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Original Article

Does *Lycium europaeum* leaf have antihyperglycemic, antihyperlipidemic and antioxidant effects

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The purpose of the present investigation is to assess, for the first time, the antidiabetic, antihyperlipidemic and antioxidant activities of *Lycium europaeum* extract in alloxan-induced diabetic rats. Diabetes was induced in adult male Wistar rats via a single subcutaneous alloxan injection (120 mg/kg). *Lycium europaeum* aqueous extract was orally administered at a dose of 20 mg/kg for 28 consecutive days. Serum concentrations of total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C) and triglycerides (TG) were assayed at the end of the experimental period in all investigated groups. Antioxidant enzymes such as glutathione peroxidase (GPx), catalase (CAT) and superoxide dismutase (SOD) were sought in the serum and pancreas. *Lycium europaeum* extract significantly increased HDL-C and reduced blood glucose, TC, LDL-C and TG as compared to the alloxan-control group. *Lycium europaeum* extract was also efficient in reducing oxidative stress in diabetic rats by increasing SOD, CAT and GPx activities both in the pancreas and the plasma of the animals. Moreover, *Lycium europaeum* extract contained considerable levels of polyphenols and flavonoids. It also exhibited an important antioxidant capacity and a remarkable ability to quench DPPH radicals and reduce irons. The obtained results highlight potentially relevant health beneficial effects of *Lycium europaeum* extract, reversing hyperglycemic, hyperlipidemic and oxidative stress effects in rats with alloxan-induced diabetes. Therefore, it may be considered as a promising alternative or complementary agent to diabetes treatment.

Keywords: *Lycium europaeum*/extract/antihyperglycemia. *Lycium europaeum*/extract/antioxidant activity. *Lycium europaeum*/extract/antihyperlipidemia.

INTRODUCTION

Diabetes mellitus (DM) is a chronic endocrine metabolic disorder that poses a major public health problem around the world. The World Health Organization (WHO) predicted it would be the 7th leading cause of death by 2030 (WHO, 2011). The progressive spreading of diabetes in both developed and developing countries has challenged scientists to conduct further investigation seeking potent therapeutic agents from natural sources for a more efficient use in the treatment and management of diabetes (Gupta *et al.*, 2012). DM is defined as a degenerative and chronic disease induced when the pancreas does not produce sufficient insulin, or when the body cannot effectively process this hormine (WHO, 2006). Diabetes frequently leads to serious complications (Deshpande, Harris-Hayes, Schootman, 2008), namely nephropathy, neuropathy and retinopathy (Winkler *et al.*, 2010), which can be delayed and reduced by maintaining tight glycemic control. Additionally, glucose homeostasis disorders also involve abnormalities in lipid metabolism characterized by a reduced cholesterol rate in high-density lipoprotein and increased triglyceride, total cholesterol and cholesterol rates of low-density lipoprotein (Capewell *et al.*, 2010). In fact, hyperlipidemia poses a major risk for the premature development of atherosclerosis and cardiovascular complications (Goldstein *et al.*, 1973).

In the past few decades, increasing evidence has connected oxidative stress to a variety of pathological diseases, including DM (El Faramawy, Rizk, 2011).

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Over time, convincing evidence has established the role of free radicals and oxidative stress in the pathogenesis and development of complications emanating from DM. The possible sources of oxidative stress in diabetes might include glucose auto-oxidation, redox balance shifts, decreased tissue concentrations of low molecular weight antioxidants (vitamin E) and impaired activities of antioxidant defense enzymes as superoxyde dismutase and catalase (Haskins, BradLey, 2003).

An effective management of diabetes requires combined multiple therapeutic approaches: exercise and diet. In fact, synthetic drugs are effectual in DM treatment but restricted by their limited action, pharmacokinetic properties, secondary failure rates and numerous side effects including hypoglycemia, damage to liver, lactic acidosis, diarrhea, abdominal pain, weight loss and loss of appetite (Dey, Attele, Yuan, 2002). Hence, safer and more effective antidiabetic drugs are deemed requisite. Nowadays, several people around the world are using natural remedies solely or in combination with their hypoglycemic drugs to manage their diabetes. As plants are safe and cost-effective (Punithavathi *et al.*, 2011; Sefi *et al.*, 2010), various herbal medicines and medicinal plants have been traditionally used for the control, management and/or treatment of diabetes (Ramachandran, Rajasekaran, Manisenthilkumar, 2012). To date, over 800 species have been investigated and their hypoglycemic effect reported (El-Abhar, Schaalan, 2014). Medicinal plants with antioxidant activity have been scrutinized for their hypoglycemic activities. Consequently, medicinal plant screening for therapeutic schemes is important in drug development, as plants may possess hypoglycemic, hypolipidemic and antioxidant activities, which may be effective in the treatment of this chronic disease (Tiwari, Rao, 2002).

