



Combined effects of laurel and olive leaf extracts on gene expression, biochemical markers, and pancreatic restoration in STZ-induced diabetes

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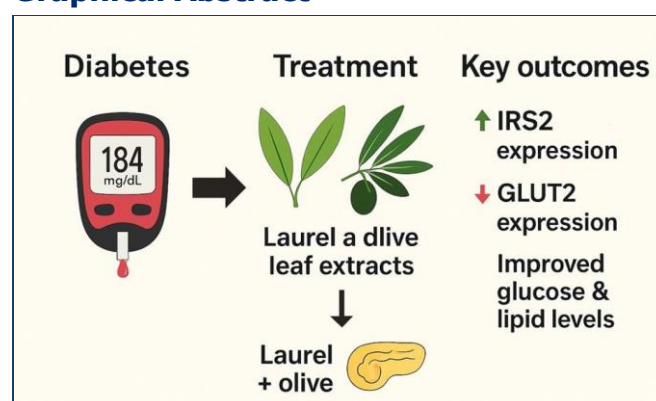
Abstract

Globally, an estimated 589 million adults aged 20–79 are living with diabetes in 2025, a number projected to reach 853 million by 2050. Diabetes mellitus (DM) is a chronic condition marked by persistent hyperglycemia due to impaired insulin secretion or action, which increase the risk of vascular complications. Developing effective treatments for DM remains a pressing challenge. This study evaluated the anti-diabetic and antioxidant effects of laurel and olive leaf extracts in streptozotocin (STZ)-induced diabetic rat models. Fifty male rats (8–10 weeks old, 180 ± 20 g) were allocated into five groups: Control, Diabetic, Diabetic + Laurel, Diabetic + Olive (*Olea europaea*), and Diabetic + Laurel and Olive. Gene expression of glucose transporter GLUT2 and insulin receptor substrate-2 IRS2, fasting blood glucose, insulin levels, lipid profiles, liver and kidney function markers, nitric oxide, and catalase (CAT) activity were assessed. Histopathological analysis of pancreatic tissues was also performed. After 28 days, all treated groups exhibited a significant increase in IRS2 and a decrease in GLUT2 expression compared to the diabetic group. Treatment improved glucose levels, lipid profiles, and liver and kidney function markers, with the most notable effects observed in the combined Laurel and Olive group. Nitric oxide levels significantly declined, while CAT activity increased in treated groups. Histopathology revealed substantial pancreatic restoration in the combined treatment group, closely resembling the control group. These results

demonstrate that Laurel and olive leaf extracts, particularly in combination, effectively manage diabetes and oxidative stress, offering potential therapeutic benefits for DM management.

Keywords: Herbal medicine. Diabetes. Anti-Oxidant. IRS2. GLUT2. Lipid profile. Pancreatic restoration. Histopathology.

Graphical Abstract



Source: Own authorship.

Introduction

Diabetes mellitus (DM) is a metabolic disorder characterized by chronic hyperglycemia due to impaired insulin secretion, insulin action, or both. It is primarily classified into two types: type 1 and type 2 diabetes [1]. According to global epidemiological data, the prevalence of diabetes has increased dramatically

over the past decades, with projections estimating that approximately 439 million individuals will be affected by 2030. This rapid increase represents a major public health concern worldwide. Managing hyperglycemia effectively is crucial to reducing the risk of long-term complications, including microvascular and macrovascular diseases [2].

Effective glycemic control provides significant advantages, including the prevention of retinopathy, nephropathy, neuropathy, and cardiovascular complications, thereby reducing morbidity and mortality associated with the disease [3]. Despite advancements in synthetic medications for DM management, achieving comprehensive control of the disease and its complications remains a challenge. The limitations of current treatments have prompted researchers to explore natural alternatives. Compounds derived from plants have shown promising potential in modulating β -cell apoptosis and enhancing insulin activity, providing a basis for new therapeutic approaches [4]. Additionally, certain natural substances have been found to inhibit enzymes involved in glucose production and absorption, further supporting their use in the management of DM [5].

