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OLIVE AND ARTICHOKE LEAVES ATTENUATE HYPERGLYCEMIA AND PROTECT LIVER IN ALLOXAN-INDUCED DIABETIC RATS

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ABSTRACT: The current study was designed to investigate the chemical composition of olive and artichoke leaves, focusing on the content of phenolic compounds, assessing the antioxidant properties of these plant leaves, and evaluating the effects of olive and artichoke leaves on the glucose levels in diabetic rats. Results indicated that the chemical composition of olive and artichoke leaves were moisture (4.98 and 3.02 %), crude fiber (13.25 and 14.4 %), ash (6.95 and 6.27 %), crude protein (10.35 and 9.41 %), total lipids (12.25 and 1.13 %) and total carbohydrate (52.22 and 65.77%). The phenolic compounds content for olive leaves were chrysin (30.1%) and oleuropein (15.17%), meanwhile phenolic compounds content for artichoke leaves were genstin (7.78%) catechol and cinamic acid (1.9 and 1.88%) respectively. The quenching of DPPH free radicals and ferric-reducing antioxidant power assay further highlighted the antioxidant potential of olive and artichoke leaves. Treatment with water and alcoholic extracts of olive and artichoke leaves decreased significantly glucose levels, ALT, AST, ALP, total cholesterol, LDL-C, and VLDL-C. It increased significantly HDL-C as compared with the hyperglycemic group.

Key words: Plant leaves, Hyperglycemia, Rats, Active compounds, Biochemical analysis

INTRODUCTION

A class of metabolic disorders known as diabetes is typified by hyperglycemia brought on by deficiencies in either the action or secretion of insulin, or both. Diabetes-related chronic hyperglycemia is linked to long-term harm, malfunction, and failure of various organs, particularly the heart, blood vessels, kidneys, eyes, and nerves (ADA, 2010). One of the main health issues in the world is diabetes mellitus (DM), a metabolic disease. It has been steadily rising to 336 million people worldwide, and by 2030, it is expected to affect 552 million people (Habtamu, *et al.*, 2018).

Diabetes damages the cardiovascular system by causing narrowing and reduced flexibility of blood vessels, impeding blood flow and oxygen delivery, which can lead to high blood pressure and damage to blood vessels. High blood sugar levels in diabetes increase the risk of macrovascular diseases such as cardiac arrest, stroke, and peripheral artery disease, impacting overall cardiovascular health. The relationship between diabetes and cardiovascular issues is significant, as all components of the cardiovascular system are susceptible to harm from high blood sugar levels, highlighting the close connection between diabetes and cardiovascular complications. Complications of diabetes can result in microvascular issues affecting the cardiovascular system, leading to problems with the eyes, kidneys, and nervous system, further exacerbating the impact of diabetes on cardiovascular health. (Dilara, *et al.*, 2023). The investigation of pharmacologically and biologically active compounds obtained from natural sources, including plant extracts, has yielded numerous therapeutically significant medications vital for the treatment of human diseases (Rastogi and Meharotra, 1990). Plants and their active ingredients are essential for enhancing overall health, particularly lowering the negative effects of diabetes and lowering blood lipid levels, according to numerous studies (Farid *et al.*, 2012; Abozid *et al.*, 2018; Sakr *et al.*, 2019). With approximately half a million plants worldwide, medicinal plants have a promising future. However, most of these plants have not yet been studied for their potential medical benefits, which could be crucial in the