Boxthorn (*Lycium europaeum*) belongs to the *Solanaceae* family and occurs spontaneously around the Mediterranean basin with three other species, namely *L. barbarum*, *L. shawii*, and *L. chinense*. These species have widely been used in traditional medicine. In fact, the *L. shawii* root decoction is widely renowned to treat mouth sores, cough, high blood pressure and diabetes. Fruit of this plant is also consumed for its antioxidant and other beneficial properties (Said *et al.*, 2002). Concerning *L. barbarum*, several studies have indicated its benefits on aging, neuroprotection, general well-being, fatigue/endurance, metabolism/energy expenditure, glaucoma, immunomodulation, antitumor activity and cytoprotection (Amagase, Farnsworth, 2011) as well as hypoglycemic and antioxidant effects in cell or animal experiments (Jin *et al.*, 2013; Zhu *et al.*, 2013; Zhao *et al.*, 2009; Luo *et al.*, 2004).

In Tunisia, the *L. europaeum* species is widely used as a functional food and as a medicinal herb in the treatment of diabetes without an adequate knowledge base. However, to the best of our knowledge, the anti-diabetic effect of *L. europaeum* leaves has not been explored. For this reason, the goal of the present study was to investigate the anti-diabetic, antihyperlipidemic and antioxidant activities of boxthorn leaves in alloxan-induced diabetic rats.

MATERIAL AND METHODS

Plant material

Fresh and mature whole boxthorn plants were collected in December 2016 from Jdaida (35° 33' 18" North, 9° 59' 1" East of Tunis, Tunisia). Botanical identification was carried out and a voucher specimen AMP 561 was deposited at the faculty of Science, University of Tunis El Manar of Tunisia. Boxthorn leaves were washed with water, dried at room temperature in the dark and then ground to a fine powder in a Mettler AE 200 (Dangoumau type) grinder.

Preparation of plant extract

75 g of the powder was soaked in 1 l boiled water at 100°C for 15 min for extraction (Ayiguli *et al.*, 2007). The water extract was filtered with N°3 Whatmann Millipore filter paper and subjected to freeze drying yielding 20.0 w/w of dry material that was stored in refrigerated amber glass containers. This procedure was repeated weekly throughout the study.

Total phenolic content

Total phenolic content of the aqueous extract from *L. europaeum* leaves was determined using the Folin-Ciocalteu reagent (Dewanto *et al.*, 2002). An aliquot (0.125 mL) of a suitable diluted leaf extract (50-400 mg/mL) was added to 0.5 mL of deionized water and 0.125 mL of the Folin-Ciocalteu reagent. The mixture was shaken and allowed to stand for 6 min, before adding 1.25 mL of 7% Na_2CO_3 solution. The solution was then adjusted with deionised water to a final volume of 3 mL and mixed thoroughly. After incubation for 90 min at 23ºC, the absorbance versus prepared distilled water blank was read at 760 nm with an UV-Visible spectrophotometer. Total phenolic content of leaves was expressed as mg gallic acid equivalents per gram of dry weight (mg GAE/g DW) through the

calibration curve with gallic acid. The calibration curve range was 50-400 mg/mL (R^2 = 0.99). All samples were analyzed in triplicate.

Total flavonoid content

Total flavonoid content of the aqueous extract from *L. europaeum* leaves was determined by a colorimetric assay according to Dewanto *et al.* (2002). An aliquot (250 mL) of a suitable leaf extract (50-500 mg/mL) was mixed with 75 mL $NaNO_2$ (5%). After 6 min, 150 mL of 10% aluminium chloride was added and 5 min later, 500 mL of NaOH (1M) was added. Finally, the mixture was adjusted to 2.5 mL with distilled water. The absorbance versus prepared distilled water blank was read at 510 nm with a UV-Visible spectrophotometer. Total flavonoid content of leaves was expressed as mg catechin equivalents per gram of dry weight (mg CE/g DW) through the calibration curve with catechin in the range of 50-500 mg/ mL. All samples were analyzed in triplicate.

Evaluation of antioxidant capacity

Total antioxidant capacity of aqueous extract from *L. europaeum* leaves was determined through the assay of the green Phosphate/ Mo^{5+} complex according to the method described by Prieto, Pineda and Aguilar (1999). An aliquot (0.2 mL) of sample extract was combined with 2 mL of reagent solution (0.3 N Sulfuric acid. 28 mM sodium phosphate and 4 mM ammonium molybdate). Methanol (80%) was used instead of sample for the blank. The tubes were capped and incubated in a boiling water bath for 90 min. Then, samples were cooled to room temperature and the absorbance was read at 695 nm against blank. Antioxidant capacity was expressed as mg gallic acid equivalent per gram dry weight (mg GAE/g DW). All samples were analyzed in triplicate.

