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RESEARCH PAPER

Investigating the Effect of Streptozotocin Induced Diabetes on the Ovarian Tissue of Nmri Mice Following the Injection of MgO and MgO NPs

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ABSTRACT

Both men and women with diabetes are known to experience reduced fertility and poor reproductive health. Diabetes can negatively affect an organ's structure and function, including the ovary. Recent investigations have identified magnesium oxide nanoparticles (MgONPs), one of the magnesium derivatives, as a strong anti-diabetic agent. In addition, the fertility hormones progesterone and estrogen are balanced by magnesium. Therefore, in the current study, the effects of MgO and MgONPs on the ovary in diabetic NMRI mice were compared. Using a randomization process, 50 female NMRI mice were placed into five groups: control, sham (physiological serum), diabetic (streptozotocin/STZ=50 mg/kg, intraperitoneal/IP), diabetic receiving MgO (25 mg/kg), and diabetic receiving MgONPs (25 mg/kg). After treatment for three weeks, every animal blood glucose and body weight were measured. The ovaries were detached following euthanasia, weighed, and then submerged in an appropriate fixative. According to the results, diabetes had no influence on the number of ovarian follicles, such as primary, secondary, and tertiary follicles, as well as follicular diameter. However, it did diminish the number of primordial follicles and corpus luteum (CL) ($p<0.05$). MgO supplementation prevented a decrease in the number of primordial follicles and CL ($p<0.05$). Administration of MgONPs prevented the loss of primordial follicles and also enhanced the quantity of CL ($p<0.05$). As a result, it is possible to draw the conclusion that MgONPs, as opposed to MgO, may have higher inhibiting and stimulating effects on folliculogenesis.

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INTRODUCTION

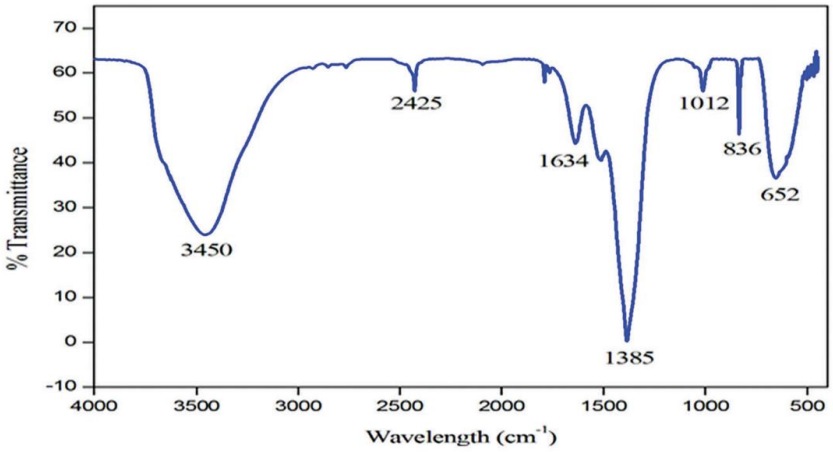
Diabetes mellitus (DM) is a condition in which blood glucose levels are not properly regulated [1]. Due to its high frequency and propensity for morbidity, DM is a potentially fatal disorder that poses a serious threat to public health [2]. Currently, 2.8% of people worldwide suffer from this metabolic condition, which is incurable [3]. It is now the most expensive chronic illness in the United States, costing $327 billion [4]. In addition to the 96 million adults in the United States who have prediabetes, which increases their risk of developing type 2 diabetes, more than 37 million Americans have diabetes [5]. Diabetes can lead to serious side effects like blindness, kidney failure, and heart disease [6–8]. Approximately 90% of women and men with diabetes experience reproductive system dysfunction in a variety of ways, including diminished fertility and libido [9]. This is one of the most serious complications of diabetes. The hypothalamic-pituitary-ovarian (HPO) axis, hormone imbalance, polycystic ovary syndrome, follicular degeneration syndrome, corpus luteum (CL) defect, immature oocyte, anovulation, and change in estrus cycles are all disorders in the histophysiology of the ovary that contribute to decreased fertility in women with diabetes [10–12]. Today, a variety of medications are used to lower blood sugar and treat or prevent diabetes. Recently, the use of nanomedicines for the treatment of diabetes has received a lot of interest [13–15]. Compared to conventional medications, nanomedicines have benefits like more selective drug action in target tissues, higher purity, fewer side effects, improved drug delivery, and lower drug toxicity. Various tests have demonstrated that one of these medications, magnesium oxide nanoparticles (MgONPs), decreases blood sugar [16–18]. High blood sugar causes an increase in the body's production of free oxygen radicals and a decrease in the activity of antioxidant enzymes due to glycosylation. On the other hand, the HPO axis is impacted by the rise in blood sugar and its normal course is disturbed. Magnesium is one of the most potent antioxidants, and a lack of it causes more oxidative harm to various organs. There is a link between elevated oxidative stress and low magnesium in diabetes patients [19]. Magnesium participates in the metabolism of proteins, lipids, carbohydrates, DNA transcription, and protein synthesis as a cofactor of more than 200 metalloenzymes [20]. Additionally, it is crucial for the production, secretion, storage, and upkeep of insulin in its crystalline form. Magnesium is an essential component for healthy fertility and is a crucial metal for reproduction [21]. MgONPs, which are inorganic nanoparticles, are among the most frequently utilized production compounds in a variety of sectors. In recent years, biological researchers and physicians have given particular attention to these compounds. By raising catalase and superoxide dismutase in the extracellular environment, MgONPs has been shown to protect live cells from oxidative stress and to have positive effects on the HPO axis [22].

