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Evaluation of antidiabetic and nephroprotective effects of *Origanum majorana* (Marjoram) Leaf Extract and its Nanoparticles on Streptozotocin-Induced diabetes in Rats

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Abstract

Background: Medicinal herbs and green manufacturing of silver nanoparticles (AgNPs) are effective treatments for diabetes. *Origanum majorana* (OM) leaf extract is known for its potential medicinal benefits, including anti-diabetic activities. The study's objective was to assess the efficiency of OML extract and nanoparticles in diabetic rats induced with Streptozotocin (STZ), as well as their histological effects on Liver organ tissue.

Methods: 25 Diabetic Rats were separated into 5gps: Group I: (n = 5) untreated (Non-diabetic Control), Group II: (n = 5) injected with Streptozotocin (STZ) untreated diabetic group, Group III: (n = 5) diabetic rats treated with the glibenclamide drug, Group IV: (n = 5) diabetic rats administration of OM leaf extract, Group V: (n = 5) diabetic rats administration of OM leaf extract nanoparticles. Various parameters including blood glucose levels, insulin, lipid profile, Liver and Kidney function test, antioxidant (MAD & GSH), and histopathological changes in the Liver were evaluated.

Results: Both *Origanum majorana* leaf extract and its nanoparticles dramatically lowered blood glucose levels when compared to the untreated diabetic group. Treatment with OM leaf extract nanoparticles showed superior effects in improving insulin sensitivity, lipid profile, and Liver and Kidney enzymes compared to the extract alone. Additionally, both formulations exhibited MDA properties, as evidenced by decreased levels and increasing GSH levels. 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.

Conclusion: OM leaf extract and its nanoparticles demonstrate promising Anti-diabetic characteristics in diabetic rats triggered by STZ, along with protective effects against histopathological alterations in the liver. The nanoparticle formulation exhibits enhanced efficacy, possibly attributed to improved bioavailabilityFurther study is needed to explain the underlying processes and investigate their potential therapeutic applicability in diabetes control, while also taking into account the histopathological consequences.

Keywords: *Origanum majorana*, Silver nanoparticles, Diabetes Mellitus, Streptozotocin, Males Albino rats, Glibenclamide drug.

1. Introduction:

Diabetes is a metabolic illness characterized by hyperglycemia, beta-cell dysfunction, insulin resistance, or both. It is a significant public health concern. Furthermore, diabetes problems may be linked to variations in the body's antioxidant defense system, oxidative stress, and dyslipidemia **[1].**

Currently, available treatments for diabetes mostly include the use of insulin or oral hypoglycemic medications. These medications are not only very expensive but also have a lot of negative side effects, such as hyponatremia, obstructive jaundice, headaches, nausea, vomiting, and weight gain **[2]**.

Because of their perceived efficacy and comparatively lower side effects when compared to conventional pharmaceuticals, herbal treatments have attracted a lot of interest for their possible therapeutic advantages in the management of diabetes **[3].**

The majority of people in both industrialized and developing nations receive their primary medical treatment from herbalists since they are frequently seen as a reasonable and well-rounded approach

to treat chronic illnesses. Many standardized herbal remedies have been authorized recently to treat diabetes mellitus and its consequences **[4]**.

Origanum majorana (OML) is a member of the Lamiaceae family and is widely utilized in the culinary, traditional medicine, and cosmetic sectors. It is a plant that grows naturally in Mediterranean regions **[5].** Many civilizations have used it medicinally for a very long time **[6]**. Many bioactive substances found in *Origanum majorana*, such as phenolic acids, flavonoids, and terpenoids, have anti-inflammatory, anti-diabetic, and antioxidant qualities **[7]**.OML extract has been shown in earlier research to be capable of increasing responsiveness to insulin and reducing hyperglycemia in diabetic animal models **[8]**.

Previous research on the pharmacological effects of *Origanum majorana* (OM) extract in vitro and in vivo has shown that it can be used to treat DM due to its strong anti-hyperglycemic effects and ability to normalize various histopathological changes caused by uncontrolled blood glucose levels through its anti-apoptotic, immunomodulatory, and antioxidant properties **[9]**. Researchers are interested in nanotechnology because it is a cutting-edge and extremely useful field that has opened up many possibilities for advancement in the medical sciences. Benefits from targeted distribution, increased bioavailability, and extended circulation times provided by nanoparticle-based drug delivery methods may improve the medicinal effectiveness of herbal extracts. The pharmacokinetic profile and bioactivity of OM leaf extract may be enhanced by encapsulating it in nanoparticles, which would increase its anti-diabetic properties.

