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Biochemical and molecular effects of the Laurel plant extract and olive leaves extract in diabetic rats induced by streptozotocin

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ABSTRACT

The global No. of people with (DM) has more than quadrupled in the last 3 decades, and it is expected that the No. will rise to 439 million by 2030, therefore many attempts were done to develop an effective therapy for diabetes. in this study we investigate the biochemical and molecular effects of Laurel and olive leaves extract in diabetic rats induced by (STZ). 50 male mature rats aged (8-10 w) weighing 180 ± 20 gm. were divided into 5 equal groups; (1) Control group: rats not receive treatment,(2) Diabetic group: rats injected IP with a single dose of STZ , and given a standard diet, (3) Diabetic + laurel: diabetic rats treated orally with Laurel plant extract for 4 weeks,(4) Diabetic + *Olea europaea* L: diabetic rats treated orally with olive leaves extract for 4 weeks,(5) Diabetic + Laurel plant and *Olea europaea* L: diabetic rats treated orally with Laurel and olive leaves extract for 4 week. Serum fasting Glucose, insulin, AST, and creatinine levels before and after induction of STZ, GLUT2 gene, plasma Glutathione and malondialdehyde were estimated. The results showed that The glucose level and the mean gene expression of GLUT2 in the diabetic group was significantly high compared to controls, with lower insulin level, elevated Malondialdehyde ($P < .001$) and reduced Glutathione levels than controls. All results were reversed with the treatment of Laurel and *Olea europaea L* extracts. In conclusion**,** laural and olive leaf extracts had shown promising anti-diabetic activity in diabetic albino rats.

1. INTRODUCTION

The by-products of the plant kingdom are abundant and distinguished by their physiological impact and biological action against human and other incurable illnesses. Over the globe, medicinal and aromatic plant and herb production has expanded, and because of their pharmacological efficacy and ability to cure diseases quickly and painlessly whether taken such as whole, powdered, or capsule form, their applications and qualities have diversified (Ali, 2020). Plants used for medicinal purposes are materials or preparations made from different plant groups or from plant sections that have been handled (Aina et al., 2020). The components that make up herbal plants, such as phenols, alkaloids, saponins, steroids, terpenoids, and tannins—are responsible for a variety of their therapeutic properties. Many qualities, including antibacterial, anti-inflammatory, and antioxidant capabilities, are found in these widely dispersed chemicals found in plants (Verawati et al., 2017). Chemicals that have been extracted from plants and created via the major or secondary metabolic processes shown below are known as natural plant products.

The process of studying natural products entails separating these chemicals in their purest form using the Soxhlet extraction method and analyzing their composition, application, and purpose using chromatography (Chahal et al., 2017). High blood sugar levels are a hallmark of diabetes mellitus, a chronic condition linked to abnormalities in the metabolism of fats and carbohydrates. Because of a complete or partial lack of insulin. It occurs when the body is unable to properly generate or use enough of the hormone insulin. This can involve abnormalities in the production of insulin, the action of insulin, or both, and can result in several serious and complicated problems (Alchalabi et al., 2020). Diabetes comprises many disorders described by hyperglycemia. According to the current classification there are two major types: type 1 diabetes (T1DM) and type 2 diabetes (T2DM). The variance between the two types has historically been based on age at the beginning, degree of loss of β cell function, degree of insulin resistance, presence of diabetes-linked autoantibodies, and requirement for insulin treatment for survival. However, none of these characteristics unequivocally distinguish one type of diabetes from the other, nor account for the entire spectrum of diabetes phenotypes (Leslie et al., 2016).

