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Original Research Article

Assessment of organ weight changes in normal and streptozotocin-induced diabetic rats treated with *Olea europaea* L. leaf extract

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Abstract

Purpose: *To evaluate the antidiabetic activity of crude extract of Olea europaea L. (CEOE) leaves in streptozotocin induced-diabetic rats and also to determine its tannin content.*
Methods: *Thirty (30) male, normoglycemic Wistar rats (170 - 250 g) were randomly divided into five groups of six rats each. Group 1, which served as the normal control group, received distilled water while groups 2 and 3, were administered 200 and 600 mg/kg of CEOE extract daily for 18 days, respectively. Group 4 was given glibenclamide (Glibil; 3 mg/kg) while group 5 served as the untreated diabetic control group. Diabetes was induced with a single intraperitoneal injection of streptozotocin (50 mg/kg) and rats with blood glucose levels ≥ 250 mg/dL were confirmed to be diabetic. The blood glucose concentration, water and feed intake, tannin content of CEOE as well as the weight of organs were determined.*
Results: *The results showed that O. europea contained a large amount of tannins (806.22 ± 0.036 µg Tannic Acid Equivalent/g Extract). Administration of the extract (200 and 600 mg/kg) significantly decreased polydipsia and polyphagia, and reversed weight loss in rats with diabetes ($p < 0.05$ and $p < 0.01$, respectively) in comparison with glibenclamide.*
Conclusion: *The antidiabetic activity of the crude extract of O. europea was higher than that observed with glibenclamide, thus validating the folkloric use of this plant in diabetes care. Further investigation of Olea europea, including thorough chemical and pharmacological studies, are required.*

Keywords: *Antidiabetic activity, Olea europea L, Polydipsia, Polyphagia, Tannins*

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INTRODUCTION

Diabetes mellitus is a metabolic disorder also known as chronic hyperglycemia. The predominant feature of this disorder is the partial or total lack of insulin. It presents several complications such as neuropathy, nephropathy, retinopathy and increased risk of cardiovascular disease [1]. The outgrowths of these complications are determined by different factors including insulin resistance, hyperglycemia, obesity and hyperlipidemia [2]. Because the majority of currently accessible medications have been derived from plants, they have long been

thought of as a source of drugs. A report containing ethnobotanical knowledge stated that over 800 plants may possess anti-diabetic properties [3]. For thousands of years, plants especially herb leaves have formed the basis of folk medicine and are mainly used for alimentary purposes [4]. Leaves of *Olea europaea L.* (Olive, zitoune; family: *Oleaceae*) have been widely used in many countries (European and Mediterranean) for traditional therapy. Herbal teas, extracts and powder of this plant have been used by human in their diet. Growing interest in biologically active substances from the leaves of olive, that play a major role in the treatment of illnesses have been observed in the past few years [5]. The plant possesses antioxidant, hypoglyceamic, antihypertensive, anti-inflammatory, anti-atherogenic and hypocholesterolemic potentials due to the presence of several potentially bioactive compounds including polyphenols, flavonoids and phenolic acids [6]. Furthermore, the plant has also been reported to be effective in the management of non-insulin-dependent (NIDDM) diabetes mellitus [7,8].

The focus of recent studies has been on elucidating the effect of natural products, particularly phenolic compounds, on a variety of diseases including diabetes, considering their potential biological activities as antioxidant and anti-inflammatory agents [9]. This study therefore explores the possibility of utilizing the crude extract of *Olea europea* in managing polydipsia and polyphagia in diabetic rats and also to ascertain the effect of its tannins content on its antidiabetic properties.

EXPERIMENTAL

Plant material

Olea europea L. leaves were collected from Ras-El-Oued (Setif) region in Eastern Algeria (35° 56′ 59″ North, 5° 02′ 09″ East) during April, 2014. The plant was authenticated by Pr. Laouer Hocine (Ferhat Abbass University, Setif-1, Algeria). A voucher specimen was placed in the Faculty Herbarium with the number Oo20bd. The leaves were air-dried in a shade and subsequently ground to a powdered form.

Animal handling and ethical approval

Thirty (30) adult male albino rats (170 - 280 g) were purchased from the Pasteur Institute (Algiers, Algeria). They were housed in an animal room with air conditioning (25 ± 2 °C) and 12/12 hours of light / dark cycle. Prior to experiments, they were provided with free access to food and water for 7 days.

The study involving the use of animals adhered to ethical guidelines outlined in the Council for International Organizations of Medical Sciences (CIOMS). Approval for these protocols was granted in line with ethical health research standards as stipulated by the Algerian Executive Directive (no. 10–90 JORA, dated 18th March 2004) and further complies with the provisions of Law No. 88 − 08 issued on 26th January 1988, addressing veterinary medicine activities and the safeguarding of animal health (approval no. JORA: 004 of 27-01-1988).