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treatment of current and upcoming research (Singh, 2015). The beneficial pharmacological effects of olive leaves are widely recognized. Extracts from olive leaves possess antimicrobial, anti-inflammatory, antioxidant, anti-hypertensive, anti-hypercholesterolemic, anti-hyperglycemic, antithrombotic, diuretic, and antitumor qualities. As a medicinal herb, olive leaves have also been used to treat infectious diseases, hypertension, and diabetic hyperglycemia. According to Komaki *et al.* (2003), they are particularly well-known in Europe as a traditional treatment for diabetes and hypertension. Artichoke leaf extract has demonstrated antioxidative, anti-HIV integrase, liver-protective, bile-expelling, antimicrobial, and lipid-lowering properties in a variety of pharmacological test systems (Wang *et al.*, 2003). Because of their high antioxidant activity and abundance of bioactive compounds, artichokes are regarded as a functional food that is good for consumers' health (Spanu, *et al.*, 2018). Other potent antioxidants of the polyphenol type that are present in artichokes may help prevent and treat leukemia, breast cancer, and prostate cancer. According to research, the antioxidants gallic acid, quercetin, and rutin present in ALE can cause apoptosis, or cell death, and slow the growth of cancer cells (Nadova *et al.*, 2008). Therefore, the current study's objective was to assess the antidiabetic effects of artichoke and olive leaves in animal models of diabetes.

MATERIALS AND METHODS

Materials

Olive and artichoke leaves were collected from the Research Center Department of Medical and Aromatic Plants. Giza Egypt. The leaves were dried and milled.

Methods

Proximate composition

The proximate composition of olive and artichoke leaves (moisture, ash, crude protein, total lipids, crude fiber, and total carbohydrates) was determined according to the methods of the Association of Official Analytical Chemists (AOAC, 1990) and (Pearson 1976).

Quantitative determination of phenolic compounds by HPLC

The approach was a modified version of (Zuo *et al.*, 2002). The prepared sample was analyzed using a Shimadzu LC 20 AT HPLC filtered with an SIL 20 A autosampler and an SPD-20 UV visible detector with a class LC 10 chromatography workstation. Using a Ryeodyne precolumn filter 7335 model, a Luna TM 5 µM C18,25cm ×4.6 mm i.e. (Phenomenex, Torrance, CA, USA) column was employed. Before being injected into an HPLC system, all solvents were degassed and filtered through a 0.45 µM Millipore membrane filter disk. The following solvent systems were used for a gradient elusion: Mobile phase A (acetonitrile, acetic acid, and double-distilled water (9/2/89 v/v/v). Acetonitrile, acetic acid, and double-distilled water (80/2/18 v/v/v) make up mobile phase B. The mobile phase composition was sustained at 60% mobile phase A and 32% mobile phase B for ten minutes in a binary gradient. The minute preceding the next injection. The column's temperature was maintained at 35 ±0.5 o C, and the mobile phase flow rate was 1 mL/min. The quantification of the catechins was conducted at a wavelength of 278 nm, utilizing a caffeine external standard accompanied by a calibration curve exhibiting an R^2 value of 0.9984. This process employed the consensus individual catechin response factor (RRF) values for caffeine, as determined on a dry matter basis. The total catechins, expressed as a percentage of mass relative to the dry matter of the sample, were presented based on the individual catechin components.

Antioxidant activity

Using the scavenging activity of the stable 1,1diphenyl-2-picrylhyorazyl (DPPH) free radical as reported by Braca *et al.* (2002), with minor adjustments, the antioxidant activity of plant extracts was assessed. Plant extracts' reducing power was assessed using the method described by (Ebrahimzadeh *et al.*, 2008).

Biological Evaluation

Animals

Thirty-six male adult albino rats a strain of Sprague Dawely weighing 150–170 grams were

acquired from the Egyptian Organization for Biological Products and Vaccines (VACSERA) Animal House in Cairo, Egypt. The cages in which the animals were housed had wire bottoms. The rats were given the diet in a special feed cup that minimizes food spills, and they were given water through a glass tube that protruded through a wire cage. One side of the cage was supported using an inverted bottle. The rats in the negative control group received a balanced diet throughout the study, whereas the rats in the other groups were administered alloxan (100 mg/kg body weight) to induce hyperglycemia during the feeding period. We began the study once the blood glucose level exceeded 200 mg/dl. A positive control group was maintained in each experiment by continuing to be fed the same diet without any supplements. 500 mg/kg body weight of plant extracts were administered to the other groups (Each group included 6 rats). Their food intake was tracked every day and before having their blood drawn, all of the rats fasted. After 30 days, blood samples were extracted from the eye plexuses. Diethyl ether was used to anesthetize the rats.