The varied mechanisms of action of traditional oral anti-diabetic medications have made treating diabetes mellitus more challenging (Ghadge and Kuvalekar, 2017). Because it is difficult to produce the present pharmaceuticals and because these chemicals have terrible side effects, it is vital to look for new and effective natural therapies against diabetes. Numerous plant species have been shown in experiments to have insulin-enhancing properties, including olive leaves, fenugreek, cloves, and laurel plants. Plants such as laurel boost insulin secretion and aid in treating diabetes. It can improve diabetes patients' glucose

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metabolism (Alchalabi et al., 2020) whereas its efficacy is linked to the existence of active ingredients such as squalene, methyl chavicol, alkaloids, tannin, eugenol, terpenoid, and flavonoids (Aljamal and Abdulrahim, 2010). Laurel is a popular and easily accessible plant that is utilized frequently in society as an alternative medicine. It is also anticipated to provide health benefits as a substitute herb (Harismah, 2017). The benefits of laurel plants extend beyond their hypoglycemic properties; they also enhance liver and renal function by improving lipid metabolism (Alchalabi et al., 2020).

Olive leaves (Olea europaea) tree is categorized as a member of the Oleaceae family which is considered as one of the oldest known agricultural plants in the world, especially in the Mediterranean region. In the Arabic language, it is acknowledged as Zaitoon, and in the English language as Olive (Abdelrahman et al., 2019). Olive leaves are agricultural waste by-products resulting from olive oil production, accounting for 10% of the weight of all harvested olive trees (Rahmanian et al., 2015). Olive leaves are a good source of valuable constituents with a variety of health-promoting effects because they contain large amounts of lipoidal and phenolic components (Özcan and Matthäus, 2017). The leaves phytoconstituents varied qualitatively as well as quantitatively as a result of numerous conditions, such as genotypes, collection times, surroundings circumstances, geographical locations, and exposition to sunlight (Losito et al., 2021).

Historically, *O. europaea* leaves have been used for the treatment of neurological and rheumatic conditions in Lebanon, as well as to relieve joint and cramps in certain areas of Iran. It was utilized in folk medicine as a traditional herbal tea with numerous curative benefits, such as gout, arteriosclerosis, and diabetes mellitus (Hashmi et al., 2015). The previous study carried out by Dekanski et al. (2011) verified that *O. europaea* leaf extracts have strong antioxidants and free radical scavenging properties, making them suitable for usage in a variety of treatments. Recently, researchers have become more interested in detailed studies on the advantages of *O. europaea* leaves as antioxidant, anti-atherosclerotic, antihypertensive, antibacterial, and anti-mutagenic agents (Martínez-Navarro et al., 2021). The current study sets out to assess the beneficial effects of olive leaves and laurel plant extract on body weight, GLUT2 gene for pancreatic tissue, AST, MDA, GSH, blood glucose, and insulin in streptozotocin-induced diabetic rats.

2. MATERIAL AND METHODS

2.1. Experimental animals

Fifty adult male Sprague-Dawley rats, weighing 180 ± 20 grams and aged 8-10 weeks, were kept in metallic cages with four rats per cage. They were kept in a climate-controlled setting with air conditioning set at 24 degrees Celsius and a 12-hour light-dark cycle. Throughout the trial, they had unlimited access to food and drink. The study was approved by the Ethical Committee of Research at the Banha University of Egypt, Faculty of Veterinary Medicine (approval no: BUFVTM 32-11-23).

2.2. Chemicals and herbal plant

2.2.1. Streptozotocin (STZ): was purchased from Sigma-Aldrich Co. (St. Louis, MO, USA), ≥75% α-anomer basis, ≥98% (HPLC), powder, DNA alkylating agent and was used to induce hyperglycemia.

2.2.2. Preparation of laurel plant (laurus nobilis) extract

Bay laurel, (Laurus nobilis), fragrant evergreen species of the family Lauraceae, the source of the cooking herb bay leaf. Bay laurel is native to the Mediterranean region but now widely cultivated in other regions of the world.

A nearby farm in Qalyubia Governorate, Egypt provided the laurel plant leaves (april 2024), which were then washed, dried at room temperature, and grounded for two minutes using an electrical grinder (Muhayyidin et al., 2018). 60 grams of leaf powder soaked in 5 Litres petroleum ether for 72-hour, 300 ml was added to Soxhlet, and then filtered through a Buchner funnel and Whatman filter paper (47 mm x 90.45 µm filters), The extract was stored at 4 °C in a dark glass container while the solvent was dried and concentrated using a rotary evaporator at 40 °C (Muhayyidin et al., 2018).

