

**Original Paper****Evaluation of the antidiabetic and hepatoprotective effect of *Origanum majorana* leaf extract and its nanoparticles in streptozotocin-induced diabetes in rats.****Mohamed O. Rateb, Afaf, D. Abdelmagid, Omnia M. Abdelhamid, Alshaimaa M. Said***Department of Biochemistry and Molecular Biology, Faculty of Veterinary Medicine, Benha University, Egypt.***ARTICLE INFO****Keywords***Origanum majorana,**Silver nanoparticles,**Diabetes Mellitus,**Streptozotocin,**Glibenclamide.**Received* 10/05/2024*Accepted* 02/06/2024*Available On-Line*

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ABSTRACT

Medicinal herbs and green silver nanoparticle manufacturing (AgNPs) are effective treatments for diabetes. *Origanum majorana* (OM) leaf extract is known for its potential medicinal benefits, including anti-diabetic activities. The study aimed to assess the efficiency of OML extract and nanoparticles in Streptozotocin (STZ) induced diabetic rats and their histopathological effects on liver tissue. 25 rats were separated into 5 groups: Group I: control non-diabetics, Group II: injected with Streptozotocin (STZ); Group III: diabetic rats were treated with glibenclamide, Group IV: diabetic rats were given OM leaf extract, Group V: diabetic rats received OM leaf extract nanoparticles. Various parameters, including blood glucose levels, insulin, lipid profile, liver function test, antioxidants (MDA and GSH), and histopathological changes in the liver, were evaluated. Both OML extract and OMLNPs dramatically lowered blood glucose levels compared to the untreated diabetic group. Compared to the extract, treatment with OMLNPs showed superior effects in improving insulin sensitivity, lipid profile, antioxidant and liver functions. Histopathological examination revealed that OM leaf extract and its nanoparticles mitigated STZ-induced damage in liver tissue, with a noticeable reduction in inflammatory infiltrates and cellular degeneration. OM leaf extract and nanoparticles show potential anti-diabetic and hepatoprotective effects, with enhanced efficacy possibly due to improved bioavailability.

1. INTRODUCTION

Chronic metabolic disease, known as diabetes mellitus (MD), is typified by hyperglycemia brought on by deficiencies in either insulin action or production, or both (Zimmet et al., 2001). Effective diabetes treatment techniques are critical given the disease's increasing worldwide incidence (Al-Maskari et al., 2011). Currently, the most common treatments for diabetes are insulin or oral hypoglycemic medications. These medications are not only very expensive but also have a lot of negative side effects (ADA, 2009). Plant sources are used in drug development because of their anti-inflammatory, antibacterial, and antioxidant properties, which can be used in the creation of new drugs without any side effects (Muntean and Vulpie, 2023). Many standardized herbal remedies have been authorized recently to treat diabetes mellitus and its consequences (Alqathama et al., 2020).

Origanum majorana (OM) is a member of the Lamiaceae family of mints. Often referred to as sweet marjoram, marjoram offers a wide range of applications and health advantages (Singletary 2010). It is a plant that grows naturally in Mediterranean regions (Tripathy et al., 2017). Many of the bioactive substances in OM, like phenolic acids, flavonoids, and terpenoids, can help with diabetes, inflammation, and free radicals (Bouyahya et al., 2021).

Research indicates that OM extract can effectively treat diabetes mellitus (DM) due to its strong anti-hyperglycemic properties and ability to normalize histopathological changes caused by uncontrolled blood glucose levels (Vujicic et al., 2015). Researchers are interested in nanotechnology due to

its potential to improve the medicinal effectiveness of herbal extracts. Adding herbal extracts to nanostructures or making them smaller can make them more effective by lowering the dose, improving bioavailability, stability, and solubility, and making it easier for cells to take them in and distribute them in the body for better targeting (Gera et al., 2017). Concisely, nano-formulations of herbal medications may increase their pharmacological activity at lower dosages than free herbal pharmaceuticals (Wani et al., 2015). The study looked at how well OML extract and its nanoparticle form worked on diabetic rats, specifically on tissue damage caused by STZ. Histopathological analysis of liver tissue was conducted to assess the potential antidiabetic and hepatoprotective effects of OML extract and nanoparticles in streptozotocin-induced diabetic rats. Understanding these properties could offer new diabetes management strategies and alternative treatments.