Streptozotocin (STZ) is commonly used to induce experimental diabetes in animal models. Its toxic effects on pancreatic β -cells lead to an autoimmune response, causing the destruction of islet β -cells within two to four days following administration of a single dose of 60 mg/kg [6]. This STZ-induced diabetes is associated with increased production of reactive oxygen species (ROS), leading to oxidative damage. Chronic hyperglycemia exacerbates this condition by depleting the antioxidant defense mechanisms and promoting the formation of free radicals [7]. Antioxidants and free radical scavengers play a significant role in protecting β -cells from oxidative damage and promoting their regeneration [8].

The olive tree (*Olea europaea* L.) from the Oleaceae family has been extensively used in traditional medicine, particularly in Mediterranean and European countries, to treat various ailments [9,10]. Olive leaf extracts possess a wide range of biological activities, including hypoglycemic, hypotensive, antiarrhythmic, anti-atherosclerotic, and vasodilatory effects [11,12]. Similarly, the laurel plant (*Laurus nobilis*) is recognized for its medicinal value and has demonstrated the ability to enhance insulin secretion and glucose metabolism [13].

Although previous studies have reported the individual anti-diabetic and antioxidant effects of olive leaf extract and laurel, limited evidence exists regarding their combined therapeutic impact, particularly in STZ-induced diabetic models.

Furthermore, the potential synergistic interaction between these two plant extracts in modulating oxidative stress and β cell protection has not been sufficiently investigated. Previous reports have mainly focused on the individual hypoglycemic properties of olive leaf [11], the glucose-lowering effects of laurel [13], and the role of plant-derived antioxidants in STZ-induced diabetes [8], without evaluating their combined efficacy.

Therefore, this study aimed to investigate the anti-diabetic and antioxidant effects of laurel and olive leaf extracts, individually and in combination, in STZ-induced diabetic rats.

Materials and Methods

Experimental Animals

Fifty mature male Sprague-Dawley rats, aged 8-10 weeks and weighing 180 ± 20 g, were used in this study. The animals were housed in metallic cages, with four rats per cage, and maintained in a controlled environment with a 12-hour light-dark cycle at a temperature of 24°C. They were provided with food and water ad libitum throughout the experiment. This study was conducted in accordance with the ARRIVE 2.0 (Animal Research: Reporting of *in Vivo* Experiments) guidelines to ensure transparency, reproducibility, and high standards in the reporting of animal research.

Ethical Approval

All procedures were conducted in accordance with the ethical guidelines approved by the Research Ethical Committee of Benha University, Egypt. (Approval No.0109325), Date:06.03.2025.

Chemicals and Herbal Plants

Streptozotocin (STZ), a DNA alkylating agent used for inducing hyperglycemia, was purchased from Sigma-Aldrich (St. Louis, MO, USA). It was $\geq 75\%$ α -anomer basis, $\geq 98\%$ purity (HPLC), and supplied in powdered form.

Preparation of Plant Extracts

Preparation of Laurel Leaves (*Laurus nobilis*)

Fresh laurel leaves were collected from a local farm in the Qalyubia governorate, Egypt. The leaves were cleaned, air-dried at room temperature, and ground into a fine powder using an electric grinder [14].

Alcoholic Extraction of Laurel Leaves

Sixty grams of the powdered laurel leaves were soaked in petroleum ether for 72 hours, followed by

extraction using a Soxhlet apparatus with 300 mL of petroleum ether for another 72 hours. The extract was then filtered using a Buchner funnel and Whatman filter paper. The solvent was evaporated using a rotary evaporator at 40°C, and the resulting extract was stored in a dark glass container at 4°C [14] (Figure 1).

Preparation of Olive Leaves (*Olea europaea L.*)

Green olive leaves were harvested from a local farm in Meet Kenana, Qalyubia governorate, Egypt. Leaves were randomly collected from various trees, air-dried, and ground into a fine powder using a rotor mill. The powder was stored at 4°C for further extraction [15].

