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Anti-diabetic activity and oxidative stress improvement of Tunisian Gerboui olive leaves extract on alloxan induced diabetic rats

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- ✓ Diabetes,
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Abstract

The purpose of this investigation was to evaluate the antidiabetic effect and stress oxidant improvement of olive leaves ethanolic extract of Gerbou variety (100 mg/kg body weight) in alloxan-induced diabetic rats (alloxan, 120 mg/kg body weight, *ip*), during 21 days. Rats were made diabetic by a single intraperitoneal injection of alloxan monohydrate. Two rats group were treated by intraperitoneal injection of olive leaves extract once a day for 21 days. Effects of olive leave treatment on tolerance glucose, plasma parameters and oxidative stress markers were determined. The olive leaves ethanolic extract of Gerboua variety exhibited at termination, a significant reduction in blood glucose an increased plasma insulin levels and hepatic glycogen in diabetic rats. The extract also prevented body weight loss in diabetic rats. The plant extract tended to reduce plasma total proteins, cholesterol, triglycerides levels and Alanine Aminotransferase (ALT) and Aspartate Aminotransferase (AST) activities toward the normal levels. Four days before animal sacrifice, olive leaves extract improved glucose tolerance test after intraperitoneal injection of glucose (2 g/kg body weight) in diabetic rats. An amelioration effect was also observed in antioxidant state in liver and kidneys of diabetic rats treated with olive leaves extract. The ethanolic extract of olive leaves from Gerboua variety showed an antihyperglycemic activity on alloxan-diabetic rats and minimized the adverse effect of oxidative response.

1. Introduction

Diabetes mellitus is the most common serious metabolic disorder that is considered to be one of the five leading causes of death in the world. It is characterized by absolute or relative deficiencies in insulin secretion and/or insulin action associated with chronic hyperglycemia and disturbances of carbohydrate, lipid, and protein metabolism [1-3]. As a consequence of the metabolic derangements in diabetes, various complications develop, such as atherosclerosis, retinopathy, nephropathy, coronary artery disease, cerebral vascular disease, and peripheral artery disease [4]. Various studies have shown that diabetes is associated with increased formation of free radicals and decrease in potential antioxidant potential [5, 6]. Implication of oxidative stress in the pathogenesis of diabetes is suggested by increasing of reactive oxygen species (ROS) production, including superoxide radical ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2), and hydroxyl radical ($OH^{\bullet}$) [3,7] and also by non-enzymatic protein glycosylation, auto-oxidation of glucose, impaired antioxidant enzyme, and formation of peroxides [7, 5, 8]. Moreover, others studies indicated an increase in lipid peroxidation [9, 10] and alteration of the glutathione redox state [11]. The formation of ROS was prevented by an antioxidant system that included non-enzymatic antioxidants (ascorbic acid, glutathione, tocopherols), enzymes regenerating the reduced forms of antioxidants, and ROS–scavenging enzymes such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxides (GPx) [12, 13].

Recently, there has been a considerable interest in finding natural antioxidants from plant materials to replace synthetic ones. Many herbal medicines have been recommended for the treatment of diabetes [14, 15]. Hypoglycemic effects have been reported for some plants that contain terpenoids, iridoid glycosides, flavonoids, and other phenolic compounds [16]. In addition, a number of secondary metabolites like flavonoids, phenolic acids, phenylpropanoids, and terpenoids have shown significant antioxidant properties [17,18-20].

O. europaea is a member of the Oleaceae family. This is a medium-sized family comprising 600 species in 25 genera distributed on all continents, except the Antarctic, from northern temperate to southern subtropical regions and from low to high elevations [21]. Historically, olive leaves have been used as a folk remedy for combating diseases, such as malaria [22]. In fact, Olive tree leaves can be regarded as a particularly rich source of polyphenolic compounds, as their polyphenolic contents may reach up to 40 g per kg of dry tissue [23].

The predominant phenolic metabolite was shown to be the secoiridoid derivative oleuropein [24-26], but other polyphenols such as rutin, verbacoside, apigenin-7-glucoside and luteolin-7-glucoside [22, 26, 27]. Olive leave extracts have been shown to have anti-oxidative, anti-inflammatory and antimicrobial activities against bacteria and fungi [28-31]. Moreover, the olive leave extract exhibits anti-viral activities against several virus like haemorragic septicaemia rhabdovirus [32]. Somova et al. reported that oleuropein and its derivatives had various biochemical roles including hypotensive, coronary dilating antiarrhythmic action [33]. Furthermore, it was reported that these polyphenols could prevent low-density lipoprotein oxidation and platelet aggregation [34, 35].