The purpose of this study is to compare the effects of MgONPs and MgO on blood sugar levels and the ovary in diabetic NMRI mice. This comparison is made in light of the blood sugar-lowering effects of MgONPs, the significance of the ovary in body physiology and fertility, the negative effects of diabetes on fertility, and the role of magnesium in glucose metabolism and fertility.

MATERIALS AND METHODS

In this study, 50 female NMRI mice weighing 18-22.5 g were purchased from Charles River (Sulzbach, Germany) and kept in an animal facility. The animals were housed for one month before being randomly separated into five groups of 10 to give them time to adjust to their new surroundings and synchronize their sexual cycles [23]. The groups were categorized as 1) Control; received no therapy and were maintained in the exact same surroundings as other groups, 2) Sham; received daily intraperitoneal (IP) physiological serum while being maintained in the same ambient settings as the other groups, 3) Diabetic; received a single IP dosage of 50 mg/kg of streptozotocin/STZ [24], 4) Diabetic; received MgO intraperitoneally at a dose of 25 mg/kg after developing diabetes due to STZ, 5) Diabetic; received MgONPs intraperitoneally at a dose of 25 mg/kg after developing diabetes due to STZ [25]. Fig. 1 shows the Fourier-transform infrared spectroscopy (FTIR), the absorption spectra and the transmission electron microscope (TEM) image of the MgONPs used in this study.

Next, the animals were weighed to determine the correct dose of the medication. Each cage included two mice. The animals were housed under settings that included 12 hrs. of light and 12 hrs. of darkness, a temperature of 25.1°C, and free access to water and commercial pelleted food



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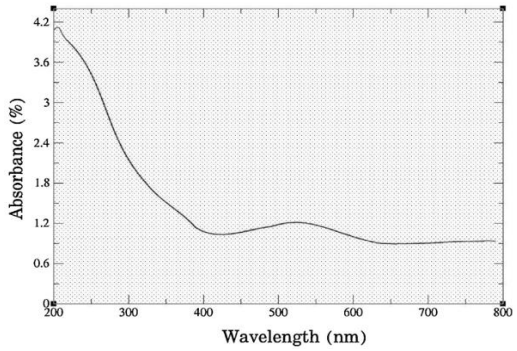

