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Original article

Antidiabetic potential of *Caralluma europaea* against alloxan-induced diabetes in mice

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ABSTRACT

Medicinal plants play an important role in the management of diabetes mellitus especially in developing countries where resources are lacking. Herbal of natural origin, unlike the synthetic compounds, are more effective, safer and have less side effects. For continuing research on biological properties of Moroccan medicinal plants, the present work was undertaken to evaluate the potential and mechanism of the antidiabetic activity of the *Caralluma europaea* methanolic extract in alloxan-induced diabetic mice. A high-performance liquid chromatography technique (HPLC) was used to identify and quantify the major phenolic compounds in the methanolic extract. The *in vitro* antioxidant property was evaluated using 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) scavenging method, reducing power and *ß*-carotene-linoleic acid assays. The acute toxicity of the extract was evaluated by giving it orally to mice at single doses of 200, 500, 1000, 2000 mg/kg body weight. The antidiabetic effect was conducted on Swiss albino mice. Diabetes was induced with single intraperitonial injection of alloxan monohydrate (200 mg/kg body weight) and animals were treated with methanol extract at a dose of 250 mg/kg and 500 mg/kg body weight. The blood glucose levels were measured and histopathological analysis of pancreas was performed to evaluate alloxan-induced tissue injuries. The main phenols identified and quantified in the extract were ferulic acid, quercetine, 3,4 dihydroxybenzoic acid, rutin, epigallocatechin, and catechin. Ferulic acid was found to be the main phenolic compound ant its proportion was up to 52% of total phenolic compounds, followed by quercetin (36%). The result showed that methanol extract exhibited an antioxidant effect. Acute toxicity studies revealed that *C. europaea* extract was safe up 2000 mg/kg body weight and approximate LD_{50} is more than 2000 mg/kg. Moreover, the methanol extract prevented the diabetogenic effect of alloxan and decreased significantly the blood glucose level ($P < 0.001$) in treated mice. Morphometric study of pancreas revealed that *C. europaea* extract protected significantly the islets of Langerhans against alloxan-induced tissue alterations.

1. Introduction

Diabetes mellitus is one of the most serious diseases mostly accompanied by characteristic long-term complications and affects a big part of the world global population (Rathmann and Giani, 2004). According to the World Health Organization, the global prevalence of diabetes is 8.5% in the adult population. The International Diabetes Federation reported that 382 million people worldwide have diabetes mellitus (IDF, 2015). In 2012 diabetes was the direct cause of 1.5 million deaths (Xu et al., 2015) and caused in additional 2.2 million indirect deaths, by increasing the risks of

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cardiovascular and other diseases. It is a chronic disorder of metabolism that characterized by high levels of glucose in the blood due to non-secretion of insulin or insulin sensitivity. It can be categorized into diabetes type 1 which is a result of insulin deficiency and diabetes type 2 is due to insulin resistance and the major factor contributing to the development of diabetes is resistance to insulin, which can result in hyperglycemia, dyslipidemia (Chen et al., 2015). Chronic hyperglycemia is associated with specific organic complications that affecting particularly eyes, kidneys, heart and blood vessels (Drouin et al., 1999). A healthy diet and regular physical activity are well known to be beneficial to subjects at high risk of diabetes. Furthermore, to prevent diabetes mellitus induced by oxidative stress, a good nutrition which is rich in antioxidants might also be helpful (Berraaouan et al., 2013). Actually, synthetic hypoglycemic agents are available for the treatment of diabetes mellitus. However, on chronic usage, most of these conventional antidiabetic agents produced several serious side effects. Therefore, the search and the identification of new safe and effective natural antidiabetic agents to treat diabetes is one of the most important ways to explore and continues to draw the attention of many researchers (Xu et al., 2015). In fact, recent studies have been reported the hypoglycemic effect of many medicinal plants in experimental diabetes (Berraaouan et al., 2013; Rathore et al., 2014; Khatun et al., 2014; Chen et al., 2015). *Caralluma europaea* is a leafless, succulent and angular plant locally known as "ddagmûs" is one of the Moroccan medicinal plants most commonly used in traditional medicine, distributed in Morocco, Egypt, Spain, Italy, Libya, Tunisia and Algeria (Meve and Heneidak, 2005). Aerial parts of this medicinal shrub are largely used as a powder or as a juice in case of diabetes and to treat cyst and goiter (Bellakhdar, 1997). Previous studies have been reported on the biological activities of extracts obtained from many species of *Caralluma* such as antinociceptive, anti-inflammatory hepatoprotective and antihyperglycemic properties (Abdel-Sattar et al., 2013; Chinenye et al., 2013; Shanmugam et al., 2013) .To the best of our knowledge, no work on the antidiabetic effect of *C. europaea* methanolic extract (CEME) has been reported to date. The safety of the plant and its reputation as antidiabetic in folk medicine, encourage us to evaluate the potential antidiabetic activity of CEME in alloxan-induced diabetic mice. The possible mechanism involved in the hypoglycemic activity was also investigated.

