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Original Article

HYPOGLYCEMIC AND HYPOLIPIDEMIC EFFECTS OF PHENOLIC OLIVE TREE EXTRACT IN STREPTOZOTOCIN DIABETIC RATS

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ABSTRACT

Objective: The aim of the present study was to determine the effects of an olive tree extract with high polyphenols content on blood glucose level and other related parameters in streptozotocin-induced diabetic rats.

Methods: Diabetes was induced in rats by intraperitoneal injection of streptozotocin (55 mg/kg bw). 72h after injection, rats with fasting blood glucose higher than 2 g/l were used for the experiments. Olive tree extract was administered for 28 d and blood glucose level was measured every 4 d. Total cholesterol, triglycerides, HDL-cholesterol, creatinine, urea, total protein, uric acid, aspartate aminotransferase and alanine aminotransferase levels, were determined at the end of the experiment.

Results: The oral administration of olive tree extract contributes to blood glucose level decreasing in diabetic rats group, which was significantly lower at 4th week compared to the diabetic control rats. Moreover, supplementation by olive tree extract decreased significantly ($p<0.05$) the values of total cholesterol, triglycerides, HDL-cholesterol, creatinine, urea, total protein, uric acid, aspartate aminotransferase and alanine aminotransferase resulting from damage caused by streptozotocin treatment. Beside this, significant reduce ($p<0.05$) in heart disease risk ratio was observed for treated group (4.1±0.14) compared to untreated group (7.64±0.36), which was quite similar to normal rats (4.50±0.36). Studied olive tree extract effects were similar to those of glibenclamide, a well-known antidiabetic drug.

Conclusion: Results herein obtained reveal the hypoglycemic effect of this olive tree extract, suggesting his potential use as a natural antidiabetic agent.

Keywords: Olive leaves, Olive fruit, Polyphenols, Antidiabetic, Streptozotocin, *In vivo*

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INTRODUCTION

There has been a tragic increase in diabetes mellitus across the world. Diabetes mellitus is caused by complete or partial deficiencies in insulin production and/or insulin action coupled with chronic hyperglycemia and metabolism disruption [1]. It is considered as one of the most important clinical risk factors involved in some disorders like nephropathy, retinopathy, neuropathy, and cardiovascular diseases, which its prevalence is predicted to be increased daily [2-6]. Therefore, it is great urgency to find better treatments and novel prevention strategies regarding this worldwide health problem. The most common treatment is insulin and drugs with hypoglycemic effects [7]. However, there is an increasing demand by patients to use natural products, due to the side effects associated with insulin and oral hypoglycemic agents [8, 9]. The study of such products may offer a natural alternative to diabetes management in the future.

Among the plants used for their antidiabetic effect, an olive tree (*Olea europeae*) is of paramount importance. Currently, the implication of the olive tree byproduct extracts in pharmacology and food industries is due to the presence of some important phenolic components. The olive tree has been recognized for a long time as a source of bioactive polyphenols, such as oleuropein, hydroxytyrosol, oleuropein aglycone, and tyrosol [10-12]. Furthermore, olive wastes are considered as a cheap raw material for extracting of high-added value products [13]. Several studies have shown that olive tree possessed a wide range of pharmacological and health-promoting properties including the reduction of coronary heart disease risk [14, 15], anti-inflammatory [16-20], antitumor, anti-proliferative [21, 22], antidiabetic [23-26], antibacterial and antifungal properties [27-30]. Many of these properties have been described as resulting from the antioxidant character of polyphenols [31]. Gonzalez *et al.* [1] have previously reported that olive polyphenols had an antihyperglycemic effect on diabetic rats, although the mechanism by which they attenuate hyperglycemia is still not well known. However, particular attention has been paid to hydroxytyrosol [32], which occurs naturally in olive byproducts. This *o*-diphenol, like the majority of the olive polyphenols such as tyrosol, has been proven to have significant antitumor, anti-proliferative [21, 22] and antiviral activities [33].

Since the waste of olive has been recommended in the literature [23-26], as a remedy for the treatment of diabetes, it was considered worthwhile to investigate the influence of an olive tree extract, administered orally during 4 w, in normal and streptozotocin-induced diabetic rats. For this purpose, serum glucose level, lipids, renal and hepatic profiles were measured during the current study.