Scavenging ability on DPPH radical

The ability of the corresponding extracts to donate hydrogen atoms or electrons was measured from the bleaching of a purple colored methanol solution of 1.1-diphenyl-2- picrylhydrazyl (DPPH), according to the method described by Hatano *et al.* (1988). 1 mL of various concentrations (1-100 µg/mL) of *L. europaeum* leaf extract was added to 250 µl of 0.2 mM DPPH radical solution in methanol. The mixture was shaken vigorously and allowed to stand for 30 min in the dark. The absorbance of the resulting solution was read at 517 nm and butylatedhydroxyl toluene (BHT) was used as positive control. Inhibition of DPPH radical was calculated as follows:

$$\text{DPPH scavenging effect (\%)} = ((A_c - A_S) / A_c)) \times 100$$

where A_c and A_S are the absorbance at 30 min of the control and the sample, respectively. The anti-radical activity was expressed as IC_{50} (µg/mL), the extract dose required to cause a 50% decrease of the absorbance at 517 nm. A lower IC_{50} value corresponded to a higher antioxidant activity. All samples were analyzed in triplicate.

Iron reducing power

Iron reducing power was determined according to the method of Oyaizu (1986). 1 mL of sample extracts at different concentrations (30, 60, 90, 120 and 150 mg/mL) was mixed with 2.5 mL phosphate buffer (0.2 M, pH 6.6) and 2.5 mL potassium ferricyanide (1g/100 mL). After incubation at 50°C for 20 min, the mixture was added to 250 µl of thichloroacetic acid (10g/100 mL) and then centrifuged at 3500 r/min for 10 min. As soon as 400 µL supernatant was aliquoted into 400 µl $FeCl_3$ (0.1 g/100 mL in distilled water), the timer was started. At 90 s, the absorbance was read at 700 nm (Unico 2100, Shangai, China) against ascorbic acid as authentic standard. Higher absorbance of the reaction mixture indicates higher reducing power. EC50 value (µg/mL) is the effective concentration of the extract at which the absorbance was 0.5 and it was obtained from linear regression analysis. All samples were analyzed in triplicate.

Experimental animals

All the experimental procedures were carried out in accordance with international guidelines for care use of laboratory animals. Male Wistar rats (15 weeks old, weighing 200-230 g each) were purchased from Pasteur Institute of Tunis (Tunisia) and housed in stainless steel cages under controlled environmental conditions (temperature 22±1°C, relative air humidity of 45% to 55% with a light/dark cycle of 12 hours). A commercial standard pellet diet (SNA, Borj Cedria, Tunisia) and water were given *ad libitum.*

Preparation of alloxan-induced diabetic rats

Induction of diabetes was carried out in rats by administering a single subcutaneous injection of 120 mg/ kg b.w. of a freshly prepared alloxan solution (alloxan monohydrate, Sigma Chemicals Co.), dissolved in acetate

buffer (pH 5.5) and prepared immediately before use. The control groups were injected only with the same volume of acetate buffer as the diabetic groups received. Alloxan induces diabetes through destructing Langerhans islands of the pancreas. Therefore, a large amount of insulin is released from the pancreas cells after the injection. In order to prevent hypoglycemic shock, during the first 24 h after the alloxan injection, the rats received 10% dextrose instead of water (Rees, Alcolado, 2005; Kwon, Song, Choi, 2003). Induction of diabetes was confirmed by measuring fasting blood glucose (FBG) three days after alloxan injection. Rats with a blood glucose level of 250 mg/kg or higher were considered to be diabetic (Ghorbani *et al.*, 2013).

Experimental design

Twenty-four rats were randomLy divided into four groups of six animals each as follows:
Group (NC): Normal control rats administered distilled water daily;
Group (DC): Diabetic control rats administered distilled water daily;
Group (CLE): Normal rats administered *L. europaeum* extract at 20 mg/kg b.w./day for 28 days (p.o.);
Group (DLE): Diabetic rats administered *L. europaeum* extract at 20 mg/Kg b.w./day for 28 days (p.o.).

During experiment, blood was collected from tip of the tail vein and fasting blood glucose level was measured once weekly by using a glucose analyzer. At the same time, the body weight of each rat was recorded. At the end of treatment (28^{th} day), overnight fasted animals were scarified by decapitation under light anesthesia and blood was collected. Serum samples were obtained by centrifugation at 2200 g for 15 min and kept at -20 °C for the biochemical assay. The pancreas was removed from each rat, homogenized and analyzed for antioxidant enzymes.

Glucose levels and lipid profile

Glucose, total cholesterol (TC), HDL-C, and triglyceride (TG) levels were assayed in serum collected at the end of the experiment and using commercial reagent kits purchased from Biomagreb Analyticals (Ariana Tunis, Tunisia). Glucose levels at J7, J14 and J21 were determined with glucometer. LDL-C was estimated by Friedwald method as follows:

LDL cholesterol = total cholesterol - HDL cholesterol - (Triglyceride ÷ 5)

All assessment assays and kits were performed in accordance with the manufacturer instructions and protocols.

Protein analysis in pancreas

Protein concentration in pancreas tissue was determined according to Lowry *et al.* (1951), using bovine serum albumin as standard.

Pancreas homogenate preparation

Animals were euthanized by an over dose of diethyl ether and immediately dissected. The operation was handLed carefully to avoid pancreas damage. The pancreas was taken out, rinsed with saline pre-cooled to 4 °C, the organ was dried with filter paper and weighed after removing adipose and connective tissue. Pancreas homogenates were centrifuged at 3000 rpm for 10 min at 4 °C and the supernatant was kept at -80 °C until use.