Nano-sizing or incorporating herbal extracts into nanostructures can improve their efficacy by reducing dosage, increasing bioavailability, stability, and solubility, and improving cellular uptake and biodistribution for better targeting **[10]**

Recent research has examined the features of plant extracts, including their nano size, antioxidant content, phenolic content, cytotoxicity, bio-activities, and safety, as well as their impact on diabetic complications **[11].**

Within this framework, the current investigation sought to determine the effectiveness of OML extract and its nanoparticle formulation in diabetic rats caused by strychnine. Furthermore, to assess OM's potential defences against tissue damage brought on by diabetes, a histological analysis of key organs, including liver tissue, was carried out. Comprehending the medicinal properties of OML

extract and its nanoparticle form may provide new perspectives on managing diabetes and open the door to the creation of supplementary and alternative treatments.

2. Materials and Methods:

2.1. Chemicals:

A) Streptozotocin

Strapozotocin (STZ): 2-Deoxy-2-(3-methyl 3-nitro shureido)-D-glucopyranose. Sigma-Aldrich (Germany) provided a reconstituted dosage of 50 mg/kg b in 0.1 M citrate buffer (pH = 4.5) (126k1174). To establish a diabetic rat model, inject intraperitoneally after dissolving for 15 minutes **[12]**

B) Glibenclamide

The medicine being promoted is glibenclamide, also known as glyburide. This oral anti-diabetic medicine treats type 2 diabetes (T2DM) **[13]** Glibenclamide was administered as normal to diabetic rats by stomach intubation, but the pills were broken up, suspended in distilled water, and given daily at a rate of 5 mg/kg. As per the FAD Guidance (2000) **[14]**

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

The procedure of **Benhalilou *et al* [15]**was used to create the OML extract, which was obtained as a green plant and dried at room temperature before being crushed into somewhat finer particles. In accordance with institutional guidelines, no further authorization was required to conduct research on the plant.

Administration of plant extract:

A plant extract dose of 200 mg/kg was given orally for 28 days. This dose was chosen for a variety of reasons. This dosage is regarded optimal and safe to rats, and it is an example of a nontoxic concentration for various plants, such as Vatairea macrocarpa **[16]**

2.3. Getting an equivalent extract from leaves of OM

A combination of 50 g dried leaf powder and 1:10 double-distilled water was heated to 100 °C for 15 minutes. The combination was then chilled for 72 hours. The filtrate was then dried by shaking it in a rotary evaporator at room temperature. Whatman Leaf fragments were removed from the extract using a No. 1 filter paper. The clear extract produced should be kept at 4 °C for subsequent use. The green residue's volume was measured after two to three hours of vacuum storage. Each rat was given the indicated dose in 1 mL of solvent from a previously prepared stock solution. Using an OME dosing rate of 200 mg/kg body weight **[17]**

2.4. Silver nanoparticles (AgNPs) and OM Leaf materials

- **Materials:**

  We purchased sodium hydroxide (NaOH) and analytical grade silver nitrate (AgNO3) from Sigma-Aldrich chemicals (98 percent purity). The N95 mask fibers and textile cotton were obtained at Cairo's local market. Whatman No. 1 was used to prepare the leaf extract. For erosion and filtration, one filter paper was utilized. Additionally, to clean glassware (beaker, pipette, etc.), distilled water and ethanol were utilized.

- **Method**

Syntheses of Silver nanoparticles at OM leaf extract (Ag /OMLE):

The Ag OMLE NPs mixture was created by co-precipitation. In this technique, a 100 mL solution containing 1 mM AgNO3 was combined with 10 g of sieved powder. The aforementioned solution was rapidly agitated and blended at 343 K for 60 minutes before being allowed to condense at 80°C for 24 hours. When the product is ready to use, it is cooled and stored in a sealed, sterile bottle. To make the extract, 2.0 g of OM leaf broth was boiled for 15 minutes, then filtered and diluted to 100

ml. The filtrate, which would be utilized as a reducing agent, was held at 10 °C in the dark for a week.

In the experimental investigation, a color shift from initial yellow to dark brown was seen as soon as the OM leaf extract was combined with the silver nitrate solution.

Characterization of silver nanoparticles

Scanning electron microscopy (SEM) examination was used to evaluate the surface morphology and form of the Ag nanoparticles. The results showed that the Ag NPs were primarily quasispherical in form. However, a handful had unusual forms, as shown in SEM pictures. (**Fig. 1a, b**).

The transmission electron microscopy (TEM) pictures (**Fig. 1c**) clearly show the synthesised Ag NPs, including their exact sizes and forms. The particles were mostly spherical and consistent in size, with an average size of around 30 ± 5 nm. Furthermore, the Ag NPs had good dispersion, and high-resolution TEM images indicated a smooth surface. (**Fig. 1d**) shows that 5 nm PdNPs have a unique spherical form. The TEM study clearly shows that the rhthtehte extract formed a protective surface layer over the Ag NPs, thereby avoiding aggregation.