Blood sampling and analysis

After 30 days, blood samples were drawn from the eye plexuses under diethyl ether anesthesia in tubes containing heparin as an anticoagulant. The plasma was then extracted by centrifuging the blood at 3000 rpm for 20 minutes, and it was stored in a freezer until analysis. Blood glucose was measured using Tinder's (1969) enzymatic method. Calorimetric analysis of total cholesterol was performed using the Richmond (1973) method. The triglycerides were examined using the method described by Fossati and Prencipe (1982). The activity of the enzymes aspartate aminotransferase (AST) and alanine aminotransferase (ALT) was assessed using the procedure outlined by Reitman and Frankel (1957). The method of Hausamen *et al.* (1967) was used to measure the activity of alkaline phosphatase (ALP). HDL cholesterol was measured using the method described by Lopez *et al.* (1977). Based on the findings of Fiedewaid *et al.* (1972), LDL-C and VLDL-C were computed.

Statistical analysis

The mean ± SE was used to express the results of the animal experiments, and Duncan's test and one-way analysis of variance (ANOVA) were used for statistical analysis. The criterion of statistical significance was always $p<0.05$.

RESULTS AND DISCUSSION

Proximate composition of olive and artichoke leaves

Proximate compositions of olive and artichoke leaves are presented in Table (1) olive leaves consisting of moisture (4.98%), crude fiber (13.25%), ash (6.95%), crude protein (10.35 %), total lipids (12.25 %) and total carbohydrate (52.22%). Meanwhile, artichoke leaves consisted of moisture (3.02 %), crude fiber (14.4%), ash (6.27%), crude protein (9.41%), total lipids (1.13%), and total carbohydrate (65.77 %). Our data are in line with that of (Delgado Pertınez 1994) who reported that chemical analysis of olive leaves indicated that it is poor in N, rich in total lipids and acid detergent fiber, and low in tannins, also Ibrahim *et al.* (2016) reported that the proximate chemical composition of whole olive leaves which contain moisture (50.5%), crude protein and ash contents that amounted in whole leaves 10.6, and 6.8% respectively. Crude fiber and total carbohydrate were 14.5 and 74.7% respectively.

Table 1: Proximate composition of olive and artichoke leaves (D/w%).

Chemical Composition of Plant	Moisture	Crude protein	Total lipids	Crude fiber	Total ash	Total carbohydrates
Olive leaves	4.98	10.35	12.25	13.25	6.95	52.22
Artichoke leaves	3.02	9.41	1.13	14.4	6.27	65.77

Phenolic compounds

Phenolic compounds in methanol extracts of leaves for olive and artichoke were analyzed by High-Performance Liquid Chromatography (HPLC), and concentrations of all tested phenolic compounds are given in Table (2).

From Table (2) it was found that olive leaf extract contains 16 phenolic compounds, analysis of olive leaves extract showed that the major compounds found in the extract, were chrysin (30.1 mg/g), oleuropein (15.17 mg/g), protocatechuic acid (3.23 mg/g), gallic acid (3.04 mg/g), benzoic acid (2.2 mg/g) and coumaric acid (1.03 mg/g).

Table (2) shows that artichoke leaves extract contains 14 phenolic compounds, the major compounds were: genstin (7.78% mg/g), catechol (1.9% mg/g), cinamic acid (1.88% mg/g), dadzine (1.45% mg/g), salycilic acid (1.35% mg/g) and ferulic acid (1.23% mg/g). The results of the chemical composition of artichoke leaf extract were following those reported by (Zug *et al.,* 2018), who studied the polyphenolic compound in olive leaves and found that oleuropein represent (24.54 %).