Antioxidant defense system assays

The activities of three antioxidant enzymes: SOD, CAT and GPx were determined in pancreas homogenate and in serum. SOD activity was determined in pancreas homogenate according to the method of Beyer and Fridovich (1987). This method is based on the capacity of SOD to inhibit the oxidation of nitroblue tetrazolium (NBT). One unit of SOD represents the amount of enzymes required to inhibit the rate of NBT oxidation by 50% at 25°C.CAT activity was measured using the method of Aebi (1984). Hydrogen peroxide (H_2O_2) disappearance was monitored kinetically at 240 nm for 1 min at 25 °C. The enzyme activity was calculated using an extinction coefficient of 0.043 Mm/cm. GPx activity was measured according to the method of Flohe and Gunzler (1984). GPx activity was quantified according to a coupled enzyme (GPx and glutathione reductase) procedure, which measures the decrease in absorbance at 340 nm as NADPH is converted to NADP (Nicotinamide adenine dinucleotide phosphate). One unit of GPx activity was recorded as the quantity of enzyme oxidizing 1 μmol NADPH per min

Statistical analysis

The data were analyzed using the package Stat Graphics plus 5.1 (Stats graphics). Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Duncan's test for comparison among groups.

All values were expressed as mean ± SE. Differences were considered significant if $p<0.05$.

RESULTS

Total phenolic and flavonoid content of Lycium europaeum leaf extract

Based on the absorbance values of the various extract solutions compared with the standard solution of gallic acid and catechin, total phenolic and flavonoid amounts were equal to 9.31±0.02 mg GAE/g DW and 6.01±17 mg CE/g DW, respectively (Table I).

Antioxidant activities of *Lycium europaeum* leaf extract

The antioxidant activity of boxthorn extract was explored using three different and complementary assays: total antioxidant capacity, DPPH free radical scavenging and reducing power (Table I). The antiradical activity evaluation of boxthorn aqueous extract displayed an IC_{50} value equal to 200 µg/mL. Besides, our results showed a strong antioxidant capacity of *L. europaeum* leaves (17 mg GAE/g DW). The third antioxidant capacity was measured in terms of the iron reducing power, showing a lower EC_{50} value equal to 92 µg/mL and thus indicating a high reducing power activity.

Effect of *Lycium europaeum* on weight body

Changes in initial and final body weight in control and experimental groups are shown in Table II. Before the experiment start, no difference was observed between studied groups in terms of body weight. The alloxan-induced diabetic rats exhibited a significant loss of the body weight as compared with control. However, boxthorn extract intake induced an increase in body weight in both control and diabetic rats.

Effect of *Lycium europaeum* on fasting blood glucose level

As shown in Table II, the administration of alloxan (DC group) led to an elevation of fasting blood glucose levels, which reached a maximum of about 353.7 ± 30 mg/dL

TABLE I - Total polyphenol, flavonoid contents and antioxidant capacity of *Lycium europaeum* extract

	Total polyphenols (mg GAE/g)	Total flavonoids (mg CE/g)	Total antioxidant capacity (mg GAE/g)	DPPH (IC50, µg/mL)	Iron reducing power (EC50, µg/mL)
Leaf extract	9.31±0.02	6.01±0.17	17.24±0.83	200±0.05	92 ± 4
BHT	-	-	-	24 ± 0.20	-
Ascorbic acid	-	-	-	-	42 ± 0.83

Total phenolic content was expressed by milligram gallic acid equivalent/gram; total flavonoid was expressed by milligram catechin equivalent/gram; total antioxidant capacity was expressed by milligram gallic acid equivalent/gram; EC_{50} value is the effective concentration at which the antioxidant activity was 50%; the absorbance was 0.5 for reducing power; 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical was scavenged by 50%; The EC_{50} value was obtained by interpolation from linear regression analysis. BHT and ascorbic acid were used as positive controls; values are the means of 3 replicates ± SD.

TABLE II - Fasting blood glucose level (mg/dL) and body weight (g) after four weeks of administration of *Lycium europaeum* extract in normal and alloxan- diabetic rats

	Fasting blood glucose (mg/dL)					Body weight (g)				
	Initial day	Week 1	Week 2	Week 3	Week 4	Initial day	Week 1	Week 2	Week 3	Week 4
NC	86.4±3.6^{a}	88.6±2.5^{a}	86±2.4^{a}	88±3.6^{a}	88.6±4.8^{a}	214.2±10.7^{a}	227.5±6.9^{a}	239.3±8.1^{a}	251±8.6^{a}	264.3±10.2^{a}
DC	344.4±35.0^{b}	353.7±30.4^{b}	308.5±14.5^{b}	332.7±20.7^{b}	342.2±23.9^{b}	217.5±6.1^{a}	200.3±6.8^{b}	203.5±6.5^{b}	196.7±5.1^{b}	186.7±10.2^{b}
CLE	85.6±3.6^{a}	87.6±3.5^{a}	85.5±4.5^{a}	84.4±3.3^{a}	85.4±7.3^{a}	205±11^{a}	217.2±11^{c}	227.5±9.3^{a}	238.7±8.6^{a}	249.5±8.3^{c}
DLE	337.2±29.8^{b}	249.7±33.7^{c}	208.5±25.6^{c}	186.6±19.0^{c}	171.0±18.7^{c}	210.0±11.4^{a}	211.8±13.2^{c}	214.5±10^{c}	216.0±8.9^{c}	221.5±10.3^{d}