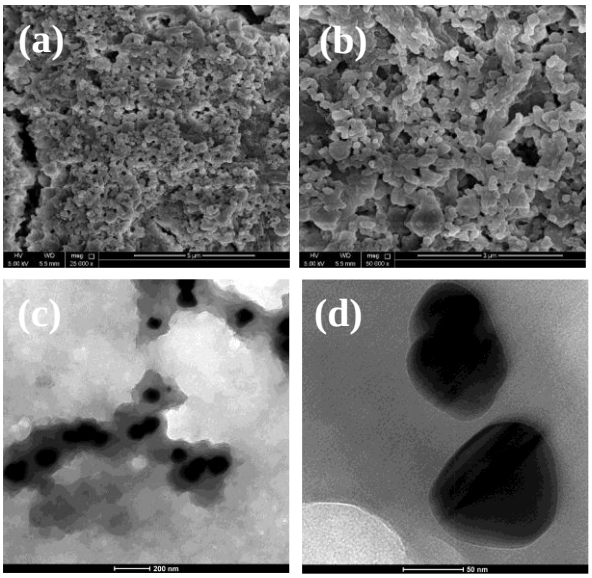

Figure (1): SEM (a & b) and TEM (c & d) for Ag/OML extract

2.5. *In-Vivo* Anti-diabetic Activity

2.5.1. Experimental Animals

This study was carried out in accordance with the Declaration of the Ethical Committee (Ethical Approval Number: **BUFVTM09-03-24**) for Institutional Animal Use and Care at the Faculty of Veterinary Medicine, Benha University. In this investigation, 25 albino male Wistar rats were employed. The weights varied from 160 to 180 g. The rats were housed in an air-conditioned animal housing with a 12-hour light/dark cycle. The temperature was kept between 21 and 23 degrees Celsius. The animals were housed for fifteen days before the study began to allow them time to acclimate.

2.5.2. Induction of diabetes

Diabetes was actuated within the rodent show by single intraperitoneal (I. A. P. infusion of 50 mg/kg body weight of STZ weakened in 0.4 ml of crisply made citrate buffer (pH 4.5) after an

overnight fasting **[18]**.Plasma glucose levels were measured 48 hours after STZ infusion to confirm the improvement in a diabetic rat model. The study included rats diagnosed with diabetes whose blood glucose levels were greater than 250 mg/dL. After two days of diabetes induction, therapy was began and was delivered for four weeks.

2.5.3. Experiment Design

Animal Groups:

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

Group I (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 become diabetic induced.

Group III (glibenclamide treated group): Rats injected with STZ (50 mg/kg) and treated with glibenclamide drug by a dose of 5 mg/kg daily for experiment period study.

Group IV (OMLE treated group**):** Rats injected with STZ (50 mg/kg) and administrated of OML extract (200 mg/ kg b.w/daily) orally for 4 week.

Group V (OMLE NP treated group): Rats injected with STZ (50 mg/kg) and administrated of OML NP extract (20 mg/ kg b.w./daily) orally for 4 week.

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

2.5.4. Collection of Blood Samples and Isolation of Organs:

Blood samples from the medial eye canthus were collected at the end of the study. Each sample was divided into two tubes: clot activator for serum and heparinized tubes for plasma. Following a 15-minute period at room temperature, the blood was centrifuged for 15 minutes at 3000 RPM. Following that, the clean serum was kept refrigerated until the analysis. Following dissection, the rats' pancreas and kidneys were quickly removed, fat-free, and cleaned with fresh tissue paper.

2.6. Biochemical assay:

Blood sugar was assessed using the colorimetric technique outlined by **Barham** and **Trinder [19]** Commercially available rat C-peptide ELISA kits (Mercodia, Uppsala, Sweden) were utilized to assay serum C-peptide. The C-peptide test has an inter-assay precision of 2.9 and an intra-assay precision of 4.4, respectively, and a sensitivity of 27.5 pmol•L-1 **[20]**The amylase was identified by means of the 2-choloro-4-nitrofenylo-α-maltrioside (CNP-G3 method), an enzymatic colorimetric technique **[21].**

Renal functions were decided by estimating blood urea, utilizing the strategy depicted by **Shrestha *et al*. [22]**and serum creatinine level as depicted by **Bartels** and **Bohmer [23]**. strategy. Sandwich enzyme-linked immunosorbent assay (ELISA) Abcam was used to detect TNF-α, IL-6, IL-1 β, and IL-10 in serum. The optical density was read on a microtiter plate reader with 450 nm (Stat fax 303 Instruments, USA) **[24]**

2.7. Tissue Sample and Histopathological Examination:

For histopathological assessment, small tissue specimens were obtained from the pancreas 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 [25]**. these sections were stained with hematoxylin and eosin. The histopathological changes in these stained sections

were examined with a Nikon Eclipse E800 light microscope and photomicrographs were taken with a digital camera.