Preparation of crude extract

Phenolic compounds extraction from *Olea europea* leaves was done according to [10], with slight modification. A portion (100 g) of powdered plant was mixed with one liter of 85 % methanol and kept for 3 days. The solution was filtered and the residue further subjected to a second extraction, this time utilizing one liter of 50 % methanol for a full day. The filtrate from both extraction steps were concentrated and subsequently evaporated using a rotary evaporator to produce a crude methanol extract of *Olea europea* (CEOE) at 40 °C and at reduced pressure. The extract was then stored at -20 ° C until used.

Induction of diabetes and experimental design

The antidiabetic activity of *Olea europea* crude extract was determined following the method used by [12] with slight modifications. To induce diabetes, rats were first fasted overnight and administered a single intraperitoneal injection of freshly-prepared streptozotocin (STZ) solution (50 mg/kg in cold sodium nitrate (0.9 %).

Rats with blood glucose levels ≥ 250 mg/dL were confirmed to be diabetic and randomly divided into five groups of six rats as follows: Group 1 (non-diabetic) served as the control group and received distilled water (1 mL/kg); Groups 2 and 3 were diabetic rats treated with 200 and 600 mg/kg of *Olea europea* crude extract (CEOE), respectively; Group 4 was also diabetic and treated with Glibenclamide (Glibil; 3 mg/kg) as reference drug and Groups 5 was untreated diabetic group. After 18 days of oral daily treatment, the animals were sacrificed and their organs were removed and measured. During the experiment, food and water intake were also monitored.

Determination of blood glucose levels

Blood glucose levels was determined using a reflecting glucometer (ACCU-CHEK, Fast Clix, Germany) with strict adherence to the manufacturer's instructions.

Determination of tannins content

The hemoglobin precipitating ability was determined by using bovine fresh blood according to the method described by [11]. In brief, a quantity of plant extract was diluted to yield a total polyphenol concentration of roughly 500 µg/mL and then combined with an equivalent volume of hemolyzed sheep blood sourced from a slaughterhouse (absorbance equivalent to 1.6). The solution was centrifuged for 20 min and the absorbance of the resulting supernatant read at 576 nm. Finally, precipitation efficiency was determined and expressed as µg tannic acid equivalent/g extract.

Statistical analysis

Data analyses were performed using GraphPad Prism version 5.00 statistical software. The *in vitro* results were expressed as mean ± standard deviation (SD), whereas the *in vivo* results were expressed as mean ± standard error of the mean (SEM). Tukey's test was conducted after analysis of variance (one-way ANOVA) to ascertain differences between groups. Values of $p < 0.05$ were considered statistically significant.

RESULTS

Tannin contents

Tannins could precipitate proteins that bind phenolic acid, therefore, the test of hemoglobin precipitation by the extract was used. The tannin content was 812 ± 0.009 mg TAE/mg extract with a yield of 22.73 %.

Blood glucose levels

Twenty-four hours after administration of STZ, blood glucose level was determined. Animals whose blood glucose levels were < 250 were excluded. Administration of STZ significantly ($p < 0.05$) increased the levels of blood glucose in the rats in Groups 2 – 5.

Administration of the extract reduced the glucose concentration to comparable levels with the positive control, Glibill.

Water and food intake

In the course of the experiment, the diabetic rats (treated and the untreated groups) showed polydipsia and polyphagia but at different degrees. The result shows a significant increase in water and food intake for the untreated diabetic group with 1070 ± 10 mL/day and 88.83 ± 0.97 g/day, respectively.

Whereas, in the normal group, these values were 496.6 ± 5.77 mL/day and 72.28 ± 1.27 g/day, respectively. The administration of 200 mg/kg and 600 mg/kg of CEOE to diabetic rats produced no significant difference in polydipsia (573.3 ± 15.07 mL/day; 650 ± 11.53 mL/day, respectively) and polyphagia (74.13 ± 0.87 g/day; 76.60 ± 0.60 g/day, respectively) compared with the normal group.

Furthermore, the rats administered the reference drug showed no significant difference in the water intake compared to the control (Table 1).