Aqueous Extraction of Olive Leaves

Dried olive leaves (200 g) were powdered and mixed with 7 L of hot water. The mixture was boiled for 30 minutes after being soaked for 3 hours. It was then cooled to room temperature and homogenized using an electric mixer for 20 minutes. The solution was filtered, and the filtrate was evaporated in an oven at 40°C to obtain dried residues containing active ingredients. The yield of the extract was approximately 20.3%. The dried extract was stored in a refrigerator for subsequent use [16] (Figure 2).



Figure 1. Extraction of laurel leaves by Soxhlet. Source: Own authorship.

Figure 2. Olive leaves extract. Source: Own authorship.

Induction of Diabetes

Diabetes mellitus was induced in overnight-fasted rats by administering a single intraperitoneal injection of STZ at a dose of 60 mg/kg body weight dissolved in saline solution. Following STZ administration, rats were provided with free access to food and water. Hyperglycemia was allowed to stabilize over four days. Rats with fasting blood glucose levels exceeding 250 mg/dL were considered diabetic and included in the study [16].

Study Design and Animal Groups

The animals were divided into five groups, each comprising 10 rats:

1. **Control Group:** Rats were maintained on a standard diet without any treatment until the end of the study.
2. **Diabetic Group:** Rats were administered a single intraperitoneal injection of STZ (60 mg/kg) and maintained on a standard diet [17].
3. **Diabetic + Laurel Plant Group:** Diabetic rats were treated orally with laurel leaf extract at a dose of 250 mg/kg body weight daily for four weeks.
4. **Diabetic + Olive Leaves Group:** Diabetic rats were treated orally with olive leaf extract at a dose of 200 mg/kg body weight daily for four weeks [16].
5. **Diabetic + Laurel + Olive Leaves Group:** Diabetic rats were treated orally with a combination of laurel leaf extract (250 mg/kg body weight) and olive leaf extract (200 mg/kg body weight) daily for four weeks.

Blood Sampling

At the end of the experiment, rats were weighed, and blood samples were collected from the orbital venous plexus under anesthesia with diethyl ether. The samples were centrifuged at 2200 RPM for 10 minutes to separate serum and plasma, which were then stored at -20°C for subsequent analyses. Parameters measured included serum glucose, insulin, cholesterol, triglycerides, HDL, LDL, VLDL, urea, creatinine, GPT, GOT, nitric oxide (NO), and catalase (CAT). Afterward, the animals were sacrificed, and their pancreases were excised and stored at -80°C for molecular analysis of GLUT2 and IRS2 gene expressions, as well as histopathological examination.

Gene Expression Assays for mRNA

Total RNA was extracted from pancreatic tissues using Trizol reagent (Invitrogen). The RNA expression levels were quantified using the StepOnePlus™ real-time PCR system and the QuantiFast SYBR Green PCR Kit (Qiagen, Germany). Quantitative real-time PCR (qRT-PCR) was conducted for glucose transporter (GLUT2) and insulin receptor substrate-2 (IRS2) genes, with β -actin serving as the normalization control. The $\Delta\Delta C_t$ method was employed to calculate mRNA expression levels [18].

Histopathological Studies

At the end of the experiment, pancreatic tissues were collected and immediately fixed in 10% neutral buffered formalin for 24 hours, as described by [19]. The tissues were then dehydrated in a graded ethanol series, cleared with xylene, and embedded in paraffin.

Sections of 5 μm thickness were prepared using a rotary microtome and stained with Hematoxylin and Eosin (H&E). A Nikon Eclipse E800 light microscope equipped with a digital camera was used for examination and imaging.

Biochemical Assay

Serum glucose levels were measured following the method of [20], while insulin was evaluated according to [21]. Cholesterol, triglycerides, HDL, and LDL levels were determined enzymatically using the methods described by [22,23]. Creatinine and urea levels were measured using the methods outlined by [24,25] respectively. GPT and GOT activities were assessed via the colorimetric method of [26]. All assays were performed using diagnostic kits obtained from Diamond Diagnostics, Egypt, following the manufacturer's protocols [27].