2. MATERIAL AND METHODS**2.1 .Chemicals****A) Streptozotocin**

Streptozotocin (STZ) (Batch No. 2), 2-Deoxy-2-(3-methyl 3-nitro shureido)-D-glucopyranose. Reconstituted in 0.1 M citrate buffer (pH = 4.5) was acquired from Sigma-Aldrich (Germany). and induced diabetes in rats via IP injection at a dose of 50 mg/kg (Brahmam et al., 2023).

B) Glibenclamide

sometimes referred to as 5-chloro N (4 [N (cyclohexyl carbamoyl) sulfamoyl] phenethyl), 2-methoxy benzamide, was purchased from Hoechst Pharmaceutical Services,

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LLC., Germany. The medication marketed as daonil is glibenclamide. It is used to treat type 2 diabetes (T2DM) with this oral anti-diabetic medication (Almulathanon et al., 2021). Glibenclamide was given as usual to diabetic rats via gastric intubation, but the tablets were broken up, suspended in distilled water, and given daily at a dose of 5 mg/kg (El-Sabawi et al., 2013).

2.2 . Collection and processing of *Origanum majorana* (OM) leaves

The procedure described by Benhalilou et al. (2019) was followed to prepare the OML extract, which was purchased from the market as a green plant and dried at room temperature before being pulverized into somewhat smaller particles.

Each rat was administered OM leaves extract orally at a dose of 200 mg/kg for 30 days. This dosage is considered optimum and harmless for rats, and it is an example of a nontoxic concentration for several plants, such as *Vatairea macrocarpa* (Oliveira et al., 2008).

2.3 . Preparation of OML Extract

A mixture of 50 g of dried leaf powder and 1:10 double-distilled water was kept at 100 °C for 15 minutes. The mixture was then refrigerated for 72 hours. Next, the filtrate was dried out by shaking the mixture in a rotary evaporator set to room temperature. Leaf fragments were eliminated from the extract using No. 1 filter paper. For later usage, the resulting clear extract is stored at 4 °C. The volume of the green residue was measured after it was vacuum-stored for two to three hours. OME was administered orally to rats at a dosage of 200 mg/kg b.wt. by Pasavei et al.(2020) .

2.4 . Synthesis of silver nanoparticles in OM leaf extract (Ag/OMLE)

The process of co-precipitation was used to create the Ag-OMLE-NPs composite. In this procedure, a 100-mL solution containing 1 mM of AgNO₃ was mixed with 10 g of powder. The aforementioned solution was vigorously stirred and mixed for 60 minutes at 343 K, and then it was allowed to condense for 24 hours at 80 °C. Once the product is ready for use, it is cooled and kept in a sterile, sealed bottle.

2.5 . Experimental Animals

This study has been conducted in compliance with the requirements of the Declaration of the Ethical Committee (Ethical Approval Number: BUFVTM09-03-24). The study involved 25 Albino male rats from Benha University, housed with a 12-hour light-dark cycle and a temperature of 21 to 23 °C. The rats were housed for fifteen days before the trial began. They were fed a balanced commercial pellet diet, including 5 percent fat, 21% protein, 55% nitrogen-free extract, and 4% fiber. Water was available at all times, and the diet included recommended amounts of minerals and vitamins. (Addass et al., 2010).

2.6 . Induction of diabetes

In the rat model, diabetes was caused by an intraperitoneal (I.A.P.) infusion of 50 mg/kg body weight of STZ diluted in 0.4 ml of freshly made citrate buffer (pH 4.5) that was given after the rats had not eaten or drunk anything for 24 hours (Rakieten et al., 1963). Plasma glucose was measured 48 hours following the STZ infusion to confirm the induction of a diabetic rat model. Rats with blood glucose levels greater than 250 mg/dL were considered diabetic and included in the study.

2.7 . Experiment Design

2.7.1 . Animal Groups

Rats were split up into five groups of five, and each group was kept in a separate cage to study the anti-diabetic and hepatoprotective effects of OML extract.

Group I (the control group): Throughout the trial, rats were fed a regular diet without any medication.

Group II (Diabetic Group) Rats were injected with STZ (50 mg/kg) to induce diabetes.

Group III (glibenclamide-treated group): Rats were injected with STZ (50 mg/kg) and treated with the glibenclamide drug at a dose of 5 mg/kg daily for 4 weeks.

Group IV (OMLE-treated group): rats were injected with STZ (50 mg/kg) and administered OML extract (200 mg/kg b.w./daily) orally for 4 weeks.

Group V (OMLE NP-treated group): rats were injected with STZ (50 mg/kg) and administered OML NP extract (20 mg/kg b.w./daily) orally for 4 weeks.