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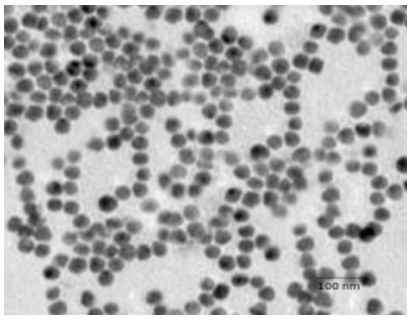

(c)

Fig. 1. Analysis results of MgONPs: (a) FTIR spectroscopy, (b) absorption spectra, and (c) TEM image.

throughout the course of the study. The groups 3, 4, and 5 received injections of STZ (50 mg/kg; IP) on the first day of the experiment to induce diabetes. All mice had their blood sugar levels checked a week after receiving STZ, and those with levels more than 250 mg/dL were classified as diabetic. In groups 4 and 5, daily medication injections began once the mice developed diabetes. The administration period lasted 28 days [26,27]. By clipping the end of the tail and using a glucometer (FreeStyle Precision Neo, Abbott), the blood sugar levels of all groups were assessed 24 hours after the last injections. The animals were euthanized in accordance with ethical standards once their body weights were determined. The abdominal cavity had to be opened in order to harvest the ovary, after which the ovary was isolated from the surrounding tissues and weighed using a digital scale examined macroscopically. Then it was immersed in a 10% formalin fixation. After ensuring that the sample was preserved, tissue sections were prepared from each mouse's ovaries using the conventional and accepted techniques. With the help of SPSS software version 23, the groups' average data on blood sugar levels, body weight, and ovarian weight were examined using the student's t-test, one-way analysis of variance (ANOVA), and least significant difference (LSD) post-test. P values < 0.05 were regarded as significant.

RESULTS AND DISCUSSION

The average body weight of diabetic NMRI mice significantly dropped after 4 weeks ($P<0.05$), as illustrated in Fig. 2. Although giving diabetic NMRI mice MgO and MgONPs prevented weight loss.

A 50 mg/kg dose of the medication STZ was able to cause diabetes in NMRI mice, and at the conclusion of the trial, blood sugar levels in this

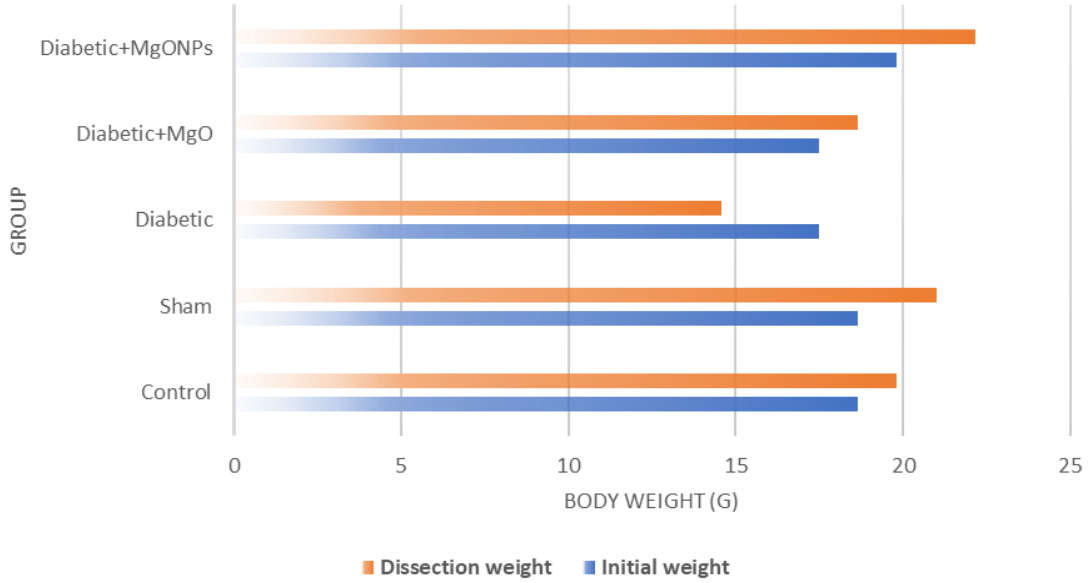

Fig. 2. Mean body weight for each group of NMRI mice.

group were remained high (P<0.05), whereas blood sugar significantly dropped with the daily injection of MgO (P<0.05). Additionally, the regular use of MgONPs resulted in a significant decrease in blood sugar (P<0.05) (Fig. 3).