2. Material and methods

2.1. Plant material

The aerial parts of *Caralluma europaea* (Guss) N.E.Br. were collected in March 2014, from Ourika Valley (High Atlas of Morocco, N 31°31′35″/W07°57′35″). The collect of plant samples has been authorized by Moroccan authority. The plant was identified by Prof. Abbad A, and a voucher specimen (CAE 023) was deposited at the Herbarium of the Laboratory of Biotechnology, Protection, and Valorization of the Plant Resources, Faculty of Sciences Semlalia, Marrakech, Morocco. The collected plant was air-dried in the shade at room temperature, ground to homogeneous powder and then subjected to extraction.

2.2. Extraction procedure

The fine dried powder of samples (100 g) was extracted by cold stirring with dichloromethane for 48 h and filtered through filter paper. The residue was then extracted by stirring with methanol for 48 h and filtered. Then the methanol solution obtained was evaporated at 45 °C under vacuum using a rotary evaporator (type Buchi R-210) and the residue was stored at 4 °C until use.

2.3. Phytochemical screening

CEME was subjected to preliminary phytochemical screening involved qualitative determinations of the following substances: alkaloids, saponins, tannins, flavonoïds, quinones, terpenes and steroids (Douhou et al., 2003).

2.4. HPLC analysis

Identification and quantification of phenolic compounds were carried out using a high-performance liquid chromatography (KNEUER) equipped with a (K-1001) pump and the sample was detected by a UV detector operating at 280 nm. A volume of 10 μl of the extract was injected over a C18 (Eurospher II 100-5) (4.6 × 250 nm) column. The flow rate was 1 ml/min and the column temperature was maintained at 25 °C. The mobile phase was composed of acidified water (A) and acetonitrile (B) with a total running time of 60 min. The phenolic compounds were identified by comparing their retention times with those of standards.

2.5. Antioxidant activity

2.5.1. DPPH free radical-scavenging activity

The stable free radical 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) was used to evaluate the free radical-scavenging activity of *Caralluma europaea* methanolic extract, according to the protocol described by Burits and Bucar (2000). Fifty microlitres of various concentrations of the methanolic extract in methanol were added to 2 ml of a 60 μM methanol solution of DPPH. After a 20 min incubation period in the dark at room temperature the absorbance of the samples was measured using a spectrophotometer at 517 nm. A blank containing the same amount of methanol and DPPH was used as negative control. Butylated hydroxytoluene (BHT) and quercetin were used as positive controls. The inhibition of the DPPH in percent (%) was calculated in the following way (Badakhshan et al., 2012):

$$I\% = [(A_{blank} - A_{Sanple}/A_{blank}) * 100]$$

where A_{blank} is the absorbance of the control and A_{sample} is the absorbance of the test compound. The sample concentration providing 50% inhibition (IC_{50}) was calculated from the graph by plotting inhibition percentages against sample concentrations.

2.5.2. β-Carotene/linoleic acid bleaching test

The ß-carotene bleaching test was performed as described by Miraliakbari and Shahidi (2008) with slight modification. A stock solution of β-carotene and linoleic acid was prepared with 0.5 mg of β-carotene in 1 ml of chloroform, 25 μl of linoleic acid and 200 mg of tween 40. The chloroform was completely evaporated under vacuum at 50 °C and 100 ml of distilled water were then added to the flask with vigorous shaking to form a clear yellowish emulsion. Then 2.5 ml of this emulsion were transferred into a series of test tubes containing 350 μl of various concentrations of the samples. Immediately after the addition of the emulsion, the test tubes were incubated in a hot water bath at 50 °C for 2 h. A blank containing all reagents except the test compound was used as negative control. After incubation, the absorbance values were measured at 470 nm. Antioxidant activities (inhibition percentage I%) of the samples were calculated using the following equation (Mallet, 2008):

$$I\% = \left[\left(A_{sample2h} - A_{blank2h}\right)/\left(A_{initial\ blank} - A_{blank2h}\right)\right] * 100$$

where $A_{sample2h}$, $A_{blank\ 2h}$ are the absorbance of the test compound and control respectively after 2 h assay and $A_{initial\ blank}$ is the absorbance of control at the beginning of the experiment.