The purpose of this investigation was to evaluate the antidiabetic effect and stress oxidant improvement of olive leaves ethanolic extract of Gerbou variety in normal and in experimentally induced diabetes rats.

2. Experimental

2.1 *Plant material*

Experiments were carried out on olive leaves of Gerboua variety which was originated from North West of Tunisia [36]. Leaves were collected on october 2010 (maturation period) in the National Agronomic Institute (Tunisia). Leaves were dried on site in a microwave oven (2500 MHz), three times for 2 min at maximum power. Dried leaves were powdered and stored in a dry place in the dark [31].

2.2 *Preparation of olive leaves extract*

A mixture of ethanol and water (20 ml, 70:30 (v/v)) was added to the olive leaves powdered (1 g). The mixture was left to stand for at least one week at room temperature in the dark. Subsequently, the solution was filtered using a 0.45 μm filter paper. The extract was dried at 45 °C in rotary evaporator to produce a semisolid mass and stored in airtight containers in refrigerator below 10 °C. The olive leaves extract of Gerboua variety contained 45.02 mg/g of dry leaves oleuropein as a major compound [31].

2.3. *Animals*

Adult male Wistar rats, weighing (150-250g), and obtained from SIPHAT Company, Tunisia, were employed in the study. The animals were kept in an environmentally controlled breeding room (temperature: 20± 2 °C, 12 h dark/light cycle). The rats were maintained on a standard chow diet and water *ad libitum*. The handling of the animals was performed in accordance of the International Guidelines regarding animal experiment.

2.4. *Induction of diabetes*

The overnight fasted rats were made diabetic by a single intraperitoneal injection of freshly prepared alloxan monohydrate (Sigma Aldrich Germany; 120 mg/kg i.p.) in sterile saline. 48 hours after Alloxan injection, rats with blood glucose level of >200 mg/dL were separated and used for the study. Blood glucose levels were measured using blood glucose test strips with elegance glucometer (Accu chek ®) twice per week until the end of study (i.e. 3 weeks). Blood glucose estimation and body weight measurement were done twice a week.

2.5. *Experimental design*

The rats were divided into three groups and for each group six animals and treated by intraperitoneal injection once a day for 21 days as follows:

Group I (n = 6). Normal healthy control: given only vehicle (saline 0,9 %).
Group II (n = 6). Diabetic control: given only vehicle (saline 0,9 %).
Group III (n = 6). Diabetic rats given olive leaves extract (100 mg/kg b.w.).

2.6. *Tolerance glucose test*

Four day after treatment period (three weeks), the glucose tolerance test was performed by intraperitoneal injection of glucose in the forme of solution (2g/kg) to three groups of rats fasted 4 hours [43]. Blood glucose levels from the tail vein were evaluated before the glucose injection and at 20, 40, 60, and 90 min after glucose injection using Glucometer.

2.7. *Analytical procedures*

Blood glucose was measured with elegance glucometer (Accu chek ®) twice a week i.e. 1, 4, 8, 11, 15, 18 and 21 day before daily administration of olive leaves extract (OLE) by intraperitoneal injection. After blood glucose estimation on day 21, the overnights fasted animals were sacrificed by decapitation, and the trunk blood was collected. The plasma was prepared by centrifugation (1500 g, 15 min, 4 °C) and the heart, the liver and kidneys were removed, cleaned of fat and weighed; all these samples were stored at -80 °C until used. Blood

glucose, Total cholesterol (TC), total protein, iron, Creatinine (SC), uric acid (SUA), alanine transaminase (ALT), aspartate transaminase (AST) and triglyceride (TG) standard kits were obtained from Biomaghreb (Tunisia) and serum insulin levels were determined using insulin ELISA kit purchased from Mercodia (Sweden). The lipid peroxidation in the testes of control and all treated groups of animals was measured by the quantification of thiobarbituric acid reactive substances (TBARS) determined by the method of Buege and Aust [37]. The activity of superoxide dismutase in control and treated rats was assayed by the spectrophotometric method of Sun and Zigman [38]. The activities of glutathione peroxidase and catalase were measured by the modified method of Flohe and Gunzler [39] and Aebi [40], respectively. The thiol disulfide status was determined by Faure and Lafond [41]. The level of total protein was determined by the method of Lowry et al. [42]. For histological studies, pieces of liver were fixed in formaldehyde (10%). Sections of 5-mm thickness were stained with hematoxyline-eosin and examined under light microscope.