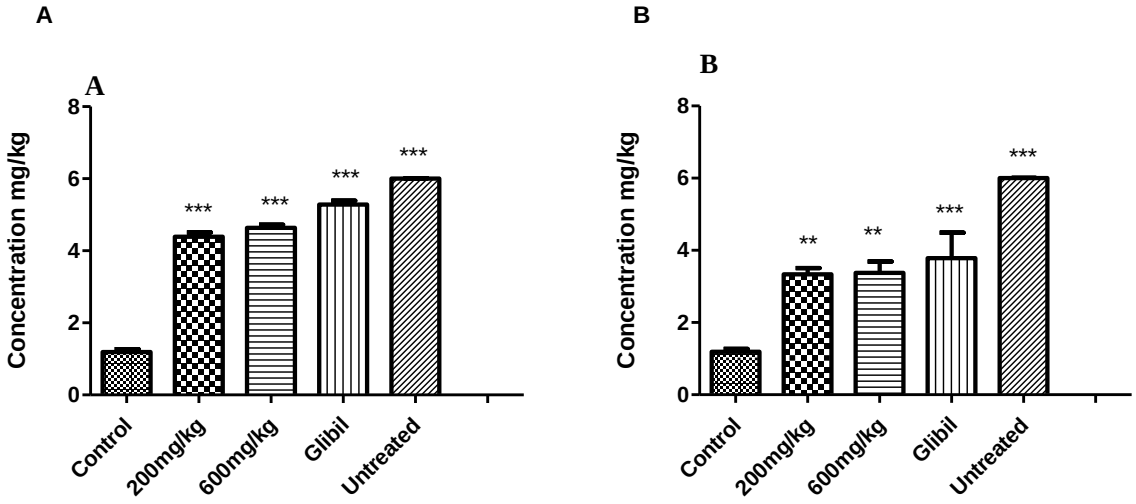

Figure 1: Effect of the crude extract of *O. Europea* on glucose level of rats (A) Pre-treatment levels and (B) Post-treatment levels. Values are given as means ± SEM (n = 6). *** $p < 0.001$; ** $p < 0.01$ vs control

Table 1: Effects of CEOE administration on water and food intake of streptozotocin-induced diabetic rats

Index	Control	CEOE (200 mg/kg)	CEOE (600 mg/kg)	Glibil (3mg/kg)	Untreated diabetic rat
Water intake (mL/day)	496.6±5.77	573.3±15.07^{ns}	650±11.53^{ns}	715±50^{ns}	1070±10*
Food intake (g/day)	72.28±1.27	74.13±0.87^{ns}	76.60±0.60^{ns}	88.83±0.97**	108.7±0.7***

Note: Values are presented as mean ± SEM for six rats in each group. Ns: no significant difference; *p < 0.05; **p < 0.01; ***p < 0.001 vs control

Table 2: Organs weight changes in streptozotocin-induced diabetic rats

Organ	Control	CEOE (200 mg/kg)	CEOE (600 mg/kg)	Glibil (3 mg/kg)	Untreated diabetic rats
Heart	0.88±0.16	0.72±0.02^{ns}	0.68±0.04^{ns}	0.67±0.11*	0.65±0.03*
Lungs	1.68±0.13	1.4±0.15^{ns}	1.48±0.13^{ns}	1.27±0.11*	1.23±0.03*
Liver	5.06±0.42	4.36±0.54*	4.40±0.59^{ns}	4.53±1.05**	4.15±0.70**
Testicles	3.14±0.22	2.44±0.39^{ns}	2.81±0.28^{ns}	2.9±0.37^{ns}	2.43±0.05^{ns}
Kidneys	1.80±0.06	1.56±0.02*	1.64±0.03^{ns}	1.61±0.06^{ns}	1.5±0.14***
Spleen	1.02±0.10	0.70±0.04*	0.72±0.08*	0.6±0.01**	0.61±0.07***
Stomach	1.55±0.19	1.59±0.03^{ns}	1.52±0.02^{ns}	1.43±0.13^{ns}	1.40±0.10^{ns}
Pancreas	0.25±0.03	0.16±0.02*	0.18±0.02^{ns}	0.14±0.02**	0.12±0.02***

Values are presented as mean ± SEM for six rats in each group. Ns: no significant difference; *p < 0.05; **p < 0.01; ***p < 0.001 vs control

Table 3: Rat organ weight (g) compared to animal weight in kilograms (kg) for treated and untreated diabetic rats

Parameter	Control	CEOE (200 mg/kg)	CEOE (600 mg/kg)	Glibil (3 mg/kg)	Untreated diabetic rats
Body weight (g)	257.14±13.16	220.2±13.18	223.32±7.74	226.87±8.12	202.40±2.68
Liver (g/kg)	19.67±5.06	19.80±4.12*	19.70±4.40^{ns}	19.76±4.33**	20.50±4.15***
Kidneys (g/kg)	7±1.80	7.08±1.56^{ns}	7.34±1.64**	7.09±1.53^{ns}	7.41±1.50***
Pancreas (g/kg)	0.97±0.25	0.72±0.16**	0.80±0.18^{ns}	0.61±0.14**	0.59±0.12***

Values are presented as mean ± SEM for six rats in each group. Ns: no significant difference; *p < 0.05; **p < 0.01; ***p < 0.001 vs control

Effect of *Olea europea* crude extract on organ weight

As shown in Table 2, oral administration of two different doses of CEOE resulted in a significant difference (p < 0.05) in the weight of the liver, kidney and pancreas compared to the control, whereas no significant difference (p > 0.05) was observed in the heart, lungs, testicles and stomach compared to the control. Also, statistically significant difference (p < 0.05) was observed in the weight of the spleen compared to the control group. On the other hand, the weight of all organs except testicles and stomach in the untreated diabetic group showed significant differences (p < 0.001) compared to the control. Furthermore, administration of the reference drug (Glibil) showed protection of the kidney with an organ weight change of 1.61 ± 0.06 g. Also, the kidney and liver weights were significantly increased in untreated diabetic rats (7.41 g/kg BW; 20.50 g/ kg BW, respectively) when compared with normal rats of the control group (7 g/kg BW; 19.67g / kg BW, respectively).