The sample concentration providing 50% inhibition (IC_{50}) was calculated by plotting inhibition percentages against the sample concentrations. BHT and quercetin were used as reference compounds.

2.5.3. Reducing power assay

The reducing power of the methanolic extract was determined according to the method of Oyaizu (1986). One ml of different concentration of samples (methanolic extract and control substance) were mixed with phosphate buffer (2.5 ml, 200 mM, PH 6.6) and potassium ferricyanide [K_3Fe $(CN)_6$] (2.5 ml, 1%). The mixture was incubated at 50 °C for 20 min. At the end of the incubation, 2.5 ml of 10% trichloroacetic acid (TCA) were added to the mixture and then centrifuged at 3000 rpm for 10 min. the upper layer solution (2.5 ml) was mixed with 2.5 ml of distilled water and 0.5 ml of 0.1% ferric chloride ($FeCl_3$). The color formed due to the reduction of Fe^{3+} was measured at 700 nm using a spectrophotometer.

The sample concentration providing 0.5 of absorbance (IC_{50}) was calculated from the graph plotting the absorbance at 700 nm against the corresponding sample concentration. BHT and quercetine were used as positive controls.

2.6. Acute oral toxicity evaluation in mice

2.6.1. Animals

Swiss albino mice (8 weeks) were used for this experiment weighing 20–30 g. The animals were supplied by the animal house of Faculty of Sciences, Semlalia, Marrakech, Morocco. Animals were housed at a constant room temperature (25 °C) and kept under 12 h light/12 h dark cycle with free access to food and water to all studied groups. After randomization into various groups according to the body weight before the experiment, the mice were acclimatized for a period of one week and treated in compliance according to guidelines of the University of Cadi Ayyad, Marrakesh (Morocco). Thus, all efforts were made to minimize the number and suffering of animals used. All the experiments were done during the daytime.

2.6.2. Acute toxicity

Swiss albino mice of both sexes were subjected to acute toxicity to determine the safe dose. Animals were divided into five groups of six in each and kept fasting for overnight providing only water. Group 1 served as control received 10 ml/kg of distilled water. Groups 2, 3, 4, and 5 were given orally at a single dose of 200, 500, 1000 and 2000 mg/kg body weight respectively of *C. europaea* extract dissolved in distilled water. Animals were observed during the first 4 h after the administration, and then once daily during the following seven days. Behavioral changes and toxicity symptoms closely observed were: Piloerection, salivation, respiratory pattern, diarrhea, tremors, convulsions, and death. Body weights of the animals were evaluated every day and observed for days following treatment.

2.7. Antidiabetic assay

2.7.1. Induction of experimental diabetes

After a week of acclimatation, animals of either sex were fasted for 14 h and then received a single intraperitoneal dose of alloxan monohydrate to induce diabetes type 1. The diabetogenic dose of alloxan was freshly prepared in normal saline (pH 4.5) with a concentration of 1% at a dose of 200 mg/kg body weight (Ahmadi et al., 2012). Twenty-four hours after the induction of diabetes, blood samples of mice were gathered from the end part of their tails. Blood glucose was measured using an electronic glucometer (On Call Plus Blood Glucose Meter and Test Strips) and the animals with marked hyperglycemia (blood glucose level of $\geq$250 mg/dl) were considered as diabetic and used for the study (Chen et al., 2015). All animals were allowed access to food and water during the experiments and maintained at room temperature in plastic cages. It is important to mention that in the beginning of the study the blood glucose of all animals was measured and animals with high blood glucose level were eliminated from the groups.

2.7.2. Assessment of Caralluma europaea methanolic extract

The mice were divided into five groups and each group consisted of six mice. Group 1 was served as normal control and received appropriate volume of normal saline (10 ml/kg body weight), Group 2: untreated diabetic mice, Group 3: diabetic mice treated with *C. europaea* methanolic extract at 250 mg/kg body weight, Group 4: diabetic mice treated with *C. europaea* methanolic extract at 500 mg/kg body weight, Group 5: diabetic mice treated with glibenclamide (Daonil) as reference drug at 20 mg/kg body weight. Methanolic extract of *C. europaea* was administered orally to mice and followed for 10 h. Blood samples were collected before the start of the treatment (t = 0) and at 2 h intervals for 10 h.