Data are expressed as mean ± SD of measurements from 6 rats. Groups are labelled as follows: Group NC: normal control, Group DC: diabetic control, Group CLE: Normal control fed with *Lycium europaeum* extract, Group DLE: Diabetic rats fed with *Lycium europaeum* extract. Different letters in the same raw indicate significant differences at $P \leq 0.05$ as determined by Duncan's multiple range tests.

during the first week, and persisted up to four weeks during the experiment. *L. europaeum* leaf extract exhibited no effect on the blood glucose level in normal rats (NC vs. CLE groups) and an effective hypoglycemic effect started after one week of treatment in diabetic rats (DLE vs DC groups) and continued for four weeks to reach 49.3% at the end of experimental period.

Effect of *Lycium europaeum* on lipid profile

As shown in Figure 1, serum TG, TC and LDL-C levels were significantly increased ($p<0.05$) in diabetic groups (DC, DLE) in comparison with controls (NC, CLE); whereas HDL-C was significantly decreased ($p<0.05$) in the diabetic groups in comparison with controls. In fact, treatment with this plant for four weeks resulted in a reduction in TG, TC and LDL-C compared to untreated diabetic rats while serum HDL-C levels were significantly ($p < 0.05$) increased in treated diabetic rats.

Antioxidant enzyme activities

Table III shows the antioxidant enzyme activities, namely SOD, CAT and GPx, in the serum and pancreatic homogenate of all the rat groups. Significantly lower pancreatic SOD, CAT and GPx activities ($p<0.05$) were observed in diabetic rats.

DISCUSSION

The alloxan-induced rat model was successfully established for the analysis of *L. europaeum* leaf extract-mediated antidiabetic, antihyperlipidemic and antioxidant effects.

Polyphenols and flavonoids are the major contributors of antioxidant activities of the herbs; therefore, they were sought in the *L. europaeum* leaf extract. Results showed that the total polyphenol and flavonoid amounts that could be extracted from *L. europaeum* leaves were 9.31 mg GAE/g DW and 6.01 mg CE/g DW, respectively. Rjeibi *et al.* (2017) showed higher content of polyphenols (15.70 mg GAE DW) and flavonoids (7.64 mg CE/g DW) in the aqueous leaf extract of *L. europaeum*. Dahech *et al.* (2013) showed that polyphenol and flavonoid contents of *L. shawii* fruit ranged from 75 to 377 mg GAE/g DW and from 2 to 11.60 mg CE/g DW, respectively. Similarly, the flavonoid content of cultivated *L. barbarum* leaves (16.65 mg CE/g DW) was much higher than that of the wild *L. barbarum* leaves (7.55 CE/g DW) (Jing *et al*., 2009). These differences in results could be due to biotic (species, organ and physiological stage) and abiotic (environmental, handLing, and solvent extraction) factors, as previously shown by Ksouri *et al.* (2008). Also, the solubility of phenol compounds is governed by temperature. In this context, Yang *et al.* (2015) reported that the total polyphenol content of *L. barbarum* berries extracted with hot water

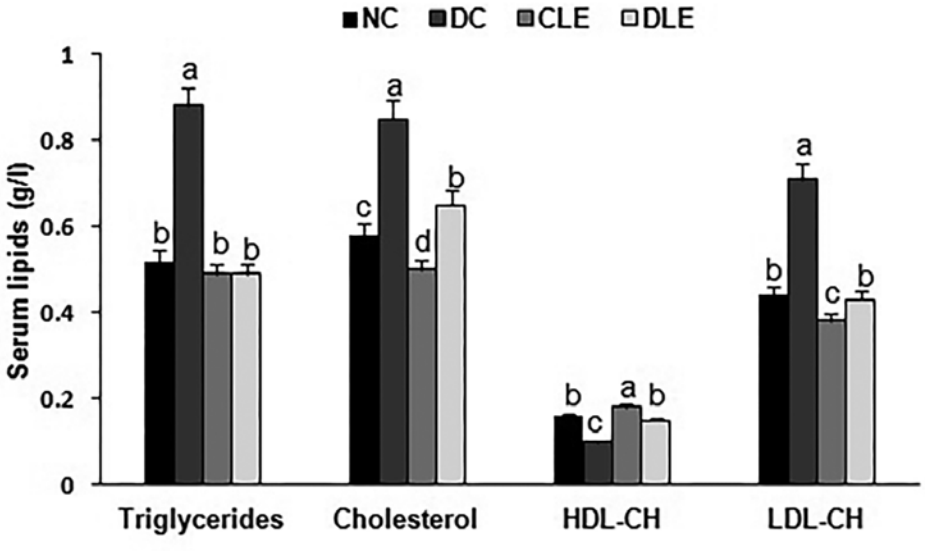

FIGURE 1 - Comparative levels of serum triglycerides, cholesterol, HDL-CH and LDL-CH in different rat groups. Groups are labelled as follows: Group NC: normal control, Group DC: diabetic control, Group CLE: Normal control fed with *Lycium europaeum* extract, Group DLE: Diabetic rats treated fed with *Lycium europaeum* extract. Data are expressed as mean ± SD of measurements from 6 rats. Means not sharing common letters are significantly different among the groups at $P \leq 0.05$ as determined by Duncan's multiple range tests.