Evaluation of Oxidant /Antioxidant Parameters

Serum nitric oxide (NO) was quantified spectrophotometrically according to [28]. Catalase (CAT) activity was assessed using the OxiSelect™ Catalase Activity Assay Kit (Colorimetric), following the protocol of [29].

Statistical Analysis

Data were analyzed using one-way analysis of variance (ANOVA) followed by least significant difference (LSD) tests to compare groups. Results were expressed as mean ± standard error, with a significance threshold of p<0.05. It was employed Stata 18 software.

Results

The diabetic group had a significantly higher mean gene expression of IRS2 compared to the control group (p<0.001), indicating an increased activity of IRS2 in the diabetic group. However, all treatment groups, including laurel's treatment, olive leaves treatment, and the mix treatment, resulted in a significant decrease in IRS2 gene expression compared to the diabetic group (p<0.001). However, the gene expression of GLUT2 significantly downregulated compared to the control group (p<0.001). On the other hand, the treatment with laurel extract, olive leaves extract, and the mixed treatment showed a significant upregulation of GLUT2 gene expression compared to the diabetic group with significant downregulation in GLUT2 gene expression compared to the control group (p < 0.05) (Table 1).

Table 1. mean values and standard errors of gene expression of GLUT2 and IRS2 for control group and experimental groups at the end of experiment.

	(control) Group	(Diabetic) Group	(laurel) administrated Group	(Olive) administrated Group	(laurel + Olive) administrated Group	p-value
β-Actin CT (cycles)	20.19	20.70±0.46	20.07±0.60	20.20±0.16	20.04±0.38	0.340
Glut2 Fold change	1.0	0.155±0.03 ^a	0.453±0.05 ^a	0.595±0.04 ^a	0.861±0.004 ^a	<0.001
IRS2 CT	22.59	26.50±0.50 ^b	23.30±0.45 ^{ab}	22.98±0.12 ^{ab}	22.40±0.33 ^b	<0.001
IRS2 Fold change	1	0.097±0.008 ^c	0.548±0.05 ^{ab}	0.809±0.01 ^{abc}	0.979±0.03 ^{abcd}	<0.001

*Data are presented as mean ± SE. mean value of the same raw with different superscript letters significantly difference a: compared to control, b: compared to Diabetic, c: compared to laurel, d: compared to Olive leaves. Source: Own authorship.

After 28 days of treatment, the diabetic group exhibited significantly higher glucose levels compared to the control group (p<0.001). In contrast, the laurel, Olive Leaves, and Mix groups demonstrated substantially reduced glucose levels relative to the diabetic group, with the Mix group approaching near-normal glucose levels. In terms of insulin levels, the diabetic group had lower insulin levels with a significant difference compared to the control group. The treatment with laurel and olive leaves extract groups showed a nearby significant increase in insulin levels compared with the control and diabetic groups. The Mix group showed significantly higher insulin levels compared to the control, diabetic, and laurel groups (p-value <0.005) (Table 2).

Liver function markers GPT and GOT were notably elevated in the diabetic group, indicating liver stress. However, the laurel, Olive Leaves, and Mix groups had significantly lower GPT and GOT levels, suggesting these treatments might offer protective effects on liver function. Creatinine and Urea levels were also significantly higher in the diabetic group, pointing to renal stress, but the laurel, Olive Leaves, and Mix groups exhibited lower Creatinine and urea levels, with the Olive Leaves and Mix groups showing the most pronounced reduction. (Table 2).

Table 2. Mean values and standard errors of Glucose, Insulin, GPT, GOT, Creatinine, and Urea for control group and experimental groups at the end of experiment.