2.7.3. Histopathological study of pancreas

After 10 h of treatment, all animals were sacrificed under chloral hydrate anesthesia and the pancreas tissues were removed from each sacrificed animal and immediately placed in a tube containing buffered formalin (10%) solution overnight at 4 °C. The histological sections were done in the Laboratory of Immunohistochemistry, Faculty of Medicine and Pharmacy, Marrakech, Morocco. Stained sections of pancreas were analyzed using Zeiss optical microscope and the photomicrographs of pancreatic islets of langerhans were taken using Nikon Coolpix p7100 camera at 400× magnification. The measurement of diameter of pancreatic islets was carried out on photomicrographs by calculating the mean of islets diameter for each section, and then the mean of diameters for many sections and finally the mean for all animals within the same group using the ImageJ Software.

2.8. Statistical analysis

Data were expressed as mean ± standard deviation (S.D.) and analyzed with two-way repeated measures analysis of variance ANOVA (for two factors: CEME and time)[CEME-time interaction $F(4, 88) = 44.863$, $P < 0.001$; CEME effect $F(4, 88) = 340.312$, $P < 0.001$; and time effect $F(4, 88) = 24.245$, $P < 0.001$] (Alamin, 2015). The values were considered statistically significant when the *P*-value was less than 0.05, 0.01, and 0.001.

3. Results

3.1. Phytochemical screening

The yield percentage of dried methanol extract was 4.2%. The preliminary phytochemical screening of CEME revealed the presence of tannins, flavonoïds, terpenes and steroids and the absence of alkaloïds, saponins, and quinones.

3.2. Analysis of phenolic compounds

A high-performance liquid chromatography technique was used to identify and quantify the major phenolic compounds of CEME. From the HPLC chromatogram (Fig. 1), it can be noticed that the studied extract contained catechin, quercetine, rutin, ferulic acid, epigallocatechin and 3,4 dihydroxybenzoic acid. Ferulic acid was the main phenolic compound identified and its proportion was up to 50% of total phenolic compounds, followed by quercetin (36%). The concentrations of the main phenols identified in CEME

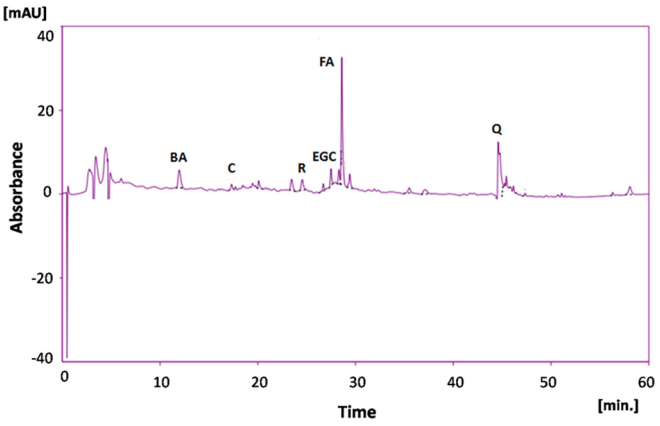

Fig. 1. HPLC chromatogram recorded at 280 nm for the main phenolic compounds identified in the methanolic extract of *Caralluma europaea*. C, catechin; Q, quercetin; R, rutine; FA, ferulic acid; EGC, Epigallocatechin; BA, 3,4-dihydroxybenzoic acid.

Table 1
Concentrations of the main phenolic compounds identified in the CEME expressed in mg EGA/100 g DM.

Phenolic compounds	Concentrations (mg EGA/100 g DM)
3,4-dihydroxybenzoic acid	12.18
Catechin	1.344
Rutin	10.08
Epigallocatechin	4.746
Ferulic acid	52.08
Quercetin	36.96

are given in Table 1. The phenols amounts found in the studied extract ranged from 1 to 52 mg EGA/100 g DM, ferulic acid exhibited the highest concentration (52.08 mg EGA/100 g DM), followed by quercetin (36.96 mg EGA/100 g DM), and then 3,4 dihydroxybenzoic acid with a concentration of 12.18 mg EGA/100 g DM.

3.3. Antioxidant activity

CEME antioxidant activity was evaluated using three complementary *in vitro* antioxidant assays namely: DPPH, reducing power assay and β-carotene-linoleic acid bleaching test. The concentrations that led to 50% of inhibition (IC_{50}) are given in Table 2. The antioxidant activities were compared with that of quercetin and of BHT. The better antioxidant activity was reflected by the lower IC_{50} values. CEME exhibited a significant antioxidant activity, the lowest IC_{50} was obtained with β-carotene-linoleic acid (IC_{50} = 48.61 ± 0.17 μg/ml), followed by DPPH (IC_{50} = 300 ± 0.005 μg/ml) and then reducing power assay (IC_{50} = 376 ± 0.003 μg/ml). This activity was found to be less potent than those of reference antioxidants butylated hydroxytoluene (BHT) and quercetine (IC_{50} values from 0.84 ± 0.04 μg/ml to 2.59 ± 0.07 μg/ml and from 0.95 ± 0.02 μg/ml to 2.62 ± 0.02 μg/ml, respectively).