MATERIALS AND METHODS

Plant material and chemicals

Streptozotocin; glucose; glibenclamide (glyburide) were purchased from Sigma-Aldrich (Paris, France). Glucometer (BIONIME blood glucose monitoring system right GM300) and strips (BIONIME blood glucose test strip rightet GS300) were purchased from Bionime distributor (Casablanca, Morocco).

The olive tree extract (OTE) was obtained from Moroccan olive fruits and leaves using an eco-extraction, free of chemical solvents or toxic additives, according to the previously described protocol [19]. OTE is marketed in the world as under the brand name OLIVIE FORCE/OLIVIE RICHE (see more in www.olivie.ma).

Animals

Male adult 'Wistar' rats (200-250 g) obtained from the animal breeding unit (located in the Faculty of Science Dhar El Mahraz-Fez–Morocco) were used in this study. The animals were housed in clean

plastic cages and maintained under environmentally controlled breeding room (temperature, 22±2 °C; humidity, 40±5%; 12 h dark/light cycle) and had free access to food and water. Housing conditions and *in vivo* experiments were approved according to the guidelines established by the European Union on Animal care (CEE Council 86/609). The animals were used after an acclimatization period of two weeks to the laboratory environment and fasted overnight before experiments.

Experimental design

Diabetes was induced in rats by single intraperitoneal injection of a freshly prepared solution of streptozotocin (55 mg/kg body weight) in citrate buffer 0.1M (pH 4.5). For three days, rats received 5% of glucose in the drinking water. 72h after injection, rats with fasting blood glucose higher than 2 g/l were used for the experiments and received daily the adequate treatment for a period of 4 w.

Rats were divided into four different groups (10 rats in each group n= 10). "Control rats": negative control rats were received orally 10 ml/kg of 0.9% NaCl solution; "Diabetic rats": positive control rats were administered orally 10 ml/kg of 0.9% NaCl solution; "Diabetic rats+glibenclamide": rats were treated by 0.3 mg/kg of glibenclamide and "Diabetic rats+OTE": rats received daily oral dose of 1 g/kg of OTE.

Biochemical analysis

During this study, blood glucose level was measured every 4 d, at the same time in the morning. Glycemia was measured in blood from the tail vein using the commercial glucometer.

After 28 d of treatments, bold samples were collected into heparinized tubes and centrifuged at 3000 tr/min for 10 min. Plasma samples were separated and transferred in Eppendorf tubes for analysis. Total cholesterol (Total CT), triglycerides (TG), high-density lipoproteins cholesterol (HDL-CT), creatinine, urea, total protein, uric acid, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels, were determined using commercial kits (Sigma-Aldrich; Paris, France) according to the manufacturer's guidelines. Low lipoproteins cholesterol (LDL-CT) level was calculated by the following Friedewald equation [34]:

$$\mathrm{LDL \cdot CT} = Total \cdot CT - HDL \cdot CT - \frac{TG}{5}$$

Statistical analysis

Statistical analyses were performed using GraphPad Prism software version 6.00 (GraphPad Inc., San Diego, California). Data were analyzed by analysis of variance (ANOVA Analysis of Variance). Values between groups were considered statistically significant for at $P<0.05$.

RESULTS

Blood glucose level

Results plotted in the graph of fig. 1 illustrates an OTE's effect on blood glucose through the period of treatment. The blood glucose levels of normal and diabetic (untreated) rats were between 0.9±0.11 and 4.5±0.62 g/l, and 1.1±0.10 and 5.23±0.66 g/l throughout the period of treatment. However, a significant decrease in blood glucose levels was observed in supplemented diabetic rats from the first intake of OTE (after 4 d). Diabetic rats in the treated (by OTE) group reach the normal blood glucose after 20 d of OTE administration (1 g/kg), while this level decreases to the same value of normal rats (~1 g/l) at the end of the study. Thus, the OTE was found to have a similar hypoglycemic effect to glibenclamide effect at the end of the study.