2.8. Statistical analysis

The SPSS software version 25.0 was used to statistically analyze the collected data in accordance with Glantz's methodology. One-way analysis of variance (ANOVA) was used to identify significant differences between the groups. The significance between the groups was then compared at p-value < 0.05 using a post hoc test that employed the Duncan test.

3. Results:

As illustrated in **Table 1** The study found that diabetic rats treated with glibenclamide showed decreased glucose levels compared to the diabetic control group. However, diabetic rats treated with OML extract showed a significant decrease in serum glucose levels comparing diabetic control group, and non significant with glibenclamide . Additionally, diabetic rats treated with OML NPs extract showed a significant decrease in glucose levels when compared with diabetic group, diabetic group treated with glibenclamide and diabetic group administrated of OML extract.

Furthermore, the study revealed that diabetic rats given with glibenclamid or OML extract showed a noticeable rise in C-peptide and a decrease in amylase activity when compared to the diabetic control group, but no significant differences when compared to each other. Additionally, diabetic rats given OML NPs had a statistically significant increase in C-peptide levels and a drop in amylase activity when compared to the diabetic control group, diabetic rats treated with glibenclamide, and diabetic rats given OML extract.

The study results showed that diabetic rats treated with glibenclamide had a substantial drop in urea and creatinine levels as compared to the diabetic group. Furthermore, urea and creatinine levels in diabetic rats given OML extract were considerably lower than in the diabetic group and non-significantly higher than in the diabetic group treated with glibenclamide medication in urea level. Concurrently, OML NPs extract revealed a significant reduction in urea and creatinine levels when compared to all treated groups.

As illustrated in **Table** (**2**), The current study found that diabetic rats treated with glibenclamide had significantly lower TNF-α, IL-1β, and IL-6 levels compared to the diabetic group. Furthermore, diabetic rats administered with OML extract showed a significant decrease in TNF-α, IL-1β, and IL-6 levels as compared to the diabetic group, and a non-significant decrease in TNF-α, IL-1β, and a significant decrease in IL-6 level when compared with the diabetic group treated with glibenclamide. Diabetic rats given with OML NPs extract had significantly lower levels of TNF-α, IL-1β, and IL-6 compared to the diabetic group, diabetic group treated with glibenclamide, and non-significant change in IL-6 compared to the diabetic group administered with OML extract.

In contrast, diabetic rats treated with glibenclamide showed a considerable rise in IL-10 levels when compared to the diabetic group. When the diabetic rats administered of OML extract shows a significant increase in IL-10 level when compared with the diabetic group and diabetic group treated with glibenclamide. the diabetic rats administered of OML NPs extract showed a significant increase in IL-10 level when compared to all diabetic groups

Table (1): Effect of Glibenclamide, OML or OML NPs extracts on Biochemical Parameters in STZ-induced Diabetic Rats:

Variable	Control group	Diabetic group control	Diabetic group treated with	Diabetic group treated with OML	Diabetic group treated with

			Glibenclamide		**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}
C-Peptide (pmol/L)	0.93±0.01^{a}	0.35±0.02^{c}	0.65±0.03^{b}	0.64±0.05^{b}	0.87±0.04^{a}
Amylase (U/L)	119.20±7.38^{d}	313.20±7.95^{a}	233.40±15.23^{b}	215.80±10.09^{b}	167.60±6.38^{c}
Urea (mmol/L)	27.20±1.16^{b}	49.40±3.37^{a}	30.40±4.23^{b}	34.00±1.64^{b}	27.80±2.42^{b}
Creatinine (mg/dL)	0.74±0.05^{d}	1.54±0.03^{a}	1.18±0.06^{b}	1.02±0.05^{c}	0.84±0.03^{d}

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 ($P<0.05$).

Table (2): Effect of glibenclamide, OML or OML NPs extract on inflammatory cytokine level, in STZ-induced diabetic rats

Variable	**Control group**	**Diabetic group control**	**Diabetic group treated with Glibenclamide**	**Diabetic group treated with MOL**	**Diabetic group treated with MOL NPs**
TNF-α (pg/mL)	32.80±1.59^{d}	57.20±2.06^{a}	47.60±1.54^{b}	43.00±1.64^{bc}	40.40±1.21^{c}
IL1β (pg/mL)	31.04±0.36^{d}	94.14±3.77^{a}	61.48±2.50^{b}	61.06±1.56^{b}	52.90±2.70^{c}
IL6 (pg/mL)	4.27±0.44^{c}	12.54±0.63^{a}	7.52±0.33^{b}	5.35±0.33^{c}	5.34±0.26^{c}
IL10 (pg/mL)	17.42±0.37^{a}	7.40±0.49^{e}	9.38±0.19^{d}	10.74±0.37^{c}	12.74±0.52^{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 ($P<0.05$).