	Glucose	Insulin	ALT	ALT	Creatinine	Urea
(Control) Group	115.60±1.91Ae	0.40±0.01Aa	32.44±1.25 ^c	88.32±2.62Ae	0.45±0.02Ae	20.65±0.51e
(Diabetic) Group	324.80±4.57Aa	0.21±0.01Aa	65.66±2.57 ^a	156.26±2.41Aa	1.03±0.04Aa	48.61±0.93a
(Laurel) Group	191.50±1.84Bb	0.26±0.02Ad	49.68±1.19 ^b	114.82±1.51Bb	0.75±0.02Ab	38.07±1.31b
(Olive) Group	176.20±1.16Bc	0.33±0.01Ac	43.40±1.21 ^c	107.04±1.51Bc	0.62±0.02Ac	32.84±1.80c
(Mix) Group	147.20±1.66Bd	0.37±0.01Ab	38.01±1.03 ^d	94.46±1.03Bd	0.52±0.02Bd	27.20±0.75d

*Data are presented as (Mean ± S.E). S.E = Standard error. Mean values with different capital superscript letters in the same column are significantly different at (p<0.05) in the same group. Mean values with different small superscript letters in the same column are significantly different at (p<0.05) in the same time. Source: Own authorship.

Significant differences were also noted in cholesterol levels across the groups, with the diabetic group having the highest levels. The laurel, Olive Leaves, and Mix groups showed markedly lower cholesterol levels, with the Mix group showing the greatest reduction, suggesting a strong lipid-lowering effect of these treatments (Table 3). Triglyceride levels were significantly lower in the laurel, Olive Leaves, and Mix groups compared to the diabetic group, highlighting their effectiveness in reducing triglycerides, similarly; LDL and VLDL levels increased in diabetic group and lowered significantly after treatment with Laurel, Olive and Mix leaves extract. HDL levels differed significantly as well; although the diabetic group had lower HDL levels than the control group, the laurel, Olive and Mix groups had significantly increase HDL levels compared to control.

Table 3. Mean values and standard errors of Lipid profile for control group and experimental groups at the end of experiment.

	Cholesterol	Triglycerides	HDL	LDL	VLDL	Risk 1	Risk 2
(Control) Group	92.80±0.94 ^e	133.02±1.51 ^e	57.16±0.84 ^e	9.04±0.38 ^e	26.60±0.30 ^e	1.62±0.01 ^e	0.16±0.01 ^e
(Diabetic) Group	125.46±2.04 ^a	164.80±1.77 ^a	33.62±1.60 ^a	43.88± 2.12 ^a	32.96±0.35 ^a	3.76±0.18 ^a	1.78±0.14 ^a
(Laurel) Group	118.60±1.07 ^b	150.68±1.69 ^b	38.52±0.67 ^b	34.95± 0.70 ^b	30.14±0.34 ^b	3.08±0.05 ^b	1.30±0.03 ^b
(Olive) Group	105.66±3.11 ^c	144.64±1.63 ^c	44.02±0.54 ^c	22.71± 3.15 ^c	28.93±0.33 ^c	2.40±0.08 ^c	0.75±0.08 ^c
(Mix) Group	99.26±2.81 ^d	138.82±1.08 ^d	51.64±1.07 ^d	14.86± 3.01 ^d	27.76±0.22 ^d	1.96±0.07 ^d	0.41±0.07 ^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). Source: Own authorship.

After 28 days of treatment, nitric oxide (NO) levels showed a significant increase in the diabetic group compared to the control group (p-value <0.001). The Mix group demonstrated the most significant reduction in NO levels compared to the diabetic group, reaching levels even near the control group. The Olive and laurel Leaves groups also showed significantly reduced NO levels compared to the diabetic group, indicating a reduction in oxidative stress and potential anti-inflammatory effects of these treatments (Table 4).

Catalase (CAT) activity was significantly lower in the diabetic group compared to the control group (p-value <0.001). The laurel, Olive Leaves, and Mix groups showed significantly higher CAT activity compared to the diabetic group, with the Mix group reaching levels comparable to the control group. This indicates that the treatments, particularly the Mix, enhanced the antioxidant defense system by increasing CAT activity (Table 4).