After the animals were slaughtered, blood samples from the treatment and control groups were drawn for examination at the end of the trial.

2.7.2 . Collection of Blood Samples and Isolation of Organs

At the end of the trial, blood samples from the medial eye canthus were collected. Every sample was separated into two tubes: A clot activator for the serum and an EDTA anticoagulant for the plasma. The blood was centrifuged for 15 minutes at 3000 rpm after being left at room temperature for 15 minutes. After that, the clean serum was kept cold until analysis. In all groups, the livers of rats were quickly removed, fat-free, and cleaned with fresh tissue paper after they had been dissected.

2.8 . Biochemical assay

Blood sugar was assessed using Barham and Trinder (1972) assay, glycosylated hemoglobin was measured following Aba et al. (2018). While, insulin was measured according to Byersdorfer et al. (2005). Liver enzymes (ALT and AST) and alkaline phosphatase (ALP) were analyzed following the protocols of Huang et al. (2006) and Belfield and Goldberg (1971) respectively. The level of lactate dehydrogenase (LDH) was assessed according to Reinhold, (1953). Total protein was measured according to Richmond (1973). The serum level of total cholesterol (TC) and total glycerolaldehyde (TG) were measured following Treitz (1995). Malondialdehyde (MDA) and glutathione were measured according to Jean et al., (1983) and Beutler et al., (1963) respectively.

2.9 . Tissue Sample and Histopathological Examination

For histopathological assessment, small tissue specimens were obtained from the pancreas, liver, and kidneys of the rats in all groups. These specimens were fixed in 10% neutral buffered formalin for 72 hours. Following proper fixation, specimens were dehydrated in ethyl alcohol before being cleared in xylene and embedded in paraffin wax. Using a rotatory microtome, tissue paraffin sections were cut at a thickness of 4–5 µm. According to Bancroft and Layton (2013), these sections were stained with hematoxylin and eosin. Histopathological changes in these stained sections were examined with a Nikon Eclipse E800 light microscope, and photomicrographs were taken with a digital camera.

2.10 . Statistical analysis

The SPSS software version 25.0 was used to statistically analyze the collected data. A one-way analysis of variance (ANOVA) was used to identify significant differences

between the groups. The significance between the groups was then compared. Data were considered significant if the P-value was < 0.05.

3. RESULTS

As presented in table (1), the diabetic rats treated with glibenclamide and OML showed a notable decrease in glucose and HbA1c levels compared to the diabetic control group. While diabetic rats treated with OMLNPs extract showed a significant decrease in serum glucose levels compared to the glibenclamide-treated group and diabetic group but was non-significant in HbA1c compared to the glibenclamide-treated group.

In diabetic rats treated with OML and OML NPs, the insulin levels also showed a significant increase compared to the control diabetic group and the glibenclamide-treated group (table, 1).

In STZ-induced diabetic rats, the study examined the effects of glibenclamide, OML, or OML NPs extract on liver enzyme levels. The diabetic rats treated with glibenclamide showed a significant decrease in ALT, ALP, and LDH activities, while there was no significant decrease in AST activity compared to the diabetic group. Furthermore, OML

and OML NP extract reduced AST and ALT activities significantly, with no significant decreases in ALP or LDH activities in comparison to the diabetic group and the diabetic group treated with glibenclamide, while no significant decrease was observed in ALP or LDH activity (table, 1).

The result of the serum biochemical analysis showed that diabetic rats treated with glibenclamide had a significantly higher total protein level than the diabetic group. While diabetic rats administered OML and OML NP extract showed no significant change in total protein level when compared to the diabetic group treated with glibenclamide and the diabetic group treated with OML extract (table, 1)

Regarding the effect of glibenclamide and OML and OML NP extract on TC and TG levels in STZ-induced diabetic rats, our findings revealed that diabetic rats treated with glibenclamide and diabetic rats administered of OML or OML NPs extract had a significant decrease in TG and TC levels when compared to the diabetic group, while OML or OML NP extract had a non-significant decrease in TC level when compared to the diabetic group treated with the glibenclamide drug (table, 1).