IL: Interleukin; **TNF**: Tumor necrosis factor

As illustrated in the histology of pancreatic tissue in normal and STZ-induced diabetic rats, The microscopic examination of the pancreas of rats in the control group revealed normal histoarchitecture of the exocrine and endocrine portions. The exocrine component comprises pancreatic acini and duct cells, whereas the endocrine portion appears as distributed masses of islets of Langerhans formed from polygonal pale cells with beta-cells in the center (Fig. 2A).

On the other hand, the majority of pancreas sections of diabetic rats showed substantial damage to the islet of Langerhans, as evidenced by necrosis and degeneration, as well as significant reductions in the sizes and number of cells (Fig. 2B). The majority of these islets had been infiltrated by mononuclear inflammatory cells. Meanwhile, the pancreatic acinar cells showed multifocal degeneration and even necrosis (Fig. 2C). The pancreatic blood arteries were severely congested, with perivascular fibrosis, edema, and inflammatory cell aggregation. Furthermore, the interlobular ducts were dilated and lined with flattened cells and filled with eosinophilic secretion.

Preadministration of glibenclamide to diabetic rats was related to an improvement in the microscopic image of the studied pancreas, with the majority of pancreatic sections demonstrating almost normal histoarchitecture of the endocrine and exocrine pancreas. However, in this group the number of Langerhans islet cell populations was smaller than in the control group (Fig. 2D). Furthermore, several pancreas sections showed congestion of the pancreatic blood vessels as well as moderate edema around the pancreatic ducts and blood vessels.
Compared to the diabetic group, marjoram extract treatment alleviated the pancreatic parenchyma where the islets of Langerhans and pancreatic acini were more or less similar to those in the control (Fig. 2E) and only congestion of the pancreatic vessels and cystic dilatation of few interlobular ducts with retained eosinophilic secretions in their lumens were seen in some examined pancreas. In addition, focal degeneration of some pancreatic acinar epithelium was infrequently observed.
Similarly, treatment with marjoram nanoparticles restored the pancreatic damage induced by the STZ in diabetic rats. Most of the islets of Langerhans and pancreatic acini appeared normal like those in the non-diabetic control group. In addition, there were no signs of inflammation or degenerative changes in the majority of the examined pancreatic sections (Fig. 2F).

The examined kidney sections of rats in the non-diabetic control group showed normal microscopic architecture of the renal cortex and medulla. The renal corpuscles were formed by glomerular capillary tufts, mesangium, and Bowman's capsules, while the renal tubules were lined by simple cuboidal epithelium with eosinophilic cytoplasm and central rounded nuclei (**Fig. 3A**).
While the examined kidney sections in non-treated STZ-induced diabetic rats revealed significant renal damage. Most kidney sections in diabetic rats had severe tubular damage in the form of degeneration and necrosis of the renal tubular epithelium. There was also glomerular shrinkage and congestion, as well as thickening of Bowman’s capsule with periglomerular mononuclear inflammatory cell aggregation (**Fig. 3B**). Furthermore, vascular injury comprising endothelial damage, tunica muscularis degeneration was seen, in addition to perivascular lymphocytic cell infiltration and edema.
In diabetic rats, glibenclamide (glibenclamide) pretreatment reduced the STZ-induced kidney damage. The microscopic findings of the kidney sections revealed an absence of glomerular lesions and inflammation. However, in some of the examined kidneys, there were a few desquamated cells in the lumens of renal tubules, with localized tubular degeneration and necrosis (**Fig. 3C**).
Interestingly, the administration of marjoram extract to diabetic rats noticeably reduced renal damage. The majority of the examined kidney sections revealed a lake of renal damage and both glomeruli and tubular epithelium were identical to those of the control group (**Fig.3E**). However, there was congestion of the glomerular tufts and renal blood vessels with perivascular edema in a few examined kidney sections.
Similarly, administration of marjoram nanoparticles to diabetic rats greatly attenuated the STZ-induced kidney damage where there was no evidence of glomerulotubular lesions and inflammatory reaction in the most examined kidney sections (**Fig.3F**). However, mild degeneration of the renal tubular epithelium was occasionally seen.