There was no significant difference in the weight of the ovaries between the various groups (P>0.05) (Table 1). The average amount of CL in the ovaries was significantly reduced as a result of diabetes (P<0.05). Diabetes-related CL loss was considerably mitigated by MgO (P<0.05). In comparison to the other study groups, the diabetic group receiving MgONPs had a larger average number of CL (P<0.05) (Table 1).

Comparing the diabetes group to the control group, the average number of primordial follicles

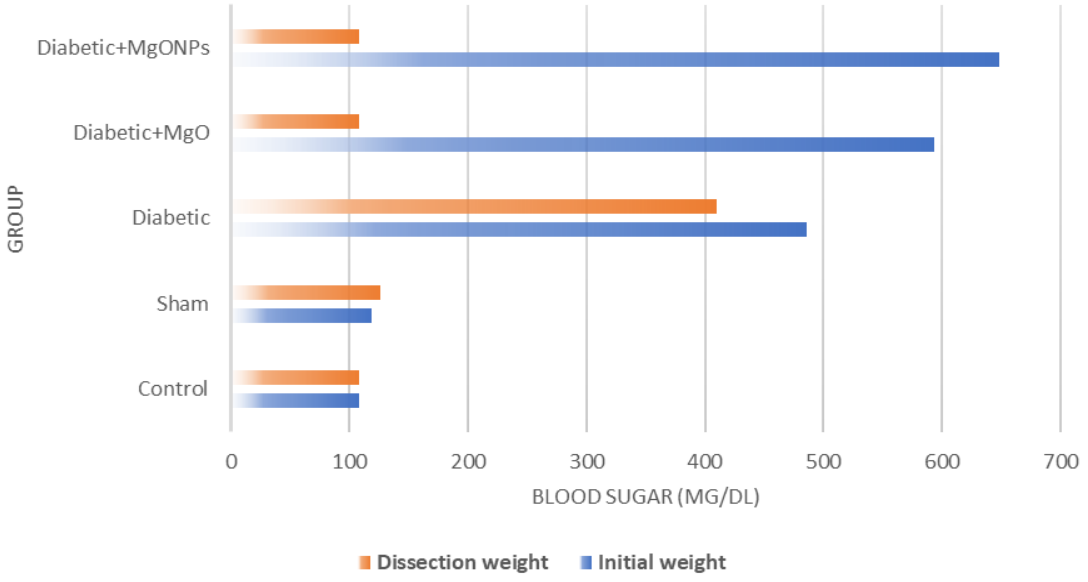

Fig. 3. Comparison of mean blood sugar in NMRI mice in each group.

Table 1. Mean weight of ovary, number of follicles and corpus luteum (CL) in different groups.

	Ovary weight (g)	Primordial follicle	Primary follicle	Secondary follicle	Tertiary follicle	Corpus luteum	Atretic follicles
Control	0.03	16.43	6.56	2.75	0.26	5.07	5.59
Sham	0.02	14.88	9.35	3.76	0.97	9.46	6.54
Diabetic	0.03	9.03	6.02	2.37	0.00	11.78	11.01
Diabetic + MgO	0.02	21.50	11.29	3.87	0.52	10.75	11.09
Diabetic + MgONPs	0.02	18.49	12.90	4.39	0.26	12.90	22.36

Table 2. Mean diameter of follicles (micrometer) in different groups.