Table 4. Mean values and standard errors of NO. And CAT. For control group and experimental groups at the end of experiment.

	(Control) Group	(Control) Group	(Control) Group	(Control) Group	(Control) Group	p- value
NO (umol/L)	17.86±2.09e	42.94±0.77a	35.09±1.55b	29.21±1.40c	24.47±0.49d	<0.005
CAT (U/L)	1.19±.04	0.48±.12 ^a	0.89±.03 ^{ab}	0.91±.05 ^{ab}	1.13±.15 ^b	<0.001

*Data are presented as mean ± SE., Mean values with different superscript letters in the same column are significantly different, a: compared to control, b: compared to Diabetic, c: compared to laurel, d: compared to Olive leaves. NO: nitric oxide, and CAT: catalase. Source: Own authorship.

The histopathological study showed that the control rats exhibited normal pancreatic histoarchitectures (Figure 4, 1A), while STZ diabetic rats showed significant endocrine and exocrine pancreatic damage, including smaller islets of Langerhans, necrosis, degeneration, and inflammatory infiltration (Figure 4, 1B-C). laurel leaf extract partially alleviated this damage, with improved islet size and cell number, though some vacuolation and pyknotic nuclei persisted (Figure 4, 1D). Olive leaf extract provided stronger protection, nearly restoring pancreatic parenchyma and increasing islet size and cell populations compared to laurel leaf extract (Figure 4, 1E). Combination treatment with laurel and olive leaf extracts demonstrated the greatest effect in restoring STZ-induced pancreatic damage, with no inflammatory or necrotic changes and islet sizes and cell populations similar to control rats (Figure 4, 1F). Minor congestion and eosinophilic secretion in interlobular ducts were infrequently observed, indicating significant amelioration of STZ-induced pancreatic injury.

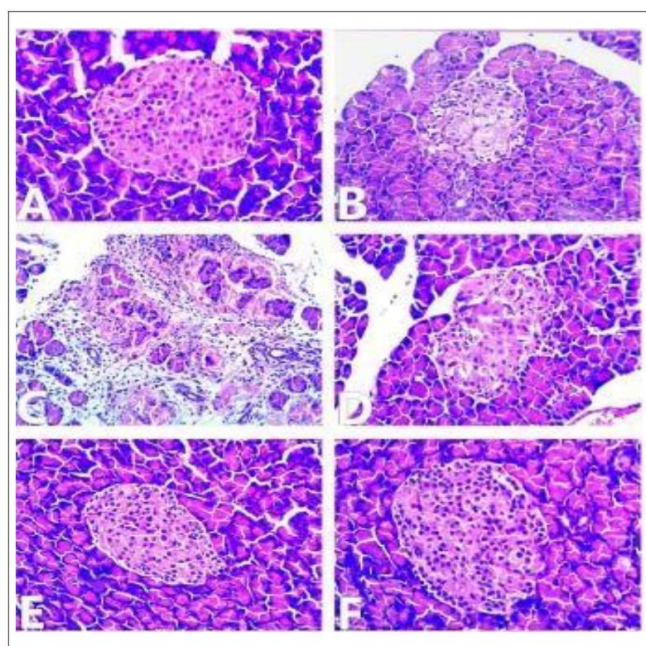


Figure 4. Representative photomicrographs of pancreatic sections of rats. Source: Own authorship. (A) Control group

showing regular well-defined pale rounded mass of islets of Langerhans in-between pyramidal acinar cells. (B-C) Diabetes group showing B-Depleted hypocellular islets of Langerhans with degenerated and necrotic cells, C- Focal hemorrhage and inflammatory edema replaced the destructed acini. (D) Laurel leaves extract treated group showing few degenerated cells within islets of Langerhans. (E) Olive leaves extract treated group showing nearly normal islets cells with less cell population in between normal pancreatic acini. (F) Combined Laurel and olive leaves extracts treated group showing large cluster of islets cells nearly identical to control group surrounded by normal pancreatic acini. H&E stain X200. Source: Own authorship.