Also (Japon-Lujan *et al.,* 2006) reported that oleuropein content in olive leaves ranges between 1% and 14%. On the other hand (Pandino, *et al.,* 2011) aimed cynarin and chlorogenic acid are the important antioxidant compounds in artichoke leaves.

Table (2): Phenolic compounds of olive and artichoke leaf extracts.

Phenolic compounds	Olive leaves (mg/g)	Artichoke leaves (mg/g)
Phenol	ND	0.49
Catechol	ND	1.9
Vanillic acid	0.05	ND
Benzoic acid	2.2	0.72
Hydroxy tyrosol	0.02	ND
Ferulic acid	0.182	1.23
Protocatechuic acid	3.23	ND
Caffeic acid	0.34	ND
Catechin	0.289	ND
Coumaric acid	1.03	0.90
Chlorogenic acid	0.52	ND
Chrysin	30.1	0.71
Rutin	0.05	ND
Gallic acid	3.04	ND
Oleuropein	15.17	ND
Salycilic acid	0.55	1.35
Quercetin	0.02	0.77
Luteolin cin	0.09	ND
Cinamic acid	ND	1.88
Euganol	ND	0.55
Cynarin	ND	0.63
Galangin	ND	1.25
Genstin	ND	7.78
Dadazine	ND	1.45

Antioxidant activity

Antioxidant function can be assessed using a variety of techniques that depend on various free radical generators and their modes of action. It is extremely difficult to assess a product's antioxidant activity using a single technique. Basic information regarding antioxidant properties can be obtained from a single method, but a combination of methods provides a more detailed description of the sample's antioxidant properties.

The quenching of DPPH free radicals further demonstrated the antioxidant potential of olive and artichoke extracts. The "stable" free radical DPPH was used to test the extracts' ability to scavenge radicals. The effective concentrations of each plant extract needed to scavenge the DPPH radical are displayed in Table (3) along with the scavenging values expressed as an inhibition percentage. Different levels of scavenging capability were demonstrated by plant extract. At 100 µg/ml, the greatest radical-scavenging effect was demonstrated by olive methanol extract (70.96%), which was less than that of ascorbic acid (93.6%), positive control. The olive water extract and artichoke methanol extract (67.78 and 67.71 percent, respectively) came next in this activity.

Table (3): Antioxidant activity of basil and thyme essential oils measured by DPPH Method.

Samples	DPPH % inhibition			
	25 µg	**50 µg**	**75 µg**	**100 µg**
Olive water extract	6.08	34.28	54.64	67.78
Olive methanol extract	15.3	40.72	59.1	70.96
Artichoke water extract	5.48	33.85	54.33	67.59
Artichoke methanol extract	5.9	34.15	54.57	67.71
Ascorbic acid	81.6	87.1	91.1	93.6

The aforementioned information is consistent with Gordon *et al.* (2001), who demonstrated that oleuropein and hydroxytyrosol found in olive leaves were also successful scavengers of the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical.

An essential mechanism in the antioxidant action of phenols is electron-donating activity, which is frequently indicated by Fe (III) reduction (Nabavi *et al.,* 2009). Reductants, or antioxidants, would reduce Fe+3 to Fe+2 in this assay by contributing an electron if they were present in the samples. The formation of Perl's Prussian blue at 700 nm can then be used to measure the amount of Fe+2 complex. Increased absorbance at 700 nm indicates a rise in reductive ability. The dose-response curves for the plant extracts' reducing powers are displayed in Fig. (1). It was found that increasing extract concentrations corresponded with enhanced reducing capabilities. Olive methanol extract exhibited the highest activity (0.073) at the highest concentration (100 µg/ml), while artichoke methanol extract showed the highest activity (0.04) at the same concentration as ascorbic acid (0.471).