Lipid profile

Total cholesterol, triglycerides and HDL-cholesterol levels were measured at the end of the experiment; obtained results are showed in the graph of fig. 2.

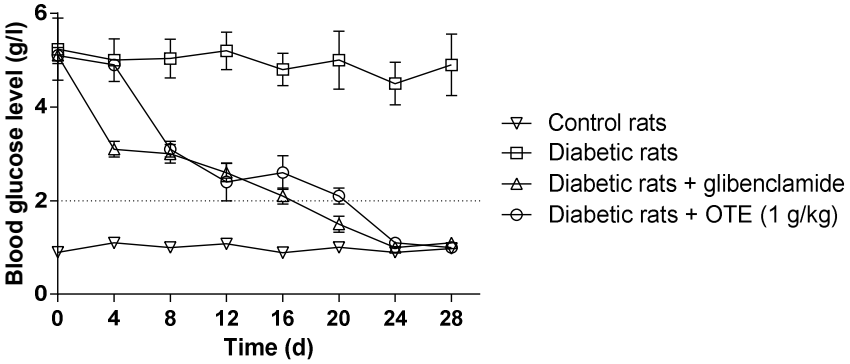

Fig. 1: Effect of OTE on blood glucose levels in diabetic and non-diabetic rats after 4 w of daily admission (n= 10)

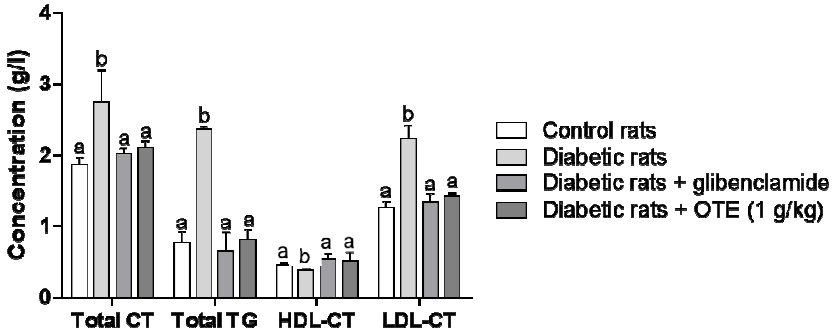

Fig. 2: Effect of OTE on total cholesterol (CT), triglycerides (TG), high-density lipoproteins cholesterol (HDL-CT) and low lipoproteins cholesterol (LD-CT) in diabetic and normal rats after 4 w of daily admission (n= 10). Different letters [a–b] indicate significant differences ($p<0.05$)

The analytical values of TC (2.75±0.44 g/l), TG (2.36±0.03 g/l) and LDL-CT (2.23±0.18 g/l) recorded in untreated diabetic rats showed a significant ($p<0.05$) increase at the end of the study compared to those of rats in control or treated groups (fig. 2). However, the values of TC, TG and LDL-CT drop significantly ($p<0.05$) to 2.1±0.01, 0.81±0.14 and 1.43±0.04 g/l, respectively, in the blood of rats supplemented by 1 g/kg of studied olive tree extract. As a net result, OTE administration was able to restore the lipid profile and correct the hypercholesterolemia associated with hyperglycemia. Nevertheless, OTE administration had no significant effect on HDL-CT of treated diabetic rats (0.51±0.12 g/l) compared to control rats (0.45±0.04 g/l) or diabetic rats treated by glibenclamide (0.54±0.07 g/l).

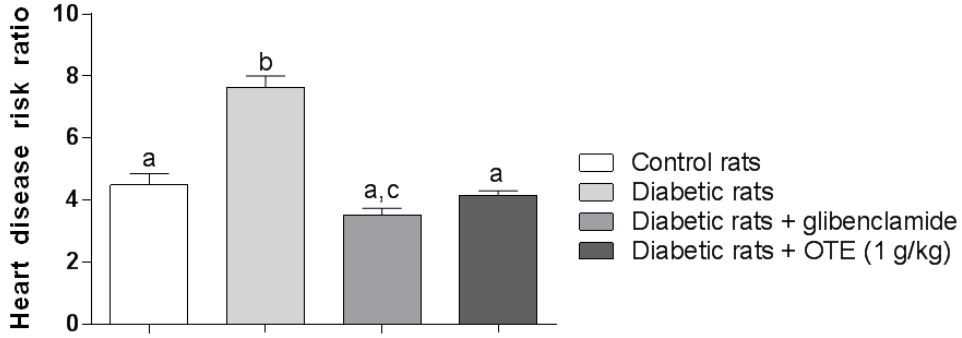

Fig. 3: Heart disease risk ratio in diabetic and normal rats after 4 w of OTE daily admission (n=10). Different letters [a–b] indicate significant differences ($p<0.05$)