Discussion

The current findings indicated that the IRS2 gene expression was significantly higher in the diabetic group compared to the control group ($p < 0.001$), reflecting increased IRS2 activity in diabetes. However, all treatment groups exhibited a significant reduction in IRS2 gene expression compared to the diabetic group ($p < 0.001$), suggesting an improvement in insulin synthesis and secretion with treatment. The insulin receptor substrate (IRS) proteins play a crucial role in insulin signaling and are involved in fundamental cellular functions such as growth, survival, and metabolism reported a significant decrease in IRS1 levels in diabetic rats on a normal diet and in those treated with low and high doses of olive leaf extract, compared to the control group [16].

Both kidney and liver, which primarily express GLUT2 as their glucose transporter, are key extrapancreatic tissues that help explain why they absorb streptozotocin (STZ) preferentially. A decrease in GLUT2 gene expression in the treatment groups suggests improved glucose homeostasis compared to the diabetic group, aligning with [29], who showed that STZ-injected rats exhibited significantly downregulated GLUT2 expression in the pancreas compared to controls. After 28 days, the diabetic group had significantly elevated glucose levels ($p < 0.001$), while treatment groups such as laurel, Olive Leaves, and Mix demonstrated notable reductions in glucose, with the Mix group approaching near-normal levels.

This improvement in glucose levels is supported by [30], who attributed the antidiabetic activity of *Laurus nobilis* to its polyphenolic compounds, which enhance insulin sensitivity, glucose uptake, and antioxidant status. [31] also reported that the diabetic group had higher glucose levels compared to controls, which were normalized with olive leaf extract treatment. The hypoglycemic effects of olive leaf extract were further attributed to leuropeide compounds, which enhance glucose-induced insulin release and increase peripheral glucose uptake [32].

Regarding lipid profile, the diabetic group showed significantly elevated cholesterol levels after 28 days. All treatment groups (laurel, Olive Leaves, Mix) displayed significantly lower cholesterol, LDL, VLDL, and triglyceride levels ($p < 0.005$), indicating potent lipid-lowering effects, particularly in the Mix group. These results are consistent with [33], who observed significant increases in cholesterol and triglycerides in diabetic rats compared to controls, and reductions with laurel treatment. Similarly, [31] reported a significant increase in cholesterol and triglycerides in the diabetic group, which was reversed with olive leaf extract treatment.

Previous researches assessed the effects of olive leaf extract on hepatic and metabolic systems in rats fed a high carbohydrate and high-fat (HCHF) diet for 16 weeks, finding reductions in TC, TG, and LDL-L levels in the olive leaf-treated group compared to placebo rats. At 28 days, the diabetic group had reduced HDL levels, while the laurel group showed a slight increase in HDL. The olive leaf and Mix groups exhibited significantly higher HDL levels than the diabetic and laurel groups, aligning with [33], who reported decreased HDL in diabetic mice compared to controls.

In this study, the diabetic group showed significantly elevated serum GPT and GOT levels compared to controls. The laurel, Olive Leaves, and Mix groups demonstrated significantly lower GOT and GPT levels, suggesting protective effects on liver function. This is in agreement with [33], who reported increased GOT and GPT levels in diabetic rats, which were reduced with laurel treatment. Furthermore, [34] observed similar alterations in liver function due to STZ induction, with significantly higher AST and ALT levels in diabetic rats compared to controls.

At 28 days, creatinine levels were higher in the diabetic group compared to controls. Treatment with laurel, Olive Leaves, and Mix extracts significantly reduced creatinine levels. These findings are consistent with [31], who reported that creatinine levels were significantly higher in diabetic rats, which were mitigated with olive leaf extract treatment. Similarly, urea levels were elevated in the diabetic group, and significantly reduced in the treatment groups, with the Olive Leaves and Mix groups showing the most reductions.