Research has indicated that a compound's reducing power capacity could be a useful predictor of its possible antioxidant activity (Sofidiya *et al.* 2006). Our findings are consistent with those of Ceccarelli *et al.* (2010), who found that the phenolic compounds in artichoke extracts have significant scavenging activity against free radicals and reactive oxygen species (ROS) and act as a barrier to prevent oxidative damage to biological molecules like proteins, lipids, and DNA.

In Vivo study of the effect of olive and artichoke leaves on hyperglycemic rats

Tables (4 and 5), which show the mean values over the entire duration, show the impact

of extracts from olive and artichoke leaves on the lipid profile and glucose level of albino rats that were hyperglycemic for 30 days. The negative control group of albino rats had a glucose level of 115.95 mg/dl, while in the hyperglycemic group was 278.41 mg/dl, meanwhile olive water and methanol extracts were 143.66 and 135.66 mg/dl respectively, on the other hand, artichoke water and methanol extracts were 155.75 and 101.33 mg/dl respectively.

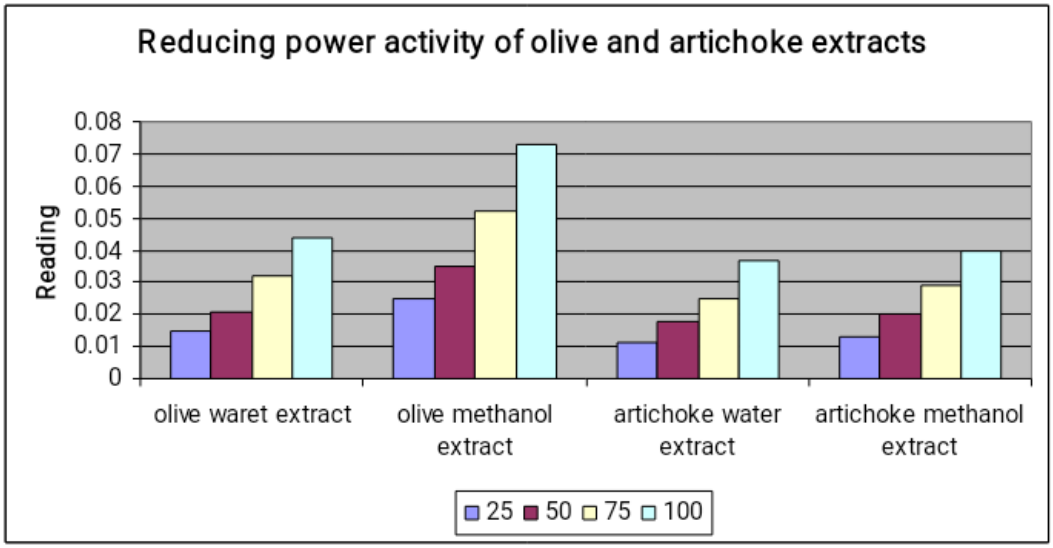

Fig. (1): Reducing power activity of olive and artichoke extracts.

Table (4): Effect of olive and artichoke leaf extracts on glucose level, triglycerides, and total cholesterol of rats.

Group	Glucose (mg/dl)	Triglycerides (mg/dl)	Total cholesterol (mg/dl)
Negative control group	115.95±3.21^{a}	60.33±1.92^{a}	91.33±0.84^{b}
Positive control group	278.41±2.67^{c}	72.66±2.07^{b}	98.33±1.14^{c}
Olive water extract	143.66±5.84^{b}	69.33±1.87^{b}	86.33±1.14^{a}
Olive methanol extract	135.66±7.78^{b}	70.33±1.64^{b}	88.33±1.14^{a}
Artichoke water extract	155.75±6.41^{b}	69.05±2.87^{b}	85.33±1.84^{a}
Artichoke methanol extract	101.33±6.78^{a}	63.66±1.30^{a}	89.33±1.00$^{a, b}$

Values represent means ± S.D obtained from (6) rats, means in the same column followed by the same letters do not differ significantly, and when the means followed by different letters differed significantly at ($p \geq 0.05$).