3.4. Acute toxicity in mice

The oral administration of a single dose (200, 500, 1000, 2000 mg/kg body weight) of CEME to mice did not cause death within the seven days of the study (Fig. 2). No mortality was observed, no signs of toxicity or significant body weight changes were observed after treatment with CEME in either sex. These observations reveal that the oral lethal dose value (LD_{50}) of the CEME is greater than 2000 mg/kg body weight in mice.

Table 2
IC_{50} values (μg/ml) of *C. europaea* methanolic extract and of reference compounds (quercetin and BHT).

	CEME (μg/ml)	Quercetin (μg/ml)	BHT (μg/ml)
DPPH	300 ± 0.005	1.98 ± 0.07	2.59 ± 0.07
β-Carotene-linoleic acid	48.61 ± 0.17	0.95 ± 0.02	0.84 ± 0.04
Reducing power	376 ± 0.003	2.62 ± 0.02	2.22 ± 0.03

Values represent means ± standard deviation for triplicate experiments.

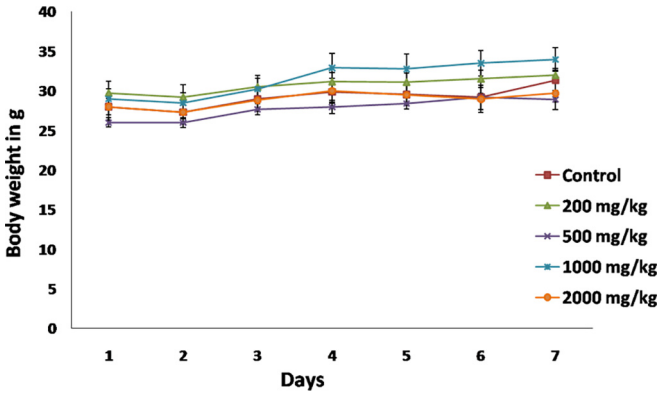

Fig. 2. Changes in the mean body weight of mice after seven days treatment with *Caralluma europaea* methanolic extract. Values were expressed as mean ± SD (n = 6), ($P < 0.05$) compared to control group.

3.5. Antidiabetic effect of Caralluma europaea methanolic extract

The effect of *C. europaea* methanolic extract administration on blood glucose level in alloxan-treated mice is presented in Fig. 3. The injection of alloxan-induced a significant increase ($P < 0.001$) in blood glucose level of alloxan group (262 ± 4.80 mg/dl) compared to normal control group (129.11 ± 5.22 mg/dl). The methanolic extract exhibited a continuous marked reduction of blood glucose levels ($P < 0.001$) particularly 6–8–10 h after treatment in diabetic mice. The extract at low dose decreased significantly the blood glucose level (from 386 ± 6.35 mg/dl to 157 ± 10.39 mg/dl at 8 h and to 87 ± 0.28 mg/dl at 10 h) in comparison with untreated diabetic mice (600 ± 0 mg/dl). The effect obtained with lower dose was comparable ($P > 0.05$) with that obtained with glibenclamide (from 326 ± 10.39 mg/dl to 143 ± 1.73 mg/dl at 8 h

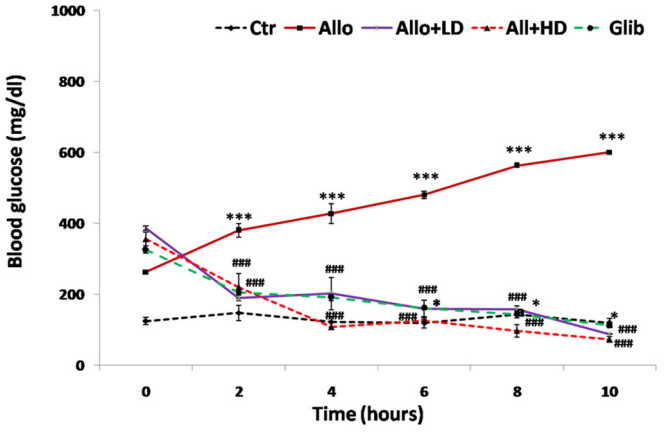

Fig. 3. Effect of *Caralluma europaea* methanolic extract on blood glucose levels in alloxan-induced diabetic mice after 10 h of treatment. Group 1 (Ctr), normal control mice treated with sterile normal saline. Group 2 (Allo), diabetic mice treated with sterile normal saline alone. Group 3 (Allo + LD), diabetic mice treated with *C. europaea* methanolic extract (250 mg/kg body weight). Group 4 (Allo + HD), diabetic mice treated with *C. europaea* methanolic extract (500 mg/kg body weight). Group 5 (Glib), positive control mice treated with glibenclamide (20 mg/kg body weight). The values were expressed as mean ± SD (n = 6), $^{*}P < 0.05$;$^{***}P <$ 0.001 compared to normal control group, $^{###}P < 0.001$ compared to alloxan group.