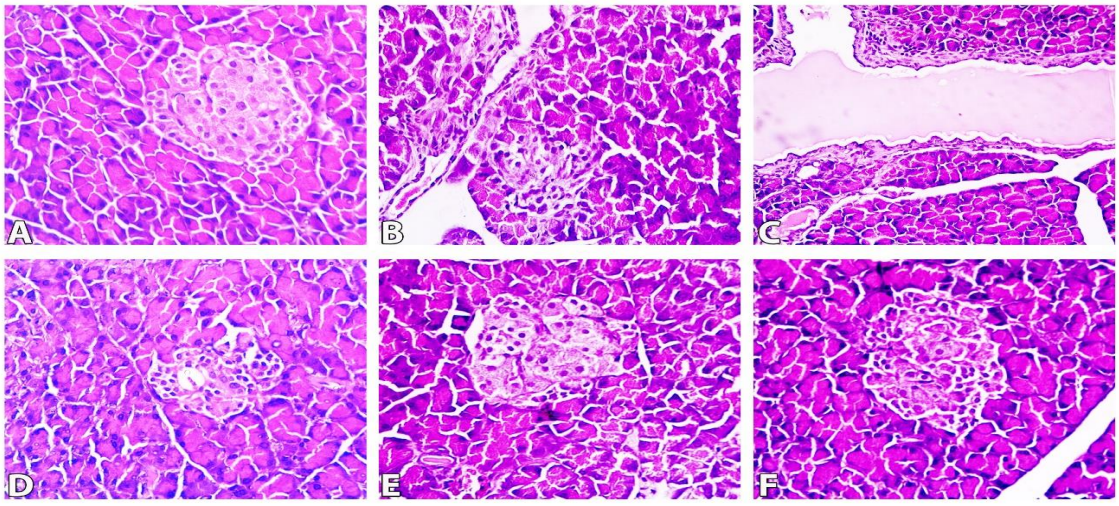

Fig. 2. Representative photomicrographs of the pancreas of rats in (A) control group showing typical histoarchitecture of islets cells and pancreatic acini (B-C) Diabetes group showing B- necrosis of islets cells, C- cystic dilatation of the pancreatic duct with retained pale eosinophilic secretion in its lumen (D) Glibenclamide treated group showing less cell population of islets cells within the pancreatic acini (E) marjoram extract treated group showing a mass of more or less normal islets cells and pancreatic acini (F) marjoram nanoparticles treated group showing a cluster of islets cells and pancreatic acini similar to those in the control group. H&E stain X200.

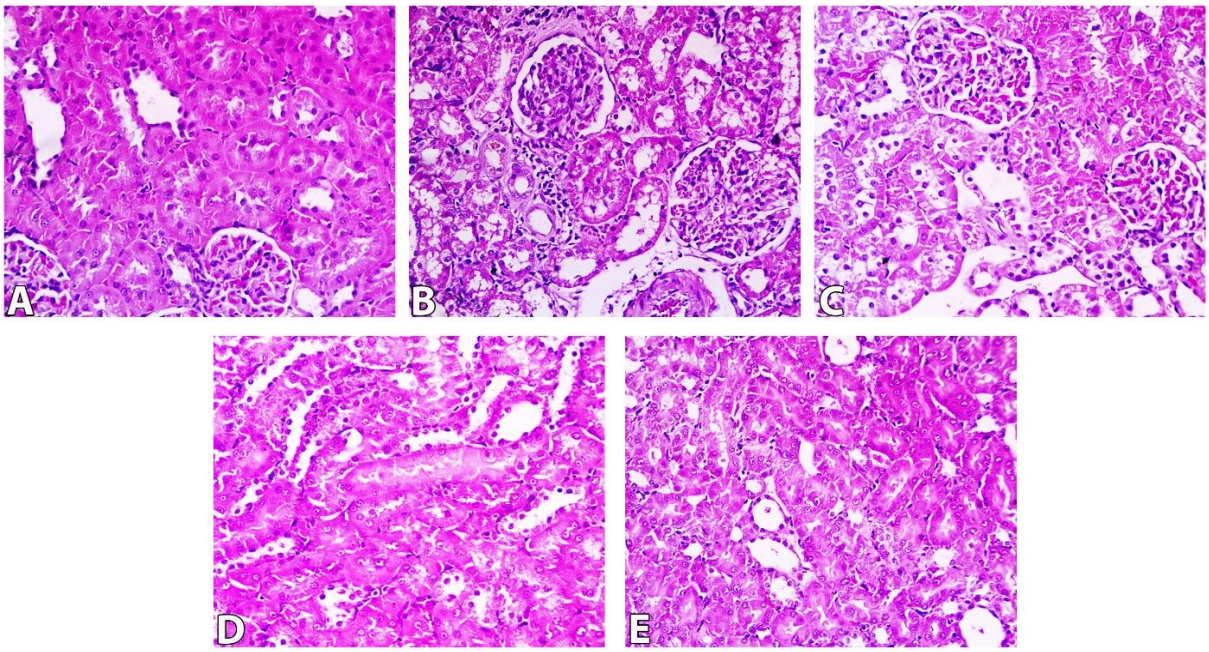

Fig.3. Representative photomicrograph of the kidney of rats in (A) control group showing normal renal tubules and glomeruli (B) Diabetes group showing thickening of Bowman's capsule with extensive tubular degeneration and necrosis (C) Glibenclamide treated group showing mild glomerular congestion and degeneration and necrosis of some tubular epithelium (E) marjoram extract treated group showing typical renal tubules lined with simple cuboidal epithelium with vesicular nuclei (F) marjoram nanoparticles treat group showing renal tubules identical to those of control. H&E stain X200.