2.2.3. Preparation of olive leaves extract

Fresh green olive leaves (*O. europaea* L. cv koroneki) was picked from the nearby farm in Qalyubia, Egypt (Meet Kenana) at April 2024. The leaves from many different trees were randomly picked, allowed to air dried, and then processed in a rotor mill to create a fine powder that was kept at 4 °C for the extraction procedure. Every two weeks, the leaf aqueous extract was made. ten liters of hot water (60º C) were mixed with 200 grams of powdered dry olive leaves. The mixture was slowly cooked for 30 minutes then the liquid was allowed to cool for three hours to ambient temperature before being carefully mixed for 20 minutes using an electric mixer. Filtration was done by Whatman filter paper (47 mm x 90.45 µm filters) on the olive leaf solutions. Ultimately, the filtrates were dried residues (active principles) by evaporating them in an oven at 40 °C. for 60 min. Regarding the samples that were powdered, the average yield of leaf extract was about 20% (200gm/10L). In addition, the extract was kept cold (4º C) for use in later studies (Al-Attar and Alsalmi, 2019).

2.3. Induction of diabetes

According to Mohammed et al, (2021) method, Intraperitoneal injection of freshly prepared STZ dissolved in 0.01 citrate buffer (PH 4.5) at a single dose of 60 mg/kg body weight was used to produce experimental diabetes mellitus in rats that had been starved overnight. The rats had unrestricted access to food and drink following injection. For three days, DM was allowed to develop and stabilize in the rats which received STZ. In these rats, diabetes mellitus was diagnosed based on measurements of fasting blood glucose (by Accu-Chek monitoring). Diabetic model rats were defined as those with blood glucose levels more than 250 mg/dl (Mohammed et al., 2021).

2.4. Experimental design and animal groups

The animals were split into five equal groups, each with ten rats; Control group (group1): normal rats act as controls. not receive treatment (serum glucose levels below 200 mg/dl, see Table 3). Diabetic group (group 2): rats induced for diabetes through a standard diet and a single IP injection of 60 mg/kg b. wt. of STZ and kept for four days. Diabetic group plus Laurel plant (group 3): diabetic rats received an oral dose of 250 mg of the plant's extract (dissolved in 540 µl saline 0.9%) per kilogram of body weight for four weeks (Abdullah and AL-Abachi, 2021). Diabetic group plus *Olea europaea* L (group 4): diabetic rats received an oral dose of 200 mg of olive leaf extract (dissolved in 500 µl saline 0.9%) per kilogram of body weight per day for four weeks. (Al-Attar and Alsalmi, 2019), and Diabetic group + Laurel plant + *Olea europaea*

L (group 5): For four weeks, diabetic rats were given oral doses of 250 mg/kg body weight of Laurel plant extract and 200 mg/kg body weight/day of olive leaf extract.

2.5. Blood sampling:

After the experiment, rats were weighed and blood samples were collected twice, two and four weeks after STZ induction. Before sacrificing, rats anesthetized with diethyl ether and blood samples were drawn via the orbital venous plexus (Al-Attar and Alsalmi, 2019). The serum was separated and kept at -20 °C to estimate the serum glucose, insulin, serum creatinine, MDA, and GSH levels after the blood samples were centrifuged for 10 minutes at 2200 rpm (Guirgis et al., 2021). After that, the animals were scarified , and the pancreas were excised immediately and rinsed with isotonic saline. the pancreas tissues were then minced and a homogenate was prepared with 10% (w/v) phosphate-buffered saline (PBS, 0.1 mol/L, pH=7.4). . until the molecular parameters were examined (Ghanbari, et al, 2016).

2.6. Evaluation of body weight changes

The rats' body weight was measured each morning at the same time using a digital balance. Throughout the research period, the experimental animals were monitored for any indications of abnormalities (Al-Attar and Alsalmi, 2019).