Table (1): Effect of Glibenclamide, OML or OML NPs extracts on the serum biochemical Parameters in Streptozotocin-induced Diabetic Rats:

Variable	Non diabetic Control group	Diabetic group control	Diabetic group treated with Glibenclamide	Diabetic group treated with OML	Diabetic group treated with OML NPs
Glucose (mg/dL)	91.60±3.20 ^d	360.60±22.69 ^a	236.00±12.97 ^b	202.60±11.96 ^b	136.80±14.88 ^c
HbA1c (mmol/L)	4.08±0.31 ^d	11.80±0.44 ^a	7.82±0.22 ^{bc}	8.04±0.17 ^b	7.00±0.22 ^c
Insulin (mIU/L)	15.18±0.29 ^a	5.54±0.23 ^c	9.80±0.32 ^d	11.36±0.61 ^c	12.78±0.58 ^b
ALT (U/L)	28.20±2.27 ^d	72.40±4.37 ^a	62.60±2.99 ^b	44.20±2.40 ^c	37.00±2.86 ^{cd}
AST (U/L)	34.40±2.25 ^b	70.20±5.32 ^a	62.00±3.81 ^a	41.00±2.55 ^b	34.60±2.68 ^b
ALP (U/L)	99.60±3.91 ^d	198.60±5.28 ^a	145.00±7.46 ^b	138.40±4.78 ^b	119.00±6.49 ^c
LDH (U/L)	116.00±12.68 ^d	275.60±16.25 ^a	216.60±7.26 ^b	195.20±5.98 ^b	160.40±10.93 ^c
Total Protein (g/dL)	7.68±0.19 ^a	5.88±0.18 ^c	6.88±0.12 ^b	6.88±0.14 ^b	7.24±0.14 ^{ab}
TC (mg/dL)	106.20±5.91 ^c	226.20±7.81 ^a	147.60±8.18 ^b	127.60±6.65 ^{bc}	123.80±7.17 ^c
TG (mg/dL)	107.40±8.66 ^b	232.60±23.68 ^a	144.20±14.94 ^b	134.80±9.72 ^b	98.60±9.51 ^b

Data are presented as (Mean ± S.E). S.E = Standard error. Mean values with different superscript letters in the same column are significantly different at (P<0.05).

HbA1c: glycated haemoglobin; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; ALP: Alkaline phosphatase; LDH: Lactate dehydrogenase; TG: Triglyceride; TC: Total cholesterol

The current study revealed that the MDA level of the diabetic rats treated with glibenclamide was significantly lower than that of the diabetic control group. When compared to the diabetic group, these rats with diabetes had higher GSH levels. In diabetic rats with OML or OML NP extracts, MDA levels decreased significantly in comparison to the diabetic group and did not change significantly when compared to the diabetic group that received

glibenclamide. Conversely, the diabetic rats given OML or OML NP extract showed a non-significant change in GSH levels when compared to the diabetic group receiving glibenclamide but a significant increase when compared to the diabetic group. Also, the GSH level dropped a lot in the diabetic rats that were treated with OML extract compared to the diabetic rats that were treated with OML NP extract (table, 2).

Table (2): Effect of Glibenclamide, OML or OML NPs on antioxidant parameters (MDA or GSH), in STZ-induced diabetic rats

Variable	Non diabetic Control group	Diabetic group control	Diabetic group treated with Glibenclamide	Diabetic group treated with OML	Diabetic group treated with OMLNPs
MDA (nmol/L)	3.33±0.12 ^c	6.14±0.09 ^a	4.91±0.23 ^b	4.91±0.11 ^b	4.57±0.21 ^b
GSH (mg/dL)	11.68±0.26 ^a	6.30±0.30 ^c	9.44±0.28 ^b	9.74±0.28 ^b	11.44±0.22 ^a

Data are presented as (Mean ± S.E). S.E = Standard error. Mean values with different superscript letters in the same column are significantly different at (P<0.05). MDA: Malonyldialdehyde, GSH: Glutathione

Histopathological findings:

Almost all liver sections of rats in the control group showed normal hepatic plate histoarchitecture. Hepatocytes were organized into hepatic plates surrounding the central veins. The cytoplasm of these liver cells was eosinophilic, and the nuclei were vesicular. The portal areas had normal bile ducts and portal veins (Fig. 1A). In contrast, almost all examined liver sections of STZ-diabetic rats displayed

substantial hepatic damage, as evidenced by severe hepatic congestion and widespread hepatic cell degeneration and necrosis (Fig. 1B). The rats showed multifocal hemorrhages, lymphocytic cell infiltration, and biliary ductal damage. Epithelial hyperplasia was seen on occasion. Meanwhile, the majority of the liver sections in diabetic rats treated with Daonil (glibenclamide) indicated a significant hepatoprotective effect of this drug. Only portal congestion

and moderate hyperplasia of the biliary epithelium were seen in some of the examined liver sections. Dilatation of the central veins with degeneration of some hepatic cells was also observed (Fig. 1C). Also, marjoram extract treatment partially reduces liver damage in diabetic rats. Mild hepatocellular degeneration and portal congestion with aggregation of lymphocytes in the portal areas were infrequently seen (Fig. 1D). Interestingly, marjoram nanoparticle treatment had a much greater hepatoprotective effect in diabetic rats than marjoram extract where there was no evidence of inflammatory reaction or hepatocellular necrosis in the majority of the examined liver sections. However, individual hepatic cell necrosis and centrilobular hepatic degeneration were occasionally seen in a few examined liver sections (Fig. 1E).