TABLE III - Effect of *Lycium europaeum* extract and/or alloxan treatment on SOD, CAT and GPx activities in plasma and pancreatic homogenates of treated rats

	Antioxidant enzymes					
	Pancreatic homogenate			Plasma		
	SOD (U/mg protein)	CAT (Mol H_2O_2/min/ mg protein)	GPx (10^{-3} mM/min/ mg protein)	SOD (U/mL)	CAT (U/mL)	GPx (U/mL)
NC	26.4±3.5[a]	56.9±4.2[a]	2.9±0.6[a]	459.5±26.3 [a]	9.9±1.2[a]	637.5±37.2[a]
DC	10.8±1.4[b]	20.4±1.8[b]	0.8±0.2[b]	228.4±15.7[b]	3.9±0.9[b]	344.6±30.9[b]
CLE	28.9±3.6[a]	55.8±3.2[a]	2.6±0.5[a]	489.7±38.2 [c]	10.6±1.4[a]	695.7±58.2[c]
DLE	22.4±1.4[a]	38.9±3.8[c]	1.9±0.3[c]	358.8±63.7 [d]	8.8±1.0[a]	590.6±112.5[d]

Data are expressed as mean± SD of measurements from 6 rats. Groups are labeled as follows: Group NC: normal control, Group DC: diabetic control, Group CLE: normal control fed with *Lycium europaeum* extract, Group DLE: diabetic rats fed with *Lycium europaeum* extract. Different letters in the same raw indicate significant differences at $P \leq 0.05$ as determined by Duncan's multiple range tests.

was notably lower than that obtained otherwise (2.55 vs. 5.32 %). Meanwhile, Yujing *et al.* (2017) indicated that the contents of bioactive compounds and the antioxidant activity of *L. barbarum* infusions increased with time and temperature. Otherwise, Gupta *et al.* (2011) reported that flavonoids act as insulin secretagogues or insulin-mimetics by influencing the pleiotropic mechanisms to attenuate diabetic complications. The presence of flavonoids in *L. europaeum* may be responsible for the stimulation of glucose uptake in peripheral tissues and regulation of the activity and/or expression of the rate-limiting enzymes involved in the metabolism of carbohydrates.

The antioxidant capacity of plant extracts is assayed through several techniques. Each method relates to the generation or use of a different radical directly involved in the oxidative process, acting through a variety of mechanisms. Hence, no single assay can measure total antioxidant capacity (Milella *et al.*, 2014). In this study, the antioxidant activities of *L. europaeum* extract were analyzed using three different and complementary assays: DPPH free radical scavenging, total antioxidant capacity and iron reducing power. The DPPH radical scavenging assay evaluated the ability of plant extracts to scavenge free radicals generated from DPPH reagent (Chung *et al.*, 2006). In fact, DPPH is a stable free radical; when an antioxidant reacts with DPPH, the electron is paired off and the DPPH solution is decolorized. The scavenging activity of the antioxidant or the bleaching of the color stochiometrically depends on the number of electrons taken up (Shirwaikar, Patel, Kamariya, 2011). The present work also involved the evaluation of the antiradical activity of aqueous *L. europaeum* extract (IC_{50} = 200 µg/mL) which was three fold higher than that obtained by Rjeibi *et al.* (2017) with IC_{50} = 65.87 µg/mL. It was observed that despite the inability of *L. europaeum* leaf extract to compete with the positive control BHT in scavenging DPPH (IC_{50} = 24 µg/mL), this extract did possess mild antioxidant activity and may be considered as a potential preservative for medical applications. Additionally, our results showed *L. europaeum* leaves to have a strong antioxidant capacity (17 mg GAE/g DW). This finding was consistent with Tawaha *et al.* (2007) who proved that the total antioxidant activity was remotely correlated to the content of flavonoids and polyphenols. The antioxidant activity of polyphenols is also due to their red-ox properties that make them act as reducing agents, hydrogen donors and singlet oxygen quenchers. The third antioxidant capacity was measured in terms of the iron reducing power. A low EC_{50} (92 µg/mL) was obtained, which indicated a higher reducing power activity. Antioxidants reducing the ferric ion/ferricyanide complex to the ferrous form of the extracts may provide a significant indication of the potential antioxidant capacity of the plant. The reducing properties are generally connected to the presence of reductones, which exert antioxidant activity by breaking the free radical chain through the donation of a hydrogen atom (Xing *et al.*, 2005). Therefore, the potent antioxidant activity of *L. europaeum* leaves might be related to the presence of secondary metabolites such as polyphenols and flavonoids. In this way, Rjeibi *et al.* (2017) reported that the chemical composition of *L. europaeum* leaf extract was rich in phenolic acids, namely caffeic (140.18 µg/g), gallic (117.17 µg/g), vanillic (26.54 µg/g) and coumaric (1.38 µg/g) acids, and flavonoids, namely naringenin (57.18 µg/g), epicatechin (33.14 µg/g), rutin (25.16 µg/g), kaempferol (12.33 µg/g),

catechin (10.66 µg/g), luteolin (9.85 µg/g), apigenin (9.47 µg/g) and quercetin (1.53 µg/g).