2.7. Biochemical assay:

Level of fasting serum insulin was determined using BIOSOURCE INS-ELISA kit according to the method of Judzewitsch et al. (1982), moreover fasting blood glucose were assessed using Barham and Trinder (1972) assay, serum creatinine according to Mohabbati-Kalejahi et al. (2012), and aspartate transaminase (GOT) in the serum according to Huang et al. (2006) were determined using a colorimetric technique using kits (DGKC-GLU-100, BK-472525D, DGKC-AST-100 codes respectively) that were provided by the manufacturer. Diamond Diagnostics, Egypt was the source of the kits (Ghanbari et al., 2016).and

2.8. Evaluation of oxidant /antioxidant parameters:

malondialdehyde (MDA) and reduced glutathione (GSH).identified and measured in blood serum. based on the procedure outlined (Sadowska-Bartosz et al., 2016). The technique described by Tawfek, et al. (2010), which used spectrophotometry.

2.9. Gene expression assays for mRNA:

Total RNAs from pancreatic tissues were isolated using Triazole (Invitrogen). Expression levels of mRNAs were quantified in total RNA using Step one plus by QuantiFast SYBR Green PCR Kit (Qiagen, Germany). Quantitative real-time PCR (qRT-PCR) was carried out using the following genes: glucose transporter GLUT2 gene. Gene transcriptions were normalized to GABDH. The mRNA expression level was calculated by the ΔΔCt method (Freitas et al., 2005) and the primer sequence was as below:

Gene	Primer sequence (5'-3')	
GLUT2	TGTGCTGCTGGATAAATTCGCCTG AACCATGAACCAAGGGATTGGACC	According to Aerni-Flessner *et al*., 2012

2.10. Statistical analysis

One-way analysis of variance (ANOVA) was used to examine the data, and then Duncan's multiple-range test (Duncan) analysis was used to assess the significant differences between the different groups. The findings were presented as mean ± standard error, with statistical significance determined by values of P<0.05 (Tawfek et al., 2010).

3. RESULTS

The results in Table (1) showed that the diabetic group had a significantly (p= 0.002) lower mean weight compared to the control group after 14 days with an insignificant decrease at 28 days. The Laura group had a significantly (P < 0.05) lower mean weight compared to the control group after 14 and 28 days. There was no significant difference between the olive leaves extract group, and mixed treatment groups compared to the control group after 14 and 28 days.

Table 1 Changes in body weight (gm) at the experiment start (Day 0), after 14 and 28 days of treatment in the control, diabetic, diabetic + Laura extract, diabetic + olive leaves extract, and mix treatment groups.

Animal groups	Day post induction		
	0	14	28
Control normal	140.3±9.9	234.1±25.6	280.8±19.5
Control Diabetic	194.3±10.0[a]	200.3±13.5[a]	263.3±30.0
Diabetic +Laura	153.3±11.0	195.2±19.0[a]	239.4±31.3[a]
Diabetic +Olive	166.4±11.5[a]	212.9±12.8	261.6±19.6
Diabetic + Laura + Olive	181.3±13.6[a]	214.4±22.3	260.4±30.4
P value	<0.001	0.002	0.056

Data are presented as mean ± SE. The mean value with different superscript letters within the same column significantly differed compared to the control.

In the diabetic group, the gene expression of GLUT2 was significantly downregulated compared to the control group (P< 0.001). On the other hand, the treatment with laura 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 2).

Table 2 Gene expression of GLUT2 for the control group compared with the diabetic group, diabetic+ Laura group, diabetic + olive leaves group, and mix group at the end of the experiment

Animal groups	Glut2 gene expression (fold change)
Control normal	1
Control Diabetic	0.155±.03[a]
Diabetic +Laura	0.453±.05[a]
Diabetic +Olive	0.595±.04[a]
Diabetic + Laura + Olive	0.861±.004[a]
P value	<0.001

Data are presented as mean ± SE. The mean value with different superscript letters within the same column significantly differed compared to the control.