On the other hand, the overall improvements in the blood lipid profile of rats treated by OTE had positively influenced heart disease risk ratio parameter (Total CT/HDL-CT) [35]. In this sense, results of fig. 3 show a significant difference ($p<0.05$) in favor of rats treated by OTE (4.1±0.14) and glibenclamide (3.53±0.21) compared to untreated diabetic rats (7.64±0.36). We should also underline that the heart disease risk parameter of rats supplemented for 28 d by the studied olive tree extract was quite similar to that of control animals (4.50±0.36).

Renal function tests

Table 1 shows the effect of OTE on creatinine, urea, total protein and uric acid in rats allocated to the study groups. The results showed that creatinine, urea, and uric acid increased in streptozotocin-induced diabetic rats compared with control rats ($p<0.05$).

However, OTE and glibenclamide administration have significantly ($p<0.05$) stabilized these parameters at the normal values compared to rats in control group. These results suggest, in fact, that supplementation by OTE has an appropriate regulating effect directed to each of the biochemical parameters associated with hyperglycemia. Moreover, serum levels of AST and ALT (and AST/ALT ratio) are commonly measured clinically as biomarkers for liver health. Results showed in table 2 (combined with those of table 1) indicate that OTE administration had no adverse effect on renal function of treated rats.

Table 1: Effect of OTE on Creatinine, urea, total protein and uric acid in diabetic rats after 4 w of daily administration

	Control rats	Diabetic rats	Diabetic rats+glibenclamide	Diabetic rats+OTE (1 g/kg)
Creatinine (mg/l)	6±1 [a]	11±1.73 [b]	6±1[a]	6±1[a]
Urea (g/l)	0.28±0.03[a]	0.45±0.06[b]	0.22±0.05[c]	0.29±0.03[a]
Total proteins (g/l)	74±4.36[a]	59±3.46[b]	77±3.60[a]	76±2[a]
Uric acid (mg/l)	40±2[a]	72±1[b]	44±3.60[a]	45±4[a]

[a–c]Values in the same row for each rats group with different superscripts are significantly different ($p<0.05$) [mean±SD, n= 10].

Table 2: Effect of OTE on aspartate aminotransferase (AST) and alanine aminotransferase (ALT) in diabetic rats after 4 w of daily administration

	Control rats	Diabetic rats	Diabetic rats+glibenclamide	Diabetic rats+OTE (1 g. kg^{-1})
AST (UI/l)	22±2.64[a]	86±1.73[b]	31±4[c]	36±2.64[c]
ALT (UI/l)	28±2.28[a]	90±5.56[b]	35±4.58[a]	40±3.60[a]

[a–c]Values in the same row for each rats group with different superscripts are significantly different ($p<0.05$) [mean±SD, n= 10].

DISCUSSION

Streptozotocin is the most prominent diabetogenic chemical agent in diabetes research. In 1963, Rakieten *et al.* reported that streptozotocin is diabetogenic [36]. Since then, it has been chosen for diabetes mellitus induction in animal models [37]. Actually, streptozotocin inhibits insulin secretion and causes a state of insulin-dependent diabetes mellitus, due to its specific chemical properties, namely its alkylating potency [37]. Streptozotocin has been known to cause specific necrosis of the pancreatic beta cells, which is similar to the feature of the later stage of type 2 diabetes [38, 39] Results of this study showed that blood glucose of diabetic rats increases significantly three days after the intraperitoneal streptozotocin injection. This was similar with the researches that have been done throughout the world for diabetes induction [37, 40, 41].