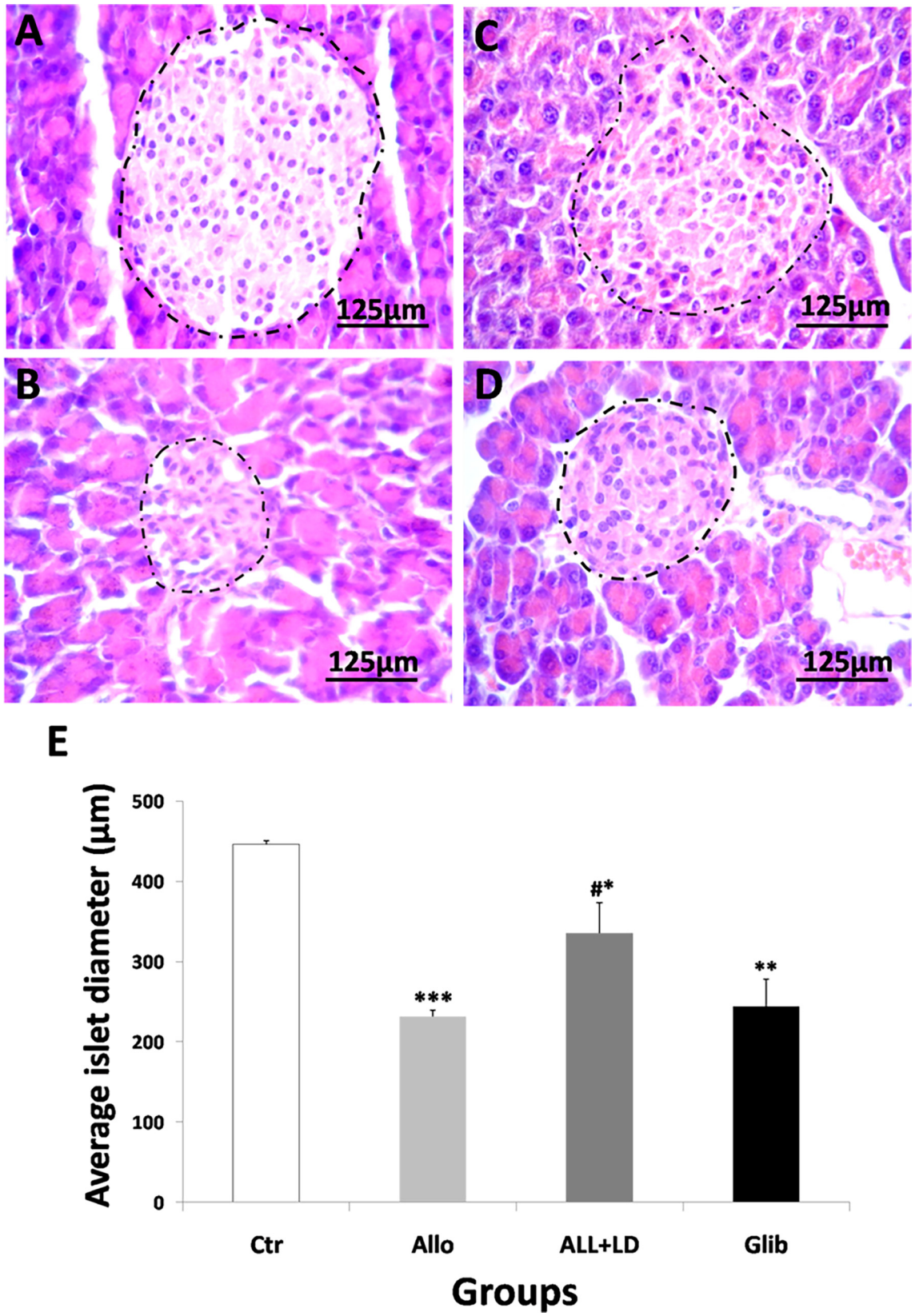

Fig. 4. Photomicrographs (hematoxylin and eosin staining) of pancreatic islets of Langerhans and average islets diameter. Normal control (A), Alloxan (200 mg/kg) (B), Alloxan + *C. europaea* methanolic extract (250 mg/kg) (C), alloxan + glibenclamide (20 mg/kg) (D), and average islets diameter (E). Microscope magnification (×400magnification). Values were expressed as means ± SD, $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$ compared to normal control group, $^{\#}P < 0.05$ compared to alloxan group.