	Primordial follicle	Primary follicle	Secondary follicle
Control	7.21	23.83	54.83
Sham	5.72	20.23	55.46
Diabetic	7.27	17.16	57.31
Diabetic+MgO	6.41	15.33	45.56
Diabetic+MgONPs	6.71	19.84	56.79

was significantly lower in the former group (P<0.05). In NMRI mice given MgO, as opposed to diabetic mice, there were more primordial follicles (P<0.05). Furthermore, animals given MgONPs treatment had more primordial follicles than those in diabetes group (P<0.05). There was no significant difference in the number of primordial follicles between the groups who received MgO and MgONPs treatments (P>0.05). There was no significant difference between the experimental groups in terms of the average number of ovarian follicles, including primary, secondary, and tertiary follicles as well as the average diameter of follicles. Additionally, there was no significant difference between primordial, primary, and secondary follicles in terms of diameter (P>0.05) (Table 2).

There is a growing need for innovative treatments with diverse effects due to the prevalence of diabetes and its numerous consequences. MgONPs is one of the medications with recently reported anti-diabetic effects [16,17]. In the current study, the influence of MgONPs on blood sugar and ovarian weight was compared to that of regular MgO four weeks after STZ-induced diabetes in NMRI mice. STZ has been used in a number of research to make lab animals develop experimental diabetes and raise their blood sugar levels. It eliminates pancreatic beta cells and raises blood sugar levels by causing oxidative stress and generating free radicals [24,27]. STZ treatment elevated blood sugar levels in female NMRI mice in the current investigation. High blood sugar levels promote the formation of free radicals. Oxidative stress, caused by the formation of oxygen free radicals, is a major contributor to the development of issues such as insulin resistance, beta cell dysfunction, glucose imbalance, and type 2 diabetes mellitus (T2DM) [19,20]. MgONPs treatment for 28 days lowered blood sugar levels in diabetic NMRI mice. A study employing different dosages of MgONPs in diabetic NMRI mice treated with STZ found that MgONPs was able to reduce blood sugar in diabetic mice after 28 days, and up to a dose of 100 mg/kg was safe in these mice with no cytotoxicity [28]. In another investigation, MgONPs drastically lowered blood sugar, increased serum insulin, and enhanced insulin receptors, and were identified as a powerful anti-diabetic medication [16]. In the present study, diabetes had no effect on ovarian weight. According to van Houtenet al. [29], diabetes did not affect the weight of the ovaries in NMRI mice. Other studies, however, have found that diabetes induces ovarian weight reduction [30,31]. Concerning the lack of ovarian weight decrease following diabetes induction in the current study, it appears that the duration of the experiment was insufficient to significantly lower ovarian weight and that additional time was required to affect the ovarian weight. While the number of primary, secondary, and tertiary follicles did not change after 28 days, hyperglycemia reduced the number of primordial follicles. Moreover, there was no difference in atretic follicle counts across the groups under study. However, according to Sinha et al. [32], streptozotocin-induced diabetes in mice results in a decrease in primordial, primary, secondary, and tertiary follicles and an increase in atretic follicles. According to Farrell et al. [33], the total percentage of follicles in the hamster ovary of

diabetic animals dropped, and there were less primary and secondary follicles in the diabetic group compared to the control group. The present study suggests that the length of the test period may be connected to the absence of change in the number of primary, secondary, and tertiary follicles following diabetes. In the ovary, hyperglycemia reduced the quantity of CL. According to Bolouki et al. [34], diabetes affects the CL analytically. Since each CL represents an ovulated follicle, it is clear that diabetes-related CL reductions are associated with decreased ovulation.

CONCLUSION

In the present study, the administration of MgO decreased blood sugar in diabetic NMRI mice. It seems that the type of magnesium salt has no effect on reducing blood sugar in diabetic animals. Despite the improved permeability and bioavailability of nanoparticles when compared to their regular solutions, there was no difference in blood sugar reduction between the groups getting MgO and MgONPs, which could be attributed to the medicine dosage. Lower doses of MgONPs may provide a greater difference, albeit their negative effects should be considered.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this manuscript.

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