Table (5) Effect of olive and artichoke leaves extracts on, HDL-C, LDL-C, and VLDL-C of rats:

Group	HDL-C (mg/dl)	LDL-C (mg/dl)	VLDL-C (mg/dl)
Negative control group	55.44±1.89^{b}	24.33±1.18^{b}	12.22±0.38^{a}
Positive control group	47.33±1.67^{a}	38.13±1.94^{c}	15.53±0.41^{b}
Olive water extract	54.33±1.84^{b}	18.13±1.58^{a}	13.86±0.37^{a}
Olive methanol extract	50.33±1.84^{b}	23.93±1.62^{b}	14.06±0.33^{a}
Artichoke water extract	51.66±1.74^{b}	19.73±1.41^{a}	14.50±0.33$^{a, b}$
Artichoke methanol extract	51.40±1.30^{b}	25.60±1.44^{b}	12.73±0.26^{a}

Values represent means ± S.D obtained from (6) rats, means in the same column followed by the same letters do not differ significantly, and when the means followed by different letters differed significantly at ($p \geq 0.05$).

The elevation of glucose levels in a hyperglycemic group may be due to a decrease in both insulin secretion and insulin action (Altan 2003), because of injection by alloxan, it is because diabetes causes lipid peroxidation that mediated damage in pancrease (Ahmed *et. al.*, 2014). According to Pereira *et al.* (2006) and Komaki *et al.* (2003), olive leaves are widely used as a traditional herbal remedy for diabetes and hypertension. Our data supports their findings. Oleuropein may also help prevent hyperglycemia and oxidative stress brought on by diabetes, which speeds up the cell's absorption of glucose, according to research by Al-Azzawie *et al.* (2006).

Plasma triglycerides and total cholesterol levels in the negative control group of albino rats were 60.33 and 91.33 mg/dl respectively, while in the hyperglycemic group were 72.66 and 98.33 mg/dl, meanwhile olive water extract recorded 69.33 mg/dl for triglycerides and 86.33 mg/dl for total cholesterol, and olive methanolic extract were 70.33 and 88.33 mg/dl. On the other hand, artichoke water extract recorded 69.05 and 85.33 mg/dl and artichoke methanolic extract was 63.66 and 89.33 mg/dl respectively.

Plasma HDL-C levels in the negative control group were 55.44 mg/dl while in the positive control group were 47.33 mg/dl meanwhile olive water and methanol extracts were 54.33 and 50.33 mg/dl respectively, on the other hand, artichoke water and methanol extracts were 51.66 and 51.40 mg/dl respectively.

Plasma LDL-C levels in the negative control group were 24.33 mg/dl while in the positive control group were 38.13 mg/dl meanwhile olive water and methanol extracts were 18.13 and 23.93 mg/dl respectively. On the other hand, artichoke water and methanol extracts were 19.73 and 25.6 mg/dl respectively.

Plasma VLDL-C levels in the negative control group were 12.22 mg/dl while in the positive control group were 15.53 mg/dl meanwhile olive water and methanol extracts were 13.86 and 14.06 mg/dl respectively, on the other hand, artichoke water and methanol extracts were 14.5 and 12.73 mg/dl respectively.

Our findings are consistent with those of Wang *et al.* (2003), who found that the extract from artichoke leaves (ALE) has lipid-lowering, liver-protective, bile-expelling, antioxidative, anti-microbial, and anti-HIV integrase properties. Additionally, (Gebhardt, 1996) demonstrated that cynarin in ALE may help support digestive health in addition to influencing cholesterol. It is well known that cyanarin promotes the synthesis of bile, which facilitates vitamin absorption and fat digestion.

Table (6), which shows the mean values over the entire duration, shows the impact of extracts from olive and artichoke leaves on the AST, ALT, and ALP activity of albino rats that were hyperglycemic for 30 days. Aspartate amino transaminase (AST) activity was found to be 190.06 IU/L in the normal group of albino rats and 193.6 IU/L in the hyperglycemic group. Artichoke water and methanol extracts were found to be 177.8 and 186.06 IU/L, respectively, while olive water and methanol extracts were 182.66 and 189.66 IU/L.