In order to evaluate the capacity of *L. europaeum* leaf extract to cure diabetes, alloxan was used to induce hyperglycemia in rats. Alloxan has been recognized as a convenient experimental model to evaluate the activity of hypoglycemic agents (Ramkumar *et al.,* 2009). It destroys the β-cells of islets in the pancreas Langerhans, resulting in a depletion of endogenous insulin secretion and leading to a decreased utilization of glucose by body tissues (Szkudelski, 2001). Obtained results showed that the administration of *L. europaeum* extract induces an increase in animal body weight in both control and diabetic rats. Additionally, *L. europaeum* leaf extract proved a potent hypoglycemic effect starting at one week of treatment in diabetic rats (DLE vs. DC groups) and continued for four weeks, reaching 49.3% at the end of the experimental period. Several studies in the last decades have shown that plant and plant-based therapies have a potential to control and treat diabetes and its complications (Marles, Farnsworth, 1995). The ability of this plant extract to reduce blood glucose level could be attributed to an improvement of the peripheral sensitivity to remnant insulin or to its capacity to induce stimulation of Langerhans islets. In fact, alloxan is known to induce free radical production and cause tissue injury. The pancreas is specifically susceptible to the action of alloxan-induced free radical damage. As reported by Akah, Uzodinma and Okolo (2011), the regeneration of islet β-cells after destruction by alloxan may be the primary recovery mechanism of alloxan-injected rats following plant extract administration. In addition, *L. europaeum* leaf extract was characterized by the presence of well-known antioxidant phytochemicals, namely phenolic acids (caffeic, gallic, vanillic and coumaric acids) and flavonoids (naringenin, epicatechin, rutin, kaempferol, catechin, luteolin, apigenin and quercetin) as mentioned by Rjeibi *et al.* (2017). Several studies have mentioned the antidiabetic effect of caffeic acid (Jung *et al.*, 2006), gallic acid (Adeniyi *et al.*, 2015), coumaric acid (Amalan *et al.*, 2016), naringenin (Ortiz-Andrade *et al.*, 2008), catechin (Liu *et al.*, 2014), rutin (Ghorbani, 2017), kaempferol (Al-Numair *et al.*, 2015), luteolin (Zang, Igarashi, Li, 2016), apigenin (Cazarolli *et al.*, 2012) and quercetin (Vessal, Hemmati, Vasei, 2003). These compounds influence glucose metabolism by several mechanisms, such as inhibition of carbohydrate digestion and glucose absorption in the intestine, stimulation of insulin secretion from the pancreatic β-cells, modulation of glucose release from the liver, activation of insulin receptors and glucose uptake in insulin-sensitive tissues and the modulation of hepatic glucose production (Hanhineva *et al.*, 2010). Therefore, *L. europaeum* leaf extract could be prescribed as therapy for DM.

The chronic hyperglycemia of diabetes is associated with the long-term damage, dysfunction and failure of various organs, especially eyes, kidneys, nerves, heart and blood vessels (Baynes, 1991). DM is also related to a hyperlipidemia and leads to serious anomalies in lipid composition and concentration (Cooperstin, Watkin, 1981). Abnormal lipid metabolism leads to the accumulation of plasma TG, LDL and TC, as well as decreased HDL-C, commonly associated with diabetes (Kondeti *et al.*, 2010). As reported by Betterridge (2002), the insulin deficiency or insulin resistance may be responsible for hyperlipidemia due to the insulin inhibiting action on the 3-hydroxy-3-methyl-methylglutaryl coenzyme-A which is a key enzyme in cholesterol biosynthesis. Due to diabetes, the hyperlipidemia may be regarded as a result of the non-inhibiting action of lipolytic hormones on adipose tissues (Goodman, Gilman, 1985). Our results showed that serum TG, TC and LDL-C levels were significantly increased ($p<0.05$) in diabetic groups (DC, DLE) in comparison to controls (NC, CLE) whereas HDL-C was significantly decreased ($p<0.05$) in the diabetic groups in comparison to control groups. On the other hand, *L. europaeum* was also able to improve some lipid metabolites. In fact, treatment with this plant for four weeks resulted in reduced TG, TC and LDL-C levels compared to untreated diabetic rats while serum HDL-C levels were significantly ($p < 0.05$) increased in treated diabetic rats. This data demonstrates for the first time that, in addition to its hypoglycemic effect, *L. europaeum* leaf extract can significantly improve the imbalance in lipid metabolism. Moreover, the reduced TC and increased HDL-C levels following *L. europaeum* administration were noteworthy, according to Wang *et al.* (2010), who reported that most drugs used in the treatment of hypercholesterolaemia reduced both total and HDL-C levels. Additionally, the polyphenols and flavonoids of *L. europaeum* extract, which contribute to its antioxidant properties, may play a synergistic role in its hypolipidemic effect. As reviewed by Bahadoran, Mirmiran and Azizi (2013), polyphenols have powerful modulator effects on many aspects of metabolic, endocrine and cellular signaling transduction of adipose tissue. They also reported that some polyphenols such as catechins increase β oxidation in adipocytes and down-regulate the enzymes and genes involved in lipogenesis, including lipoprotein lipase, fatty acid synthase complex, peroxisome proliferators-activated