2.8. *Statistical analysis*

Statistical analysis of data was performed using a one-way analysis of variance (ANOVA) for comparison between groups followed by the Fisher test. Values for $p < 0.05$ were considered statistically significant. The data are shown as mean±standard error of the mean (S.E.M).

3. Results and discussion

3. 1. *Effects of OLE on body weight and glucose metabolism*

The alloxane-induced diabetic rat is one of the animal models of human diabetes mellitus. Alloxan acts as diabetogenic by irreversible destruction of pancreatic ß-cells, causing a massive reduction in insulin release and inducing hyperglyceaemia [44]. Table 1 summarizes the changes in body weight, the levels of blood glucose and insulin, and hepatic glycogen concentration. There was a significant elevation in glucose 48 h after administration of alloxan. After 21 days, no significant change in glucose was noted in normal rats (Group I), while there was a significant elevation in glucose and decrease in insulin in alloxan-induced diabetic rats (Group II). Insulin deficiency can lead to various metabolic alterations in the diabetic rats [47-48]. The administration of OLE (Groups III) significantly decreased glucose and significantly increased insulin levels in diabetic rats as compared with diabetic control rats. The hepatic glycogen level of diabetic rats was significantly different from the non-diabetic rat ($p < 0.01$). In rats treated with OLE, a significant increase in hepatic glycogen level was observed ($p < 0.05$). In fact, the bioactive compounds present in the olive leaves extract like flavonoids were reported to stimulate insulin secretion by pancreatic beta-cells with numerous mechanisms such as exertion distal to K+-ATP channels and L-type Ca2+ channels [49], activation of the cAMP/PKA signaling [50], and antioxidant activities [51]. The other possible mechanism of action of olive leaves extract could be mediated through liver by affecting gluconeogenesis, glycogenesis or glycogenolysis. The table 1 showed the changes in body weight in normal and experimental rats. Treatment with alloxan (120 mg/kg, i.p) had significantly decreased the body weight at the end of 21st days as compared to normal animals. This decrease could be due to the loss or degradation of structural proteins that were known to contribute to the body weight and impairment in insulin action in the conversion of glucose into glycogen and catabolism of fats [45-46]. We noted an increase in body weight in OLE-treated groups. Repeated administration of OLE (100 mg/kg) had prevented the reduction in body weight on 21st day in diabetic rats.

3. 2. *Effect of OLE on blood glucose level in hyperglycaemic rats induced by ip injection of glucose*

Fig. 1 shows the blood glucose levels of normal control, diabetic control, and OLE-treated rats after intraperitoneal injection of glucose (2 g/kg body weight). In diabetic control rats, the peak increase in blood glucose concentration was observed after 20 min and remained high over the 90 min. OLE (100 mg/kg) caused a significant decrease in blood glucose concentration at 20 min after injection when compared to the diabetic control rats. Mean glucose concentration calculated from the area under the response curves revealed a significant increase in glucose concentration of untreated diabetic rats (35650 ± 196.29 mg/dL) as compared to control rats (12983.75 ± 456.67 mg/dL). Administration of the plant extract during glucose load markedly reduced glucose concentration 14535 ± 1460.69 mg/dL ($p < 0.05$) for the 100 mg/kg dose.

3. 3. *Effects of olive leave treatment on plasma parameters*

At the end of the experimental period, various plasma parameters were measured in all the groups. we noted, in untreated diabetic animals, lipid abnormalities such as hypertriglyceridemia and hypercholesterolemia. In addition, liver enzymes e.g. AST and ALT level increased which is responsible for the liver damage. We also observed impairment in renal function by increasing serum creatinine and uric acid (Table 1).

Treatment with olive leaves extract (100 mg/kg) significantly reduced plasma total proteins, ALAT, ASAT levels as compared with untreated diabetic rats. Olive leaves extract exhibited hypolipidemic effect that may be due to decreased cholesterolgenesis and fatty acid synthesis [52]. Plasma iron levels were significantly decreased in untreated diabetic animals as compared to the control rats. The administration of olive leaves extract (100 mg/kg) increase iron levels significantly.

Table 1. Effect of OLE on rat body weight, metabolism glucose, plasma total proteins, cholesterol, triglycerides, ALAT, ASAT, uric acid, creatinine and iron levels in alloxan diabetic rats after 3 weeks of treatment.