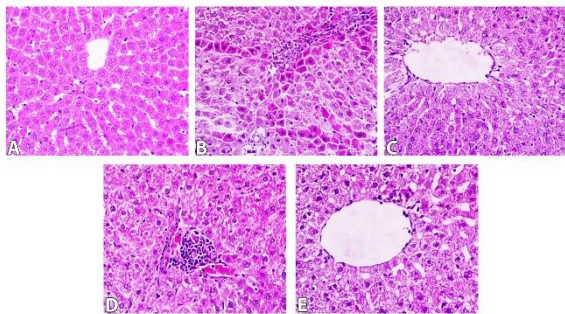


Fig. 1. Representative photomicrographs of the liver of rats in (A) control group showing well-organized hepatocytes arranged in hepatic cords surrounding the central vein (B) Diabetes group showing periportal hepatocellular degeneration and necrosis (C) glibenclamide treated group showing degenerated hepatic cells surrounding dilated central vein (D) marjoram extract treated group showing mononuclear cells infiltration of the portal area with congested blood vessels (E) marjoram nanoparticles treated group showing a few necrotic hepatic cells scattered in between degenerated hepatocytes around central vein. H&E stain X200.

4. DISCUSSION

Diabetic hepatic damage is a serious complication of uncontrolled diabetes mellitus. Unfortunately, the efficient antidiabetic drug that preserves the normal liver parenchyma is unavailable. The present study evaluated the antidiabetic and hepatoprotective effects of *Origanum majorana* leaf extract and its nanoparticles on streptozotocin-induced diabetes in rats. The results of serum biochemical analysis found that diabetic rats treated with glibenclamide had lower glucose and HbA1c levels and higher insulin levels compared with the control group. While diabetic rats treated with OML and OML NPs extract showed significant decreases in glucose and HbA1C levels when compared to the diabetic group, there was no significant change in HbA1C when compared to the diabetic group treated with glibenclamide. The diabetic rats treated with OML and OML NPs also showed an increase in insulin levels compared to both the diabetic control group and the diabetic group treated with glibenclamide. These results were consistent with the findings of Farag et al. (2022). Also, Ghudhaib and Khaleel (2024) found that diabetic rats given OML or OML NP extract had significantly lower blood glucose and HbA1C levels and significantly higher insulin levels compared to diabetic rats given glibenclamide medication. Mohamoud et al. (2020) found that administration of OML extract, a traditional medicinal plant, to diabetic rats diminished their increased HbA1C and blood glucose levels. Furthermore, Cakar et al. (2023) discovered that AST, ALT, LDH, and ALP levels rose in diabetic rats without therapy while decreasing dramatically in the treated groups. Our results revealed that the diabetic rats treated with glibenclamide had a significantly higher total protein level than the diabetic control group. While diabetic rats

administered OML and OML NP extract showed no significant change in total protein level when compared to the diabetic group treated with the glibenclamide drug and the diabetic group treated with OML extract, these results were consistent with the findings of Farag et al. (2022). Previous experimental studies conducted by Tripathy et al. (2018) demonstrated the protective impact of OML or OML NP extract and glibenclamide administered shortly after diabetes diagnosis, which reduces blood TC and serum TG levels.

The results also revealed that diabetic rats treated with glibenclamide, OML, and OMLNP extracts had significantly lower MDA and higher GSH levels compared to the control diabetic group; however, there were non-significant differences between them. These results were consistent with those of Güllü et al. (2023).

The histopathological examination of the liver of diabetic rats showed alterations and disruptions caused by STZ injection. Treatments with glibenclamide and OML and OMLNP extracts restored the liver's structure to a more or less normal histoarchitecture.

5. CONCLUSIONS

These findings have demonstrated that OML and OML NP extracts can shield the hepatic tissues from the negative effects of diabetes mellitus. This effect could be attributed to the strong antioxidant activities of these extracts.

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