Alanine amino transaminase (ALT) activity in the normal group was 70.4 IU/L while in the positive control group was 91.66 IU/L meanwhile olive water and methanol extracts were 71.33 and 84.33 IU/L respectively, on the other hand, artichoke water and methanol extracts were 91.66 and 79.33 IU/L respectively. ALP activity in the normal group was 125.8 IU/L, while in the positive control group was 135.66 IU/L meanwhile olive water and methanol extracts were 123.66 and 137.66 IU/L respectively. On the other hand, artichoke water and methanol extracts were 125.66 and 122.33 IU/L respectively.

This outcome may be explained by the findings of Xia *et al.* (2016), who showed that the oleuropein in olive leaf extract has indirect membrane stabilization capabilities by preventing the production of reactive oxygen species and preserving the structural integrity of the membrane. Our findings are consistent with those of Kulza *et al.* (2010), who described the hepatoprotective action of ALE concerning its active compounds and backed the use of

artichokes as an antioxidant and hepatoprotective agent.

CONCLUSION

In conclusion, the study demonstrated that olive and artichoke leaves contain valuable bioactive compounds, including phenolics with strong antioxidant properties. Their extracts exhibited significant hypoglycemic and lipid-lowering effects in diabetic rats, suggesting their potential as natural therapeutic agents for managing diabetes and associated metabolic disorders. Further research is recommended to explore the underlying molecular mechanisms of these effects, conduct long-term clinical studies to assess their safety and efficacy in humans, and investigate the potential synergistic effects of combining these extracts with other natural or pharmaceutical agents for diabetes management.

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أوراق الزيتون والخرشوف لخفض سكر الدم وحماية كبد الفئران المصابة بمرض السكرى الناتج عن الألوكسان

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الملخص العربى

تهدف هذة الدراسة إلى دراسة التركيب الكيميائى لأوراق الزيتون والخرشوف ، دراسة محتوى الاوراق من المركبات الفينولية ، تقييم مستخلصات الاوراق كمضادات أكسدة ومدى تأثير تلك المستخلصات على مستوى سكر الدم فى الفئران المصابة بداء السكرى. وسجلت أوراق الزيتون والخرشوف رطوبة ٤,٩٨ و ٣,٠٢ % - الياف خام ١٣,٢٥ و ١٤,٤ % - رماد ٦,٩٥ و ٦,٢٧ % - بروتين ١٠,٣٥ و٩,٤١ % - ليبيدات ١٢,٢٥ و ١,١٣ % وكربوهيدرات ٥٢,٢٢ و ٦٥,٧٧ % . وبتحليل المركبات الفينولية لأوراق الزيتون سجل chrysin ٣٠,١ % وسجل oleuropein ١٥,١٧% وبتحليل أوراق الخرشوف سجل genstin ٧,٧٨ % وسجل كلا من catechol و cinamic acid ١,٩ و ١,٨٨ % على التوالى. تم دراسة النشاط المضاد للأكسدة لمستخلصات الزيتون والخرشوف من خلال تثبيط الجذور الحرة DPPH واختبار قدرتهم على إختزال الحديديك. وأشارت الدراسة إلى أن المعاملة بالمستخلصات المائية والكحولية لأوراق الزيتون والخرشوف أدت إلى انخفاض معنوى فى مستوى سكر الدم وكذلك نشاط أنزيمات الكبد ALT , AST , ALP والكولستيرول الكلى و , LDL-C VLDL-C وزيادة معنوية فى HDL-C وذلك بالمقارنة بالمجموعة المصابة بارتفاع سكر الدم.

الكلمات المفتاحية : أوراق النبات - أرتفاع سكر الدم - فئران التجارب - المركبات الفعالة - التحليلات الكيميائية الحيوية.

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