The findings in Table (3) show that after 3 days of induction with STZ there was a significant increase in glucose levels among the groups. The diabetic, Laura, Olive Leaves, and Mix groups all show significantly elevated glucose levels compared to the control group (P< 0.001). Insulin, GOT, and creatinine levels did not show statistically significant differences among the groups.

After 14 days of treatment, the glucose levels remained significantly elevated in the diabetic group compared to the control group (P<.001). However, Laura, Olive Leaves, and Mix exhibited significantly reduced glucose levels compared to the diabetic group with no significant difference compared to the control group (Table 3). In terms of insulin levels, the diabetic group had lower insulin levels with insignificant differences compared to the control group. The treatment with Laura and mix group showed no significant difference in insulin levels with the control and diabetic groups. The Olive Leaves extract group showed significantly higher insulin levels compared to the control, diabetic, and Laura groups (P<.001) (Table 3). No significant differences were found in GOT levels among the groups (P<0.593). Similarly, creatinine levels did not show significant differences across the groups (P= 0.458) (Table 3).

After 28 days of treatment, the glucose levels remained significantly elevated in the diabetic group compared to the

control group (P<.001). However, Laura, Olive Leaves, and Mix exhibited significantly reduced glucose levels compared to the diabetic group with no significant difference compared to the control group (Table 3). In terms of insulin levels, the diabetic group had lower insulin levels with insignificant differences compared to the control group. The treatment with Laura extract and mix group showed a nearby significant increase in insulin levels compared with the control and diabetic groups. The Olive Leaves extract group showed significantly higher insulin levels compared to the control, diabetic, and Laura groups (P<.001). (Table, 3). Also, The treatment with Laura, olive leaves extracts and mixed group showed a significant decrease in GOT levels compared with the control and diabetic groups (P<.001). Similarly, creatinine levels show significant lowering across laura, olive leaves extract and mixed group compared with control and diabetic groups (P <.001) (Table 3).

The findings on oxidant and antioxidant levels after 14 days highlight significant differences among the groups, Malondialdehyde (MDA) level was significantly elevated in the diabetic group compared to the control group (mixed P <.001). However, the Laura, Olive Leaves extract, and mixed groups showed significantly lower MDA levels compared to the diabetic group, with the mixed group exhibiting the lowest level overall with a significant decrease compared to the control and Laura groups. In addition, Glutathione (GSH) levels were significantly reduced in the diabetic group compared to the control group (P<.001). The Laura and Olive Leaves groups exhibited significantly higher GSH levels compared to the diabetic group. However, the Mix group showed GSH levels that were lower than those of the Laura group but still significantly higher than those of the diabetic group (Table 4).

Table 3 Glucose, Insulin, GOT and Creatinine after 3, 14 and 28 days of STZ induction and treatment compared with the control group

Animal group	Day 3				Day 14				Day 28			
	Glucose (mg/dl)	Insulin (uIU/ml)	GOT (U/L)	Creatinine (mg/dl)	Glucose (mg/dl)	Insulin (uIU/ml)	GOT (U/L)	Creatinine (mg/dl)	Glucose (mg/dl)	Insulin (uIU/ml)	GOT (U/L)	Creatinine (mg/dl)
Control normal	124.1±6.8	0.31±0.07	127±6.4	0.74±0.12	118.8±6.2	0.253±0.05	121.1±13.5	0.93±0.23	113.8±11.1	0.197±0.01	131.2±22	0.86±0.04
Control Diabetic	308.4±5.3^{a}	0.29±0.09	131.4±7.8	0.79±0.1	337.3±29.9^{a}	0.231±0.04	122.2±6.8	0.99±0.07	307.4±19.6^{a}	0.184±0.04	125.9±7	1±0.06^{a}
Diabetic +Laura	302.6±7.4^{a}	0.32±0.08	133.6±5.2	0.72±0.09	178.4±16.5^{b}	0.254±0.05	120±6.6	0.903±0.13	172.3±10.7^{b}	0.272±0.04^{b}	112.8±6.6^{a}	0.88±0.07^{b}
Diabetic +Olive	252±4.1^{a}	0.29±0.06	129.5±4.2	0.78±0.11	179±10.9^{b}	0.408±0.05^{abc}	115.1±11.7	0.87±0.1	168.7±6.6^{b}	0.477±0.08^{abc}	111.2±8.8^{a}	0.85±0.05^{b}
Diabetic +Laura + Olive	306.3±5.4^{a}	0.30±0.03	127.7±4.3	0.73±0.09	165.1±5.6^{b}	0.248±0.03^{b}	117.8±10.5	0.85±0.25	131.22±8.5^{b}	0.27±0.06^{bd}	107.7±7.3^{ab}	0.79±0.1^{b}
P value	<.001	0.365	0.643	0.178	<.001	<.001	0.593	0.458	<.001	<.001	<.001	<.001