A significant decrease in blood glucose of diabetic rats treated by OTE –compared with that of the diabetic rats– was observed from the first day of the study to the 28 d (first OTE intake). The OTE was found to have a similar hypoglycemic effect to glibenclamide at the end of the study. The OTE's hypoglycemic activity may result from two mechanisms: (i) potentiation of glucose-induced insulin release, and (ii) increasing peripheral uptake of glucose [1]. Beside this, OTE –like olive leaf extract– might produce its hypoglycemic effect is

through the inhibition of pancreatin amylase activity [24]. In this sense, olive leaf extract was found to inhibit the activities of α-amylases from human saliva and pancreas [24]. In Animal models studies, the hypoglycemic effect of OTE could be facilitated by the reduction of starch digestion and absorption. Moreover, hydroxytyrosol, oleuropein and their Seco iridoids derivatives –major phenolic compounds of OTE [42] – had a hypoglycemic and antioxidant *in vitro* and in rats [23]. These compounds may (i) protect pancreatic cells from progressive damage caused by streptozotocin, (ii) enhance insulin secretion by several mechanisms [43], (iii) active some enzymes – hexokinase and pyruvate kinase– implicated in glucose metabolism [23], and (iv) protect pancreatic cells from oxidative damage –through their strong antioxidant activity– caused by the increase of insulin secretion [44].

The lipid profile levels are usually raised in diabetics, which represents a risk factor for coronary heart disease [45]. It has been showed that high levels of total cholesterol and LDL-cholesterol are cardiovascular risk factors. However, increased level of HDL-cholesterol assured anti-inflammatory properties [46]. The present results showed that OTE exhibited a significant decrease in the level of lipid parameters in diabetic rats. OTE improves lipid profile because of high concentration of phenolic compounds having a lipid lowering action and prevented LDL-cholesterol oxidation. Epidemiological studies also suggested that the Mediterranean diet, rich in polyphenol, decreases the cardiovascular disease risk factors [47-49].

The total protein level was decreased in diabetic rats. This may lead to muscle wasting and an increased release of purine, the main source of uric acid as well as in the activity of xanthine oxidase. Moreover, the increase of uric acid level may be due to a metabolic disturbance in diabetes reflected in the high activities of xanthine oxidase, lipid peroxidation, and triglycerides and cholesterol increasing [50]. However, results herein presented show that the OTE decreases the creatinine, serum urea, and uric acid levels and increase total protein level in diabetic rats. In fact, elevation of the serum's urea and creatinine, as significant markers, are related to renal dysfunction in diabetic hyperglycemia [51].

Serum enzymes, including AST and ALT, are studied to evaluate the hepatic profile. An increase in these enzyme activities reflected liver damage. High transaminases levels are caused by hepatocellular inflammation [52]. Streptozotocin treatment has a significant role in the alteration of liver functions since the activity of AST and ALT were significantly higher than those of normal values. In the present study, a reduction in AST and ALT levels were found in diabetic rats treated by OTE. It may be the result of the olive polyphenols anti-inflammatory activity, which allowed OTE to regulate transaminases levels [19, 20].

By the end of the study period, all of the evaluated parameters in rats treated by OTE exhibited a significant restoration, which was similar to the normal control rats. In our previous studies, we found that OTE was endowed with important antioxidant and anti-inflammatory activities [19, 42]. These properties allow OTE to be efficient in the protection against some metabolic diseases related to oxidative stress such as diabetes. In fact, it has been demonstrated that antioxidant-based therapy is promising to minimize the complications associated with oxidative stress in diabetes mellitus [42, 53-56].

CONCLUSION

Based on the findings of this study, we demonstrate that olive tree extract with high polyphenols content has the same effects –in comparison with glibenclamide– regarding blood glucose and other related parameters regulation in diabetes illnesses. The current findings are in agreement with those obtained *in vitro* or *in vivo* in several pre-clinical and clinical studies about anti-diabetic effects of olive polyphenols and/or olive tree extracts, suggesting the potential role of these natural compounds for "functional foods" conception to help in diabetes management.

CONFLICTS OF INTERESTS

All authors have none to declare

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