and to 112 ± 1.73 mg/dl at 10 h). Treatment with a high dose of CEME (500 mg/kg) caused a maximum reduction in blood glucose (from 355 ± 19 mg/dl to 96 ± 17.03 mg/dl at 8 h and to 72 ± 8.37 mg/dl at 10 h; $P < 0.001$) compared to alloxan group. Furthermore, this effect was more potent than those of the reference drug.

3.6. Histopathological study of pancreas

Photomicrographs of hematoxylin and eosin stained sections of the pancreas and average islet diameter are grouped in Fig. 4. Photomicrographs of the pancreas from normal control group showed normal architecture with normal acini and a normal population of the islets of Langerhans containing α and β cells (A). Pancreas from alloxan untreated mice showed degeneration and necrosis of pancreatic tissue and exhibited damage to islets of Langerhans (B). The obtained results showed that administration of *C. europaea* methanolic extract attenuated the destructive effect of alloxan on pancreatic islets and induced a restoration of the normal cellular population of β-cells (C). Group treated with glibenclamide showed less restoration of cells of islets of Langerhans and partial regeneration of islets cells (D). The results of morphometric analysis of pancreas showed that alloxan administration induced a significant reduction in the islet dimension especially in diameter (231.94 ± 7.14 μm), compared to normal control group (446.17 ± 4.65 μm). Whereas the oral administration of *C. europaea* methanolic extract prevented significantly the alloxan-induced pancreas damages by preserving islet diameter (335.64 ± 32.73 μm), and this effect was more effective than those of glibenclamide with a islet diameter of (244.44 ± 33.26 μm).

4. Discussion

C. europaea has been used traditionally in Moroccan folk medicine to treat several diseases including cyst, goiter, and diabetes mellitus (Bellakhdar, 1997). The present study was designed, mainly, to evaluate the potential and mechanism of the antidiabetic activity of *C. europaea* methanolic extract in alloxan-induced diabetic mice.

The phytochemical screening of the aerial parts of *C. europaea* showed that the plant is rich in tannins, flavonoïds, terpenes and steroids followed by a quantification of phenolic compounds and revealed significant levels of polyphenols in the CEME. The high performance liquid chromatography analysis of phenolic compounds revealed the presence of six phenolic compounds: catechin, quercetine, rutin, ferulic acid, epigallocatechin and 3,4 dihydroxybenzoic acid, with the prevalence of quercetin and ferulic acid. The antioxidant activity of CEME was assessed by three complementary *in vitro* antioxidant assays and showed that the extract exhibited a significant antioxidant property.

Acute oral toxicity study revealed the non-toxic nature of the methanol extract of *C. europaea*. There was no lethality or any toxic reactions found with the selected doses until the end of the study period. The LD_{50} value of CEME was inferred to be greater than 2000 mg/kg.

The difference observed between the initial and final blood glucose levels of CEME treated hyperglycemic mice revealed that the oral administration of CEME prevented the diabetogenic effect of alloxan and showed the hypoglycemic effect of CEME throughout the period of study. CEME attenuated pancreatic islets histological changes and decreased significantly the blood glucose levels for diabetic animals. The marked antidiabetic effect could be observed up to 120 min after the administration of the extract and during the period of treatment, this further indicates that CEME is effective in controlling the elevated blood glucose levels. The *C. europaea* hypoglycemic effect was comparable and sometimes higher than that obtained with the reference standard, glibenclamide.

Alloxan is an unstable organic compound, widely used in studies of experimental diabetes. Alloxan is well-known to destroy the beta cells of the pancreas and causes hyperglycemia in mice (Szkudelski, 2001) and also it is selectively toxic for pancreatic β-cells of the islets of Langerhans by induction of necrosis (Etuk, 2010). Alloxan is a cytotoxic glucose analog which presents a molecular shape analogy with glucose (Berraaouan et al., 2015) and has two distinct pathological effects: Selective inhibition of glucose-induced insulin secretion through specific inhibition of glucokinase, and generation of free radicals.