Data are presented as mean ± SE. with different superscript within the same column means significant difference i The mean value with different superscript letters within the same column significantly differed compared to the control."

Table 4 Malondialdehyde and Glutathione values in the diabetic, diabetic + Laura, diabetic + olive leaves, and mix (Diabetic +Laura + Olive) groups.

Animal groups	Malondialdehyde (nmol/ml)	Glutathione (mg/dl)
Control normal	4.87±0.16	3.79±0.13
Control Diabetic	10.83±1.4^{a}	2.98±0.11^{a}
Diabetic +Laura	4.98±0.32	4.70±0.1^{a}
Diabetic +Olive	3.74±0.48	4.46±0.34
Diabetic +Laura + Olive	2.60±0.35^{a}	3.59±0.35
P value	<.001	<.001

Data are presented as mean ± SE. a: significant difference compared to control in Duncan at p< 0.05.

4. DISCUSSION

The metabolic condition known as diabetes mellitus (DM) has numerous etiologies and is typified by persistent hyperglycemia along with disruptions in the metabolism of carbohydrates, proteins, and fats. These disruptions can arise from deficiencies in either insulin production, insulin action, or both. Measurements of abnormal hyperglycemia are used to validate the clinical diagnosis of diabetes, which is frequently suggested by symptoms such as polyuria, polydipsia, and unexplained weight loss (WHO and IDF, 2006). By 2025, the World Health Organization (WHO) predicts that more than 300 million individuals globally will suffer from diabetes (Park et al., 2011). The current study's findings demonstrated that, following diabetes induction, the mean weight of the diabetic group was considerably lower than that of the control group ($p < 0.001$). There was no statistically significant difference between the control group ($p < 0.05$) and the Laura, olive leaf, and combination groups. (Abdullah and AL-Abachi, 2021) demonstrated that there was no significant difference between the control group and the Laura-treated group, and that the diabetic-induced group had considerably lower weight than the control group. These findings are consistent with our findings. When compared to the control group and the diabetic group treated with olive leaf extract, the diabetic groups' initial body weight was lower, which was related to a deficiency in insulin concentration that causes the breakdown of protein and fatty acids. This is because the diabetic groups' excess protein degradation raises blood levels of amino acids due to insulin hormone deficiency. However, when the diabetic group is treated with Laura leaves extract, their initial body weight increases. These findings suggest that the extract plays a critical role in preventing protein degradation and weight loss by stimulating insulin secretion (Qian et al., 2015). Consistently, Abunab et al. (2016) declared that although there was a significant reduction in body weight in the diabetic group, the treatment with olive leaves extract did not produce a significant effect regarding body weight. The fact that the kidney and liver are two of the few extra-pancreatic mammalian tissues that express GLUT2 as their primary glucose transporter isoform may help to explain why these organs preferentially absorb STZ (Guirgis et al., 2021). The decrease in GLUT2 gene expression suggests a potential improvement in glucose homeostasis in the treatment groups compared to the diabetic group (Guirgis et al., 2021) Which came in agreement with those of Guirgis et al. (2021) who showed that Rats injected with STZ exhibited a significant downregulation in the expression level of the GLUT2 gene in the pancreas when compared with the normal control group.