		Normal	Diabetic control	Diabtic+OLE
Glucose (g/l)	Initial	1.15 ± 0.01	4.28 ± 0.83**	4.35 ± 0.63
	Final	1.21 ± 0.20	2.91 ± 0.28*	1.86 ± 0.10§§
Insulin (µg/l)		1.62 ± 0.04	0.67 ± 0.09**	1.02 ± 0.11§
Hepatic glycogen (mg/g tissue		19.66 ± 0.52	12.82 ± 1.29 **	25.16 ± 2.53§
Change in body weight$^{\#}$		5.89 ± 0.80	-16.16 ± 6.60**	12.72 ± 5.95§
Total proteins (g/l)		67.54 ± 9.46	140.37 ± 3.93**	107.32 ± 1.09§
Cholesterol (g/l)		0.74 ± 0.05	1.26 ± 0.07*	0.95 ± 0.003§
Triglycerides (g/l)		0.64 ± 0.08	1.48 ± 0.12**	0.56 ± 0.06§§
ALAT (U/I)		54.83 ± 0.33	78.63 ± 3.88*	45.93 ± 5.25§§
ASAT (U/I)		118.22 ± 5.88	178.50 ± 11.11*	118.80 ± 4.69§
Uric acid (mg/l)		21.79 ± 2.02	48.60 ± 6.57*	28.39 ± 7.91
Creatinine (mg/l)		6.35 ± 0.24	13.86 ± 1.75**	11.78 ± 1.70
Iron (mg/l)		1.29 ± 0.18	0.46 ± 0.18*	4.52 ± 0.61§§

Values are given as mean ± S.E.M for six rats in ach group. *p<0.05 and **p<0.01 as compared with normal rats; §p<0.05 and §§p<0.01 as compared with Diabetic control rats. OLE: ethanolic extract of olive leaves. $^{\#}$: percentage change from the initial weight.

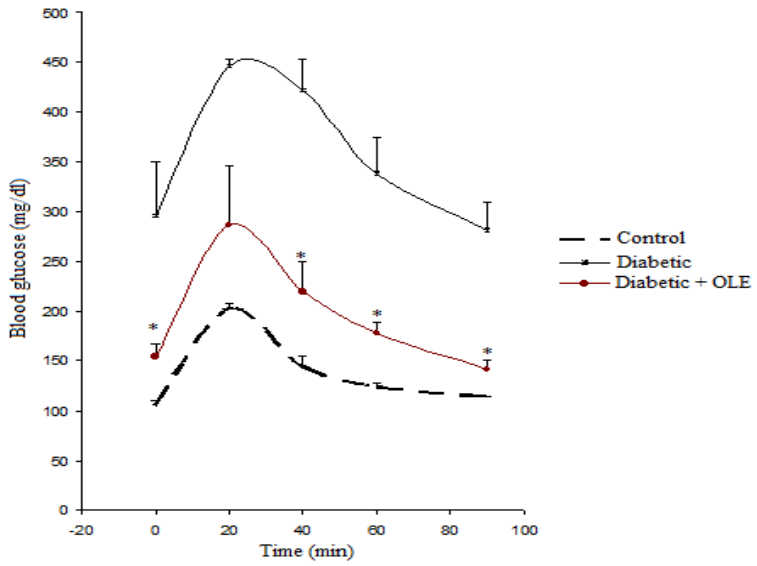

Figure 1. Effect of olive leaves extract on blood glucose level after intraperitoneal injection of glucose. Values are means±S.E.M., n=6. *p < 0.05 compared with untreated diabetic rats.

3. 4. *Effects of OLE on heart, liver and kidneys thiol groups, MDA, SOD, CAT and GPx*

Various studies have shown that diabetes is associated with increased formation of free radicals and decrease in antioxidant potential. Our data showed a decrease of SOD, CAT, and GPx activities and thiol groups amount in the liver and kidneys and an increase of MDA amount in both tissues in diabetic rats. The administration of olive leaves extract improved oxidative response. In fact, activity of SOD was restored in liver and kidneys. However, GPx activity was improved only in kidneys. Olive leaves extract increased thiol groups amount and decreased MDA content in liver and kidneys. The antioxidant effect of olive leaves extract could be explained by two mechanisms. Firstly, the olive leaves extract prevented against protein glycosylation and peroxydation by interaction with free radicals and minimised their damaging effects. Secondly, olive leaves extract induced protein synthesis of the antioxidant enzymes. In fact, previous studies reported that polyphenolic compounds increased enzyme expression of SOD and GPx in transcriptional level [53, 56].

Table 2. Thiol groups (nM/mg protein) and MDA (nM/mg protein) levels and enzyme activities of SOD (U/mg protein), CAT (U/mg protein/min) and GPx (U/mg protein/min) activities in liver and kidneys after 3 weeks of treatment in all groups.