The mechanism for alloxan induced diabetes is that free radicals generated by alloxan initiates damage that leads to the β-cells death; Alloxan infiltrated to the pancreatic β-cells through the GLUT2 transporter (Elsner et al., 2002), in cytosol, alloxan is reduced in the presence of different cellular reducing agents to dialuric acid. The reduction of alloxan leads to the establishment of redox cycle and production of superoxide radicals ($O^{\cdot}$), then the dismutation of this superoxide radicals induces a generation of hydrogen peroxide (H_2O_2), which reacts with ferrous (Fe^{2+}) to produce hydroxyl radical ($OH^{\cdot}$), a high oxidative agent (Szkudelski, 2001). Oxidative agents provoke necrosis in pancreatic β-cells which lead to the diabetes mellitus type 1 (Lenzen et al., 1996).

It is previously reported that methanolic extract of aerial parts of *C. tuberculata*, another species of the same genus *Caralluma*, exhibited an interesting hypoglycemic activity in streptozotocin induced-diabetic rats (Abdel-Sattar et al., 2013). The hypoglycemic activity of *C. tuberculata* has been attributed to its nonacetylated and acetylated pregnane glycosides (Ahmad et al., 1988; Wadood et al., 1989; Abdel-Sattar et al., 2008). As *C. europaea* is closely related to *C. tuberculata*, pregnane glycosides-like compounds may be present in the aerial parts of *C. europaea* and showing the hypoglycemic activity.

The phytochemical screening of *C. europaea* extract and the quantification of phenols revealed the presence of phenolic compounds. A positive correlation was found between antioxidant property and polyphenols. In fact, phenolic compounds are well known to possess a high antioxidant activity (Villano et al., 2007). CEME exhibited significant radical scavenging activity. Therefore, the hypoglycemic effect was possibly due to the presence of antioxidant compounds in the tested extract, antioxidant agents act by inhibiting the production of free radicals induced by alloxan injection. Also, the phenolic compounds are known to help in the regulation of blood glucose concentration (Olmedilla et al., 1997) and to possess marked antidiabetic activity (Kumari et al., 2012). The phytochemical studies carried out on the extract revealed the presence of flavonoïds, and the flavonoïds are well known to regenerate the pancreatic beta cells in alloxan diabetic animals (Chakkravarthy et al., 1980). The HPLC analysis of phenols showed that ferulic acid was the main simple phenolic compound identified in the extract followed by quercetin. The hypoglycemic activity of the studied extract may be attributed to the presence of ferulic acid. In fact, many studies have shown the significant antidiabetic effect of ferulic acid in experimental diabetes (Ohnishi et al., 2004; Choi et al., 2001; Narasimhan et al., 2015). Moreover, quercetin was previously reported to possess antidiabetic potential (Vessal et al., 2003; Hatware and Annapurna, 2014). Furthermore, it has been shown in previous studies that phenolic compounds could also involve in the protecting action against alloxan cytotoxicity (Kumari et al., 2012; Berraaouan et al., 2013; Kumar et al., 2015). Phenolic compounds in the methanolic extract act by inhibiting the lipid peroxidation in brown adipose tissue of diabetic mice, by increasing the insulin sensitivity in diabetic animals, and by their protective and therapeutic effects on diabetes by reducing oxidative stress (Ohnishi

et al., 2004; Choi et al., 2001). Also these compounds with their antioxidant properties participate in the regeneration of the pancreactic islets of Langerhans in diabetic animals (Vessal et al., 2003). Furthermore, it was previously reported that polyphenols shows their effect either by the insulinomimetic activity and increasing the insulin secretion (Hatware and Annapurna, 2014). It is also interesting to note that the total antidiabetic activity is not necessarily due to the compounds that are present in highest concentrations; less abundant compounds should also be considered. Thus, the significant antidiabetic activity of CEME in this study may be attributed to the synergism between their antioxidant compounds in quenching free radicals and in protecting β-cells of the islets of Langerhans against damage and necrosis.

5. Conclusion and future recommendation

Data provided through the present investigation sustained an obvious and potent anti-hyperglycemic activity of *C. europaea* extract at a dose of 500 mg/Kg in alloxan-induced diabetes in mice. Such effect was concomitant with an enhanced histological features of pancreatic lesions, leading to a possible pancreatoprotective potential of the plant, and therefore, may be recommended as a new therapeutic agent for diabetes management.

Further studies are needed to assess the antidiabetic effect of CEME in a long term, to evaluate this activity in other diabetes mellitus models and to identify the active principle(s) responsible for the hypoglycemic effect and evaluate its mechanism of action.

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Authors' contribution

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Conflict of interest

There is no conflict of interest.

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