Finally, it has been manifested that the deterioration of insulin-stimulated glucose transport is responsible for impedance to insulin-stimulated glycogen synthesis in muscle in subjects with type 2 diabetes (Cline et al., 1999). So, impaired glucose transport plays a major role in the pathogenesis of type 2 diabetes. Regarding our results, glucose levels in the diabetic groups treated with leaves extract of Laura, Olive, and Mix (laura and olive leaves) remained significantly high compared to controls. After 14 days, glucose levels in the Laura, Olive Leaves, and mixed groups significantly reduced, showing improvement and no significant difference from the control group, indicating effective glucose-lowering benefits over time. After 28 days, the diabetic group maintained significantly elevated glucose levels compared to the control group (P value <.001). However, the Laura, Olive Leaves, and mixed groups showed markedly reduced glucose levels compared to the diabetic group, with the mixed group approaching near-normal levels. Supporting our results, (Abdullah and AL-Abachi, 2021) showed that there was a significant increase in glucose levels in the diabetic group compared to the control group.

An additional noteworthy discovery is that the administration of olive leaf extract is comparable to the use of metformin, a popular antidiabetic medication that lowers

blood sugar levels by reducing intestinal and hepatic glucose production as well as by increasing peripheral glucose uptake and utilization. Other investigations have reported similar mechanisms of action of olive leaf extract (El-Amin et al., 2013, Moghaddam et al., 2021). Additionally, Aljamal (2010) clarified that the polyphenolic molecule presents in Laurus nobilis altered insulin sensitivity, glucose absorption, and antioxidant status, which in turn affected the plant's antidiabetic action.

Moreover, Eidi et al. (2009) reported that the diabetic group had significantly higher glucose levels compared to the control group. The treatment with olive leaves extract brings serum glucose to the normal level. The leuropoeside is the chemical responsible for the mechanism of olive leaf extract. This chemical may have two mechanisms behind its hypoglycemic action: enhanced peripheral glucose absorption and potentiation of glucose-induced insulin release (Wainstein et al., 2012). In the present study, Olive Leaves extract significantly showed an increase in insulin level compared to the control, diabetic, and Laura groups ($P < .001$), This indicates that Olive Leaves might have a distinct effect on insulin secretion or metabolism. However, the mixed group had significantly lower insulin levels than the Olive Leaves group. Abunab et al. (2016) conducted a meta-analysis that giving diabetic rats extract supplementation resulted in hyperinsulinemia and decreased hyperglycemia. Consequently, in diabetic rats induced with alloxan and STZ, oral treatment of an olive leaf extract resulted in a considerable drop in serum glucose levels and an increase in serum insulin. Olive leaf extract was found to have a greater beneficial impact on diabetic rats than glibenclamide, a medication that lowers blood sugar levels by stimulating the pancreas to produce more insulin. Blood sugar levels are lowered by olive leaf extract (Boaz et al., 2011). Moreover, Wainstein et al. (2012) examined the effects of treating individuals with type 2 diabetes mellitus (T2DM) with olive leaf extract in addition to measuring glucose levels in diabetic rats. Supplementing with olive leaf extract for 14 weeks significantly reduced HbA1c levels when compared to placebo; nevertheless, there was no significant difference in fasting or postprandial insulin and glucose levels between the human experimental and control groups. About the serum AST enzyme, which is measured and used to assess hepatic diseases. Increased activity of these enzymes indicates ongoing liver injury. Transaminase levels are markedly raised in inflammatory hepatocellular diseases (Contreras-Zentella and Hernández-Muñoz, 2016). These results are consistent with Yazdi et al. (2019) observation that streptozotocin therapy considerably alters liver functioning, as seen by the significantly higher than normal levels of AST and ALT. Furthermore, Abdullah and AL-Abachi, (2021) found that following treatment with Laura, there was no significant difference in GOT between the diabetic group and the control group, while there was a substantial rise in GOT in the former. Moreover, Eidi et al. (2009) showed that diabetic rats had significantly higher GOT level compared to the control group. The treatment with Olive leaves extracts significantly decreased the GOT level compared to the diabetic group. Hepatic cell injury is manifested by elevated serum transaminase activity before the appearance of clinical symptoms and signs. AST and ALT used in the diagnosis of hepatic cell injury are aminotransferases in the mitochondria of rats. Comparable elevations of both enzymes often reflect liver damage (Kurup & Mini 2017). Usually, the liver enzyme levels in the blood are low. When the liver is damaged, it will release more AST and ALT into the blood, and the enzyme levels will rise (Eidi et al. 2009, Kurup & Mini 2017). The focus of our study was on one of the most common liver enzymes, AST. This enzyme level of all the groups in the current study was measured at the end of 28 days. According to our results, the serum AST levels had no significant difference in diabetic rats when compared with the untreated rats. The increase in these enzyme values may be due to the injection of STZ that has a significant role in the change of liver functions. The increased AST levels were dramatically decreased in the presence of Laura and Olive leaves extracts compared with the control and diabetic groups. In the current study, at 14 days, creatinine levels did not show significant differences across the groups. At 28 days, the diabetic group had higher creatinine levels compared to the control. The extract of Laura, Olive Leaves, and mixed groups had significantly lower creatinine levels compared to the diabetic group. In line with our results, Eidi et al. (2009) highlighted that the diabetic rats had significantly higher creatinine level compared to normal rats, this increase was mitigated with Olive Leaves extracts treatment. Overproduction of reactive oxygen species such as hydrogen peroxide and molecular oxygen modulates biological function of all biomolecules, being lipids target to oxidation to generate MDA, a marker of lipid damage (Maritim, et al., 2003 , Pisoschi and Pop, 2015). The higher levels of oxidative stress in diabetic rats were due to autoxidation of glucose, protein glycation, lipid peroxidation, and low activities of antioxidant enzymes (Giugliano , et al, 1996). The depletion of GSH level in diabetic rats might be due to its utilization to alleviate the oxidative stress in diabetes