4. Discussion:

Origanum majorana (OM) leaf extract shown anti-diabetic action in STZ-induced rats in a variety of *in vitro* and *in vivo* experiments. In vitro, OM has been demonstrated to significantly suppress the production of advanced glycation end products. The impact was greater than the typical antiglycation drug, aminoguanidine (**Campbell *et al.*, 2012**) **[3].** Studies have demonstrated that OML extract can lower blood glucose levels in diabetic rats **[26].**
Furthermore, its long-term use reduced STZ-induced diabetic tissue damage **[27].** As a result, the current work explored and assessed the efficiency of OML extract and its nanoparticles by examining their histological effects on liver organ tissue of STZ-induced diabetic rats.

The current study found that diabetic rats treated with glibenclamide and OML extract had lower glucose levels compared to the diabetic group. Furthermore, diabetic rats administered with OML NPs extract shown a substantial decrease in glucose when compared to all treatment groups.These experimental results were consistent with the findings of **Farag *et al***. **[27]**
Similarly, **Ghudhaib** and **Khaleel [28]** discovered a substantial drop in blood glucose, HA1c levels, and an increase in insulin levels among diabetic rats administered with OML or OML NP extract when compared to the diabetic group treated with glibenclamide medication.
In both severe and moderate diabetic models, glibenclamide therapy reduced blood glucose concentrations while increasing insulin levels **[29]**.
Shortly before treatment began, diabetic rats' serum levels significantly increased, according to **Kamel** [30]. *In vivo* study revealed that all treatment groups had significantly decreased blood glucose levels, with nano-cupsomal (NC) formulations showing the greatest reduction. The values tended to revert to almost normal once glibenclamide and OML were administered. At the conclusion of the study, the blood glucose levels of those with diabetes treated with OM were 11.43 mmol/L, as opposed to those treated with glibenclamide were 10.13 mmol/L. The author demonstrated OM oil's hypoglycemic activity in comparison to glibenclamide, a common hypoglycemic medication. Since years, sulfonylureas like glibenclamide have been used to treat diabetes. They promote insulin production from pancreatic β-cells by blocking ATP-sensitive K+ (K ATP) channels in the plasma membrane.
Mohamoud *et al*,[31] found that giving OML extract, a traditional medicinal plant, to diabetic rats diminished their increased blood glucose levels. It was also harmless, as shown by the lack of growth impairment, clinical changes, or death.
The study looked at the effects of glibenclamide, OML, and OML NPs extracts on C-peptide levels in STZ-induced diabetic rats. The diabetes groups had lower C-peptide levels than the control group. Glibenclamide therapy raised C-peptide levels, additionally OML extract and OML NP extract also boosted C-peptide levels.
the diabetic rats after 21 days of treatment, the serum insulin and C-peptide levels of the treated diabetic rats were significantly enhanced compared to the untreated diabetic rats group**[26]**.
Espinoza -Hernández FA *et al*, [32]found that the Plant extracts function as anti-hyperglycemic agents by suppressing glucosidase, increasing glucose absorption, and increasing insulin secretion.
Our study found that diabetic group showed an increase in amylase activity, while glibenclamide, OML extract and OML NPs treatment showed a significant decrease in amylase activity. **Habtemariam, S.,[33]** said that Carvacrol, a key ingredient in oregano essential oil, has been shown to have a hypoglycemic impact. Studies on mice and rats have shown that carvacrol has a function in regulating blood glucose levels. Similar findings were reported in rainbow trout on a

diet enriched with carvacrol **[34]**. oregano essential oil therapy decreased serum amylase activity, perhaps due to carvacrol's hypoglycemic effects **[35]**.