Oxidative stress markers	Tissues	Groups		
		Normal	Diabetic control	Diabetic+OLE
Thiol group (nM/mg protein)	Liver	35.88 ± 5.70	11.99 ± 1.51*	27.66 ± 3.79§
	Kidneys	45.56 ± 8.17	25.19 ± 3.69	50.71 ± 5.18§
MDA (nM/mg protein	Liver	3.57 ± 0.11	5.48 ± 0.04***	3.07 ± 0.30§§
	Kidneys	2.22 ± 0.21	5.33 ± 0.36*	3.08 ± 0.27§
SOD (U/mg protein/min)	Liver	1.13 ± 0.08	0.44 ± 0.06***	1.36 ± 0.12§§§
	Kidneys	0.98 ± 0.09	0.49 ± 0.05*	1.33 ± 0.11§§
CAT (U/mg protein/min	Liver	61.12 ± 2.29	31.25 ± 5.81*	54.61 ± 15.12
	Kidneys	12.87 ± 0.56	9.20 ± 0.79*	9.21 ± 5.24
GPx (U/mg protein/min	Liver	7.41 ± 0.36	5.59 ± 0.27*	5.60 ± 1.14
	Kidneys	28.58 ± 4.45	15.46 ± 2.78**	22.97 ± 3.67§

Values are given as the mean ± S.E.M for groups of six animals each. Diabetic control rats were compared with normal rats *p<0.05, **p<0.01, ***p<0.001; OLE-treated diabetic rats were compared with diabetic control §p<0.05, §§p<0.01 and §§§p<0.001. OLE: ethanolic extract of olive leaves. One unit of superoxide dismutase (SOD) is defined as the amount of enzyme required to inhibit the epinephrine auto-oxidation. One unit of catalase (CAT) is defined as µmol of H_2O_2 decomposed min^{-1}. One unit of gluthatione peroxidase (GPx) is defined as 1 nmol GSH consumed min^{-1}.

3. 5. *Histological changes*

Histopathological examinations of liver showed normal hepatic cells with well preserved cytoplasm, nucleus, nucleolus (Figure 2A). In case of diabetic rat, hepatocytes were hypertrophied with clear cytoplasm after usual coloration (HE). The heptocytes have a vegetal cell shape, the enclaves were optically empty represented the steatosis which defined hepatic lesions (Figure 2B). The administration of OLE (100mg/kg) restored the damaged hepatic tissues (Figure 2C). In addition, olive leaves extract was non-toxic effect and regenerated the toxic effect of alloxan.

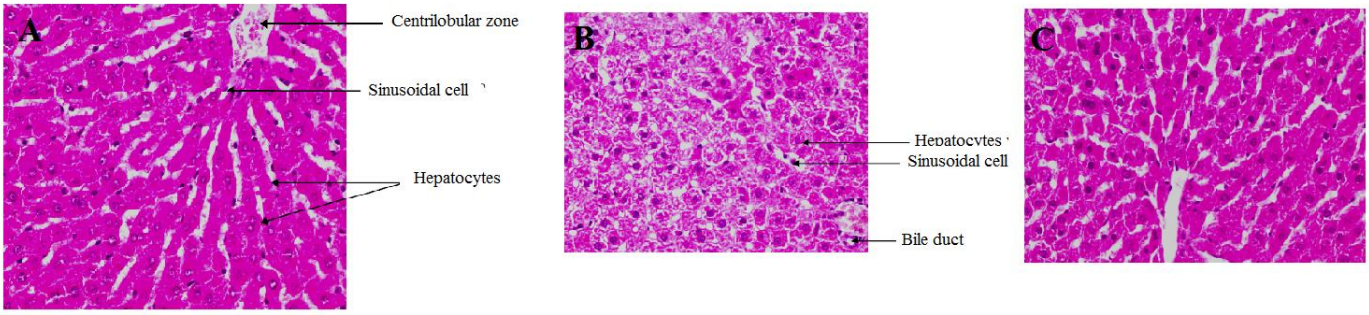

Figure 2. Effect of combined olive leaves extract on the histological morphology of rats' liver by HE staining (400×) (A: Control; B: diabetic rats; C: diabetic rats treated with olive leaves extract).

Conclusions

In conclusion, the present study showed that the antihyperglycemic action of the ethanolic extract of olive leaves from Gerboua variety on alloxan-diabetic rats was associated with an increased plasma insulin concentration and hepatic glycogen and improved tolerance glucose, and hepatic function markers and minimized the adverse effect of oxidative response.

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