In the present study, at 14 days of treatment, it revealed that Malondialdehyde (MDA) levels were significantly elevated in the diabetic group compared to the control ($P < .001$) Similar results were reported by Khashana and Al-Turfib,(2017). , while Laura, Olive Leaves, and Mix groups showed significantly lower MDA levels. The mixed group exhibited the lowest levels overall. At 28 days, MDA levels remained significantly elevated in the diabetic group, Similar results were reported by Khashana and Al-Turfib (2017), while the Laura, Olive Leaves, and mixed groups continued to show significantly lower levels, particularly the Olive Leaves and Mix groups. This indicates an effective reduction of oxidative stress by these treatments, especially the Mix group.

This agreed with Guex et al. (2019) who demonstrated that the MDA concentration was significantly decreased with olive leaves extracts treatment compared to the diabetic group.

In the current study, Glutathione (GSH) levels were significantly reduced in the diabetic group ($P < .001$), Similar results were reported by Khashana and Al-Turfib (2017). However, Laura and Olive Leaves extract groups showed significantly higher GSH levels compared to the diabetic group, indicating improved antioxidant capacity. The mixed group had lower GSH levels than Laura but higher than the diabetic group. The Laura and Olive Leaves extract groups showed the highest GSH levels, followed by the mixed group. Al-Attar and Alsalmi, (2019) also observed a statistically significant decrease in the levels of serum SOD, GSH, and CAT in diabetic rats when compared to control values. Following treatment with both low and high dosages of olive leaf extract, the levels rose once again. Rats with diabetes who were given an extract of olive leaves had much lower serum MDA levels than the control group, which had significantly higher MDA levels.

5. CONCLUSIONS

Laurel and olive leaves extracts, particularly in combination, offer a significant therapeutic potential in managing diabetes and its associated complications by improving glucose homeostasis, lipid profile, renal and liver function, and enhancing antioxidant defense.

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