for the ZnO nanoparticles diabetic group, the results indicated that the pre-test mean weight of 261.8 increased to 304.9 in the post-test phase, which managed to curtail the mean weight as compared to the nanoparticles diabetic group. In the diabetic group with *Mentha spicata* butanol extract, the results showed that the pre-test mean weight of

260.6 increased to 289.8 in the post-test phase, which managed to curtail the mean weight as compared to the *Mentha spicata* butanol extract diabetic group. Moreover, the *Mentha spicata* butanol extract was more successful than zinc oxide nanoparticles in reducing the weight of rats. As for the *Mentha spicata* butanol extract diabetic group, the results indicated that the pre-test mean weight of 260.1 increased to 304.9 in the post-test phase, which managed to curtail the mean weight as compared to the *Mentha spicata* butanol extract diabetic group. Compared with zinc oxide nanoparticles diabetic group, the *Mentha spicata* butanol extract and zinc oxide nanoparticles diabetic group managed to effectively reduce body weight of rats. Compared to the *Mentha spicata* butanol extract diabetic group, however, it failed to reduce body weight of rats.

In relation to the glucose level of diabetic rats, the results showed that the pre-test glucose of 104.9 increased to 186.25 after the test in streptozotocin-induced diabetic rats. As for the ZnO nanoparticles diabetic group, the results indicated that the pre-test glucose of 95.6 increased to 141.4 in the post-test phase, which managed to curtail the glucose as compared to the nanoparticles diabetic group.

In the diabetic group with *Mentha spicata* butanol extract, the results showed that the pre-test glucose of 101 increased to 149.3 in the post-test phase, which managed to curtail the glucose as compared to the *Mentha spicata* butanol extract diabetic group. Moreover, the *Mentha spicata* butanol extract was less successful than zinc oxide nanoparticles in reducing the glucose level of rats. As for the *Mentha spicata* butanol extract diabetic group, the results indicated that the pre-test glucose of 96.6 increased to 129.1 in the post-test phase, which managed to curtail the glucose as compared to the *Mentha spicata* butanol extract diabetic group. Compared with zinc oxide nanoparticles diabetic group and *Mentha spicata* butanol extract diabetic, the *Mentha spicata* butanol extract and zinc oxide nanoparticles diabetic group managed to effectively curtail the glucose level in rats.

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