In addition, administration of low dose of *Olea europea* crude extract (200 mg/kg) showed maximum protection on the organ weight in treated diabetic rats. Furthermore, Glibil and *Olea europea* crude extract (600 mg/kg) had similar effects on the liver weight (19.70 g/kg and 19.76 g/kg, respectively) as shown in Table 3.

DISCUSSION

The effect of administration of the crude extract of *Olea europea* on some parameters in STZ-induced diabetes in rats were evaluated in this study. Streptozotocin (STZ) is a natural chemical extracted from *Streptomyces achromogenes* which toxic to pancreatic beta cells in mammals. Due to its structural similarity with glucose, it is rapidly transported into the beta cells as a result of the presence of high concentration of glucose transporter 2 (GLUT2) in these cells. It has a similar structure with glucose so its uptake into beta cells because of the presence of high concentration of and it is considered as a nitrosourea alkylating agent [13].

In the present study, diabetes was induced by a single intraperitoneal administration of

streptozotocin (50 mg/kg body weight). This dose was effective in inducing extreme hyperglyceamia in the experimental animals in agreement with [14,15]. The use of higher doses of streptozotocin in inducing hyperglyceamia in rats have been reported but this usually leads to animal mortality [16]. This result from this study demonstrates that there is an association between the decrease in body weights and diabetes in streptozotocin (STZ)-induced diabetic rats. A similar study showed that the reduction in body weight observed in an uncontrolled diabetic may be as a result of protein degradation for use as a source of energy because of lack of carbohydrate [16].

The findings showed no improvement in pancreatic weight in relation to body weight in animals of the untreated diabetic and control group. Rats' body weight and pancreas weight both proportionately reduced in the untreated diabetic group. The decrease in pancreatic weight may be due to the selective disappearance of insulin-producing cells [14,15]. Also, there was an increase in kidney weight (hypertrophy) in proportion to body weight in the untreated diabetic rats compared with the control group even though the weight of untreated diabetic rats was decreased. Over expression of transforming growth factor (TGF) – beta 1 in the kidney, particularly in proximal convoluted tubules (PCT) cells and glomerular mesangial cells, is linked to the development of renal hypertrophy in insulin-dependent diabetic mellitus (IDDM) [5].

It has been shown that decreased activities of hepatic antioxidant enzymes, such as superoxide dismutase (SOD) and catalase (CAT) found in diabetic rats, were restored by the use of oleuropein and hydroxytyrosol, thereby attenuating the oxidative stress associated with diabetes [15]. In addition, previous studies have shown the anti-diabetic effect of *Olea europea* on body weight loss and polydipsia in streptozotocin-induced diabetic animals [17].

Tannins have also been described as anti-hyperglycemic agents in diabetic rats. Tannins as reported can delay the absorption of glucose from the intestine and the start of insulin-dependent diabetes mellitus by providing an insulin-like impact on sensitive tissues of insulin, which reduces glucose levels and regulates the oxidative environment of pancreatic cells [15]. In agreement with the present results, result shows the presence of tannins in *O. europea*. Tannins have also been shown to effectively reduce intestinal α-glucosidase activity, similar to synthetic inhibitors such as acarbose and voglibose, which are currently used to treat non-insulin-dependent diabetic mellitus [18]. Tea extract tannins have been shown to have insulin-stimulating characteristics and to control hepatic glucose production [15], which could aid diabetic patients. In addition, Tannins have recently been shown to improve glucose absorption and decrease adipogenesis, making them suitable medications for the treatment of non-insulin dependent diabetes mellitus [19].

CONCLUSION

The antidiabetic activity of the crude extract of *O. europea* is better than that observed with Glibenclamide, thus validating the common use of this plant in diabetes care. There is, however, a need for further investigation of this plant extract, including chemical and pharmacological studies.

DECLARATIONS

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Ethical approval

None provided.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. Bencheikh Dalila: Conceptualization, validation; visualization; formal analysis; writing-review and editing, writing-original draft. Seddik Khennouf:

Project administration; Validation; Visualization; Writing-review & editing. Saliha Dahamna: Conceptualization; visualization; writing-original draft; writing-review and editing.

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