Serum nitric oxide (NO) levels remained elevated in the diabetic group, while the Mix group exhibited the most significant reduction, approaching control levels. Olive Leaves and Laurel groups also significantly reduced NO levels, suggesting reduced oxidative stress and potential anti-inflammatory effects. The catalase (CAT) levels in the diabetic group were lower, which

was reversed by treatments, especially in the Mix group. These results are supported by [35], who found increased CAT activity in olive leaf-treated diabetic rats, and [16] who reported that serum CAT levels were significantly reduced in diabetic rats but increased with olive leaf extract treatment.

Histopathological examination revealed that control rats had normal pancreatic architecture, while STZ-treated diabetic rats exhibited significant damage, including smaller islets, necrosis, degeneration, and inflammatory infiltration. Laurel leaf extract partially alleviated this damage, improving islet size and cell number, although some vacuolation and pyknotic nuclei persisted. Olive leaf extract offered stronger protection, nearly restoring pancreatic structure and increasing islet size and cell populations compared to laurel extract. These findings are consistent with [16], who noted that normal control rats had intact pancreatic islet cells, while hyperglycemia-induced structural changes were observed in diabetic rats [36].

The combination of laurel and olive leaf extracts showed the greatest effect in restoring STZ-induced pancreatic damage, with no inflammatory or necrotic changes and islet sizes similar to control rats. Minor congestion and eosinophilic secretion in interlobular ducts were occasionally observed, indicating significant improvement in pancreatic injury. These results align with [37], who reported that laurel and olive leaves, used since ancient times, possess antioxidant, anti-inflammatory, antiseptic, antispasmodic, and wound-healing properties [16]. Also observed mild degeneration in diabetic pancreatic islets treated with olive leaf extract.

Conclusion

The present study demonstrated that laurel and olive leaf extracts, individually and in combination, effectively improved hyperglycemia, lipid abnormalities, oxidative stress, and pancreatic damage in STZ-induced diabetic rats. The combined extract produced the strongest benefits, reflected in better glucose control, lipid profile improvements, enhanced antioxidant activity, and greater restoration of pancreatic structure. These findings suggest that co-administration of laurel and olive leaf extracts may offer a promising, low-cost complementary approach for diabetes management.

Limitation

This study was conducted only on male rats, limiting generalizability to other sexes or species. The STZ model mainly represents type 1-like diabetes, which may not fully reflect human type 2 diabetes. The

28-day duration may be insufficient to assess long-term metabolic or pancreatic effects. Only two gene markers (GLUT2 and IRS2) were evaluated, providing a limited view of molecular mechanisms. Finally, the study used crude plant extracts, and the specific active compounds responsible for the therapeutic effects were not identified.

CrediT

Author contributions: **Conceptualization**- All authors. **Data curation**- Mohamed G. Elharrif, Afaf D. Abdelmaged; **Formal Analysis**-All authors; **Investigation**- All authors; **Methodology**- All authors; **Project administration**- Mohamed G. Elharrif, Afaf D. Abdel-Maged, Hussein A. Abdel-Maksoud; **Supervision** - Mohamed G. Elharrif; **Writing - original draft**-All authors; **Writing-review & editing**-All authors.

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Ethical Approval

All procedures were conducted in accordance with the ethical guidelines approved by the Research Ethical Committee of Benha University, Egypt. (Approval No.0109325), Date:06.03.2025.

Informed Consent

Not applicable.

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Data Sharing Statement

The datasets generated and/or analyzed during the current study are not publicly available due to institutional policy restrictions but are available from the corresponding author upon reasonable request. All materials and methodological details used in this study are fully described within the manuscript. If additional documentation or raw data files are required, they can be provided by the corresponding author for academic and non-commercial use upon request. No third-party datasets were used in this work.

Conflict of Interest

The authors declare no conflict of interest.

Similarity Check

It was applied by Ithenticate®.

Application of Artificial Intelligence (AI)

Not applicable.

Peer Review Process

It was performed.

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