receptor γ, regulatory element-binding protein 1-c and fatty acid binding protein. To the best of our knowledge, this is the first report on the hypolipidemic effect of *L. europaeum* leaf extract. Thus, it is reasonable to conclude that *L. europaeum* leaf extract could modulate blood lipid abnormalities by acting as a potential hypolipidemic agent.

Oxidative stress, due to increased free radical formation and reduced antioxidant status, is widely believed to be a key factor in the pathogenesis and progression of diabetes (Punithavathi *et al.*, 2011). Available evidence strongly suggests that excessive free radicals, particularly reactive oxygen species (ROS) generated from hyperglycemia-induced glucose auto-oxidation and protein glycosylation, play a critical role in diabetes (Masjedi, Gol, Dabiri, 2013). Reddy *et al.* (2005) showed a close relationship between the increase of free radicals, blood glucose and lipid peroxidation in the induction of diabetes. Increased lipid peroxidation damages membrane function by decreasing membrane fluidity and changing the activity of membrane-bound enzymes and receptors (Verma, Itankar, Arora, 2013). Enzymatic antioxidants, such as SOD, CAT and GPx, play a vital role in preventing oxidative damage to the cells. Because of the unbalanced oxidant/antioxidant system in DM and its involvement in the mechanism of various pathological complications, the effect of the aqueous leaf extract of *L. europaeum* on oxidative stress was investigated. Our results corroborated previous works that have demonstrated that an increased blood glucose level induces a depletion of the antioxidant system (Punitha, Manoharan, 2006; Vina *et al.*, 2006; Shabeer, Srivastava, Singh, 2009). The enzyme activity reductions observed in the diabetic control group suggest their excessive utilization in attenuating free radicals generated during the metabolism of alloxan, as it was already reported in diabetic animals (Onyeka, Nwakanma, Bakare, 2013). *L. europaeum extract* prevented this decrease, especially for SOD activity in pancreatic homogenate and CAT activity in serum, which became comparable to normal control values. SOD scavenges the superoxide radicals by converting them to H_2O_2 and molecular oxygen. The observed decrease in SOD activity in diabetic control rats could result from inactivation by H_2O_2 or by glycosylation of the enzyme, which have been reported to occur in diabetes. Meanwhile, the increase in its activities is an indication of its ability to scavenge ROS, thus contributing to the protective effect against oxidative stress and preventing further damage to membrane lipids. Natural antioxidant agents fundamentally originate from plants in the form of secondary metabolites (Marjorie, 1996). The presence of different types of bioactive components, like phenolic acids and flavonoids in *L. europaeum* extract, could be responsible for the antidiabetic activities (Sharma, 2012) since they could scavenge free radicals, quench electronically excited compounds, reduce hydro-peroxide formation, and attenuate production of ROS through the modulation of several pro-oxidant enzymes involved in the development of ROS, including xanthine oxidase, cyclooxygenase, lipoxygenase, microsomal monooxygenase, NADH oxidase and mitochondrial succinoxidase (Dembinska-Kiec *et al.*, 2008). In fact, these bioactive components decreased lipid peroxidation and increased plasma total antioxidant capacity; they also attenuated stress-sensitive signaling pathways, pro-oxidant enzymes, and inducted antioxidant enzymes including SOD, CAT and GPx (Crespy, Williamson, 2004). Thus, the antidiabetic effect of *L. europaeum* extracts might be due to these bioactive constituents.

CONCLUSION

Currently, medical prevention and treatment of diabetic complications are mainly based on the optimized control of blood glucose. According to our findings, *L. europaeum* leaf extract has beneficial effects on blood glucose in diabetic rats, thus revealing its antidiabetic potential. *L. europaeum* extract was also able to restore altered levels of serum lipids and endogenous antioxidant enzymes revealing its antihyperlipidemic and antioxidant potential. These putative effects may be due to the additive effects of bioactive constituents present in the aqueous extract. Moreover, the consumption of *L. europaeum* leaf extract could be considered a good complementary source of natural antioxidant and as an adjunct therapy in the management of diabetes. However, intensive investigations must be conducted on the extract fractions to identify pharmacological active compound(s), to establish the possible mechanism(s) of action and to set appropriate doses.

CONFLICTS OF INTEREST

All contributing authors declare no conflicts of interest.

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