The experimental result explained that the diabetic rats treated with glibenclamide drug demonstrated a significant decrease in urea and creatinine levels when compared with diabetic group. This pertains to the effect of glibenclamide and OML and OML NP extract on kidney function (Urea and creatinine level) in STZ-induced diabetic rats. Furthermore, OML or OML NPs showed significant decrease in renal functions parameters compared to diabetic control group.
Khan et al. [36] found that oregano had antiurolithic action in vitro and in vivo, as well as antioxidant and renal epithelial cell protecting properties. These findings are consistent with our findings.
The study findings presented in **Table** (**2**); Regarding the investigation of the effect of glibenclamide, OML or OML NP extracts on inflammatory cytokines in STZ-induced diabetic rats, the study found that diabetic rats treated with glibenclamide or OML or OML NPs extract showed significant decreases in TNF-α, IL-1β, and IL-6 levels compared to the diabetic group and a significant increase in IL-10 levels.These findings were agreed with the study of **Vujicic *et al.* [9].**

The findings from the study of **Bouyahya *et al*. [7]** highlight the potential of Glibenclamide and OML/OML NP extracts as anti-inflammatory agents in the management of diabetes. By reducing pro-inflammatory cytokines like TNF-α, IL-6, and IL-1β, and increasing the anti-inflammatory cytokine IL-10, these treatments may help mitigate the inflammatory component of diabetes, potentially improving outcomes for patients. This dual action—suppressing inflammation while enhancing anti-inflammatory responses—could be particularly beneficial in managing the chronic inflammation associated with diabetes. Furthermore, Plant extracts control blood glucose and reduce lipid oxidation, which can contribute to insulin resistance. They also have antioxidant and anti-inflammatory properties **[37].** The primary ingredients of O. majorana's methanolic extract may be responsible for its anti-inflammatory properties.

Regarding the effect of glibenclamide drug, OML, and OML NP extracts on the histological examination for the pancreatic and renal tissue cells in STZ-induced diabetic rats.
The pancreas of diabetic rats showed normal exocrine and endocrine components, with pancreatic acini and duct cells. However, diabetic rats showed significant damage to the islet of Langerhans, necrosis, degeneration, and reduced cell sizes. The pancreatic blood arteries were severely congested, and interlobular ducts were dilated and filled with eosinophilic secretion.
Glibenclamide treatment improved pancreatic microscopic images in diabetic rats, with normal histoarchitecture. However, Langerhans islet cell populations were smaller and blood vessel congestion was present. OML extract treatment alleviated pancreatic parenchyma, with islets and acini similar to the control group. Treatment with OML NP extract restored pancreatic damage induced by STZ, with most islets appearing normal and no inflammation or degenerative changes observed. These findings were agreed with the study of **Stojanovi´c *et al*,[38]**

The kidney sections of rats in a non-diabetic control group showed normal renal architecture, with renal corpuscles and tubules lined by simple epithelium. However, diabetic rats showed severe renal damage, including tubular degeneration, necrosis, glomerular shrinkage, congestion, and thickening of Bowman's capsule. Vascular injury was also observed.

Glibenclamide pretreatment reduced STZ-induced kidney damage in diabetic rats, with no glomerular lesions or inflammation. However, some kidney sections showed desquamated cells and tubular degeneration. OML extract administration also reduced renal damage, with most sections showing normal glomeruli and epithelium.

OML NP extract significantly reduced STZ-induced kidney damage in diabetic rats, preventing glomerulotubular lesions and inflammatory reactions, but occasionally causing mild degeneration of renal tubular epithelium. These findings are consistent with other earlier research by **Khan *et al* , [36]** who reported that oregano had antioxidant and renal epithelial cell-protective properties,

Ghosian Moghaddam *et al.* [39] demonstrated that taken together, long term treatment of diabetic rats with OM can partially protect the renal tissue via attenuation of oxidative stress and glomerular expansion.

In the study of **Ghosian Moghaddam *et al*, [39]** the increase in renal tissue levels of MDA was documented and oral administration of OM resulted in decreasing the levels of oxidative stress markers in the renal tissue. Correspondingly, some positive attributes of OM in this study may be the ability to lower the oxidative stress. On this matter, it was shown that some flavonoids can boost the activity of some free-radical-fighting mechanisms of anti-oxidants and enzymes **[40]**Accordingly, flavonoids can lower the occurrence of lipid peroxidation as well **(Toklu *et al.*, [41]** which can lead to lower levels of MDA in the renal tissue. According to earlier research, the anti-apoptotic and antioxidant qualities of the Origanum species are responsible for this trait **[42].**

5. CONCLUSION:

Our purpose in this study was to determine how glibenclamide, OML, and OML NP extract helped treat STZ-induced diabetes in rats. In conclusion, the administration of OML and OML NP extract resulted in a significant reduction in glucose, amylase, urea, creatinine, and proinflammatory indicators. C-peptide levels were also improved, as was anti-inflammatory IL10. OML and OML NP extract can protect pancreatic tissues from the detrimental effects of diabetes mellitus while also protecting renal tissues. These findings showed that the high antioxidant activity of OML and OML NPs extract conferred anti-diabetic benefits in diabetic rats. More study is needed to completely understand the molecular mechanisms behind these effects.

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